UNIVERSITAT DE BARCELONA

p53 i p16 en el carcinoma escamós de penis. Correlació molecular i pronòstica

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p53 I p16 EN EL CARCINOMA ESCAMÓS DE PENIS.

CORRELACIÓ MOLECULAR I PRONÒSTICA.

Memòria de tesis doctoral presentada per Isabel Trias i Puig-Sureda

per optar al grau de doctor por la Universitat de Barcelona Dirigida per

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Agraïments

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Índex

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LLISTAT D'ABREVIATURES I ACRÒNIMS

ADN: Àcid desoxiribonucleic.

AJCC: American Joint Comittee on Cancer.

CEP: Carcinoma escamós de penis.

EAU: European Association of Urology.

HSIL: High Grade Squamous Intraepithelial Lesion

ISUP: International Society of Uropathology.

NIP: Neoplàsia intraepitelial del penis.

NIV: Neoplàsia intraepitelial de vulva.

NIVd: Neoplàsia intraepitelial de vulva diferenciada.

OMS: Organització mundial de la salut.

TNM: Tumor, Node, Metastasis.

UICC: Union for International Cancer Control.

VPH: Virus del papil·loma humà.

1. Articles inclosos a la tesi

ARTICLE NUMERO 1

Autors: **Isabel Trias**, Adela Saco, Lorena Marimon, Ricardo López Del Campo, Carolina Manzotti, Oriol Ordi, Marta Del Pino, Francisco Pérez, Naiara Vega, Silvia Alós, Antonio Martínez, Leonardo Rodriguez-Carunchio, Oscar Reig, Pedro Jares, Cristina Teixidó, Tarek Ajami, Juan Manuel Corral-Molina Ferran Algaba, Maria José Ribal, Inmaculada Ribera-Cortada, Natalia Rakislova.

Títol: **p53 in Penile Squamous CellCarcinoma: Pattern Based Immunohisto-chemical Framework with Molecular Correlation.**

Revista: **Cancers (Basel). 2023 May 11;15(10):2719.**
doi: 10.3390/cancers15102719.

Factor d'impacte: **5.2**

Quartil – rànking (any) - Categoria: **Q2 - 72/241 (2022)- Oncology**

Tipus de publicació: **Investigació original**

ARTICLE NUMERO 2

Autors: **Isabel Trias,** Ferran Algaba, Inés de Torres, Adela Saco, Lorena Marimon, Núria Peñuelas, Laia Diez-Ahijado, Lia Sisuashvili,, Katarzyna Darecka, Alba Morató, Marta del Pino, Carla Ferrándiz-Pulido, María José Ribal, Tarek Ajami, Juan Manuel Corral, Josep Maria Gaya, Oscar Reig, Oriol Ordi, Inmaculada Ribera-Cortada, Adriana García -Herrera, Natalia Rakislova .

Títol**: p53 Immunohistochemistry Defines a Subset of Human Papillomavirus-Independent Penile Squamous Cell Carcinomas with Adverse Prognosis**

Revista: **CIMB** (En revisió) Factor d'impacte: **3.1**

Quartil – rànking (any) - Categoria: **Q3 -(2022)- Biochemistry and Molecular Biology**
Tipus de publicació: **Investigació original**

ARTICLE NUMERO 3

Autors: José Guerrero, **Isabel Trias**, Luis Veloza , Marta Del Pino, Adriana Garcia, Lorena Marimon, Sherley Diaz-Mercedes, María T Rodrigo-Calvo, Silvia Alós, Tarek Ajami, Rafael Parra-Medina, Antonio Martinez , Oscar Reig, Maria José Ribal, Juan Manuel Corral-Molina, Jaume Ordi, Inmaculada Ribera-Cortada, Natalia Rakislova.

Títol: **HPV-negative Penile Intraepithelial Neoplasia (PeIN) With Basaloid Features.**

Revista: **Am J Surg Pathol. 2022 Aug 1;46(8):1071-1077.**

doi: 10.1097/PAS.0000000000001885.

Factor d'impacte: **5.6**

Quartil – rànking (any) - Categoria: **Q1 - 12/76 (2022)- Pathology**

Tipus de publicació: **Investigació original**

2. Introducció

2.1. CARCINOMA ESCAMÓS DE PENIS: ASPECTES GENERALS

El carcinoma escamós és la neoplàsia maligna més freqüent del penis, constituint el 95% de càncers d'aquesta localització (1). La incidència del carcinoma escamós del penis (CEP) presenta una gran variabilitat geogràfica (Figura 1). Aquesta variabilitat es considera deguda a la diferent exposició de la població a determinats factors de risc, entre els quals hi trobem la manca de higiene, antecedents de lesions inflamatòries del penis com la balanitis, la fimosi o el liquen esclerós, l'obesitat, exposició a fototeràpia i la infecció pel virus del papil·loma humà (VPH) (2). La implicació del VPH en el desenvolupament de neoplàsies és un fet epidemiològicament ben establert. A més del carcinoma de penis, el VPH està també relacionat amb els carcinomes de cèrvix uterí, vagina, vulva, anus i orofaringe (3) .

En països desenvolupats la prevalença del CEP és de 0,1-1/100.000 habitants amb tendència a incrementar (4) tal i com mostra un estudi fet a Gran Bretanya on s'observa un increment fins 0,45-1,33/ 100.000 habitants durant el període 1979-2009 i que els autors atribueixen a canvis de conducta sexual amb més exposició a VPH (5). Per contra, a l'Àfrica subsahariana, Brasil i algunes zones d'Àsia el CEP pot arribar a constituir el 10% de totes les neoplàsies malignes (1).

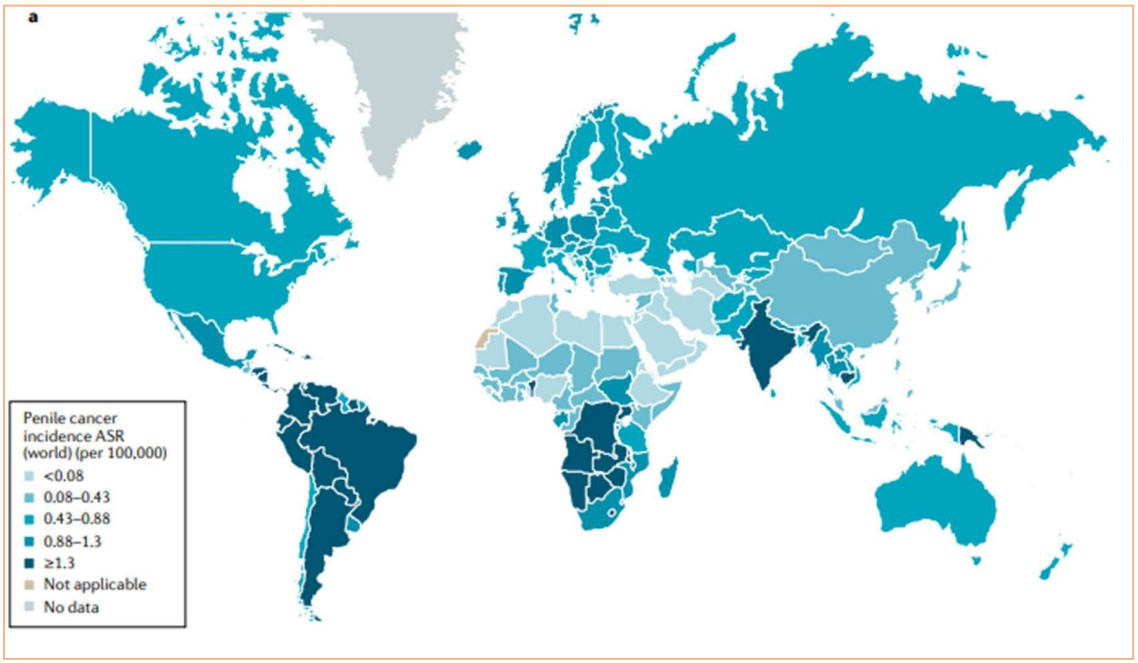

Figura 1. Incidència mundial del carcinoma escamós de penis. *Nat Rev Dis Prim. 2021;7(1):11-34.*

Espanya té una incidència una mica més elevada (2.5/100.000 habitants) respecte als països del seu entorn amb notables variacions geogràfiques entre comunitats autònomes (Figura 2) (6).

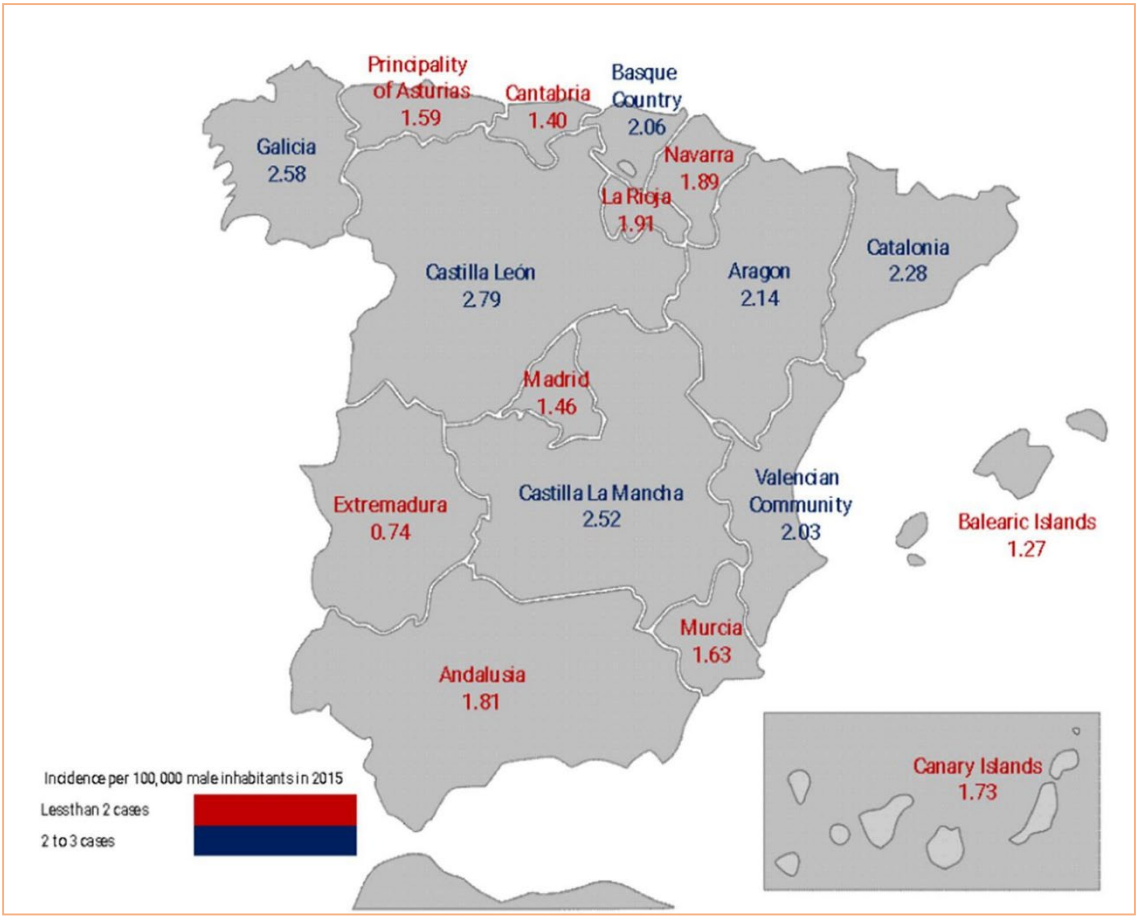

Figura 2. Incidència de carcinoma escatós de penis a l'estat espanyol. *Cancers 2023;15:616-628*

Considerat de manera global, s'estima que fins a un 50,8% dels CEP estan relacionats amb la presència del VPH (7). No obstant això, aquesta xifra varia molt en funció del país. Així, a Europa, al voltant del 30% dels CEP corresponen a casos associats a VPH mentre que a d'altres àrees geogràfiques aquesta proporció pot arribar al 70-90% (8,9). Això significa que en el nostre àmbit, la gran majoria dels casos de CEP son VPH-independents.

Morfològicament s'han descrit una sèrie de subtipus de CEP, alguns dels quals es consideren associats a la presència de VPH, entre els quals destaquen els tipus basaloide, condilomatós, el carcinoma de cèl·lules clares i el carcinoma

limfoepitelial. Per altra banda, alguns tipus morfològics es consideren independents del VPH; entre ells destaquen el carcinoma escamós "usual", el berrugós, el papil·lar i el sarcomatoide. No obstant, la morfologia no sempre és concordant amb la presència de VPH per la qual cosa la classificació del llibre de la Organització Mundial de la Salut (OMS) de l'any 2022 (10) recomana agrupar els CEP tant en funció de la morfologia com de la presència o no de VPH. El motiu principal per utilitzar aquest criteri és ser coherent amb les classificacions emprades per els carcinomes de cèrvix i vulva que també comparteixen la infecció per VPH com factor de risc (11).

Els mecanismes oncogènics relacionats amb el CEP i les seves lesions precursores son encara bastant desconeguts, especialment per als CEP VPH-independents (12). Tanmateix, tant els CEP relacionats amb VPH com els CEP VPH-independents comparteixen el mateix maneig clínic i tractament (13) que segons les guies europees de la European Association of Urology (EAU) es basen en un abordatge bàsicament quirúrgic amb penectomies parcials o radicals i un seguiment estricte en funció de l'estadiatge seguint el sistema del Tumor Node and Metastasis (TNM) establert per la Union for International Cancer Control (UICC) juntament amb la American Joint Comittee on Cancer (AJCC) (14).

2.2. LESIONS PRECURSORES DEL CEP

De manera similar al que passa a vulva, hi ha lesions precursores anomenades neoplàsia intraepitelial del penis (NIP) que estan presents abans de l'aparició del CEP i que son diferents en funció de si es tracta del grup relacionat amb VPH o si és el grup VPH-independent (15). La classificació de OMS descriu diferents subtipus morfològics relacionats amb VPH que afecten tant les lesions precursores com a les neoplàsies infiltrants. Pel que fa a la NIP associada a VPH, també anomenada lesió intraepitelial d'alt grau o HSIL per les seves inicials en anglès (High Grade Squamous Intraepithelial Lesion) (16), es descriuen moltes variants morfològiques entre les que destaca la basaloide o indiferenciada, la condilomatosa i les menys freqüents de tipus pagetoide amb cèl·lules clares (17).

En quan a les NIP independents de VPH només se'n descriu una anomenada neoplàsia intraepitelial diferenciada (15).

A nivell mundial, les lesions relacionades amb VPH constitueixen el 80% de totes les NIP (7). No obstant, la seva incidència a Europa és baixa amb un 0.47 casos per 100.000 habitants i any tot i que es constata una tendència a augmentar (1). La soca més freqüent relacionada amb NIP és VPH16 (18) i es considera que al voltant del 30% dels casos diagnosticats de NIP associada a VPH progressaran a CEP (12,19).

La morfologia de les NIP associats a VPH és variable amb molts possibles patrons. Els més freqüents són el basaloide que mostra una proliferació mitòticament molt activa de cèl·lules indiferenciades que ocupen tot el gruix de l'epiteli, i el condilomatós amb cèl·lules pleomòrfiques amb atípia coilocítica i abundants mitosis , en ocasions és possible identificar patrons mixtes on l'epiteli basal mostra una morfologia indiferenciada (basaloide) que canvia cap a coilocítica en estrats més superficials (19). L'expressió immunohistoquímica relacionada amb les NIP associades a VPH reflecteixen la seva oncogènesi i es caracteritzen per una positivitat en bloc a p16, una elevada expressió de Ki-67 en estrats suprabasals i negativitat a p53 (19).

La NIP diferenciada es relaciona amb el liquen esclerós i atròfic de llarga evolució i acostuma trobar-se en la part interna del prepuci en homes no circumcidats i d'edat més avançada comparats amb els pacients amb NIP associats a VPH (20). Com que no estan relacionats amb el VPH, solen ser p16 negatius i poden donar lloc als tumors de tipus habitual i verruciforme. (10). Tot i que no es coneix la incidència de la progressió d'aquesta lesió a CEP (19), alguns estudis suggereixen que només un 2-8% (21) dels casos de NIP diferenciada associada a liquen esclerós poden evolucionar a CEP amb un període llarg de 12-17 anys (10).

Morfològicament es caracteritza per presentar una hiperplàsia epitelial amb elongació de les crestes interpapil·lars, que es poden unir entre elles creant un patró reticular, i amb híper/paraqueratosi. Amb freqüència s'associen a espongiosi i es poden identificar cèl·lules disqueratòsiques. Habitualment les cèl·lules atípiques es limiten a la capa basal i parabasal lo qual dificulta la

interpretació de la lesió al ser observada a petit augment, ja que pot donar la impressió d'estar observant un epiteli que madura (20) ,no obstant està descrit un espectre de canvis histològics que oscil·len de menys a més atípia amb casos que mostren molt pleomorfisme sense canvis coilocítics (22). La majoria de les NIP diferenciades estan associades a CEP concomitant ja que quan es presenten de manera aïllada, el diagnòstic morfològic és difícil degut a que les alteracions descrites poden ser molt similars als canvis reactius secundaris a inflamació crònica com els que es veuen en el liquen esclerós i atròfic, la hiperplàsia pseudo-epiteliomatosa o el liquen simple crònic (23).

Seguint les indicacions de la OMS 2022, és recomanable demostrar la presència de VPH en totes les lesions precursores per tal de classificar-les correctament, ja sigui utilitzant mètodes moleculars o immunohistoquímics (10). En aquest sentit l'expressió immunohistoquímica en bloc de p16 està acceptada com un indicador de la presència de soques de VPH d'alt risc oncogènic (24) i per tant es pot utilitzar per classificar les lesions de NIP associades a VPH. Per altra banda les NIP independents del VPH, a diferència de les NIP associades a VPH, no expressen p16 i mostren amb una elevada freqüència mutacions de gen *TP53* (25). La presència de mutació es relaciona amb una expressió immunohistoquímica anòmala de la proteïna p53 (26) lo qual ens permet classificar millor els NIP independents de VPH que amb freqüència seran positius a p53.

Malgrat que les diferències morfològiques de les NIP associades a VPH i les VPH-independents estan ben descrites (10), hi ha casos discordants amb morfologies suggestives d'estar relacionades amb VPH però que son VPH independents. Aquest solapament morfològic ha estat descrit per el nostre grup i probablement indica que no es pot relacionar de manera automàtica la presència de VPH amb una morfologia concreta (27). Aquest fenomen està també descrit a vulva on la dissociació morfològica de les lesions precursores en relació a la presència del VPH ha estat també publicada pel nostre grup en un estudi on es van avaluar les característiques histològiques i immunohistoquímiques de les lesions intraepitelials utilitzant les tincions per p16 i p53. En aquest treball es va demostrar que el 33% de casos de carcinoma vulvar presentaven lesions premalignes adjacents de les quals el

73% tenien NIVd, 9% tenien acantosi vulvar amb diferenciació alterada i el 18% tenien lesions que imitaven el HSIL tot i ser VPH independents (28,29).

2.3. CLASSIFICACIÓ DEL CEP

El CEP és la forma més freqüent de neoplàsia maligna en aquesta localització (10). Per topografies, la gran majoria dels carcinomes apareixen en el gland (65%), seguit del prepuci (17%) i el solc balano-prepucial (14%), la resta de localitzacions son anecdòtiques (6) (Figura 3).

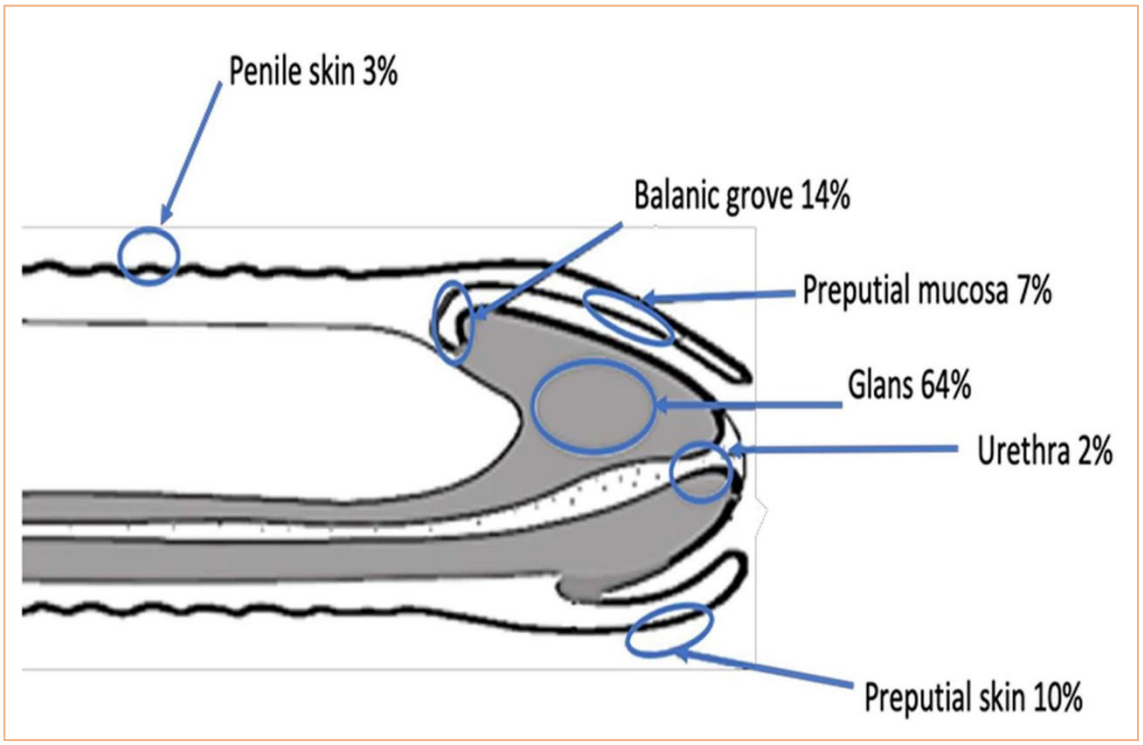

Figura 3. Distribució topogràfica dels carcinomes en el penis. *Cancers (Basel). 2023;15(3):616*

En les dues últimes edicions (2016 i 2022) la OMS classifica els CEP basant-se en la seva relació amb el VPH igual que es fa amb els carcinomes de cèrvix i vulva (10,11,30). El principal motiu per fer-ho així és mantenir una coherència amb el criteri utilitzat en d'altres carcinomes de zona genital en el que el VPH hi té un paper oncogènic conegut (11).

Amb aquest enfocament, el llibre de la OMS 2022 classifica els carcinomes agrupant- los per subtipus en funció de la seva relació amb el VPH:

2.3.1 Carcinomes associats a VPH

Els pacients amb carcinomes relacionats amb el VPH, especialment carcinomes condilomatosos i els basaloides, són uns 10 anys més joves que aquells amb neoplàsies no relacionades amb el VPH. Els tumors tendeixen a afectar preferentment el gland i s'estenen al prepuci en aproximadament un 20% dels casos (32). Tots ells han de ser positius per a p16 o demostrar la presència de ADN víric amb mètodes moleculars (31). Els tipus histològics descrits son els següents:

- Carcinoma basaloide: es localitza al glans i prepuci i pot afectar el solc balano-prepucial i és un dels més freqüents. Amb freqüència hi ha metàstasis ganglionars en el moment del diagnòstic (33). Histològicament es caracteritzen per una proliferació de cèl·lules de tipus basaloide, agrupades en nius que mostren necrosis o queratinització abrupta central. Hi ha una elevada activitat mitòtica i tendència a la invasió vascular i perineural. Tot i que majoritàriament son tumors sòlids poden tenir un creixement papil·lar (34). El pronòstic és dolent (10).
- Carcinoma condilomatós (*warty*): Son tumors exofítics que es caracteritzen per un creixement papil·lomatós amb un revestiment per un epiteli amb coilocitosi molt atípica que afecta tot el gruix epitelial i s'acompanya de hiperqueratosi i paraceratosi. La interfície tumor- estroma és irregular i dentada, almenys de forma focal (35).
- Carcinoma de cèl·lules clares: Neoplàsia constituïda per cèl·lules de citoplasmes amples i clars, positius per la tinció de PAS resistent a la diastasa i s'associen a una extensa invasió vascular i perineural. Amb freqüència es presenten amb metàstasis ganglionars (36).
- Carcinoma de tipus limfoepitelial: Es tracta d'un tumor poc freqüent amb una morfologia de carcinoma escatós pobrament diferenciat

amb un marcat component limfocitari i amb cèl·lules de tipus sincitial similars a les que es troben en el carcinoma de càvum. No s'ha demostrat cap relació amb el virus d'Epstein-Barr (37).

- Carcinomes mixtes: En alguns casos és possible veure neoplàsies que barregen patrons, sobre tot el basaloide i el condilomatós. Aquesta nomenclatura està acceptada tot i que es recomana indicar el % dels diferents tipus presents (10).

2.3.2 Carcinomes independents de VPH:

Aquestes neoplàsies afecten a homes entre els 50 i els 70 anys, una edat més avançada que els casos associats a VPH (38), i es relacionen amb alteracions com la fimosi, la balanopostitis o liquen esclerós i atròfic. Totes aquestes patologies es consideren un factor de risc ja que s'associen a inflamació crònica, condició relacionada amb l'aparició del CEP independent del VPH (39). La majoria dels CEP independents del VPH apareixen al gland, exceptuant alguns casos molt relacionats amb el liquen esclerós i atròfic amb més tendència a presentar-se en el prepuci (10). Els tipus histològics descrits son els següents:

- Carcinoma escamós habitual/usual: La gran majoria apareixen al gland al voltant dels 60 anys i és el subtipus més freqüent (45-60% de tots els carcinomes de penis) (6,40). Morfològicament hi ha un espectre de lesions que poden variar des de les més ben diferenciades a les més pobrament diferenciades. Els carcinomes més ben diferenciats son infreqüents i els més mal indiferenciats són poc queratinitzants amb nius i cordons de patró infiltrant. La gradació cal fer-la seguint el sistema recomanat per la ISUP/OMS (41).
- Carcinoma verrucós: Habitualment afecta al gland i s'estén al solc balano- prepucial i prepuci. És infreqüent (3-7%) i afecta a homes d'edat avançada (10). Morfològicament es caracteritza per ser un tumor de creixement exo i endofític i molt ben diferenciat però sense atípia coilocítica (42). Dins d'aquest grup s'inclou el carcinoma cuniculatum (10).
- Carcinoma papil·lar: Tumor infreqüent que es presenta amb un creixement exofític i granular. Histològicament es caracteritza per un

creixement amb disposició papil·lar complexa, sense atípia coilocítica. Es poden identificar zones de queratinització irregulars (43).

- Carcinoma sarcomatoide: L'edat mitja de presentació és als 59 anys. Es tracta d'una neoplàsia infreqüent, molt agressiva amb metàstasis ganglionar en el 75-89% dels casos i amb elevada mortalitat. Morfològicament pot presentar diverses morfologies simulant sarcomes amb proliferacions de cèl·lules fusiformes. Precisa estudis immunohistoquímics per demostrar la seva naturalesa epitelial en la majoria dels casos (44).

2.3.3 Altres carcinomes.

La OMS reconeix l'existència d'altres carcinomes (carcinoma adeno-escamós o muco-epidermoide, Paget extra-mamari i carcinomes neuroendòcrins) que són extraordinàriament infreqüents i no comparteixen cap factor de risc amb els carcinomes escatosos prèviament descrits (10).

La correlació entre les variants morfològiques i la presència o no de VPH depenen en gran mesura de l'experiència de l'observador. La correlació és moderada en dues terceres parts dels casos diagnosticats (10) i les discordances existeixen, especialment en el cas del carcinoma habitual, on hi ha molta variabilitat reportada amb casos relacionats amb VPH que oscil·len entre 24-59% (1). En aquest context, la OMS recomana utilitzar la tinció immunohistoquímica de p16 com el mètode més fàcil i fiable davant de casos que no siguin clars i/o no es disposin de mètodes moleculars per a la detecció del VPH. Finalment es recomana que els informes d'anatomia patològica incloguin el subtipus histològic de CEP i també el grup de VPH al qual pertanyen (10).

2.4. ALTERACIONS GENÒMIQUES I MOLECULARS DEL CEP

2.4.1 Via molecular oncogènica associada a VPH

L'exposició persistent i/o recurrent del VPH a l'epiteli del penis pot comportar la

integració de l'ADN del VPH en el genoma de l'hoste i eventualment, a la transformació de la cèl·lula hoste en una cèl·lula maligne. La sobre expressió de les onco-proteïnes virals E6 i E7 que es produeixen com a conseqüència d'aquesta integració, provoca la desregulació del cicle cel·lular a través de la interacció amb p53 i pRb (45).

L'onco-proteïna E7 s'associa i inhibeix pRB, alliberant el factor de transcripció E2F que activa gens implicats en la síntesi d'ADN i resulta en una progressió incontrolada del cicle cel·lular (46). Aquest procés condueix a la sobre expressió de $p16^{ink4a}$, a causa de la interrupció del bucle de retroalimentació negativa entre $p16^{ink4a}$ i pRB (47,48) Per aquest motiu la sobre expressió de $p16^{ink4a}$ es pot utilitzar en casos de CEP com a marcador substitutiu de la infecció per VPH amb transcripció activa (48,49).

Els efectes cancerígens d'E6 resulten de la inhibició de p53 entre altres proteïnes, que provoca la inhibició de l'apoptosi i afavoreix la inestabilitat cromosòmica (50). Això provoca una manca de la reparació de l'ADN i predisposa la cèl·lula infectada a l'acumulació d'esdeveniments genètics secundaris com mutacions que finalment condueixen al càncer (51).

2.4.2 Mutacions somàtiques a la via associada a VPH

La presència de mutacions somàtiques en els CEP associats a VPH estan descrites però amb una freqüència molt menor comparat amb els CEP independent de VPH (35% vs 90%). A més, les mutacions que es troben en els casos associats a VPH estan compartides amb els CEP VPH-independents i son les que afecten als gens *PIK3CA, FGFR3, PTEN i FBXW7.* Curiosament aquestes alteracions tenen una incidència similar en els dos grups (52). En la Figura 4 es pot veure la distribució de les mutacions somàtiques en el grup de CEP associats a VPH i la seva relació amb les principals dades clínico-patològiques.

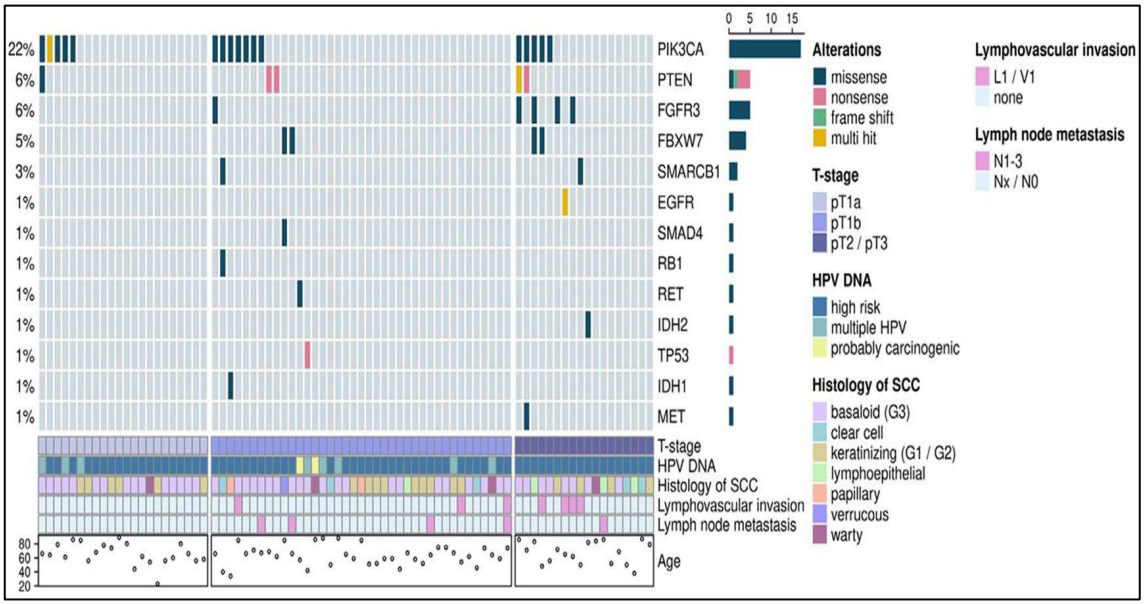

Figura 4. Distribució de les mutacions somàtiques en els carcinomes associats a VPH. *Mod Pathol. 2023;36(10):100250*

2.4.3 Via molecular oncogènica independent del VPH

Les vies moleculars en els casos independents de VPH són bastant desconegudes, no obstant sabem que patologies inflamatòries cròniques del penis, incloent balanopostitis, fimosi i liquen esclerós i atròfic, són factors de risc per al desenvolupament del CEP. Totes aquestes entitats comparteixen un mecanisme comú mitjançant l'expressió de la ciclooxigenasa 2 (COX2), que s'ha descrit associada en la NIP diferenciada així com en els CEP, tant en formes localitzades com disseminades (53).

Els estudis suggereixen que la sobreexpressió de COX2 impulsa la sobreproducció de prostaglandines (sobretot prostaglandina E2) i tromboxans, donant lloc a angiogènesi, proliferació i invasió a través de diverses vies moleculars compartides també en el CEP associat a VPH.

2.4.4 Mutacions somàtiques a la via independent de VPH

Les alteracions genètiques somàtiques són una altra via genòmica no mediada

pel VPH i que ha estat relacionada amb la carcinogènesis del CEP. En aquest cas es produeixen amplificacions del nombre de còpies gèniques, delecions i mutacions (54). En general els casos VPH independents s'associen a mutacions en múltiples gens a diferència dels associats al VPH, entre els que destaquen les mutacions de *TP53, CDKN2A* i *HRAS*. Amb freqüència la mutació de *TP53* és concomitant a la de *CDKN2A* (52). La mutació de *TP53* es relaciona amb una major expressió de la proteïna p53 anòmala (90%) o amb una absència total d'expressió (10%) (39). Això es pot detectar amb tècniques immunohistoquímiques de tal manera que entre un 45 a un 89% dels CEP expressen p53 i, a més, s'associa a mal pronòstic (39,47). Altres mutacions freqüents descrites en el CEP VPH independents, algunes compartides amb els CEP associats a VPH, son les de l'oncogen *PIK3CA* que està mutat en un 8,9% de CEP (55,56) i les mutacions activadores de *RAS*, que s'han trobat en un 1-19% de CEP (56,57). D'altra banda, la disminució de la expressió de PTEN, un gen supressor de tumors i repressor de la via PI3K, és un esdeveniment comú en CEP present en el 62-75% dels casos (58). En la Figura 5 es pot veure la distribució de les mutacions somàtiques en el grup de CEP independents de VPH i la seva relació amb les principals dades clínico-patològiques.

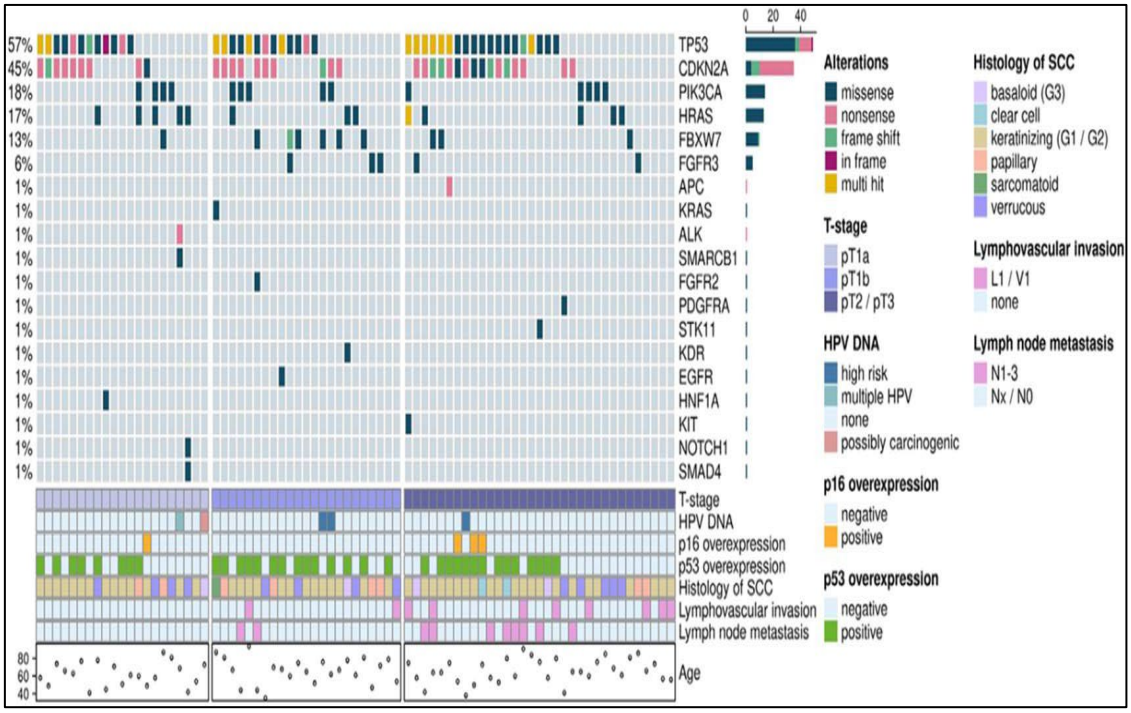

Figura 5. Distribució de les mutacions somàtiques en els carcinomes

independents del VPH. *Mod Pathol. 2023;36(10):100250.*

Tot i que els camins oncogènics son diferents per als CEP en funció de la seva relació amb el VPH (veure figura 6), sabem que hi ha alteracions compartides. En aquest aspecte son especialment interessants les que incorporen potencials dianes terapèutiques que podrien ser utilitzades en els dos grups (52).

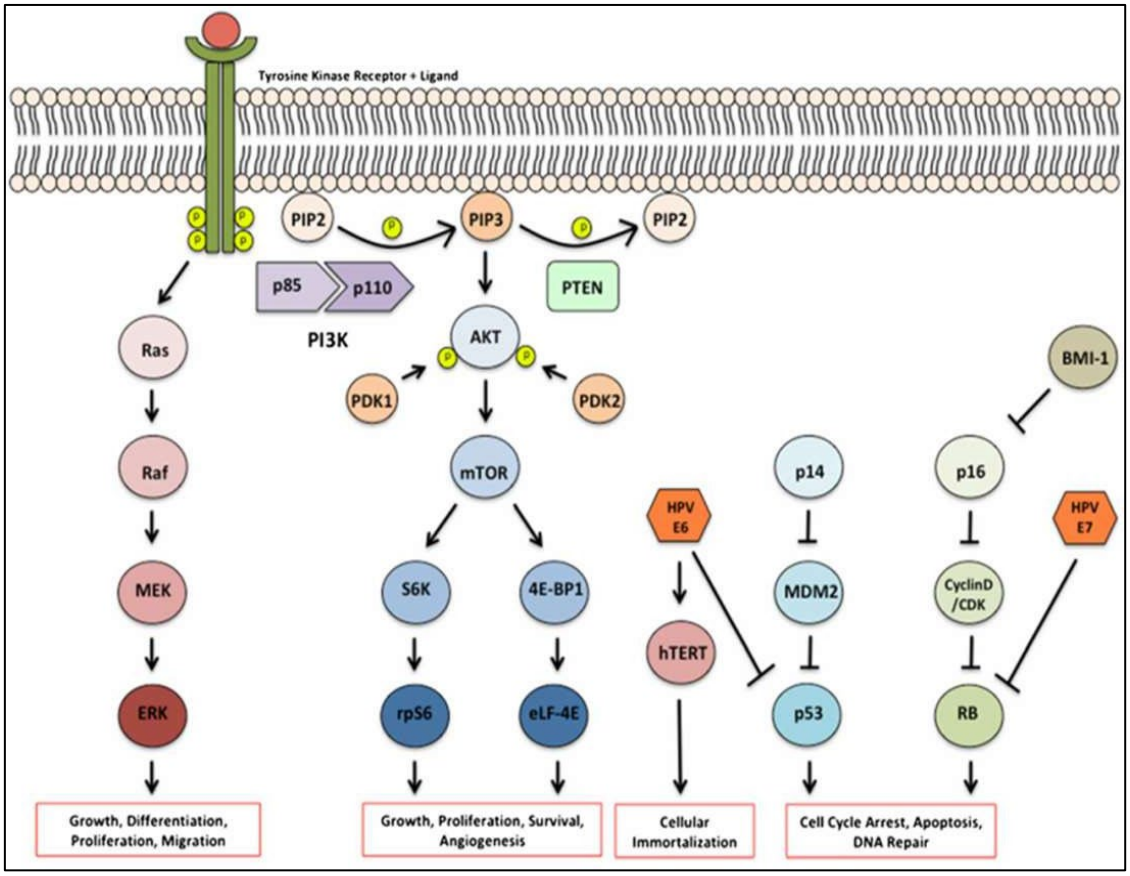

Figura 6. Vies oncogèniques generals del CEP. *Virchows Arch. 2019;475(4):397–405.*

2.5. FACTORS PRONÒSTICS DEL CEP.

2.5.1 Estadiatge.

Com en totes les neoplàsies malignes el factor pronòstic que té més impacte és l'estadiatge. El TNM de penis en la seva última edició, ha modificat alguns criteris respecte de la classificació anterior que afecten especialment als tumors classificats com T1. En la 8ena edició del TNM de la UICC juntament amb la

AJCC, es reconeix que el tipus histològic, el grau, el nivell de invasió, la invasió limfovascular i la perineural actuen com factors patològics de mal pronòstic i son criteris per estratificar el pT1 en dos: el pT1a (CEP grau 1 o 2, sense invasió vascular ni perineural) i el pT1b (CEP grau 3 o pobrament diferenciats, amb invasió perineural o limfovascular) (59).

La inclusió de paràmetres morfològics per definir un estadiatge T és excepcional, tanmateix, hi ha autors que qüestionen el valor d'aquesta subdivisió ja que consideren que l'estadiatge pT1b es comporta més com una neoplàsia no localitzada suggerint una revisió crítica en la classificació del TNM (60)

Altres canvis que han aparegut en l'última classificació afecten al T2 i el T3. En aquest cas és de destacar la consideració de la invasió de cos esponjós com un T2 i el de cossos cavernosos com un T3 independentment de l'afectació uretral. Aquesta classificació en estadis localment avançats estratifica millor els malalts de més risc de progressió (61).

2.5.2 Invasió vascular i perineural.

Tal i com molts estudis han demostrat, el risc de metàstasi a distància augmenta significativament si s'associa amb invasió limfovascular perquè se sap que la infiltració de cèl·lules tumorals al sistema limfàtic, és un requisit per a la disseminació tumoral. Per tant no és estrany que sigui un paràmetre de mal pronòstic amb un impacte especialment significatiu en pacients sense metàstasi ganglionar en el moment de la cirurgia (60).

La invasió perineural també es considera un factor de mal pronòstic i sovint es troba associada a la limfovascular. En un estudi fet sobre 554 pacients de CEP T1 grau 2, tant la presència de invasió perineural con la limfovascular s'associen a metàstasis ganglionar (62).

La inclusió d'aquests dos paràmetres morfològics en l'estadiatge T1 ha emfatitzat el seu valor i obliga als patòlegs a tenir-los presents en el moment del diagnòstic.

2.5.3 Subtipus histològics i grau del CEP..

La OMS 2022 recomana estudiar els carcinomes en funció de la seva relació amb el VPH (10), no obstant, tal i com s'ha presentat abans, també es reconeixen subtipus histològics alguns dels quals tenen un impacte pronòstic especialment agressiu i que per tant, han de ser considerats en l'estadiatge T1. Entre els subtipus amb pitjor pronòstic cal destacar la variant basaloide de CEP associada a VPH. (60)

A banda dels subtipus histològics, el grau és una variable important especialment en els carcinomes habituals/usuals ja que també participa en l'estratificació dels pacients en fase de malaltia localitzada (T1) (60). Alguns estudis (63) demostren de manera convincent l'impacte en la supervivència del grau dels carcinomes (Figura 7). Mentre que en d'altres apunten que el grau podria també ser utilitzat per estratificar millor casos T2/T3 (64).

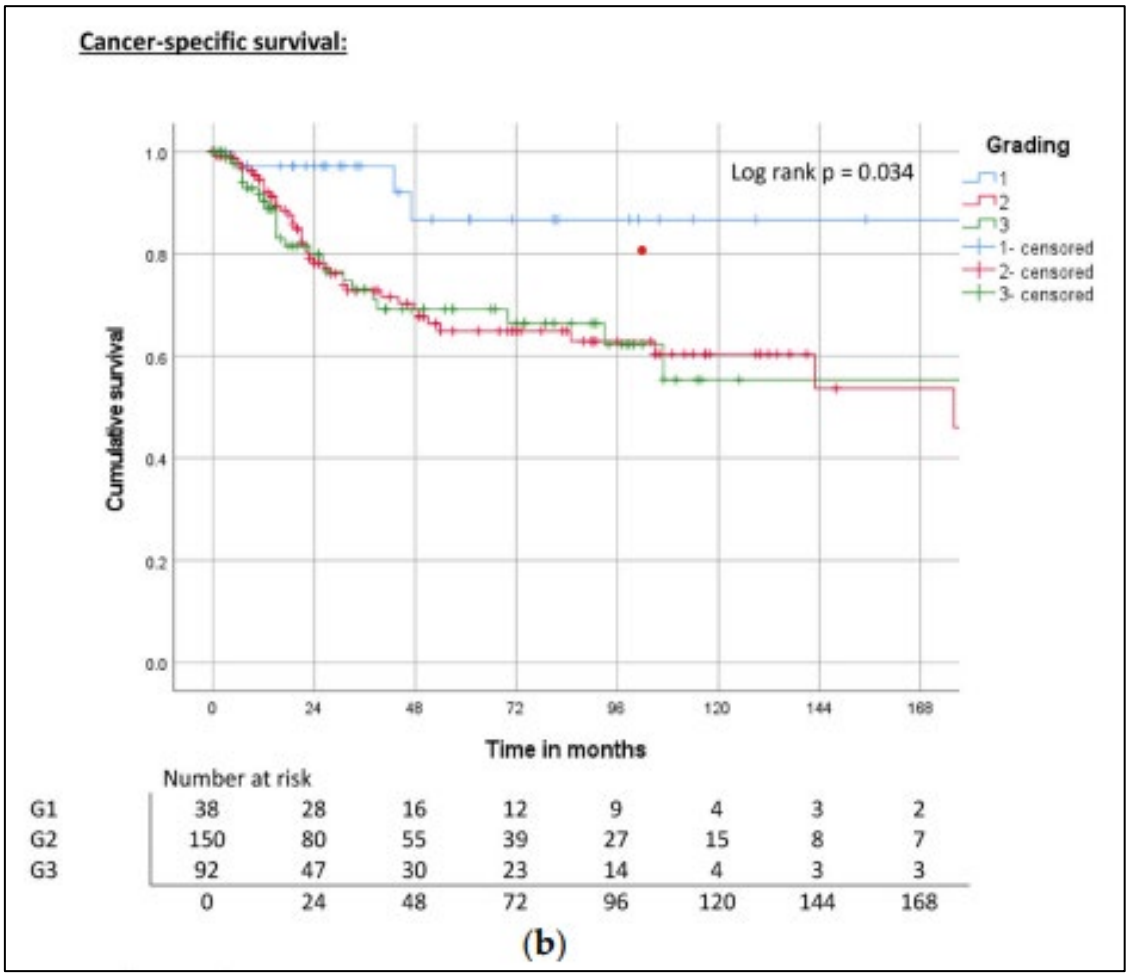

Figura 7. Valor pronòstic dels graus de diferenciació en el CEP

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2.5.4 Paper del VPH.

És ben sabut que en els càncers de cap i coll, els tumors associats al VPH mostren una millor taxa de supervivència que els carcinomes independents . (65). Cal remarcar que el millor pronòstic en els tumors de cap i coll associats a VPH no s'atribueix a una millor diferenciació o menor agressivitat, sinó a una major sensibilitat a la quimioteràpia i la radioteràpia, que juguen un paper bastant menor en el tractament curatiu del càncer de penis . ,(66)

Alguns estudis retrospectius recents mostren que els casos de CEP associat a VPH amb metàstasis ganglionar, tenen millor pronòstic que els VPH independents 67. Tanmateix en altres treballs les troballes son més incongruents ja que tot i que la presència de VPH es relaciona amb un estadiatge clínic inferior i menys incidència de invasió limfovascular respecte dels CEP independents, també es descriuen més casos de metàstasis ganglionar .(68)

Fins a l'actualitat, les dades relacionades amb el valor pronòstic del VPH en el CEP segueixen sent poc clares a causa de resultats contradictoris .

2.5.5 Altres factors pronòstics: p53.

El gen p53 és un dels gens més mutats associats al càncer (69). Hi ha diverses maneres en què poden sorgir mutacions de p53. Les formes germinals es poden heretar, donant lloc al desenvolupament de la síndrome Li-Fraumeni (70). Aquesta síndrome comporta el desenvolupament primerenc del càncer a en edats joves, juntament amb un mal pronòstic, ja que els càncers són d'alt risc, generalment difereixen del teixit d'origen amb lo que la detecció inicial i el tractament poden ser difícils (71).

Les mutacions més freqüents son les de tipus "miSsense" i quan es produeixen, poden alterar la funció posterior de p53 (72) A més, algunes proteïnes virals poden actuar disminuint o anul·lant la funció de p53, no per mutació, sinó per desregulació en els mecanismes de control de la proteïna.

Les mutacions de p53 estan implicades, encara que no exclusivament, en càncers d'ovari, esòfag, colon i recte, cap i coll, laringe i pulmó, així com en la

leucèmia primària, càncer testicular i melanoma maligne. (73)

En altres tipus de càncer es pot desregular o inactivar p53 per promoure la malignitat, sense evidència de mutació. Alguns exemples d'això inclouen sarcomes, mielomes i càncers impulsats pel VPH (74) .

Hi ha diversos mecanismes de desregulació de p53 que inclouen delecions, metilació, mutacions, microARN (miARNs) o isoformes. També la sobreexpressió de reguladors negatius de p53 como el “murine double minute 2" (MDM2) poden interferir la funció de la proteïna p53 (figura 8) (74).

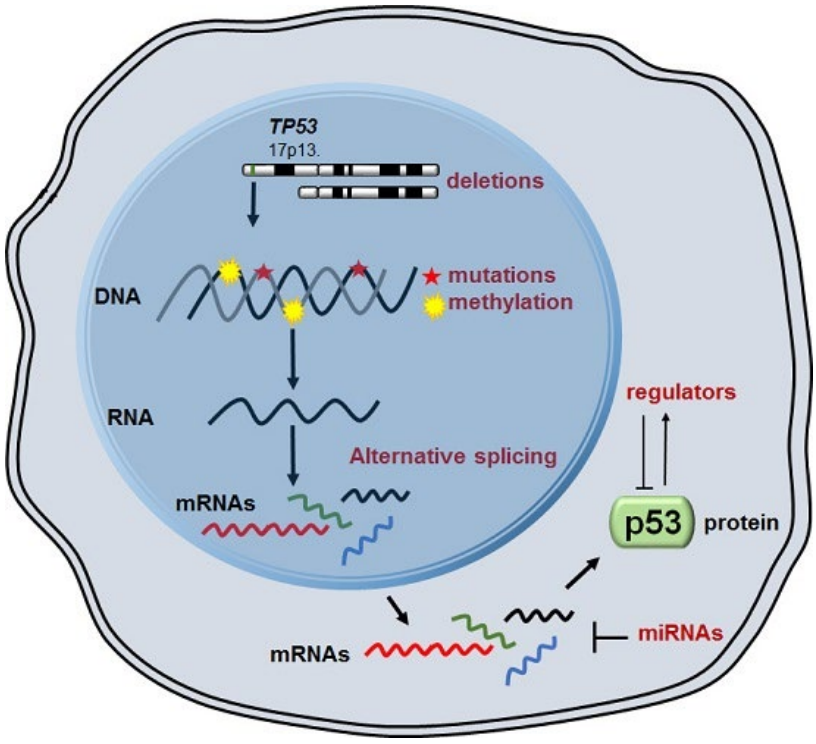

Figura 8 Alteració de les vies moleculars de *TP53* i p53. *Int. J. Mol. Sci.* 2016;*17*(12):2003

L'alteració de *TP53*, és un factor important de carcinogènesi del CEP especialment en casos independents de VPH (75) . Sembla que les alteracions

en els gens supressors de tumors són bastant infreqüents en la carcinogènesi induïda pel VPH, però no és tan rara en la carcinogènesi dels casos independents de VPH. A tall d'exemple crida l'atenció que la taxa de mutació de TP53 és només del 5% en el càncer de coll uterí (molt relacionat a l'exposició de VPH), molt menor que en qualsevol altre càncer (75). Per contra, estudis exhaustius fets amb sistemes de seqüenciació massiva en series amplies de CEP confirmen l'elevada incidència de mutació de TP53 (52).

Per alguns autors l'expressió immunohistoquímica positiva de p53 es correlaciona amb la presència de mutacions de TP53 i es postula que podria ser bon marcador pronòstic en CEP ja que en alguns estudis han trobat que la positivitat a p53 amb tècniques immunohistoquímiques en casos de CEP, està relacionada amb la presència de ganglis limfàtics positius (76–78).

En els carcinomes vulvars amb un etiopatogènia dual molt similar al penis, la incidència de mutació de TP53 és també molt més freqüent en els casos independents de HPV on sembla que té un impacte pronòstic.(79)

És especialment interessant un dels estudis sobre carcinoma de vulva, on s'han descrit sis patrons immunohistoquímics relacionats amb la presència de mutació de TP53.

Dos dels patrons es relacionen amb absència de mutació (TP53 nadiu) i quatre s'associen a mutació de TP53. (80)

En els dos patrons no mutats s'observen positivitats nuclears disperses i aïllades (patró més freqüent) o bé una positivitat de distribució difusa però respectant la basal dels nius infiltrants (positivitat suprabasal). Els quatre patrons mutats es caracteritzen per presentar una positivitat difusa en tots els nius infiltrants (patró més freqüent), absència de positivitat (patró nul), positivitat citoplasmàtica i, finalment, sobreexpressió basal (figura.9) (80).

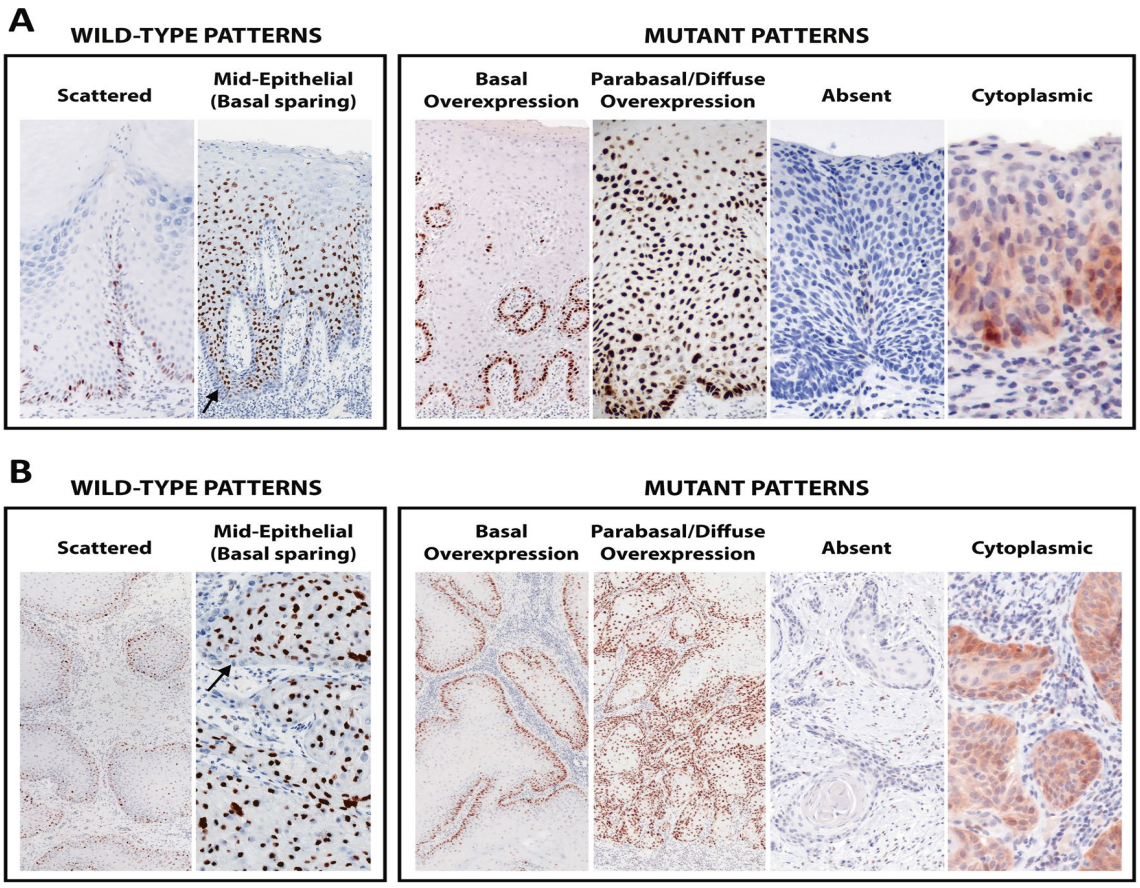

Figura 9. Patrons immunohistoquímics de p53. *Modern Pathology (2020) 33:1595–1605.*

Aquests patrons es van estudiar en les neoplàsies infiltrants i en les lesions precursores associades (VINd), on es van reconèixer els mateixos patrons de tinció, amb molt bona correlació amb la presencia de mutació.

En l'esquema adjunt (figura 10), es pot apreciar el nivell de concordança entre tinció i mutació tant per els carcinomes infiltrants com en les neoplàsies in situ (lesions precursores vulvars o NIV) (59).

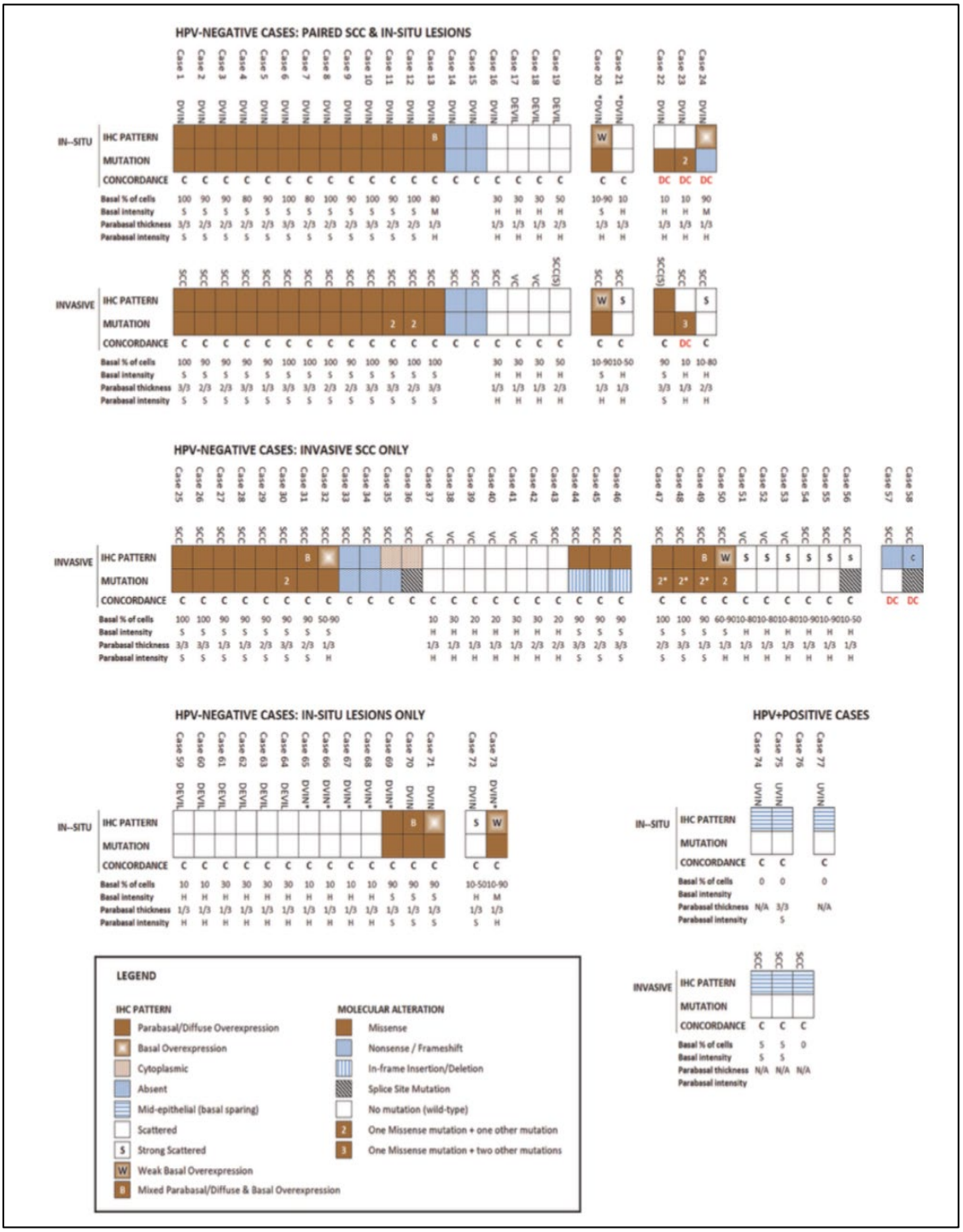

Figura 10. Correlació de mutació de *TP53* i p53. *Modern Pathology (2020) 33:1595–1605.*

Es molt possible que aquests patrons es puguin reproduir en altres neoplàsies essent el penis, per la seva localització a zona genital i la seva reconeguda oncogènesi dual molt similar a la de la vulva (12), un candidat idoni.

Si aquesta troballa es confirma i les tincions immunohistoquímiques per p53 valorades per patrons es poden utilitzar també en el CEP, és molt probable que aquesta dada contribueixi a definir grups de risc.

La relació de la mutació de TP53 amb dades de mal pronòstic en el CEP està molt ben documentada en un dels treballs recentment publicats, on l'existència de mutació es troba incrementada en estadis localment avançats especialment en els casos independents de VPH, amb una incidència marcadament elevada en els pT2/pT3 (52).

Aquests casos mostren també un increment d'expressió immunohistoquímica per p53 sense que s'especifiqui de manera detallada quina metodologia s'ha utilitzat per determinar la positivitat (52), no obstant la relació amb estadis avançats és una evidència més de la relació pronòstica de p53. Aquestes troballes son concordants amb les prèviament descrites per altres autors (81).

Conseqüentment, si s'aconseguís demostrar una bona correlació entre la presència de mutació de TP53 i la tinció immunohistoquímica per p53, s'afegiria informació pronòstica rellevant utilitzant una metodologia assequible a pràcticament tots els laboratoris d'anatomia patològica.

2.6. TRACTAMENT DEL CEP

Malgrat que la classificació de la OMS relaciona els CEP a la presència o no de VPH, actualment els tractaments no són diferents per a tots dos grups. De manera similar al que passa en altres localitzacions on també es desenvolupen carcinomes relacionats amb el VPH (83), els pacients amb CEP VPH-independents semblen tenir un pitjor pronòstic (15) comparats amb pacients amb CEP associat al VPH (81), no obstant, el principal factor pronòstic, com en la gran majoria de neoplàsies malignes, és l'estadificació. En el cas del penis es segueix el TNM de la UICC/AJCC (taula 2) (59).

La supervivència global per al CEP localitzat, és aproximadament del 90% als 5 anys (82,83) i el seu tractament es basa en una cirurgia conservadora amb intenció curativa (14) però un cop el tumor és metastàsic, el pronòstic empitjora dràsticament i, per tant, per als casos amb malaltia localment avançada o progressió de la malaltia, es recomana un abordatge quirúrgic i sistèmic que pot ser multimodal (84). La supervivència global de la malaltia metastàtica és d'aproximadament el 80% quan l'afectació dels ganglis limfàtics inguinals és unilateral, del 10-20% quan és bilateral o implica els ganglis limfàtics pelvians i <10% en els casos d'invasió extra-nodal (1).

L'estratègia terapèutica agrupada per estadis i basada en les guies internacionals (1) és la següent:

- **Estadi 0** (Tis, N0, M0) Els tumors són lesions no invasives limitades a l'epiteli que no solen fer metàstasi i per tant, l'exploració clínica dels ganglis inguinals combinada amb el diagnòstic d'anatomia patològica, és suficient per estadificar la malaltia. Es poden tractar amb extirpació quirúrgica, ablació i/o teràpia tòpica.
- **Estadi I** (T1a, N0, M0) Els tumors envaeixen l'estroma però no s'acompanyen dels factors de risc descrits per la OMS i la UICC/AJCC, (invasió limfo-vascular o perineural i els carcinomes d'alt grau) (10,85). Aquestes lesions es poden tractar amb cirurgia conservadora, ablació amb làser o radioteràpia. En els casos de carcinomes de baix grau és suficient un examen clínic de l'engonal per fer estadificació ganglionar, mentre que en els casos de grau intermedi la EAU recomana valorar cas a cas la necessitat d'utilitzar procediments quirúrgics, inclòs estudi de gangli sentinella, per fer un estadificació ganglionar inguinal (86).
- **Estadi II** (T1b–T3, N0, M0) Els tumors envaeixen el teixit sub-epitelial amb característiques d'alt risc (T1b: invasió vascular o perineural o carcinomesd'alt grau), el cos esponjós (T2) o els cossos cavernosos (T3), sense ganglis limfàtics palpables. Aquests tumors tenen un alt risc de micro- metàstasi i els pacients s'han de sotmetre

a una estadificació inguinal quirúrgica o estudi amb gangli sentinella. El tumor primari es pot tractar amb cirurgia conservadora, penectomia parcial, penectomia radical o radioteràpia.

- **Estadi III** (T1–3, N1–2, M0) Els tumors es caracteritzen per la presència de metàstasi ganglionar inguinal sense extensió extra-capsular però encara es consideren candidats a tractament amb intenció curativa. Els casos amb ≤2 ganglis limfàtics sense extensió extra-capsular (N1) habitualment es tracten només amb cirurgia. Per la resta el maneig és sovint multimodal, incloent cirurgia dels ganglis limfàtics inguinals i, en casos seleccionats, radioteràpia adjuvant opcional (evidència dèbil segons EAU). La quimioteràpia neoadjuvant és una opció en la malaltia N2 tot i que té una evidència dèbil segons les guies de la EAU (86).
- **Estadi IV** (T4 i/o N3 i/o M1) La malaltia es caracteritza per tumors localment avançats que infiltren estructures circumdants (com l'escrot o l'os púbic), ganglis inguinals voluminosos i/o fixos o ganglis inguinals amb extensió extra-pelviana, afectació de ganglis pelvians o metàstasi a distància. El tractament dels pacients en aquest estadi de la malaltia es basa en tractament quimioteràpic combinat seguint l'esquema TIP (paclitaxel, ifosfamida, i cisplatí). Encara que alguns d'aquest pacients es poden curar amb estratègies terapèutiques multimodals radicals que incloguin cirurgia o radioteràpia de forma seqüencial a la quimioteràpia, la majoria tenen molt mal pronòstic i l'atenció de suport és primordial. La inclusió en assaigs clínics que investiguen noves opcions terapèutiques és una opció per a pacients seleccionats.

El seguiment recomanat en funció del risc de cada cas està resumit en la Taula 1.

Recommendation	Surveillance period		Examinations and investigations	MinimumFU duration
	Years 1–2	Years 3–5		
Primary tumour FU				
Penile-preserving treatment	3-monthly	6-monthly	Regular PEx or self-examination. Repeat biopsy after topical or laser treatment for PeIN (optional).	5 yr
Amputation	3-monthly	Annually	Regular physician or self-examination.	5 yr
Inguinal node FU				
Surveillance	3-monthly	6-monthly	Regular PEx or self-examination. US ± FNAC optional.	5 yr
pN0	3-monthly	Annually	Regular PEx or self-examination. US ± FNAC optional.	5 yr
pN+	3-monthly	6-monthly	Regular PEx or self-examination. US ± FNAC, CT chest/abdomen/pelvis or ^{18}FDG-PET/CT optional.	5 yr

CT = computed tomography; ^{18}FDG = ^{18}F-fluoro-2-deoxy-D-glucose; FNAC = fine-needle aspiration cytology; FU = follow-up; PeIN = penile intraepithelial neoplasia; PEx = physician examination; PET = positron emission tomography; US = ultrasound.

Taula 1. Seguiment dels malalts en funció dels risc. *Eur Urol 2023;83:548–560*

La metàstasi ganglionar constitueix la frontera entre la malaltia curable i la d'alt risc de progressió. Per aquest motiu el maneig dels casos de CEP de risc intermedi o d'alt risc sense ganglis palpables inclou l'estudi de gangli sentinella o una estadificació inguinal quirúrgica ja que no es disposa de cap marcador fiable predictiu de metàstasis ganglionar (1,14,86) .

Alguns autors han trobat que la positivitat a p53 amb tècniques immunohistoquímiques està relacionada amb la presència de ganglis limfàtics positius (77,78). Si es confirma aquesta troballa els estudis immunohistoquímics de p53 poden contribuir a definir pacients de risc.

TAULA 2. TNM EN NEOPLASIES DE PENIS SEGONS LA UICC.

CLASSIFICACIÓ CLÍNICA

T – Tumor primari

- TX Tumor primari no valorable
- T0 Sense evidència de tumor primari
- Tis Carcinoma in situ (Neoplàsia intraepitelial del penis-NIP)
- Ta Carcinoma no invasor (berrucós)
- T1 Invasió del teixit sub-epitelial
    - T1a El tumor envaeix el teixit sub-epitelial. No hi ha invasió vascular ni perineural i el tumor no és mal diferenciat.
    - T1b El tumor envaeix el teixit sub-epitelial. Hi ha invasió vascular o perineural o el tumor és mal diferenciat.
- T2 Invasió de cos esponjós amb o sense invasió de la uretra
- T3 Invasió de cossos cavernosos amb o sense invasió de la uretra
- T4 Invasió d'estructures adjacents

N – Ganglis limfàtics regionals

- cNX Els ganglis no es poden valorar
- cN0 Ganglis engonals no palpables i/o no visibles.
- cN1 Ganglis engonal unilateral palpable i mòbils
- cN2 Múltiples ganglis palpables o ganglis engonals bilaterals
- cN3 Massa engonal fixa o limfadenopatia pelviana unilateral o bilateral.

M Metàstasis a distància

- cM0 Absència de metàstasis
- cM1 Presència de metàstasis a distància

CLASSIFICACIÓ PATOLÒGICA

Les categories pT corresponen a les T.

- Les categories pN es cataloguen en funció de la biòpsia o extirpació quirúrgica. pN – Ganglis limfàtics regionals
- pNX Els ganglis no es poden valorar
- pN0 Sense metàstasi en ganglis limfàtics regionals pN1 Metàstasis en un o dos ganglis regionals
- pN2 Metàstasis en més de dos ganglis unilaterals or metàstasis en ganglis engonals bilaterals
- pN3 Metàstasis en ganglis (o gangli) pelvians, unilateral o bilateral o amb extensió extra-nodal dels ganglis limfàtics regionals

pM – Metàstasis a distància

- pM1 Metàstasis confirmada microscòpicament.

Grau histològic segons la OMS 2022

- GX Grau no valorable
- G1 Ben diferenciat
- G2 Moderadament diferenciat
- G3 Mal diferenciat

2.7. ARTICLE PRELIMINAR: REVISIÓ SOBRE LES ALTERACIONES MOLECULARS DEL CEP

Amb l'objectiu de contextualitzar a nivell molecular els dos treballs que formen part d'aquesta tesi doctoral, es va fer una revisió de la literatura relacionada amb els canvis moleculars del CEP i que seguidament s'adjunta.

Títol: **Pathogenesis of Penile Squamous Cell Carcinoma: Molecular Update and Systematic Review.**

Autors: Ribera-Cortada I, Guerrero-Pineda J, **Trias I,** Veloza L, Garcia A, Marimon L, Diaz-Mercedes S, Alamo JR, Rodrigo-Calvo MT, Vega N, López Del Campo R, Parra-Medina R, Ajami T, Martínez A, Reig O, Ribal MJ, Corral-Molina JM, Jares P, Ordi J, Rakislova N.

Revista: International Journal of Molecular Sciences, 2021 Dec 27;23(1):251. DOI: 10.3390/ijms23010251.

Factor d'impacte: 6,208

Quartil – rànking (any) - Categoria: Q1 – 69/297 (2021) - Biochemistry & molecular biology.

Tipus de publicació: Revisió sistemàtica de la literatura

Resum:

Fonaments: El carcinoma escatós de penis (CEP) és un tumor infreqüent amb dues vies carcinogèniques descrites, una associada al virus del papil·loma humà (VPH) i l'altre independent del VPH. Les vies oncogèniques moleculars con poc conegudes i l'objectiu del treball és fer una revisió de les publicacions sobre alteracions moleculars associades a CEP.

Metodologia: Durant el mes de juny del 2021 es va fer una cerca mitjançant la plataforma Medline Pubmed, dels treballs originals centrats en la seqüenciació del genoma del CEP publicats fins al 30 de juny de 2021 i amb les dades extraïbles per el seu anàlisi.

Resultats: Es van incloure 7 estudis amb un total de 268 mostres de CEP dels quals en 4 es van valorar només mutacions somàtiques, en 1 alteracions numèriques i mutacions somàtiques i en 2 només alteracions numèriques. En 5 treballs es va fer servir sistemes de seqüenciació massiva i en els dos restants altres sistemes, no obstant cal remarcar que el número de mostres analitzades és escàs i que tant les tècniques utilitzades com el material son heterogenis. En tots els treballs es va estudiar la presència de VPH amb tècniques de PCR. En 4 dels treballs es van fer relacions pronòstiques amb seguiments dels pacients que oscil·len entre els 27 i els 96 mesos. Les mutacions somàtiques més freqüents son TP53,CDKN2A,NOTCH1,PIK3CA,FAT1,CASP8 i FBXW7. TP53

estava mutada en el 32% de les mostres (rang 10-48%). Pel que fa les alteracions numèriques, els guanys de MYC van ser l'alteració més reportada seguida d'alteracions de EGFR. També es detecta una menor càrrega mutacional en els casos associats a VPH en relació als VPH independents. Les vies oncogèniques més afectades son les de Notch, RTK-RAS i Hippo. Les alteracions més relacionades amb mal pronòstic varen ser una elevada carga mutacional de CDKN2A, EGFR, MYC, HRAS i TP53 i guanys a MYC i CCND1.

Conclusions: Els estudis existents són limitats en número de mostres, heterogeneïtat sociodemogràfica i variabilitat en la metodologia de seqüenciació de l'ADN. Especialment hi ha manca de coneixement en la caracterització dels

perfils moleculars en relació amb l'estat del VPH i per tant, es necessiten estudis multicèntrics per continuar en el camí de la caracterització molecular de CEP.

International Journal of
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MDPI

Review

Pathogenesis of Penile Squamous Cell Carcinoma: Molecular Update and Systematic Review

Inmaculada Ribera-Cortada [1], José Guerrero-Pineda [1], Isabel Trias [1], Luis Veloza [2], Adriana Garcia [1], Lorena Marimon [1,3], Sherley Diaz-Mercedes [1], José Ramon Alamo [1], Maria Teresa Rodrigo-Calvo [1], Naiara Vega [1], Ricardo López del Campo [1], Rafael Parra-Medina [4], Tarek Ajami [5], Antonio Martínez [1,6], Oscar Reig [6,7], Maria J. Ribal [5,6], Juan Manuel Corral-Molina [5], Pedro Jares [1,6], Jaume Ordi [1,3,†] and Natalia Rakislova [1,3,*,†]

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Abstract: Penile squamous cell carcinoma (PSCC) is a rare but aggressive neoplasm with dual pathogenesis (human papillomavirus (HPV)-associated and HPV-independent). The development of targeted treatment is hindered by poor knowledge of the molecular landscape of PSCC. We performed a thorough review of genetic alterations of PSCC focused on somatic mutations and/or copy number alterations. A total of seven articles have been identified which, overall, include 268 PSCC. However, the series are heterogeneous regarding methodologies employed for DNA sequencing and HPV detection together with HPV prevalence, and include, in general, a limited number of cases, which results in markedly different findings. Reported top-ranked mutations involve *TP53, CDKN2A, FAT1, NOTCH-1* and *PIK3CA*. Numerical alterations involve gains in *MYC* and *EGFR*, as well as amplifications in HPV integration loci. A few genes including *TP53*, *CDKN2A*, *PIK3CA* and *CCND1* harbor both somatic mutations and copy number alterations. Notch, RTK-RAS and Hippo pathways are frequently deregulated. Nevertheless, the relevance of the identified alterations, their role in signaling pathways or their association with HPV status remain elusive. Combined targeting of different pathways might represent a valid therapeutic approach in PSCC. This work calls for large-scale sequencing studies with robust HPV testing to improve the genomic understanding of PSCC.

Keywords: genomic landscape; molecular analysis; next generation sequencing; penile cancer; penile squamous cell carcinoma; whole-exome sequencing; HPV

1. Introduction

Malignant tumors of the penis are rare but impose a major challenge due to their high morbidity and mortality [1]. They occur predominantly in elderly men and their frequency increases with age, reaching its peak between the sixth and the seventh decades of life [2]. Low-income countries in South America and Africa register the highest incidences of penile squamous cell carcinoma (PSCC) [1]. PSCC accounts for around 95% of all malignancies of this organ [3]. The tumor originates most commonly from the epithelium of the glans, inner prepuce and coronal sulcus [4].

Two different etiopathogenic pathways have been described in PSCC [2]: one associated with human papillomavirus (HPV) and the other one independent of this infection. HPV-associated PSCC is more prevalent in relatively young males, who commonly refer to a high number of sexual partners and smoking history [3]. HPV-associated PSCC shows frequently basaloid or warty features and develops on high-grade squamous intraepithelial lesions (HSIL), also known as HPV-associated penile intraepithelial neoplasia (PeIN), Bowen disease or erythroplasia of Queyrat [3]. Immunohistochemical (IHC) overexpression of the p16 protein has been shown to be an accurate surrogate marker of HPV-associated PSCC [4], similar to squamous cell carcinomas of other anatomical sites of the anogenital tract and head and neck region [5]. HPV-independent PSCC is predominant in high-income countries and affects mainly older men [6]. The etiopathogenesis of HPV-independent PSCC is less well understood; however, phimosis, chronic inflammation, poor personal hygiene and trauma seem to be associated factors [3]. Histologically, these tumors are frequently keratinizing and develop from a special type of precursor lesion known as differentiated PeIN (dPeIN) [7]. Both HPV-independent PSCC and its precursor, dPEIN, are almost always negative for p16 [8,9] and frequently show p53 overexpression by IHC [9]. Figure 1 shows a characteristic example of each of the two types of PSCC, HPV-associated and HPV-independent, including the histological features, as well as the p16 and p53 IHC typical patterns of staining. Due to these remarkable epidemiological and clinico-pathological differences, the International Society of Urological Pathology (ISUP) modified, in 2016, its World Health Organization (WHO) classification and categorized PSCCs based on their HPV status and not only on pure histological features [10]. However, in contrast with other anatomical sites where HPV-associated tumors show better prognosis than HPV-independent carcinomas, it remains unclear whether HPV-associated PSCC has a better outcome [11]. Moreover, there are no differences in treatment based on HPV status to date.

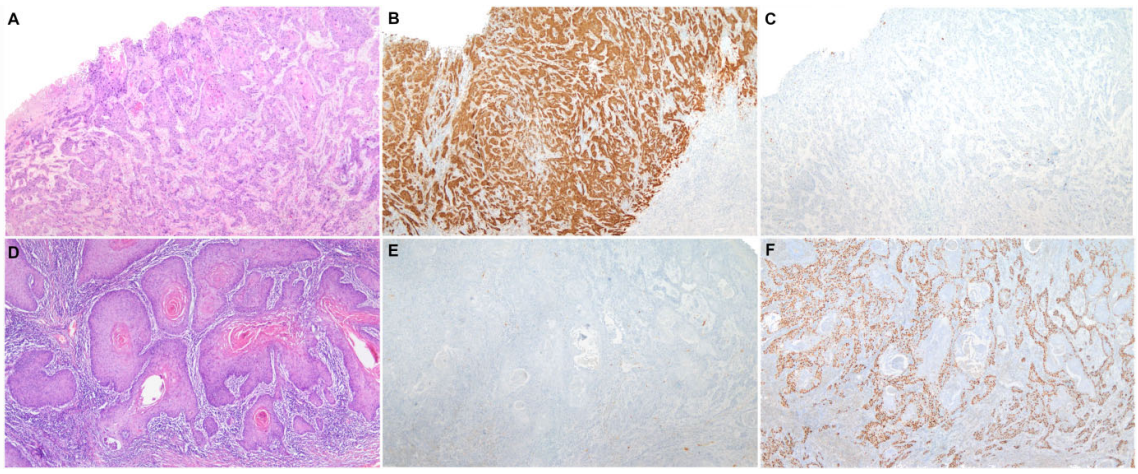

Figure 1. A characteristic example of each of the two types of penile squamous cell carcinoma, HPV-associated and HPV-independent. (**A**) Penile squamous cell carcinoma (H&E 40×) with positive p16 (**B**) and wild-type p53 immunohistochemical stainings (**C**) (40×); (**D**) Penile squamous cell

carcinoma (H&E 40×) with negative p16 (**E**) and mutated pattern (diffuse overexpression) of p53 immunohistochemical stainings (**F**) (40×).

Patients with PSCC frequently develop early loco-regional and angiolymphatic spread and have a devastating prognosis [1]. The management of lymph node-negative disease is primarily dependent on risk stratification based on clinico-pathological parameters [12], whereas the management of advanced disease is hampered by partial and short-term response to chemotherapy [13]. These current limitations highlight the need for novel therapeutic options. Regrettably, the low tumor mutation burden [14] and the rare *CD274* (*PD-L1*) amplifications observed in PSCC [15] hint at low responsiveness to immunotherapy [16]. The development of novel biomarkers and therapeutic options is hampered by a limited knowledge of the genomic landscape of PSCC. Remarkably, most of the genetic studies focus on the analysis of single genes (mainly *TP53*, *CDKN2A*, *EGFR*, *PIK3CA* and *MYC*) or analyze a limited number of samples and systematic and extensive genomic analyses of PSCC have not taken place yet.

Since 2014, increased access to next generation sequencing (NGS) has pushed forward the molecular characterization of prevalent neoplasms such as breast or lung cancer. Unfortunately, molecular progress on rare cancers such as PSCC or vulvar cancer [17] has been much slower. We undertook this review to summarize and discuss the findings of the available studies on the genomic landscape of PSCC.

2. Methodology

2.1. Literature Revision and Criteria of Selection

Relevant studies on genomic alterations (somatic mutations and/or copy number alterations) were captured through a search of Pubmed Medline database using the terms "penis", "penile", "cancer", "carcinoma", "molecular", "genomic", "genetic" and "mutation". We also conducted a manual search of reference lists from the identified papers. Those original articles focused on genome sequencing in PSCC published until 30 June 2021 and having openly published and extractable datasets were deemed eligible.

After the first search results, we excluded papers in languages other than English and those with unavailable full text. Then, we screened titles and abstracts excluding misclassified non-original studies (reviews, meta-analyses, editorials or comments), those not focused on human PSCC or not involving DNA sequencing, and other types of documents (books, congress abstracts). After reviewing the full text and examining methodology, we discarded studies not focused on genomic sequencing of PSCC and those assessing less than 10 genes or using non-tissue samples. The results of the eligible studies (tables, figures and Supplementary Materials) were further screened in terms of availability and completeness for each studied gene.

The researchers extracted the relevant data including the number of PSCC samples and patients studied, the type of DNA sequencing method, the number and prevalence of genomic alterations and the type and results of HPV testing. The genes involved in copy number alterations were searched among those with somatic mutations to identify genes with both types of abnormalities.

2.2. Study Selection

The flowchart of the study with the outline of the search results and the study selection process is shown in Figure 2. The search in Medline Pubmed (accessed on 30 June 2021) rendered 434 articles. Of these, 398 were English-written articles with available full text. After discarding non-original articles, those not focused on human penile cancer and those not applying genomic analysis, 152 studies were further evaluated. More than half of them (86; 57%) were excluded due to the primary focus on HPV prevalence, genotyping or viral integration patterns. Sixty-six articles (43%) were excluded due to the inclusion of non-squamous penile carcinomas, their primary focus on transcriptomic or proteomic

analysis or because of a limited number of assessed genes. After evaluating the DNA sequencing results of seven selected articles and reviewing the list of references, one study was not included in the analysis due to the impossibility of reliably extracting the exact numbers from the genomic results [15], while an additional study was captured from the reference list and included in the analysis.

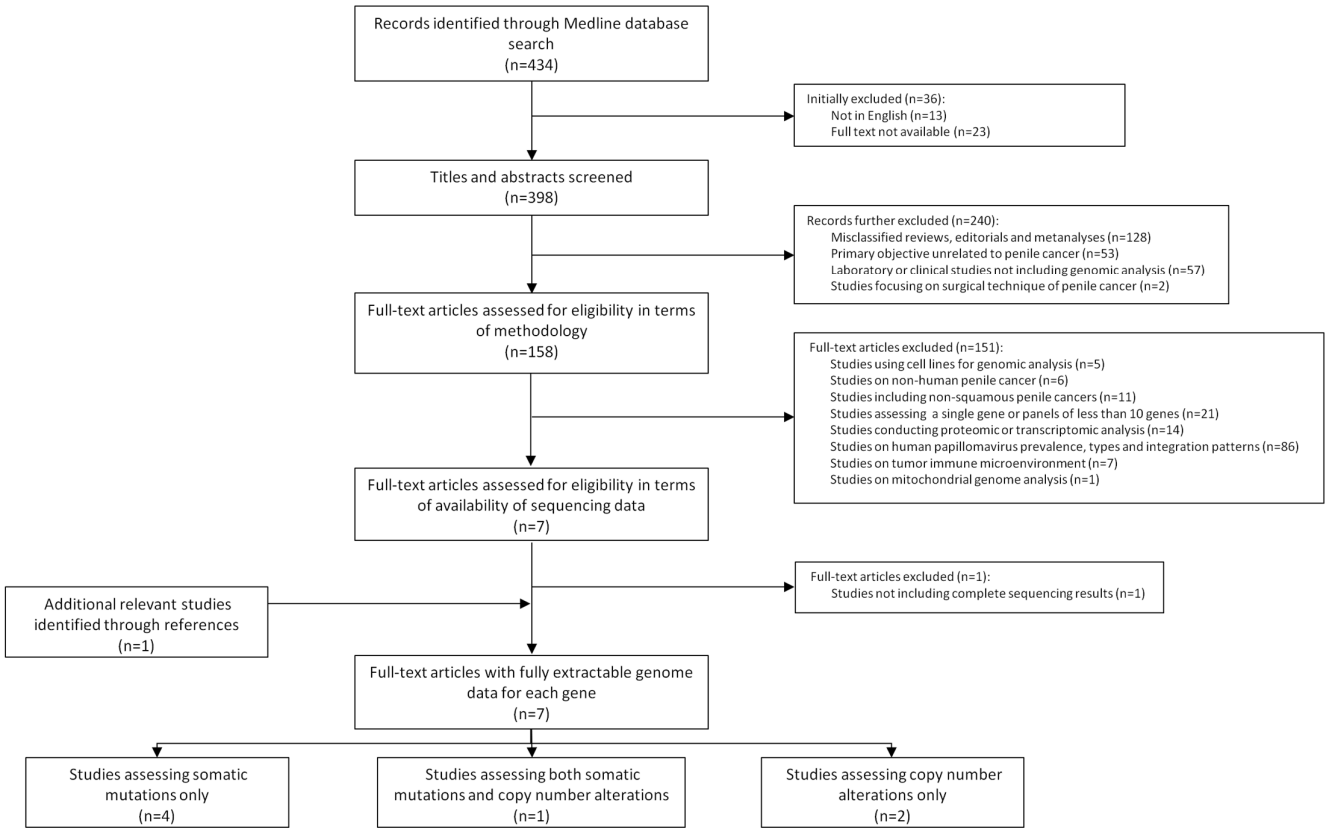

Figure 2. Flowchart with outline of search results and study selection process.

2.3. Methodological Features of the Studies

Table 1 summarizes the main methodological features and HPV testing results of the seven included study series. Adding all cases reported in the seven series included in the analysis, a total of 268 PSCC samples from 251 patients were analyzed. The studies were published between 2015 and 2021. Two studies (29%) were conducted in the United States, two (29%) in China, one (14%) in the United Kingdom, one (14%) in Brazil and one (14%) in Spain. The geographical distribution of the selected study series is shown in Figure 3.

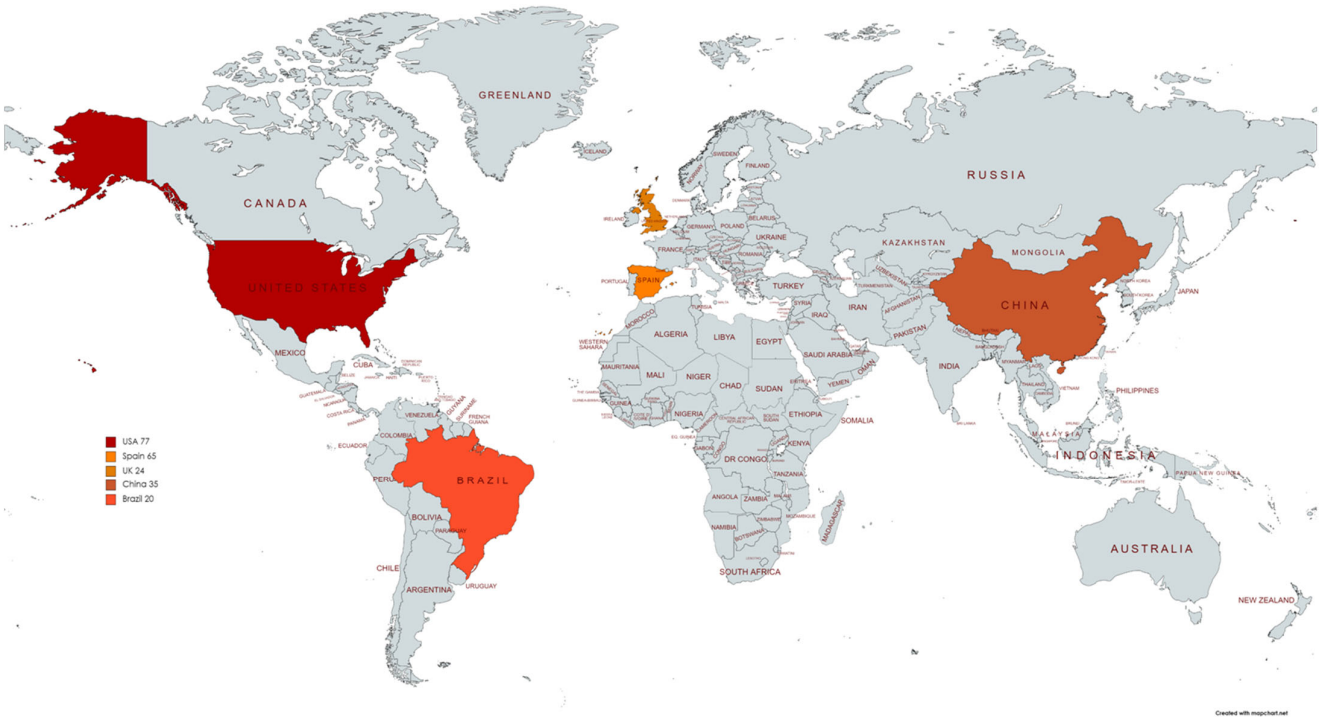

Figure 3. Geographical distribution of the selected study series and the number of patients from each country involved.

Table 1. Main characteristics of the studies analyzing the genomic alterations in Penile Squamous Cell Carcinoma.

Author, Year and Reference	Country	Number of Patients	Number of Samples	Characteristics of the Sample	HPV Test	HPV Prevalence	Type of Genomic Analysis	Gene Panel	Number of Targeted Genes	Platform/Sequencing Depth (for NGS Studies)
Studies assessing only somatic mutations (*n* = 4)										
Ferrándiz-Pulido (2015) [21]	Spain	65	65	FFPE	Unspecified PCR and p16	28%	Targeted mass spectrometry sequencing	Oncocarta mutation panel v1.0	19	N/A
Feber (2016) [18]	UK	24	24	Not specified	Unspecified qPCR	37%	Whole exome sequencing	N/A	Whole exome	Hi-Seq 2000/60x
Wang (2019) [19]	China	30	30	Fresh frozen	PCR-reverse dot blot assay	20%	Whole exome sequencing	N/A	Whole exome	Hi-Seq 2500/130x
Chahoud (2021) [20]	USA	34	34	Fresh frozen	Cobas HPV assay and p16	29%	Whole exome sequencing	N/A	Whole exome	Hi-Seq 4000/141x
Studies assessing both somatic mutations and copy number variations (*n* = 1)										
McDaniel (2015) [24]	USA	43	60*	FFPE	GP5+/GP6+ My09/11 and p16	12%	Multiplex-based targeted NGS	Oncomine Compreh	126	PGM/535x

							ensive Panel			
Studies assessing only copy number variations (*n* = 2)										
Macedo (2020) [22]	Brazil	20	20	FFPE and fresh frozen	My09/My11	96%	aCGH; TaqMan copy number assay in the genes of PI3K/AKT pathway	N/A	N/A	N/A
Yongbo (2020) [23]	China	35	35	Fresh frozen	PCR-reverse dot blot assay	20% **	Whole exome sequencing	N/A	Whole exome	Hi-Seq2500/120x

* A subset of matched primary/metastatic tissue was assessed; ** frequency based on 30 out of 35 samples; aCGH: comparative genomic hybridization; FFPE: formalin fixed paraffin embedded; N/A: not applicable; HPV: human papillomavirus; NGS: next generation sequencing; PCR: polymerase chain reaction; UK: United Kingdom; USA: United States of America.

Four studies (57%) [18–21] evaluated only somatic mutations, two (29%) [22,23] assessed only copy number profiling and one (14%) [24] included assessment of both somatic mutations and copy number alterations. NGS was applied in five studies (72%), four of them used whole exome sequencing (WES) analysis and one applied a targeted approach using a commercial panel. Three out of the four WES series focused on somatic mutations [18–20] and one [23] on copy number alterations. Two studies used other, non-NGS-based methods of genomic analysis: array comparative genomic hybridization [22] and mass spectrometry-based DNA sequencing [21]. One study [24] assessed a subset of matched primary/metastatic tissue. Two studies [20,24] compared their findings of PSCC with those of other types of squamous cell carcinomas using The Cancer Genome Atlas. Four studies, all of them performing WES, analyzed the implicated pathways. IHC or gene expression analysis to validate identified mutations in tissue was conducted in four studies: one used IHC alone [24], one both IHC and Western-Blot [19], one both IHC and PCR [22] and one used only PCR [21].

Four of the five NGS studies included an analysis of non-tumor samples, most commonly blood [19,23] or normal penile tissue [20]. The largest WES series included 35 PSCC cases and was focused on copy number DNA analysis. The mean sequencing coverage depth of the WES studies in the tumor samples ranged from 60× [18] to 141× [20], whereas the targeted NGS study [24] was sequenced at 535x. Three out of four WES studies [18–20] additionally explored mutational signatures.

HPV testing based on PCR was performed in all seven studies, however, only five correlated HPV status and molecular results. The HPV tests included PCR-reverse dot blot assay (two series), unspecified PCR (two series), PGMY9/11 (two series) and Cobas HPV assay (one study). Only three studies, including one of the WES series [20], additionally conducted p16 IHC. The HPV positivity rates ranged from 12% in the American cohort [24] to 96% in the Brazilian study [22].

Four out of seven studies (57%) evaluated the prognostic implications of the genomic alterations identified in PSCC. The follow-up ranged from 27 [21] to 96 months [24].

3. Results

3.1. Somatic Mutations

Table 2 features the results of the five studies [18–21,24] on somatic mutations in PSCC. Top-ranked somatic mutations comprised *TP53*, *CDKN2A*, *NOTCH1*, *PIK3CA*, *FAT1*, *CASP8* and *FBXW7*. *TP53* was mutated in 32% (48/148) of the assessed samples, with frequencies ranging from 10 to 48%. Strikingly, few recurrent somatic alterations were reported in the two WES series (17% and 11%) [18,19].

Table 2. Frequencies of somatic mutations identified in individual genes, stratified by most frequently altered genes, in penile squamous cell carcinomas (PSCC).

Gene	Studies Evaluating the Gene	Studies Identifying Alterations in the Gene	Total Number of PSCC Assessed	Number of PSCC with Alterations in the Gene	Overall Frequency (%)	Frequency Range (%)
Genes identified in more than one study						
TP53	4	4	148	48	32.4	10–48
NOTCH1	4	4	148	26	17.6	7–44
PIK3CA	4	4	189	25	13.2	9–21
HRAS	4	4	179	20	11.2	6–17
CDKN2A	3	3	118	30	25.4	4–32
FAT1	3	3	88	22	25.0	13–35
CASP8	3	3	88	15	17.0	13–24
FBXW7	3	3	118	13	11.0	8–15
NFE2L2	3	3	114	12	10.5	8–12
TTN	2	2	64	14	21.9	10–32
MUC17	2	2	58	8	13.8	13–15
FLG	2	2	54	7	12.9	10–17
EP300	2	2	58	6	10.3	4–15
KRAS	2	2	125	8	6.4	3–9
KIT	2	2	89	4	4.5	3–8
BRAF	2	2	125	4	3.0	3–3
Genes identified in a single study						
NBPF1	1	1	24	13	54.2	N/A
MLL3	1	1	24	9	37.5	N/A
HLA-B	1	1	24	5	20.8	N/A
MUC4	1	1	34	7	20.6	N/A
DNAH6	1	1	34	6	17.6	N/A
GXYLT1	1	1	24	4	16.7	N/A
AHNAK2	1	1	34	5	14.7	N/A
LAMA1	1	1	34	5	14.7	N/A
MUC2	1	1	34	5	14.7	N/A
XRP2	1	1	24	3	12.5	N/A
NSD1	1	1	24	3	12.5	N/A
IL7R	1	1	24	3	12.5	N/A
DNAH12	1	1	24	3	12.5	N/A
WASF3	1	1	24	3	12.5	N/A
TSC1	1	1	24	3	12.5	N/A
SETDB1	1	1	24	3	12.5	N/A
NF1	1	1	24	3	12.5	N/A

COL5A3	1	1	24	3	12.5	N/A
CHD4	1	1	24	3	12.5	N/A
ANK3	1	1	24	3	12.5	N/A
ALK	1	1	24	3	12.5	N/A
ZNF462	1	1	24	3	12.5	N/A
ZBTB5	1	1	24	3	12.5	N/A
NID1	1	1	24	3	12.5	N/A
IQGAP2	1	1	24	3	12.5	N/A
INSR	1	1	24	3	12.5	N/A
HEXA	1	1	24	3	12.5	N/A
CNTLN	1	1	24	3	12.5	N/A
PFAS	1	1	24	3	12.5	N/A
PAPLN	1	1	24	3	12.5	N/A
CENPJ	1	1	24	3	12.5	N/A
C2CD3	1	1	24	3	12.5	N/A
ATP10D	1	1	24	3	12.5	N/A
ASXL1	1	1	24	3	12.5	N/A
HHAT	1	1	24	3	12.5	N/A
AK302511	1	1	34	4	11.8	N/A
ARPP21	1	1	34	4	11.8	N/A
BIRC6	1	1	34	4	11.8	N/A
CACNA1C	1	1	34	4	11.8	N/A
CSPG4	1	1	34	4	11.8	N/A
FAT4	1	1	34	4	11.8	N/A
FHAD1	1	1	34	4	11.8	N/A
FRG1	1	1	34	4	11.8	N/A
FRY	1	1	34	4	11.8	N/A
FSIP2	1	1	34	4	11.8	N/A
GRIN2B	1	1	34	4	11.8	N/A
KMT2B	1	1	34	4	11.8	N/A
MYO188	1	1	34	4	11.8	N/A
PDE4DIP	1	1	34	4	11.8	N/A
PKD1	1	1	34	4	11.8	N/A
SLITRK2	1	1	30	3	10.0	N/A
TRRAP	1	1	30	3	10.0	N/A
CCDC168	1	1	30	3	10.0	N/A
SACS	1	1	24	2	8.3	N/A
NUP210L	1	1	24	2	8.3	N/A
MGA	1	1	24	2	8.3	N/A
USP31	1	1	24	2	8.3	N/A
TM9SF1	1	1	24	2	8.3	N/A
TGM1	1	1	24	2	8.3	N/A
SNX19	1	1	24	2	8.3	N/A
SMG6	1	1	24	2	8.3	N/A
SLC7A6OS	1	1	24	2	8.3	N/A
PITPNM2	1	1	24	2	8.3	N/A
PIGT	1	1	24	2	8.3	N/A

NCF2	1	1	24	2	8.3	N/A
MTHFR	1	1	24	2	8.3	N/A
IQCG	1	1	24	2	8.3	N/A
INADL	1	1	24	2	8.3	N/A
GPS1	1	1	24	2	8.3	N/A
FAM72D	1	1	24	2	8.3	N/A
DFNA5	1	1	24	2	8.3	N/A
CX3CR1	1	1	24	2	8.3	N/A
CREB3L4	1	1	24	2	8.3	N/A
CPNE1	1	1	24	2	8.3	N/A
CHPT1	1	1	24	2	8.3	N/A
BRCA1	1	1	24	2	8.3	N/A
ZFHX3	1	1	24	2	8.3	N/A
TXNDC8	1	1	24	2	8.3	N/A
TNFRSF14	1	1	24	2	8.3	N/A
TGFBR2	1	1	24	2	8.3	N/A
TET2	1	1	24	2	8.3	N/A
TDRD10	1	1	24	2	8.3	N/A
SNX25	1	1	24	2	8.3	N/A
SELP	1	1	24	2	8.3	N/A
PRDM1	1	1	24	2	8.3	N/A
OTUD7A	1	1	24	2	8.3	N/A
NTN4	1	1	24	2	8.3	N/A
NCOR1	1	1	24	2	8.3	N/A
HLA-A	1	1	24	2	8.3	N/A
CREBBP	1	1	24	2	8.3	N/A
BRE	1	1	24	2	8.3	N/A
ATM	1	1	24	2	8.3	N/A
PDGFA	1	1	65	3	4.6	N/A
ZRANB3	1	1	24	1	4.2	N/A
ZNF180	1	1	24	1	4.2	N/A
TIMM17A	1	1	24	1	4.2	N/A
STK19	1	1	24	1	4.2	N/A
SPEN	1	1	24	1	4.2	N/A
OR52N1	1	1	24	1	4.2	N/A
OR4A16	1	1	24	1	4.2	N/A
MYOCD	1	1	24	1	4.2	N/A
MORC4	1	1	24	1	4.2	N/A
MICALCL	1	1	24	1	4.2	N/A
ITPKB	1	1	24	1	4.2	N/A
FAM166A	1	1	24	1	4.2	N/A
DIS3	1	1	24	1	4.2	N/A
CTCF	1	1	24	1	4.2	N/A
C3orf70	1	1	24	1	4.2	N/A
BCLAF1	1	1	24	1	4.2	N/A
ALPK2	1	1	24	1	4.2	N/A
NRAS	1	1	65	2	3.1	N/A
SMARCB1	1	1	60	1	1.7	N/A

ABL	1	1	65	1	1.5	N/A
EGFR	1	1	65	1	1.5	N/A
MET	1	1	65	1	1.5	N/A
RET	1	1	65	1	1.5	N/A

The most frequent (but not the most studied) somatic mutations were identified in *NBPF1* (13/24; 54%), followed by *MLL3* (9/24; 37.5%). Both mutations were identified in a single WES study [18].

3.2. Copy Number Variations

Table 3 shows the results of the three studies on copy number variations in PSCC. The chromosome region analyzed, the type of event detected, the targeted genes, the total number of tumors analyzed, the number of tumors showing alterations in each region and the overall frequency and range of alterations are shown. The most common copy number variations included gains in 8q24 (*MYC* locus). Two studies identified copy number variations involving the locus of *EGFR* in 10 to 70% of cases [22,24]. The Brazilian series [22] showed correlation of *EGFR* variations with increased tumor size. McDaniel et al. [24] also showed high heterogeneity in copy number variations between matched primary tumors and metastasis by finding only 42% of concordance.

Table 3. Frequencies observed in copy number alteration studies of identified alterations in individual genes, stratified by most frequently altered genes, in penile squamous cell carcinomas (PSCC). The genes showing both somatic mutations and copy number alterations are highlighted in bold.

Chromosome Region Studied	Event	Targeted Genes	Studies Identifying Alterations in the Gene	Number of PSCC Assessed	Number of PSCC with Gene Alteration	Overall Frequency (%)	Frequency Range (%)
Copy number alterations identified in more than one study							
8q24	Gains	*MYC*	2	80	26	32.5	18-75
7p12.1 to 11.2	Gains	***EGFR***	2	80	20	25.0	10-70
Copy number alterations identified in one study							
14q32.33	Amplifications	*ADAM 6, KIAA0125, LINC00226, LINC00221 miR7641-2*	1	20	20	100	N/A
2p12-p11.2	Gains	*REEP, CTNNA2, LRRTM1, ATOH8, DNAH6, FABP1, CD8A, CD8B, C2orf3, FAM176A, SUCCLG1, ELMOD3, USP39, VAMP8, FOXI3, FAM176A, SMYD1*	1	20	20	100	N/A
10q26.13	Gains	*MGMT, EBF3, JAKMIP3, INPP5A, KNDC1, GLRX3, PPP2R2D, BNIP3, DPYSL4, LRRC27, ADAM8, PRAP1, PTER, PAOX, MTG1, CALY, SPRN, UTF1*	1	20	17	85.0	N/A
8p23.1	Losses	*MCPH1, SGK223, SOX7, GATA4, PINX1, TDH, FAM66A*	1	20	17	85.0	N/A

10q11.22	Losses/deletions	*ZNF488, GDF2, SYT15, MAPK8, RBP3*	1	20	15	75.0	N/A
1p36.3	Gains	*VWA1, CCNL2, MIB2, ATAD3A, GNB1, HES5, TP73*	1	20	15	75.0	N/A
14q11.2	Losses/deletions	*CHD8, TOX4, APEX1, SALL2*	1	20	14	70.0	N/A
15q11.2-q13.3	Gains	*OCA2, CYFIP1, TRPM1, BCL8*	1	20	13	65.0	N/A
8q11.1-q24.3	Gains	*TOX, WISP1, IL7, STK3, SOX17, RP1, MAFA*	1	20	13	65.0	N/A
10p11.23	Deletions	*Bmi1*	1	35	22	62.9	N/A
1q43	Gains	*IRF2BP2, ARID4b, LYST, GGPS1, FMN2*	1	20	12	60.0	N/A
7q21.11	Gains	*CD36, GNAT3, TMEM60, PHTF2*	1	20	12	60.0	N/A
2p24.3	Amplifications	*MYCN*	1	35	20	57.1	N/A
15q11.1-q11.2	Losses/deletions	*BCL8, CYFIP1, NIPA1, NIPA2*	1	20	11	55.0	N/A
17p13.1	Deletions	***TP53***	1	35	19	54.3	N/A
8q24.3	Amplifications	*PTK2 (FAK)*	1	35	19	54.3	N/A
12q15	Deletions	*MDM2*	1	35	18	51.4	N/A
22q11.21	Gains	*BID, CECR2, TBX1, GSC2, HIRA*	1	20	10	50.0	N/A
2p25.3-p11.1	Gains	*SOX11, REL, FOXI3, EGR4, SIX3, TLX2,* ***BIRC6****, CD8B*	1	20	10	50.0	N/A
3q26.1	Gains	*MECOM, SI, PDCD10*	1	20	10	50.0	N/A
17q21.33	Deletions	*NGFR (p75NTR)*	1	35	17	48.6	N/A
15q26.2-q26.3	Gains	*CHD2, IGF1R, RGMA, MEF2A, PTER, SYNM*	1	20	9	45.0	N/A
4q13.2	Losses	*UGT2B4, STAP1, TMPRSS11D*	1	20	9	45.0	N/A
5q13.2	Losses/deletions	*SKP2, BRIX1,* ***IL7R****, GDNF, RAD1, BRIX1, SPEF2*	1	20	9	45.0	N/A
7p21.3	Gains	*PHF14, COL281A, RPA3, ARL4A*	1	20	9	45.0	N/A
14q12	Gains	*REC8, TINF2, PRKD1, IL25, FOXG1*	1	20	8	40.0	N/A
16p11.2-p11.1	Gains	*ZNF689, ZNF668, YPEL3, PYCARD, MAZ, IL27, CD19*	1	20	8	40.0	N/A
1q21.2	Losses	*POLR3C, BCL9,* ***PDE4DIP****, TXNIP, CD160, ACP6*	1	20	8	40.0	N/A
20q13.32-q13.33	Gains	*BIRC7, DIDO1, ZGPAT, CTCFL, GNAS, SPO11,*	1	20	8	40.0	N/A

9p21	Gains	*SLC2A4RG, GATA5, TAF4, PTK6 CDKNB, ACO1, TAF1L, TEK, IFT74, IFNK, TUSC1, MLLT3*	1	20	8	40.0	N/A
1q23.1	Amplifications	*NTRK1(TRKA)*	1	35	13	37.1	N/A
2p16.3	Gains	*MSH2, MSH6, FOXN2, NRXN1, FSHR*	1	20	7	35.0	N/A
1p36.13	Deletions	*ZBTB17(Miz1)*	1	35	12	34.3	N/A
11q24-q25	Gains	*ST14, CDON, OPCML, BARX2, ETS1, ADAMTS15, PTER*	1	20	5	25.0	N/A
14q31-q31.3	Gains	*GPR65, STON2, FLRT2*	1	20	5	25.0	N/A
18p11.31 -p11.21	Gains	***LAMA1***, *APCDD1, TGIF1, MC5R*	1	20	5	25.0	N/A
7p22.3-p11	Gains	*PMS2, ADAP1, FAM126A, TSPAN13, MAFK*	1	20	5	25.0	N/A
Yp11.3-p11.2	Gains	*CD99, ZBED1, TSPY1*	1	20	5	25.0	N/A
9p21.3	Losses	***CDKN2A***	1	60	13	21.6	N/A
14q23.3	Amplifications	*Max*	1	35	7	20.0	N/A
11q13.3	Gains	***CCND1***	1	60	8	13.3	N/A
3q26.33	Gains	*SOX2*	1	60	8	13.3	N/A
3q26.33	Gains	*ATP11B*	1	60	5	8.3	N/A
5p15.33	Gains	*TERT*	1	60	4	6.7	N/A
3q26.33	Gains	*DCUN1D1*	1	60	3	5.0	N/A
10p14	Losses	*GATA3*	1	60	2	3.3	N/A
11p13	Gains	*CD44*	1	60	2	3.3	N/A
22q12.2	Losses	*NF2*	1	60	2	3.3	N/A
3q26.32	Gains	***PIK3CA***	1	60	2	3.3	N/A
11q22.2	Gains	*BIRC3*	1	60	1	1.7	N/A
12q14.1	Gains	***CDK4***	1	60	1	1.7	N/A
20q11.21	Gains	*BCL2L1*	1	60	1	1.7	N/A
4q31.3	Losses	***FBXW7***	1	60	1	1.7	N/A

N/A: not applicable.

Amplifications or gains at HPV integration sites 14q32.33 (loci of noncoding RNAs (*IncRNAs*, *ADAM6*, *LINC00226*, *LINC00221* and *KIAA0125*), 2p12-p11.2, 10q26.13 and 8q23.1 were identified with high frequencies (85–100%); however, all of them were reported in a single study [22]. Somatic mutations, in addition to copy number alterations, have been reported in a few genes including *TP53*, *CDKN2A*, *PIK3CA*, *CCND1*, *ALK*, *BIRC6*, *IL7R*, *PDE4DIP* and *LAMA1*.

3.3. Relationship with HPV Status

Among the five studies that have compared the genomic alterations identified in HPV-associated and HPV-independent PSCC, two [18,24] reported a markedly lower mutational load (number of non-silent and driver mutations) in HPV-associated than in HPV-independent PSCC. Contrarily, a WES study [20] found only minimal, negligible mutation load differences between the two etiopathogenic types. Two studies [20,24] identified a strong inverse correlation between HPV positivity and *TP53* and *CDKN2A* mutations. In

one of them [20], HPV-associated tumors were significantly associated with somatic mutations in *ARPP21*, *CMYA5*, *RPGRIP* and *CSPG4*.

Regarding copy number alterations, one study [24] showed low frequency in HPV-associated PSCC, whereas the Brazilian series, with 95% of the tumors being HPV-associated [22], reported high rates of copy number alterations in HPV integration sites (2p12-p11.2 and 14q32.33), as well as in inflammation-related genes (*EGFR* and *COX2*).

3.4. Mutational Signatures and Signaling Pathways in PSCC

Transition mutations, including mostly C>T alterations, mediated by the APOBEC family of cytosine deaminases [25] were reported in three studies [18–20]. Feber et al. [18] identified HPV-associated APOBEC mutation signatures and NpCgP signatures in HPV-negative PSCC, in which C>T alterations correlated with decreased DNA methylation. Chahoud et al. [20] additionally reported a subset of PSCC with a defective DNA repair system (*BRCA1*, *BRCA2*, *ARID1A*, *ATR*, *CHEK2*, *PARP1*, *FANCA*, *PALB2*, and *RAD51*).

At least 10 signaling pathways have been identified as disrupted in PSCC in four WES studies. Of them, two [19,20] (50%) reported the Notch, RTK-RAS and Hippo signaling pathways as the three most implicated. The WES study conducted in China [19] showed alterations in these three pathways in more than half of the samples. By contrast, McDaniel et al. [24] showed the predominance of the p53 pathway deregulation. On the other hand, one of the studies on copy number variants [23] highlighted the role of the MYCN/Max pathway.

3.5. Prognostic Implications of Mutations and Copy Number Alterations in PSCC

McDaniel et al. [24] noticed that patients with *MYC* and *CCND1* gains, and those with negative p16 IHC, showed shorter time to progression or survival. The same study reported that high mutational burden in the five most frequently mutated genes (*CDKN2A*, *EGFR*, *MYC*, *HRAS* and *TP53*) correlated with an advanced stage.

Amplifications in *MYCN* and *FAK* correlated significantly with worse survival in one study [23]. Chahoud et al. [20] showed a trend towards worse overall survival for patients with mutations in the Notch pathway, whereas patients with the PI3K pathway mutated genes had improved outcomes. High APOBEC scores correlated with shorter overall survival, higher tumor mutational burden and the presence of lymph node metastasis [20].

3.6. Potential Therapeutic Targets to Treat PSCC

Most of the studies suggest potential actionable targets on the basis of the identified genomic alterations. McDaniel et al. [24] proposed targeting amplifications of *EGFR* and cell cycle kinase *CDK4*, as well as somatic mutations in *KRAS*. Ferrándiz-Pulido et al. [21] indicated that patients with concomitant KRAS and PIK3CA mutations might benefit from a combination of mTOR and tyrosine-kinase inhibitors. The same authors proposed imatinib for patients with *PDGFA*-mutated tumors. Chahoud et al. [20] hypothesized that patients with APOBEC-enriched tumors might benefit from immune checkpoint inhibitors, whereas those with a defective DNA repair system and microsatellite instability might be treated with PARP inhibitors alone or combined with immune checkpoint inhibition. The same study provided an extensive list of druggable genes using the Drug Gene Interaction database, which included genes such as *TP53*, *CDKN2A*, *NOTCH1*, *PIK3CA*, *FBXW7*, *CASP8*, *LAMA1* and *TTN*, among others.

The Brazilian series, which mostly included HPV-positive tumors [22], proposed targeting *ADAM6* alterations involved in the Notch pathway, although there is little knowledge of their role in cancer. The same authors proposed using *EGFR* and *COX2* inhibitors.

4. Discussion

Considerable insight on the genomic landscape of PSCC has been acquired over the last six years (2015–2021), as evidenced by the seven studies identified. However, these studies are highly heterogeneous in terms of sociodemographic characteristics, methodology (tissue analyzed, frozen or paraffinized, type of genomic analysis) and include a limited number of samples, which may hamper the validity of some of the conclusions. As a result, the series are also highly heterogeneous in terms of their findings.

Somatic mutations in cancer-related genes *TP53*, *CDKN2A*, *FAT1*, *NOTCH1* and *PIK3CA* are consistently identified in PSCC. Copy number alterations have also been reported in three of these genes (*TP53*, *CDKN2A* and *PIK3CA*) [20,21], which speaks to the relevance of these genes in PSCC carcinogenesis. *TP53* and *CDKN2A* are well-known tumor suppressor genes [15,20,24]. *NOTCH1* and *FAT1* mutations are consistently featured in PSCC [15]. However, little is known on the role and mechanisms of both types of mutations in PSCC and other cancers [26].

Another intriguing and frequent finding of the recent studies [18–20] includes the identification of *CASP8* and *FBXW7* alterations. *CASP8* is known for its involvement in apoptosis cascade, whereas *FBXW7* acts as a promoter of tumorigenesis through ubiquitin degradation of cell cycle regulators, including p53 [27]. As occurs with *FAT1* mutations, the contribution and clinical relevance of both genes in PSCC remain to be elucidated. Curiously, patients with *TP53* wild-type tumors of oral cavity harboring both *CASP8* and *HRAS* mutations showed improved outcomes [28]. It is also interesting to further explore the role of the *NBPF1* gene in PSCC, identified with high frequency but only in a single study. *NBPF1* is known to deactivate the PI3K signaling pathway leading to tumor growth inhibition [29].

The genomic profile of PSCC also typically contains numerical alterations in *MYC*, *EGFR* and *CCND1*. The high numbers of *MYC* and *EGFR* variations in PSCC are in concordance with previous evidence reported in head and neck cancers squamous cell carcinoma, a similar tumor with dual pathogenesis [30].

Notch represents the most involved signaling pathway in the studies exploring the whole exome. Curiously, the PI3K pathway is not among the three most frequently involved signaling pathways, despite frequent identification of *PIK3CA* and *EGFR* alterations [19,20]. It is of note, however, that both genes are also implicated in the Hippo pathway, among the three most implicated pathways in this review.

Although PSCC has been divided into two different etiologic pathways (HPV-associated and -independent), the overall mutational profile of HPV-associated PSCC is not considerably different from HPV-independent tumors in the published studies. However, a marked variability in HPV prevalence hampers comparability of findings among studies. Indeed, whereas the HPV prevalence ranged from 12 to 37 in six of the studies [18–20,23,24], which is in keeping with the numbers reported in most studies on PSCC [15,31,32], one of the series [22] reports an unusually high percentage (96%) of HPV-associated tumors.

The prognostic role of most molecular alterations in PSCC also remains elusive. Remarkably, only a single study in this review [20], based on WES, finds prognostic association for PI3K pathway mutations, *NOTCH1* mutations or APOBEC scores. The association of MYC gains with adverse prognosis was also shown in a single study [24], in accordance with only one available publication [33]. The prognostic relevance of *MYCN* and *FAK* variations described by Yongbo et al. [23] certainly warrant further research using a similar approach based on WES.

Unfortunately, the genes most frequently altered in PSCC, including *TP53*, *CDKN2A*, *PIK3CA*, *MYC*, and *EGFR*, have proven to be challenging to target separately [34–36]. Thus, it might be worth exploring combinations of treatments based on an interaction between implicated signaling pathways. For example, mutant p53 is highly oncogenic through the stimulation of the PI3K signaling pathway, which suggests the utility of mTOR inhibitors in *TP53*-mutated patients [37]. Patients with defective DNA repair and

APOBEC systems might respond to PARP and checkpoint immune inhibition [20]. Lastly, since both *NOTCH1* and *PIK3CA* mutations are frequent in PSCC [20], vulvar [17] and head and neck squamous cell tumors [38], there is rationale to enroll such patients in clinical trials focused on PI3K/mTOR inhibitors in *NOTCH1*-mutated patients.

High heterogeneity in findings among the studies might be due to methodological differences in DNA sequencing. Indeed, the targeted NGS study [24], which explores 126 genes, prioritizing recurrently altered and tumor suppressor genes, cannot be compared with WES studies covering around 20,000 genes. Nevertheless, even the three WES studies are heterogenous in terms of results and methods. The low number of mutations (only 12 genes) reported by the Chinese WES study [19], in contrast with at least double the number of mutations detected in the other WES series, is striking [18,20]. Indeed, while the earliest WES study [18] reports 60x coverage using Hi-Seq2000, the most recent WES series [19,20] use a more advanced (Hi-Seq2500 or Hi-Seq4000) sequencer, with coverages ranging from 130x to 141x.

In conclusion, there is still limited understanding of molecular abnormalities involved in PSCC. There is a lack of evidence regarding the association of molecular abnormalities with main clinico-pathological variables. The existing studies are limited in sample size, sociodemographic heterogeneity and variability in DNA sequencing methodology. There is a particular gap of knowledge in the characterization of molecular profiles in relation to HPV status. Given the rarity of PSCC, especially in high-income countries, a number of genomic studies regarding this disease face challenges in acquiring enough samples. Therefore, large multicenter studies are urgently needed to continue on the path of the molecular characterization of PSCC.

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References

1. Cardona, C.E.M.; García-Perdomo, H.A. Incidence of penile cancer worldwide: Systematic review and meta-analysis. *Rev. Panam. Salud Pública* **2017**, *41*, 1–10. https://doi.org/10.26633/RPSP.2017.117.
2. Emmanuel, A.; Nettleton, J.; Watkin, N.; Berney, D.M. The molecular pathogenesis of penile carcinoma—current developments and understanding. *Virchows Archiv* **2019**, *475*, 397–405. https://doi.org/10.1007/s00428-019-02607-8.
3. Crispen, P.L.; Mydlo, J.H. Penile Intraepithelial Neoplasia and Other Premalignant Lesions of the Penis. *Urol. Clin. North Am.* **2010**, *37*, 335–342. https://doi.org/10.1016/j.ucl.2010.04.003.
4. Chaux, A.; Pfannl, R.; Lloveras, B.; Alejo, M.; Clavero, O.; Lezcano, C.; Muñoz, N.; José, F.X.B.; Bosch, X.; Hernández-Pérez, M.; et al. Distinctive Association of p16INK4a Overexpression with Penile Intraepithelial Neoplasia Depicting Warty and/or Basaloid Features: A Study of 141 Cases Evaluating a New Nomenclature. *Am. J. Surg. Pathol.* **2010**, *34*, 385–392. https://doi.org/10.1097/PAS.0b013e3181cdad23.
5. Jouhi, L.; Hagström, J.; Atula, T.; Mäkitie, A. Is p16 an adequate surrogate for human papillomavirus status determination? *Curr. Opin. Otolaryngol. Head Neck Surg.* **2017**, *25*, 108–112. https://doi.org/10.1097/MOO.0000000000000341.
6. Alemany, L.; Cubilla, A.; Halec, G.; Kasamatsu, E.; Quirós, B.; Masferrer, E.; Tous, S.; Lloveras, B.; Hernández-Suarez, G.; Lonsdale, R.; et al. Role of Human Papillomavirus in Penile Carcinomas Worldwide. *Eur. Urol.* **2016**, *69*, 953–961. https://doi.org/10.1016/j.eururo.2015.12.007.
7. Canete-Portillo, S.; Sanchez, D.F.; Cubilla, A.L. Pathology of Invasive and Intraepithelial Penile Neoplasia. *Eur. Urol. Focus* **2019**, *5*, 713–717. https://doi.org/10.1016/j.euf.2019.06.013.
8. Cubilla, A.L.; Lloveras, B.; Alejo, M.; Clavero, O.; Chaux, A.; Kasamatsu, E.; Monfulleda, N.; Tous, S.; Alemany, L.; Klaustermeier, J.; et al. Value of p16(INK)4(a) in the pathology of invasive penile squamous cell carcinomas: A report of 202 cases. *Am. J. Surg. Pathol.* **2011**, *35*, 253–261. https://doi.org/10.1097/PAS.0b013e318203cdba.

9. Chaux, A.; Pfannl, R.; Rodríguez, I.M.; Barreto, J.E.; Velazquez, E.F.; Lezcano, C.; Piris, A.; Netto, G.J.; Cubilla, A.L. Distinctive immunohistochemical profile of penile intraepithelial lesions: A study of 74 cases. *Am. J. Surg. Pathol.* **2011**, *35*, 553–562. https://doi.org/10.1097/PAS.0b013e3182113402.
10. Moch, H.; Cubilla, A.L.; Humphrey, P.A.; Reuter, V.E.; Ulbright, T.M. The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs—Part A: Renal, Penile, and Testicular Tumours. *Eur. Urol.* **2016**, *70*, 93–105. https://doi.org/10.1016/j.eururo.2016.02.029.
11. Kidd, L.C.; Chaing, S.; Chipollini, J.; Giuliano, A.R.; Spiess, P.E.; Sharma, P. Relationship between human papillomavirus and penile cancer-implications for prevention and treatment. *Transl. Androl. Urol.* **2017**, *6*, 791–802. https://doi.org/10.21037/tau.2017.06.27.
12. Hakenberg, O.W.; Dräger, D.L.; Erbersdobler, A.; Naumann, C.M.; Jünemann, K.P.; Protzel, C. The Diagnosis and Treatment of Penile Cancer. *Dtsch. Aerzteblatt Int.* **2018**, *115*, 646. https://doi.org/10.3238/ARZTEBL.2018.0646.
13. Stecca, C.E.; Alt, M.; Jiang, D.M.; Chung, P.; Crook, J.M.; Kulkarni, G.S.; Sridhar, S.S. Recent Advances in the Management of Penile Cancer: A Contemporary Review of the Literature. *Oncol. Ther.* **2021**, *9*, 21–39. https://doi.org/10.1007/S40487-020-00135-Z.
14. McGregor, B.A.; Sonpavde, G.P. New insights into the molecular profile of penile squamous cell carcinoma. *Clin. Cancer Res.* **2021**, *27*, 2375–2377. https://doi.org/10.1158/1078-0432.CCR-21-0139.
15. Jacob, J.; Ferry, E.; Gay, L.; Elvin, J.A.; Vergilio, J.A.; Ramkissoon, S.; Severson, E.; Necchi, A.; Killian, J.K.; Ali, S.M.; et al. Comparative Genomic Profiling of Refractory and Metastatic Penile and Nonpenile Cutaneous Squamous Cell Carcinoma: Implications for Selection of Systemic Therapy. *J. Urol.* **2019**, *201*, 541–548. https://doi.org/10.1016/J.JURO.2018.09.056.
16. Vanthoor, J.; Vos, G.; Albersen, M. Penile cancer: Potential target for immunotherapy? *World J. Urol.* 2020 395 **2020**, *39*, 1405–1411. https://doi.org/10.1007/S00345-020-03510-7.
17. Carreras-Dieguez, N.; Guerrero, J.; Rodrigo-Calvo, M.; Ribera-Cortada, I.; Trias, I.; Jares, P.; López Del Campo, R.; Saco, A.; Munmany, M.; Marimon, L.; et al. Molecular Landscape of Vulvar Squamous Cell Carcinoma. *Int. J. Mol. Sci.* **2021**, *22*, 7069. https://doi.org/10.3390/IJMS22137069.
18. Feber, A.; Worth, D.C.; Chakravarthy, A.; de Winter, P.; Shah, K.; Arya, M.; Saqib, M.; Nigam, R.; Malone, P.R.; Tan, W.S.; et al. CSN1 Somatic Mutations in Penile Squamous Cell Carcinoma. *Cancer Res.* **2016**, *76*, 4720–4727. https://doi.org/10.1158/0008-5472.CAN-15-3134.
19. Wang, Y.; Wang, K.; Chen, Y.; Zhou, J.; Liang, Y.; Yang, X.; Li, X.; Cao, Y.; Wang, D.; Luo, L.; et al. Mutational landscape of penile squamous cell carcinoma in a Chinese population. *Int. J. Cancer* **2019**, *145*, 1280–1289. https://doi.org/10.1002/ijc.32373.
20. Chahoud, J.; Gleber-Netto, F.O.; McCormick, B.Z.; Rao, P.; Lu, X.; Guo, M.; Morgan, M.B.; Chu, R.A.; Martinez-Ferrer, M.; Eterovic, A.K.; et al. Whole-exome Sequencing in Penile Squamous Cell Carcinoma Uncovers Novel Prognostic Categorization and Drug Targets Similar to Head and Neck Squamous Cell Carcinoma. *Clin. Cancer Res.* **2021**, *27*, 2560–2570. https://doi.org/10.1158/1078-0432.ccr-20-4004.
21. Ferrándiz-Pulido, C.; Hernández-Losa, J.; Masferrer, E.; Vivancos, A.; Somoza, R.; Marés, R.; Valverde, C.; Salvador, C.; Placer, J.; Morote, J.; et al. Identification of somatic gene mutations in penile squamous cell carcinoma. *Genes Chromosomes Cancer* **2015**, *54*, 629–637. https://doi.org/10.1002/gcc.22274.
22. Macedo, J.; Silva, E.; Nogueira, L.; Coelho, R.; da Silva, J.; Dos Santos, A.; Teixeira-Júnior, A.A.; Belfort, M.; Silva, G.; Khayat, A.; et al. Genomic profiling reveals the pivotal role of hrHPV driving copy number and gene expression alterations, including mRNA downregulation of TP53 and RB1 in penile cancer. *Mol. Carcinog.* **2020**, *59*, 604–617. https://doi.org/10.1002/mc.23185.
23. Yongbo, Y.; Chengwen, G.; Yuanbin, C.; Wang, M.; Zhang, J.; Ma, X.; Liu, S.; Yuan, H.; Li, Z.; Niu, H. Copy Number Analysis Reveal Genetic Risks of Penile Cancer. *Front. Oncol.* **2020**, *10*, 596261. https://doi.org/10.3389/fonc.2020.596261.
24. McDaniel, A.S.; Hovelson, D.H.; Cani, A.K.; Liu, C.-J.; Zhai, Y.; Zhang, Y.; Weizer, A.Z.; Mehra, R.; Feng, F.Y.; Alva, A.S.; et al. Genomic Profiling of Penile Squamous Cell Carcinoma Reveals New Opportunities for Targeted Therapy. *Cancer Res.* **2015**, *75*, 5219–5227. https://doi.org/10.1158/0008-5472.CAN-15-1004.
25. Rebhandl, S.; Huemer, M.; Greil, R.; Geisberger, R. AID/APOBEC deaminases and cancer. *Oncoscience* **2015**, *2*, 320–333. https://doi.org/10.18632/ONCOSCIENCE.155.
26. Peng, Z.; Gong, Y.; Liang, X. Role of FAT1 in health and disease. *Oncol. Lett.* **2021**, *21*. https://doi.org/10.3892/OL.2021.12659.
27. Wang, L.; Ye, X.; Liu, Y.; Wei, W.; Wang, Z.; Wang, L.; Ye, X.; Liu, Y.; Wei, W.; Wang, Z. Aberrant regulation of FBW7 in cancer. *Oncotarget* **2014**, *5*, 2000–2015. https://doi.org/10.18632/ONCOTARGET.1859.
28. Ciriello, G.; Miller, M.L.; Aksoy, B.A.; Senbabaoglu, Y.; Schultz, N.; Sander, C. Emerging landscape of oncogenic signatures across human cancers. *Nat. Genet.* **2013**, *45*, 1127–1133. https://doi.org/10.1038/ng.2762.
29. Qin, Y.; Tang, X.; Liu, M. Tumor-suppressor gene NBPF1 inhibits invasion and PI3K/mTOR signaling in cervical cancer cells. *Oncol. Res.* **2016**, *23*, 13–20. https://doi.org/10.3727/096504015X14410238486766.
30. Rehmani, H.S.; Issaeva, N. EGFR in head and neck squamous cell carcinoma: Exploring possibilities of novel drug combinations. *Ann. Transl. Med.* **2020**, *8*, 813–813. https://doi.org/10.21037/atm.2020.04.07.
31. Busso-Lopes, A.F.; Marchi, F.A.; Kuasne, H.; Scapulatempo-Neto, C.; Trindade-Filho, J.C.S.; de Jesus, C.M.N.; Lopes, A.; Guimarães, G.C.; Rogatto, S.R. Genomic profiling of human penile carcinoma predicts worse prognosis and survival. *Cancer Prev. Res.* **2015**, *8*, 149–156. https://doi.org/10.1158/1940-6207.CAPR-14-0284.

32. Kuasne, H.; Barros-Filho, M.C.; Busso-Lopes, A.; Marchi, F.A.; Pinheiro, M.; Muñoz, J.J.M.; Scapulatempo-Neto, C.; Faria, E.F.; Guimarães, G.C.; Lopes, A.; et al. Integrative miRNA and mRNA analysis in penile carcinomas reveals markers and pathways with potential clinical impact. *Oncotarget* **2017**, *8*, 15294–15306. https://doi.org/10.18632/oncotarget.14783.
33. Peter, M.; Rosty, C.; Couturier, J.; Radvanyi, F.; Teshima, H.; Sastre-Garau, X. MYC activation associated with the integration of HPV DNA at the MYC locus in genital tumors. *Oncogene* **2006**, *25*, 5985–5993. https://doi.org/10.1038/sj.onc.1209625.
34. Allen-Petersen, B.L.; Sears, R.C. Mission Possible: Advances in MYC Therapeutic Targeting in Cancer. *BioDrugs* **2019**, *33*, 539–553. https://doi.org/10.1007/s40259-019-00370-5.
35. Adimonye, A.; Stankiewicz, E.; Kudahetti, S.; Trevisan, G.; Tinwell, B.; Corbishley, C.; Lu, Y.J.; Watkin, N.; Berney, D. Analysis of the PI3K-AKT-mTOR pathway in penile cancer: Evaluation of a therapeutically targetable pathway. *Oncotarget* **2018**, *9*, 16074–16086. https://doi.org/10.18632/oncotarget.24688.
36. Wykosky, J.; Fenton, T.; Furnari, F.; Cavenee, W.K. Therapeutic targeting of epidermal growth factor receptor in human cancer: Successes and limitations. *Chin. J. Cancer* **2011**, *30*, 5. https://doi.org/10.5732/CJC.010.10542.
37. Cordani, M.; Oppici, E.; Dando, I.; Butturini, E.; Dalla Pozza, E.; Nadal-Serrano, M.; Oliver, J.; Roca, P.; Mariotto, S.; Cellini, B.; et al. Mutant p53 proteins counteract autophagic mechanism sensitizing cancer cells to mTOR inhibition. *Mol. Oncol.* **2016**, *10*, 1008–1029. https://doi.org/10.1016/J.MOLONC.2016.04.001.
38. Shah, P.A.; Huang, C.; Li, Q.; Kazi, S.A.; Byers, L.A.; Wang, J.; Johnson, F.M.; Frederick, M.J. NOTCH1 Signaling in Head and Neck Squamous Cell Carcinoma. *Cells* **2020**, *9*, 2677. https://doi.org/10.3390/cells9122677.

3. Hipòtesi

Les evidències actuals indiquen que hi ha dues vies carcinogèniques diferents relacionades amb el carcinoma esacmós de penis, una associada al virus del papil·loma humà i una altra independent del virus del papil·loma humà, una situació similar a l'etiopatogènia dels carcinomes de la vulva. D'acord amb aquesta categorització etiològica, la versió actual de de la Organització Mundial de la Salut 2022 classifica els carcinoma escamós de penis segons la presència o absència de virus del papil·loma humà i recomana l'ús de la tinció immunohistoquímica per p16 com marcador subrogat de la presència del virus del papil·loma humà. En la majoria de sèries d'Europa occidental, s'observa un marcat predomini de carcinomes escamosos independents del VPH i una baixa freqüència de tumors associats al virus del papil·loma humà.

Els mecanismes moleculars implicats en els carcinomes escamosos de penis associats als virus del papil·loma humà es caracteritzen per una inestabilitat genòmica secundària a la sobre expressió de les onco-proteïnes E6 i E7 que condueix a una activació incontrolada del cicle cel·lular. Per contra, la patogènesi del carcinoma escamós e penis independent del virus del papil·loma humà és poc coneguda. Les mutacions TP53 son freqüents en aquest grup de carcinoma escamós de penis i diversos estudis consideren que les mutacions d'aquest gen podrien estar associades amb una alta freqüència de metàstasis ganglionars, un conegut factor de mal pronòstic. Aquests estudis suggereixen que l'estat mutacional de TP53 podria afegir informació pronòstica rellevant en el carcinoma escamós de penis. No obstant, l'anàlisi molecular de l'estat mutacional de TP53 és tècnicament difícil i no està disponible en molts laboratoris de d'anatomia patològica. Així, l'avaluació immunohistoquímica de p53 s'ha proposat com una alternativa més factible. Malauradament, hi ha dades contradictòries sobre la correlació entre l'expressió immunohistoquímica de p53 i l'estat mutacional de TP53. Aquests resultats discrepants podrien ser deguts a una falta d'estandardització en l'avaluació immunohistoquímica de p53. De fet, en la majoria d'estudis publicats, l'avaluació immunohistoquímica de p53 s'ha basat en el percentatge de nuclis positius a les capes basal i para basals, però sense establir els punts de tall i, per tant, es desconeix el significat real d'aquests percentatges. Recentment s'ha definit un sistema basat en patrons d'expressió de immunohistoquímica de p53 en tumors vulvars, que ha

mostrat una estreta correlació amb l'estat mutacional de TP53. A més, combinant la presència o no de virus del papil·loma humà i el tipus de tinció immunohistoquímica de p53 s'han pogut determinar tres subtipus pronòstics de tumors a la vulva.

Seguint l'exemple iniciat a la vulva, les hipòtesis plantejades en el carcinoma escamós de penis son les següents:

1. A causa de les marcades similituds etiopatògeniques entre el carcinoma escamós de penis i el carcinoma vulvar es planteja la possibilitat que els patrons immunohistoquímics d'expressió de p53 descrits a la vulva també podrien ser aplicats al carcinoma escamós de penis i presentarien una bona correlació amb la presència de mutació del gen TP53.
2. L'aplicació d'aquests patrons immunohistoquímics d'expressió de p53, juntament amb la detecció de p16 com a marcador surrogat de la infecció per virus del papil·loma humà, pot separar diversos tipus moleculars de carcinoma escamós de penis, cadascun dels quals tindria unes característiques clíniques, patològiques i de pronòstic específiques i diferenciades.
3. L'aplicació d'aquests patrons d'expressió es poden utilitzar també en les lesions precursores. En aquest context clínic l'expressió anòmala de p53 associada a la tinció de p16, és molt útil per classificar adequadament aquelles neoplàsies intraepitelials amb morfologia no concordant.

4. Objectius

1. Determinar si el sistema d'avaluació en sis patrons immunohistoquímics de tinció de p53 descrits prèviament en el càncer de vulva pot ser també aplicat a càncer de penis.
2. Avaluar el grau de correlació entre aquests patrons d'expressió immunohistoquímica de p53 i la presència de mutacions del gen *TP53.*
3. Determinar si el nou sistema d'avaluació en sis patrons mostra o no una millor correlació amb les mutacions del gen *TP53* que l'avaluació clàssica en dos patrons *(*nuclis aïllats i sobre-expressió difusa).
4. Determinar la proporció de tumors associats al virus del papil·loma humà (VPH) i independents del VPH a la població de la nostra àrea geogràfica i, dintre d'aquest segon grup, la proporció de tumors amb i sense mutació de *TP53.*
5. Determinar si l'avaluació immunohistoquímica de p53 té alguna significació en els pacients amb carcinoma de penis.
6. Avaluar si la classificació dels càncers de penis basada en la relació amb VPH i la presència o no d'anormalitats de p53 té valor pronòstic.
7. Determinar si el nou sistema d'avaluació en sis patrons de la tinció immunohistoquímica per a p53 aporta algun avantatge en relació als sistemes d'avaluació clàssics en dos patrons.
8. Verificar si existeixen al penis lesions precursores intraepitelials VPH-independents, amb característiques morfològiques indistingibles de les lesions precursores associades a VPH prèviament descrites pel nostre grup a la vulva.
9. Determinar el valor de les tincions immunohistoquímiques per a p16 i p53 en el diagnòstic de les lesions precursores del carcinoma escamós de penis i en el càncer invasor d'aquest òrgan.

5. Mètodes i resultats

5.1 Primer estudi

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Resum

Objectius: L'objectiu d'aquest estudi és determinar la correlació de l'estat mutacional de *TP53* amb la tinció immunohistoquímica de p53 definida per un sistema de patrons descrits recentment en carcinomes de vulva, en una sèrie de 40 tumors de penis.

Mètodes: Es revisen 40 casos de CEP diagnosticats i tractats a l'Hospital Clínic dels quals res recuperen blocs de parafina representatiu del carcinoma. Es van revisar utilitzant la classificació de la OMS del 2022. Tres observadors van valorar la tinció immunohistoquímica de p53 segons els següents patrons descrits com mutats: tinció difusa, tinció basal, tinció citoplasmàtica o "null" és a dir cap tinció, o patrons no mutats: positivitat aïllada o positivitat suprabasal. Finalment es va fer un estudi de l'estat mutacional de *TP53* en tots els casos utilitzant la tècnica de "whole exome sequencing" (WES) que ens va permetre valorar tant les mutacions puntuals com les alteracions numèriques.

Resultats: Es van detectar mutacions de *TP53* en 22 tumors i en els altres 18 el gen *TP53* era nadiu. La majoria dels casos mutats es troben en el grup dels CEP independents de VPH i és ocasional en els associats a VPH. Utilitzant el sistema de patrons la correlació entre les tincions immunohistoquímiques de p53 i l'estat de *TP53* va ser excel·lent tant en el cas de *TP53* mutat (21/23; 91,3%) com nadiu (16/17; 94,1%). La sensibilitat, especificitat i precisió utilitzant aquest sistema, van ser del 95,5% (IC 95% 77,2–99,9%), 88,9% (IC 95% 65,3–98,6%) i 92,5% (IC 95% 79,6–98,4%), respectivament.

Conclusions: El nostre estudi mostra que l'avaluació de les tincions immunohistoquímiques de p53 basada en patrons prediu amb precisió l'estat mutacional de *TP53* en el CEP, millorant el rendiment dels mètodes prèviament reportats d'avaluació utilitzant aquesta tècnica. Aquest nou marc reconeix tres nous patrons (basal, nul i citoplasmàtic) en el CEP que no s'haurien reconegut com patrons mutats seguint criteris convencionals.

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Article

P53 in Penile Squamous Cell Carcinoma: A Pattern-Based Immunohistochemical Framework with Molecular Correlation

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Simple Summary: Penile squamous cell carcinomas harbouring mutations of *TP53* have an increased risk of lymph node metastases and an impaired prognosis, but the mutational analysis of the *TP53* gene is not available in many pathology laboratories. Although p53 immunohistochemistry (IHC) has been proposed as an alternative to the molecular analysis, the current method of evaluation of p53 IHC has many inaccuracies. The aim of our study was to determine, in a series of 40 penile tumours, if a recently described pattern-based framework of p53 IHC evaluation correlates better than the classical method with the *TP53* mutational status. Our results show that the new method has a very good correlation with *TP53* mutations (95% sensitivity; 92% specificity), higher than that of the classical method, and can be considered as a reliable surrogate of the *TP53* mutational status. This new framework can help clinicians to better define risk groups and refine treatment strategies.

Abstract: p53 immunohistochemistry (IHC) has been proposed as a surrogate for *TP53* mutations in penile squamous cell carcinomas (PSCC). We aimed to evaluate the performance of a pattern-based evaluation of p53 IHC in PSCC. Human papilloma virus (HPV) DNA testing, p16 and p53 IHC, and whole exome sequencing were performed in a series of 40 PSCC. p53 IHC was evaluated following a pattern-based framework and conventional p53 IHC evaluation. Out of 40 PSCC, 12 (30.0%) were HPV-associated, and 28 (70.0%) were HPV-independent. The agreement between the p53 IHC pattern-based evaluation and *TP53* mutational status was almost perfect (k = 0.85). The sensitivity and accuracy of the pattern-based framework for identifying *TP53* mutations were 95.5% and 92.5%, respectively, which were higher than the values of conventional p53 IHC interpretation (54.5% and 70.0%, respectively), whereas the specificity was the same (88.9%). In conclusions, the pattern-based framework improves the accuracy of detecting *TP53* mutations in PSCC compared to the classical p53 IHC evaluation.

Keywords: p53 immunohistochemistry; *TP53* mutations; penile squamous cell carcinoma; surrogate marker; pattern-based framework

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1. Introduction

Penile squamous cell carcinoma (PSCC) is an unusual neoplasm, with incidence rates that range from 0.5 to 1.6 per 100,000 inhabitants in different European regions [1]. Several risk factors have been identified as possibly implicated in the development of PSCC, including local chronic inflammatory conditions and sexual behaviour, especially exposure to human papillomavirus (HPV) [2].

Two distinct pathways seem to be involved in the carcinogenesis of PSCC: one driven by HPV (HPV-associated) and another independent of HPV infection (HPV-independent) [1]. In keeping with this etiological categorization, the current version of the World Health Organization (WHO) classification of urological tumours [2] divides PSCC according to the presence or absence of HPV. As a consequence, the use of immunohistochemical (IHC) staining for p16, a surrogate marker of the presence of HPV, has become a recommended biomarker to accurately classify these tumours [3]. In Western Europe, a marked predominance of HPV-independent and a low frequency of HPV-associated tumours (70% vs. 30%) has been reported in most studies [4].

The molecular mechanisms involved in HPV-associated PSCC are characterized by genomic instability secondary to the overexpression of the oncoproteins E6 and E7, which lead to uncontrolled activation of the cell cycle [5,6]. In contrast, the pathogenesis of HPV-independent PSCC is less well understood [1]. *TP53* mutations [7–10] have frequently been reported in this HPV-independent subset of PSCC. Moreover, several studies [11,12] have suggested that *TP53* mutations might be associated with a high frequency of nodal metastases, a factor known to be strongly correlated with impaired prognosis [13,14]. These studies have suggested that *TP53* mutational status could add relevant prognostic information in PSCC [8,11]. However, molecular analysis of the *TP53* gene is technically challenging and not available in many pathology laboratories. Thus, the evaluation of p53 IHC has been proposed as a more feasible alternative [15]. Although a few studies have shown a correlation between p53 IHC overexpression and impaired prognosis in PSCC [16,17], unfortunately, there is a lack of standardization in the evaluation of p53 IHC. Indeed, in most published studies, the assessment of p53 IHC has been based on the percentage of positive nuclei at the basal and parabasal layers [15], and p53 is considered abnormal when diffuse overexpression is identified. However, the cut-off levels have not been clearly established, and thus, the actual meaning of these percentages is not known. Not surprisingly, the studies analysing the correlation between p53 IHC expression and *TP53* mutational status in PSCC have shown discrepant results [18].

Interestingly, the histological features of PSCC as well as the two pathogenic pathways are very similar to the pathology and etiopathogenesis of vulvar carcinomas [19]. Recently, a well-defined, pattern-based framework of p53 IHC evaluation showing a close correlation with *TP53* mutational status has been described in vulvar tumours [20,21]. This pattern-based framework includes four main abnormal p53 IHC patterns that strongly correlate with *TP53* mutations and two normal patterns that reflect a wild-type protein [20,21]. Moreover, based on a combination of HPV status and p53 IHC, three prognostic subtypes of tumours have recently been identified in vulvar squamous cell carcinomas [22].

Due to the marked similarities between PSCC and vulvar carcinoma [23], we hypothesized that this pattern-based framework of p53 IHC interpretation [21] could also be applied to PSCC and have similar implications to those defined in the vulva. Thus, we aimed to explore the correlation between p53 IHC evaluated as described in vulva [21] and the *TP53* mutational status in a series of PSCC from a single institution in Spain, comparing its performance with the conventional interpretation of p53 IHC that generally includes only diffuse overexpression.

2. Material and Methods

2.1. Case Selection

All PSCCs diagnosed and surgically treated at the Hospital Clinic de Barcelona from 2000 to 2021 were retrieved, and all the available material was reviewed. The initial

inclusion criteria for this study were: (1) the presence of invasive PSCC, (2) sufficient material available for HPV testing and IHC evaluation, and (3) available tissue for whole-exome sequencing of the invasive carcinoma. The study was approved by the Ethics Committee of the Hospital Clinic de Barcelona (ref HCB/2020/1207).

A total of 51 PSCC complied with the initial inclusion criteria.

2.2. Histological Revision

All of the haematoxylin and eosin sections of the 51 tumours were carefully reviewed. The histological revision aimed to confirm the presence of invasive carcinoma, which was further classified according to the 2022 WHO classification of tumours of the urinary system and male genital organs [2].

In the histological review, a block of formalin-fixed, paraffin-embedded tissue including both the PSCC, and the adjacent skin was selected for HPV testing and IHC staining. In this evaluation, two blocks were also selected for whole-exome sequencing, one representative of the invasive tumour, containing at least 50% of tumour cells (tumour purity estimated by morphology), and another of normal skin or a reactive lymph node, which was selected as control tissue.

2.3. HPV Testing, p16 IHC, and Criteria for Classifying a Tumour as HPV-Associated or HPV-Independent

DNA extraction was performed on 10-µm whole tissue sections using a commercial kit (QIAamp DNA FFPE Tissue Kit; Qiagen, Hilden, Germany) as previously described [24]. HPV DNA genotyping was performed using the short polymerase chain reaction (PCR) fragment (SPF10) amplified through the INNO-LiPA HPV Genotyping Extra II Amplification (Fujirebio, Gent, Belgium) [25].

All IHC analyses were performed with the Roche platform. p16 IHC was conducted with the CINtec Histology Kit (clone E6H4). Only the "block" staining pattern with diffuse and intense positivity (nuclear and cytoplasmic) in a group of contiguous cells located in basal and parabasal layers, except in the areas of keratosis and parakeratosis, were considered positive [2].

To categorise a tumour as HPV-associated or -independent, both p16 IHC staining and HPV testing were considered. Any tumour showing a positive p16 IHC and/or HPV DNA testing result was considered as HPV-associated. Tumours negative for both techniques were considered as HPV-independent [2].

2.4. p53 IHC Staining and Evaluation

p53 IHC was performed with the anti-p53 (DO-7) monoclonal antibody (Roche, Vienna, Austria), a widely used antibody that detects both wild-type and mutant p53 [21,22]. Staining in the invasive tumour was evaluated according to the p53 pattern-based interpretation framework recently described for vulvar tumours [21,26]. This framework consists of six patterns grouped into two major categories: normal and abnormal. The "normal" category, which is suggestive of wild-type protein, includes two patterns: (1) occasional positive nuclei in the basal and/or parabasal layer (scattered pattern), and (2) moderate to strong nuclear p53 IHC staining in the parabasal layers with an absence of expression in the basal cells (mid-epithelial pattern). The "abnormal" category, suggestive of mutant protein, includes four p53 IHC staining patterns: (1) continuous, strong nuclear staining of the basal layer (basal overexpression pattern), (2) continuous and strong nuclear basal staining with suprabasal extension of the positive cells (diffuse overexpression pattern), (3) cytoplasmic staining with or without nuclear positivity (cytoplasmic pattern), and (4) complete absence of staining in the tumour, with evidence of intrinsic positive control in the adjacent skin, stromal, or inflammatory cells (null pattern). Figure 1 shows a representative example of the six staining patterns of PSCC.

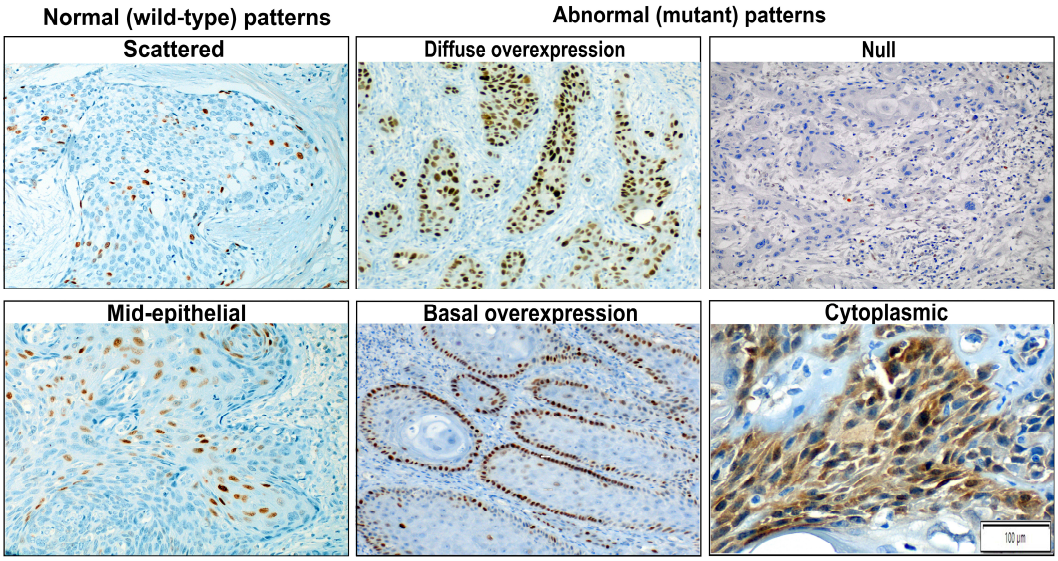

Figure 1. Examples of the six evaluated patterns of p53 immunohistochemical expression in penile squamous cell carcinoma: two normal (wild-type) patterns include scattered and mid-epithelial pattern. Four abnormal (mutant) patterns comprise basal (continuous, strong staining of the nuclei in the basal layer) and diffuse overexpression (continuous and strong nuclear basal staining with suprabasal extension) patterns, null pattern (complete absence of staining in the tumour with positivity in the background inflammatory and stromal cells), and cytoplasmic pattern (with or without nuclear staining).

p53 IHC slides were independently evaluated by three pathologists (I.T., C.M. and N.R.) blind to the molecular results. All pathologists were asked to assign the p53 IHC status (normal vs. abnormal) and pattern for each PSCC. Discordant cases were reviewed between the three evaluators in a meeting where consensus was reached.

In addition to this six-pattern framework, we evaluated the performance of the conventional interpretation of p53 IHC (only diffuse overexpression considered as abnormal) conducted blindly to the molecular results by a fourth pathologist (A.S.) not involved in the evaluations described above.

2.5. Whole Exome Sequencing and Bioinformatic Analysis

DNA was isolated as described above from invading tumour and matched normal tissue (skin or lymph node). For the DNA isolation, between 20 and 200 ng of gDNA were sheared using a Covaris™ LE220-Plus (MA, USA) and underwent quality control on an Agilent 2100 Bioanalyzer (CA, USA). The adaptor-modified end library was amplified by 10, 15 or 18 cycles of pre-capture PCR with the 2x KAPA HiFi HotStart ReadyMix PCR Kit (Roche). Pools of eight indexed libraries of a combined mass of 1.5 microgram were set up for hybridization (55 °C; 16 h). After washes, the pooled libraries were PCR-amplified. The libraries were sequenced on NovaSeq 6000 (Illumina, San Diego, CA, USA) in paired-end mode with a read length of 2 × 151 bp.

Reads were mapped to the human genome (hs37d5) using the Burrows-Wheeler Alignment and processed using Picard tools version 1.110. The Genome Analysis Tool Kit [27] was used for local indel realignment and base recalibration. Somatic variant calling was performed with GATK v4.1.9.0 Mutect2 and Strelka2 v2.8.3, and annotation with SnpEff v.4.3.e and SnpSift. Copy number variants were predicted with Control-FREEC [28] and annotated with SnpEff.

Cases with tumour and/or non-tumour samples with low coverage depth (<20×) were excluded from the analysis.

2.6. Attribution of TP53 Mutational Status

TP53 was considered mutated if a somatic mutation/s and/or loss in copy number were identified. Only the variants with an allele frequency >4% [29], predicted by both the Mutect2 and Strelka2 databases, and that had passed the quality filters of each program were considered as driver mutations. Tumours with no identified *TP53* somatic mutations or variants with allele frequency <4%, and tumours showing only gains in the *TP53* gene, in the absence of somatic mutations, were classified as *TP53* wild-type [30]. The pathogenicity level (clinical significance) for each identified *TP53* somatic variant was retrieved from the National Centre of Biotechnology Information ClinVar database [31].

2.7. Statistical Analysis

StataC/v15.0.591 (StataCorp, College Station, TX, USA) was used for all the data analyses. The clinical and histopathological data were compared using Chi-square tests (categorical data) and analysis of variance (numerical data). The diagnostic test performance of p53 IHC evaluation against *TP53* mutational status (gold standard), as well as inter-observer agreement, was calculated using the Fleiss' kappa test. The strength of agreement of kappa values was evaluated following the Landis-defined categories: 0, none beyond chance; 0–0.20, slight; 0.21–0.40, fair; 0.41–0.60, moderate; 0.61–0.80, substantial; and 0.81–1.00, almost perfect [32]. The sensitivity, specificity, positive and negative predictive values, and accuracy of p53 IHC evaluation, with 95% confidence intervals (95%CI), were also calculated. A two-sided p-value < 0.05 was considered statistically significant.

3. Results

3.1. Cases Included in the Study and Association with HPV

Eleven tumours were excluded from the study due to insufficient coverage depth (<20×) of the tumour or the matched control tissue (8 and 3 samples, respectively). Forty tumours fulfilled the inclusion criteria and were included in the study. Twelve out of the 40 PSCC (30.0%) were classified as HPV-associated PSCC, with seven cases showing p16 overexpression and HPV-DNA and five cases being only positive for p16 IHC with a negative HPV testing result. In all seven HPV-DNA positive cases, HPV16 was the only type identified. Twenty-eight out of 40 tumours (70.0%) were negative for p16 and HPV testing and were classified as HPV-independent.

3.2. Characteristics of the Overall Series

Table 1 shows the main clinic-pathological characteristics of the PSCC patients, categorised into the two major groups, HPV-associated and HPV-independent, as well as their stage at diagnosis. Patients with HPV-associated PSCC were slightly younger (mean 65.2 years; range 45–94) than the HPV-independent patients (mean 69.1 years; range 40–86) (p = 0.23).

Table 1. Clinical and pathological characteristics of the penile squamous cell carcinomas (PSCC) included in the study categorized in the two main pathological types.

	HPV-Associated PSCC (n = 12)	HPV-Independent PSCC (n = 28)	p
Mean age (years) ± standard deviation	65.2 ± 14.9	69.1± 13.1	0.23
Histological subtype			<0.001
Usual/verrucous	3 (25.0%)	27 (96.4%)	
Basaloid/warty/lymphoepithelioma-like	9 (75.0%)	1 (3.6%)	
Vascular invasion	1 (8.3%)	3 (10.7%)	0.8
Perineural invasion	1 (8.3%)	2 (7.1%)	0.8
Lymph node metastases	3 (25.0%)	8 (28.6%)	0.8
Stage			1
Initial (I)	6 (50.0%)	14 (50.0%)	
Advanced (II/III/IV)	6 (50.0%)	14 (50.0%)	
Treatment			1
Surgery	10 (83.3%)	21 (75.0%)	
Surgery + radiation and/or chemotherapy	2 (16.6%)	7 (25.0%)	

HPV: human papillomavirus.

3.3. TP53 Mutations

The mean percentage of tumoral cells in the samples (tumour purity) was 58% (range 0.50–0.89). The average tumour sequencing depth was 81×, ranging from 23× to 314×. *TP53* mutations (somatic and/or copy number alterations) were identified in 22 PSCC (52.5%), whereas 18 tumours (47.5%) were *TP53* wild-type. Nineteen PSCC harboured 21 somatic variants, with two of them additionally showing *TP53* copy number alterations. Three tumours showed only *TP53* copy number loss. The mean allele frequency of the 21 identified somatic *TP53* variants was 0.21 (range 0.04–0.79). *TP53* missense variants were the most prevalent (14/21; 66.6%), followed by nonsense (4/21; 19.0%), splice-site (2/40; 5.0%) and frameshift (1/4; 2.5%).

3.4. Agreement between p53 IHC and TP53 Mutational Status and Interobserver Agreement

The agreement between the p53 IHC status and *TP53* mutational status was substantial for two observers, and moderate for the third observer (k = 0.64, 0.75 and 0.50, respectively; $p < 0.0001$ for each). After the consensus meeting, in which the twelve discordant cases were discussed, the agreement between the final p53 IHC pattern-based evaluation and the *TP53* mutational status increased to almost perfect (k = 0.85; 95% CI = 0.68–1). In this consensus evaluation of p53 IHC, 17 tumours (42.5%) were classified as normal. Fifteen of them showed scattered and two mid-epithelial staining. Twenty-three tumours were classified as showing an abnormal p53 IHC pattern. Of these, 14 (60.8%) showed diffuse overexpression, six (26.0%) null pattern, two (8.6%) cytoplasmic staining, and one (4.3%) basal overexpression. All but one tumour assigned as p53 IHC normal were *TP53* wild-type (16/17; agreement: 94.1%), and all but two tumours evaluated as p53 IHC abnormal in the consensus were *TP53*-mutant (21/23; agreement: 91.3%). The agreement of the p53 IHC pattern-based evaluation (normal vs. abnormal) between three observers was moderate (k = 0.59). A flowchart showing the results of the pattern-based framework evaluation of p53 IHC, their correlation with the classic IHC evaluation of p53, and with the results of the genomic analysis of *TP53* are shown in Figure 2. The differences between the pattern-based and the conventional p53 IHC evaluation were related to a misclassification as abnormal expression of the mid-epithelial pattern and a misclassification as normal expression of the cases showing cytoplasmic, null, or basal overexpression.

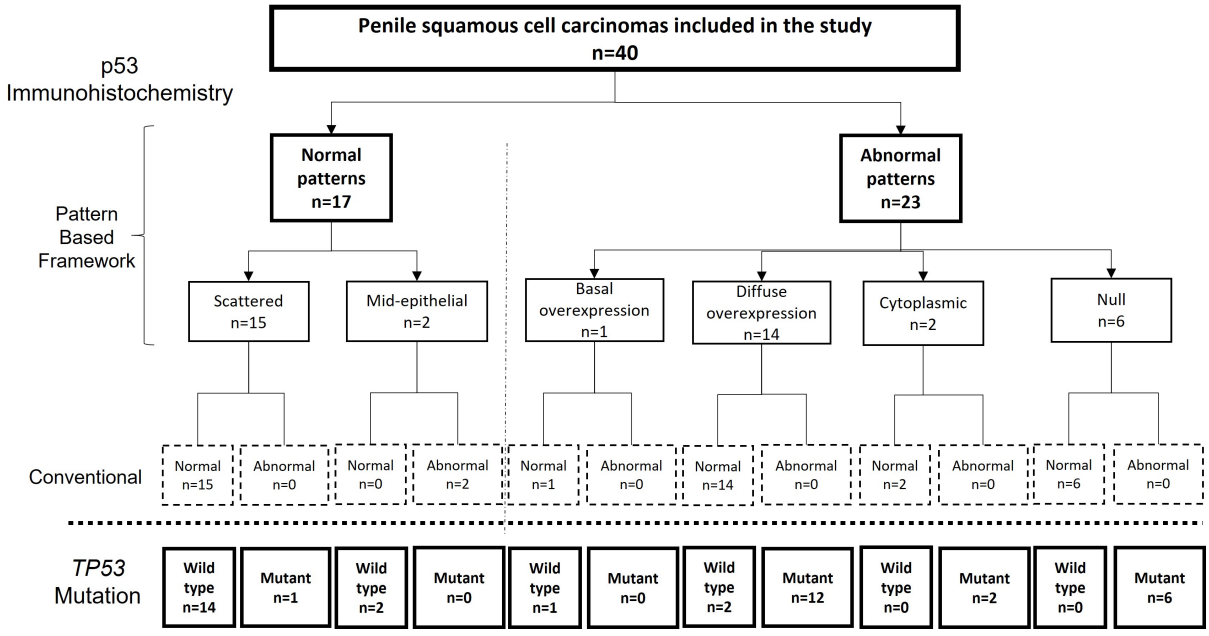

Figure 2. Flow chart showing the results of the pattern-based framework evaluation of p53 immunohistochemistry (IHC), their correlation with the classic IHC evaluation of p53 and with the results of the genetic analysis.

The sensitivity, specificity, and accuracy of each ratter and of the consensus evaluation, as well as the figures for the evaluation using conventional criteria are shown in Table 2. The final p53 IHC pattern-based evaluation sensitivity, specificity and accuracy were 95.5% (95%CI 77.2–99.9%), 88.9% (95%CI 65.3–98.6%) and 92.5% (95%CI 79.6–98.4%), respectively.

Table 2. The p53 immunohistochemical (IHC) evaluation in penile squamous cell carcinomas (PSCC) for each of the observers and after consensus meeting.

	p53 Immunohistochemistry Evaluation										
	Pattern-Based *								**Conventional ****		***TP53* Status**
	Observer 1		**Observer 2**		**Observer 3**		**Consensus**		**Observer 4**		
	Normal	**Abnormal**	**Normal**	**Abnormal**	**Normal**	**Abnormal**	**Normal**	**Abnormal**	**Normal**	**Abnormal**	
TP53 wild-type	15	3	14	4	13	5	16	2	16	2	18
TP53 mutant	2	20	6	16	2	20	1	21	10	12	22
Total	17	23	20	20	15	25	17	23	26	14	40
Sensitivity	90.9 (70.8–98.9)		72.7 (49.8–89.3)		90.9 (70.8–98.9)		95.5 (77.2–99.9)		54.5 (32.2–75.6)		
Specificity	83.3 (58.6–96.4)		77.8 (52.4–93.6)		72.2 (46.5–90.3)		88.9 (65.3–98.6)		88.9 (65.3–98.6)		
Accuracy	87.5 (73.2–95.8)		75.0 (58.8–87.3)		82.5 (67.2–92.7)		92.5 (79.6–98.4)		70.0 (53.5–83.4)		

Sensitivity, specificity, and accuracy are shown as percentage and (95% confidence interval); * The pattern-based evaluation considers two normal p53 IHC patterns (scattered and mid-epithelial) and four abnormal patterns (null, cytoplasmic, basal overexpression and diffuse overexpression); ** The conventional evaluation includes only diffuse overexpression as abnormal, and all other types of staining are classified as normal.

A detailed description of the p53 IHC patterns and *TP53* mutational status of the 40 PSCC and their relationship with HPV status is shown in Table 3.

Table 3. Summary of immunohistochemical (IHC) and molecular features for each of the 40 penile squamous cell carcinomas.

Case	**HPV Status**	**p53 IHC Status**	**p53 IHC Pattern**	***TP53* Status**	***TP53* Variant (Protein Change)**	**Type of *TP53* Mutation**	**VAF**	**Clinical Significance of *TP53* Variant**	***TP53* Copy Number Status**
1	Pos	Normal	Scatt	Wt	-	-	-	-	Normal
2	Pos	Normal	Scatt	Wt	-	-	-	-	Normal
3	Pos	Normal	Scatt	Wt	-	-	-	-	Normal
4	Pos	Normal	Scatt	Wt	-	-	-	-	Normal
5	Pos	Normal	Scatt	Wt	-	-	-	-	Normal
6	Pos	Normal	Scatt	Wt	-	-	-	-	Normal
7	Pos	Normal	Scatt	Wt	-	-	-	-	Gain
8	Pos	Normal	Scatt	Wt	-	-	-	-	Gain
9	Pos	Normal	Mid-ep	Wt	-	-	-	-	Normal
10	Pos	Normal	Mid-ep	Wt	-	-	-	-	Normal
11	Pos	Abnormal	Diff OE	Mut	c.637C>T (R213*)	Nonsense	0.05	Pathogenic	Loss
12	Pos	Abnormal	Diff OE	Wt	-	-	-	-	Normal
13	Neg	Normal	Scatt	Wt	-	-	-	-	Normal
14	Neg	Normal	Scatt	Wt	-	-	-	-	Normal
15	Neg	Normal	Scatt	Wt	-	-	-	-	Normal
16	Neg	Normal	Scatt	Wt	-	-	-	-	Normal
17	Neg	Normal	Scatt	Wt	-	-	-	-	Normal
18	Neg	Normal	Scatt	Mut	c.251C>T (A84V)	Missense	0.04	Likely benign	Normal
19	Neg	Normal	Scatt	Wt	-	-	-	-	Gain
20	Neg	Abnormal	Null	Mut	-	-	-	-	Loss
21	Neg	Abnormal	Null	Mut	-	-	-	-	Loss
22	Neg	Abnormal	Diff OE	Mut	-	-	-	-	Loss
23	Neg	Abnormal	Diff OE	Wt	-	-	-	-	Normal

Table 3. *Cont.*

Case	HPV Status	p53 IHC Status	p53 IHC Pattern	*TP53* Status	*TP53* Variant (Protein Change)	Type of *TP53* Mutation	VAF	Clinical Significance of *TP53* Variant	*TP53* Copy Number Status
24	Neg	Abnormal	Basal	Mut	c.530C>T (P177L)	Missense	0.27	Likely pathogenic/uncertain	Normal
25	Neg	Abnormal	Cyt	Mut	c.574G>A (A192T)	Missense	0.04	Uncertain	Normal
26	Neg	Abnormal	Diff OE	Mut	c.1082G>A (G361E)	Missense	0.07	Uncertain	Normal
27	Neg	Abnormal	Diff OE	Mut	c.919+1G>A (-)	Splice-site	0.06	Likely pathogenic	Normal
28	Neg	Abnormal	Diff OE	Mut	c.527G>T (C176F)	Missense	0.12	Pathogenic/likely pathogenic	Normal
29	Neg	Abnormal	Diff OE	Mut	c.843C>G (D281E)	Missense	0.16	Uncertain/likely pathogenic	Normal
30	Neg	Abnormal	Cyt	Mut	c.991C>T (Q331*)	Nonsense	0.11	Pathogenic	Normal
31	Neg	Abnormal	Diff OE	Mut	c.335G>A (S112N)	Missense	0.79	Uncertain	Normal
32	Neg	Abnormal	Diff OE	Mut	c.524G>A (R175H)	Missense	0.12	Likely Pathogenic/uncertain	Gain
33	Neg	Abnormal	Diff OE	Mut	c.799C>T (R267W)	Missense	0.05	Pathogenic/likely pathogenic	Gain
34	Neg	Abnormal	Diff OE	Mut	c.844C>T (R282W)	Missense	0.26	Pathogenic/likely pahogenic	Normal
35	Neg	Abnormal	Null	Mut	c.637C>T (R213*)	Nonsense	0.32	Pathogenic	Normal
36	Neg	Abnormal	Null	Mut	c.273dupT (-)	Frameshift	0.65	Not found	Normal
37	Neg	Abnormal	Null	Mut	c.817C>T (R273C)	Missense	0.05	Pathogenic/likely pathogenic	Normal
38	Neg	Abnormal	Null	Mut	c.461G>A (G154D); c.276+1G>C (-)	Missense; Splice-site	0.05; 0.64	Uncertain; Not found	Normal
39	Neg	Abnormal	Diff OE	Mut	c.517G>A (V173M); c.637C>T (R213*)	Missense; Nonsense	0.21; 0.1	Pathogenic; Pathogenic	Normal
40	Neg	Abnormal	Diff OE	Mut	c.524G>A (R175H)	Missense	0.44	Likely Pathogenic/uncertain	Normal

Cyt: cytoplasmic; Diff OE: diffuse overexpression; Mid-ep: mid-epithelial; Mut: mutant; Neg: negative; Pos: positive; Scatt: scattered; VAF: variant allele frequency; Wt: wild-type; (-) protein unknown.

Frequencies and numbers of *TP53* variants in other type of cancers (found to be discordant with pattern-based p53 IHC evaluation for at least one of the three observers) are shown in the Supplementary Table S1.

3.5. p53 IHC Patterns and TP53 Status in HPV-Associated PSCC

Ten out of 12 (83.3%) HPV-associated tumours showed normal p53 IHC pattern. All of them were *TP53* wild-type. Eight tumours showed scattered p53 staining, and two showed a mid-epithelial pattern. In seven tumours, the p53 IHC normal pattern was unanimously assigned by the three observers, whereas in three cases, at least one of the observers diagnosed a p53 IHC abnormal pattern.

An abnormal p53 IHC pattern was identified in 2/12 (16.7%) HPV-associated tumours, the two of them showing diffuse p53 overexpression pattern, which was independently assigned by each of the three pathologists. In the sequencing analysis, one tumour showed a pathogenic *TP53* c.637C>T nonsense mutation (p.Arg213Ter) with a variant allele frequency of 0.05 accompanied by a loss in the *TP53* copy number. Figure 3 shows the histological and IHC features of this tumour.

Contrarily, in the second case, the exome sequencing did not reveal any *TP53* alteration. Figure 4A,A' illustrates the latter discordant case. None of the HPV-associated PSCC tumours showed cytoplasmic, null, or basal overexpression pattern.

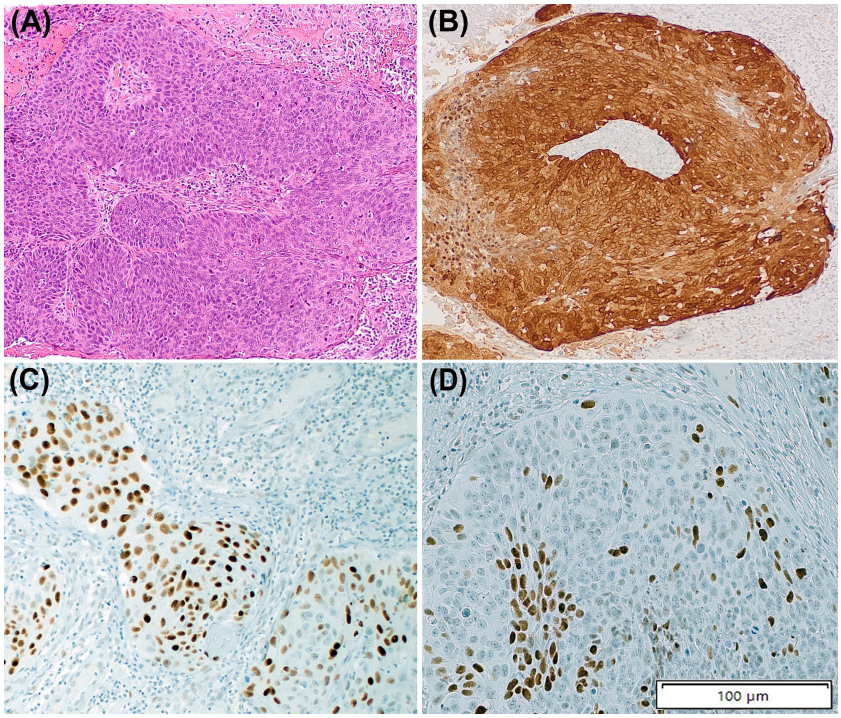

Figure 3. The case of Human Papillomavirus (HPV)-associated penile squamous cell carcinoma (PSCC) with basaloid features (**A**). Immunohistochemistry for p16 is positive (**B**) and p53 staining shows an abnormal pattern of diffuse overexpression (**C**). However, in some areas, the tumour shows scattered p53 staining (**D**). A pathogenic *TP53* nonsense mutation (c.637C>T [R213*]) and a loss in *TP53* copy number have been identified in this tumour.

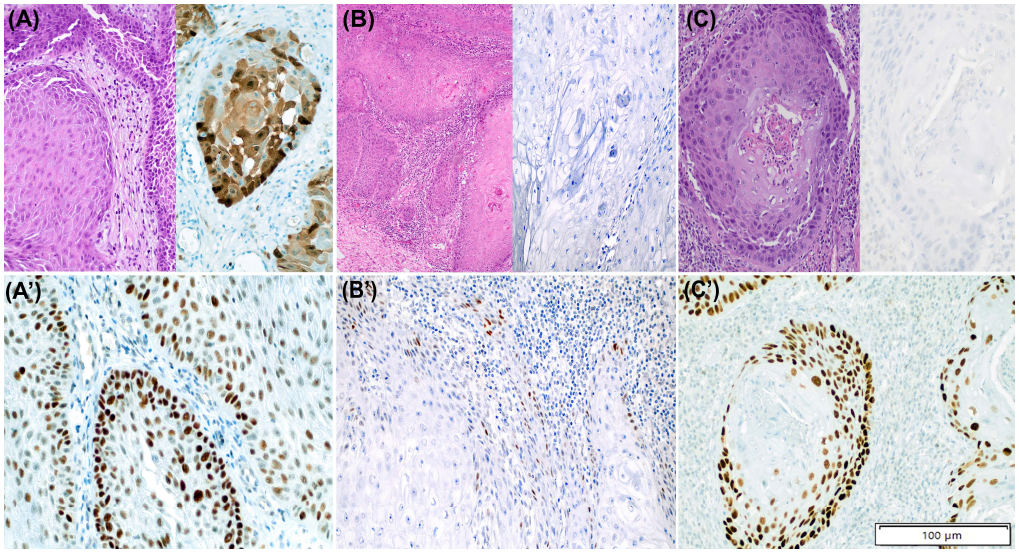

Figure 4. Three discordant cases between p53 immunohistochemistry (IHC) and *TP53* mutational status in penile squamous cell carcinomas (PSCC). (**A**–**C**) show H/E staining together with p16 IHC staining and (**A′**–**C′**) outline p53 IHC staining for each case. (**A**,**A′**): HPV-associated PSCC with p53 abnormal pattern (diffuse overexpression) and no evidence of *TP53* alterations. (**B**,**B′**): HPV-independent tumour with normal (scattered) p53 IHC pattern and presence of likely benign missense *TP53* mutation (c.251C>T [A84V]). (**C**,**C′**): HPV-independent PSCC with p53 abnormal pattern (diffuse overexpression) and no evidence of *TP53* alterations.

3.6. p53 IHC Patterns and TP53 Status in HPV-Independent PSCC

Seven out of the 28 (25.0%) HPV-independent tumours showed normal p53 IHC; all of them displayed scattered pattern. Six showed *TP53* wild-type status in the sequencing analysis and one harboured a likely benign *TP53* c.251C>T missense mutation (p. Ala84Val), with a variant allele frequency of 0.04. Figure 4B,B′ illustrates the latter case. In four cases, the normal p53 IHC status was assigned by the three observers (full concordance), whereas in three cases, including the *TP53*-mutated case, one of the observers suggested an abnormal p53 pattern. No tumours with mid-epithelial pattern were identified in the HPV-independent PSCC group.

An abnormal p53 IHC pattern was identified in 21/28 (75.0%) HPV-independent tumours. Twelve of them (57.1%) showed a diffuse overexpression pattern, six (28.6%) a null pattern, two tumours (9.5%) showed cytoplasmic and one (4.8%) basal staining. Twenty out of the 21 (95.2%) PSCC with abnormal p53 IHC were *TP53*-mutant. Of them, 17 tumours (85.0%) showed at least one somatic *TP53* alteration and three (15.0%) only *TP53* copy number loss. In 15/21 tumours (71.4%), all three observers assigned p53 abnormal IHC (including a *TP53* wild-type tumour), whereas in six cases, an abnormal p53 IHC was diagnosed by at least one observer. Figure 4C,C′ shows the PSCC with abnormal p53 IHC and absence of *TP53* mutations.

Of the 17 cases with *TP53* somatic mutations and abnormal p53 IHC, 11 (64.7%) harboured at least one pathogenic or likely pathogenic variant, five (29.4%) showed only a variant of uncertain significance and in one (5.9%) case the mutational variant was not found in ClinVar database (frameshift c.273dupT codifying p.Glu131fs protein). Eight out of 10 (80.0%) PSCC with diffuse overexpression pattern were enriched in pathogenic/likely pathogenic variants of *TP53*, followed by those with null pattern (2/4; 50.0%), cytoplasmic pattern (1/2; 50.0%), while the only tumour with basal overexpression harboured the variant of uncertain significance.

Among the three tumours with only *TP53* copy number loss, two showed p53 IHC null pattern and one diffuse overexpression p53 IHC pattern. Finally, the tumour with abnormal p53 IHC and *TP53* wild-type status showed diffuse overexpression (with full concordance between 3 observers).

4. Discussion

In this study, we evaluated the correlation between p53 IHC expression and *TP53* mutations in PSCC, using for the first time the pattern-based p53 IHC evaluation framework recently described in vulvar tumours. In keeping with the data reported in the vulva [21,26], this pattern-based p53 IHC evaluation framework reliably predicted the *TP53* mutational status of the PSCC (95.7% sensitivity, 88.9% specificity, 92.5% accuracy).

Several studies have shown that *TP53* mutational status is clinically relevant in patients with PSCC because mutations are associated with an increased risk of lymph node metastases and impaired prognosis [8,10–13]. However, *TP53* sequencing is technically challenging to implement in the routine. Consequently, several investigators have proposed using p53 IHC staining as a surrogate of *TP53* [14], with conflicting results in the correlation between both techniques [8,18]. This poor correlation can be attributed to several factors. Firstly, previous studies have considered diffuse p53 IHC overexpression as the only abnormal pattern suggestive of *TP53* mutation, which has shown a limited sensitivity in most series [8]. Secondly, there is a lack of standardisation in the evaluation of p53 staining: although p53 IHC assessment is usually based on the percentage of positive nuclei at the basal and parabasal layers [14], the threshold of positivity suggesting mutation has not been clearly defined. While some studies consider as abnormal p53 staining a positivity in at least 20% of the nuclei [33], other investigators have used a combination of intensity and extent of the positivity [34]. Remarkably, the sensitivity and accuracy of the pattern-based framework of p53 IHC expression to detect *TP53* mutation (95.5% and 92.5%, respectively) were much higher than the classical criteria considering only diffuse positive staining as

abnormal (9) (54.5% and 70.0%, respectively). However, the specificity did not vary at all between the two methods of evaluation (88.9%).

The correlation between normal p53 IHC using the pattern-based evaluation framework and wild-type *TP53* status was excellent in our study (16/17; 94.1%). Interestingly, the only tumour with normal p53 IHC staining but *TP53*-mutated status harboured a *TP53* mutation classified as likely benign, probably not involved in the pathogenesis of this neoplasm. Thus, if only the pathogenic variants were used to define *TP53*-mutated status, this case would have been reclassified as p53 IHC-*TP53* concordant, which would increase the correlation between normal p53 IHC and *TP53* wild-type status to 100%. The two p53 IHC patterns described as normal in vulvar carcinomas, scattered and mid-epithelial pattern [21], were identified in our series. The mid-epithelial pattern, previously described in HPV-associated vulvar cancers [21,26,35,36], and identified in two HPV-associated PSCC, is of particular interest. This type of staining probably reflects senescence of high-risk HPV-infected neoplastic cells and represents a potential diagnostic pitfall with p53 overexpression [36], if the pathologist only notices the strong staining in the centre of the tumoral nests.

The excellent correlation between normal p53 IHC and wild-type *TP53* status is in contrast with the data reported by Kashofer et al. [8], who, applying the conventional p53 IHC evaluation criteria, showed high frequency of *TP53*-mutated tumours with normal p53 staining. It should be noted that some of the p53 patterns considered as abnormal in the pattern-based p53 IHC evaluation framework, especially the cytoplasmic and the null patterns, are considered as normal in the conventional evaluation.

We also observed an excellent correlation between abnormal p53 IHC using the pattern-based evaluation framework and *TP53*-mutant status (21/23; 91.3%). However, if only pathogenic or likely pathogenic variants were considered to define *TP53* mutant status, the correlation would drop to 65.2% (15/23). The most frequent abnormal p53 IHC pattern was diffuse overexpression (61%), the only pattern previously considered as abnormal in previous studies on PSCC [8]. It is also the most frequent pattern in vulvar tumours [20,21,26], stomach [37], and ovary [38], usually associated with a missense *TP53* mutation. In addition to this common pattern, two additional abnormal patterns of p53 IHC expression (null and cytoplasmic) were identified in as many as one-third of HPV-independent tumours. These two patterns have been identified in HPV-independent vulvar tumours [21,39] but, to our knowledge, have not been previously described in PSCC. Finally, basal overexpression pattern was the most uncommon pattern in our series, observed in only one tumour. As reported in the vulva [21] the distinction between wild-type expression and basal overexpression is often challenging. In addition, this case had a *TP53* variant with uncertain pathogenicity and thus might as well be classified as wild-type if only pathogenic variants had been used to define *TP53*-mutated status.

The findings of our study are consistent with previously reported data [29], showing that *TP53* mutations are much more frequent in HPV-independent than in HPV-associated PSCC [7]. Indeed, 75% of the HPV-independent and 8.3% of the HPV-associated PSCC in our series had *TP53* alterations ($p < 0.001$). These differences were also observed for p53 IHC using the pattern-based framework: abnormal patterns were identified in 75% of the HPV-independent and 16.6% of the HPV-associated PSCC ($p = 0.005$).

The case of HPV-associated tumour with diffuse p53 overexpression and a pathogenic nonsense *TP53* variant (c.637C>T codifying for p.Arg213Ter protein) is certainly interesting, as *TP53* mutations are highly uncommon in HPV-related neoplasm [7,29,40]. Remarkably, the same mutational variant of *TP53* was identified in two additional cases in our series, both HPV-independent, one with diffuse overexpression and one with null p53 pattern. The mutation has been reported to cause a truncated or absent *TP53* protein [31], thus correlating with diffuse overexpression and null IHC patterns identified in our study, respectively. The variant was occasionally reported in PSCC [7,41] but was never correlated with p53 IHC staining.

The main strength of our study is that we analysed the *TP53* gene by exome sequencing, targeting both somatic mutations and copy number alterations, which has allowed us to obtain an accurate correlation between the findings of IHC and the molecular analysis. In addition, we have used a well-defined pattern-based framework of p53 IHC evaluation that has shown a good correlation in vulvar squamous cell carcinoma, a neoplasm with similar etiopathogenic background [21]. This framework allowed the identification of several abnormal patterns of expression not previously identified in PSCC and showed better correlation with the *TP53* molecular status than the conventional criteria. The main limitation is the small sample size, particularly of HPV-associated tumours, which precludes obtaining a reliable distribution of p53 IHC patterns. A second limitation is the use of formalin-fixed, paraffin-embedded tissue for sequencing, which might have resulted in under- or over-identification of *TP53* alterations. Finally, the tumour purity of the samples was relatively low (58%), which could have impacted the somatic variant calling conducted in this study. Lastly, although we obtained strong correlation between p53 IHC and mutational status, we identified a proportion of tumours with *TP53* variants of uncertain significance, which introduced a challenge in attribution of *TP53* mutational status.

5. Conclusions

In conclusion, our study shows that the pattern-based framework of p53 IHC evaluation accurately predicts *TP53* mutational status in PSCC, improving the performance of previously reported methods of p53 IHC evaluation. This new framework recognises three new patterns (mid-epithelial, null and cytoplasmic) in PSCC that would be misclassified by conventional criteria, while the existence of basal pattern is questionable. Further molecular studies are warranted to validate our findings in larger cohorts of PSCC.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cancers15102719/s1, Table S1: Frequencies of identified *TP53* variant among other TP53 mutations and number and types of cancers in which the variant was reported for five penile squamous carcinomas (PSCC) in which there was discordance between p53 immunohistochemistry (IHC) evaluation (for at least one of the three observers) and *TP53* mutational status.

Author Contributions: I.T.: study design, investigation, methodology, writing of the first draft, writing and editing; A.S.: investigation, methodology, writing of the first draft, writing and editing; M.d.P.: investigation, writing and editing, software, statistical analysis; F.M.P.: investigation, writing and editing; R.L.d.C.: investigation, writing and editing; C.M.: investigation, writing and editing; O.O.: investigation, writing and editing; N.V.: investigation, writing and editing; S.A.: investigation, writing and editing, L.M.: investigation, methodology, writing and editing; L.R.-C.: investigation, writing and editing; A.M.: study supervision, investigation, writing and editing; P.J.: investigation, methodology, writing and editing; O.R.: study design, resources, investigation, methodology, writing and editing, project management; C.T.: investigation, methodology, writing and editing; T.A.: investigation, methodology, writing and editing; J.M.C.-M.: investigation, resources, writing and editing; F.A.: study design, investigation, resources, writing and editing; M.J.R.: study design, investigation, resources, writing and editing; I.R.-C.: study design, investigation, methodology, writing of the first draft, writing and editing, project management; N.R.: study design, resources, investigation, methodology, statistical analysis, writing of the first draft, writing and editing, project management. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Emmanuel, A.; Nettleton, J.; Watkin, N.; Berney, D.M. The molecular pathogenesis of penile carcinoma—Current developments and understanding. *Virchows Arch.* **2019**, *475*, 397–405. [CrossRef]
2. Amin, M.B.; Moch, H. Tumours of the penis and scrotum: Introduction. In *WHO Classification of Tumours: Urinary and Male Genital Tumours*; International Agency for Research on Cancer: Lyon, France, 2022.
3. Cubilla, A.L.; Velazquez, E.F.; Amin, M.B.; Epstein, J.; Berney, D.M.; Corbishley, C.M. Members of the ISUP Penile Tumor Panel The World Health Organisation 2016 classification of penile carcinomas: A review and update from the International Society of Urological Pathology expert-driven recommendations. *Histopathology* **2018**, *72*, 893–904. [CrossRef]
4. Alemany, L.; Cubilla, A.; Halec, G.; Kasamatsu, E.; Quirós, B.; Masferrer, E.; Tous, S.; Lloveras, B.; Hernández-Suarez, G.; Lonsdale, R.; et al. Role of Human Papillomavirus in Penile Carcinomas Worldwide. *Eur. Urol.* **2016**, *69*, 953–961. [CrossRef]
5. Chaux, A.; Velazquez, E.F.; Algaba, F.; Ayala, G.; Cubilla, A.L. Developments in the pathology of penile squamous cell carcinomas. *Urology* **2010**, *76*, S7–S14. [CrossRef]
6. Gross, G.; Pfister, H. Role of human papillomavirus in penile cancer, penile intraepithelial squamous cell neoplasias and in genital wartsitle. *Med. Microbiol. Immunol.* **2004**, *193*, 35–44. [CrossRef]
7. McDaniel, A.S.; Hovelson, D.H.; Cani, A.K.; Liu, C.-J.; Zhai, Y.; Zhang, Y.; Weizer, A.Z.; Mehra, R.; Feng, F.Y.; Alva, A.S.; et al. Genomic Profiling of Penile Squamous Cell Carcinoma Reveals New Opportunities for Targeted Therapy. *Cancer Res.* **2015**, *75*, 5219–5227. [CrossRef]
8. Kashofer, K.; Winter, E.; Halbwedl, I.; Thueringer, A.; Kreiner, M.; Sauer, S.; Regauer, S. HPV-negative penile squamous cell carcinoma: Disruptive mutations in the TP53 gene are common. *Mod. Pathol.* **2017**, *30*, 1013–1020. [CrossRef]
9. Feber, A.; Worth, D.C.; Chakravarthy, A.; de Winter, P.; Shah, K.; Arya, M.; Saqib, M.; Nigam, R.; Malone, P.R.; Tan, W.S.; et al. CSN1 Somatic Mutations in Penile Squamous Cell Carcinoma. *Cancer Res.* **2016**, *76*, 4720–4727. [CrossRef]
10. Wang, Y.; Wang, K.; Chen, Y.; Zhou, J.; Liang, Y.; Yang, X.; Li, X.; Cao, Y.; Wang, D.; Luo, L.; et al. Mutational landscape of penile squamous cell carcinoma in a Chinese population. *Int. J. Cancer* **2019**, *145*, 1280–1289. [CrossRef]
11. Stoehr, R.; Weisser, R.; Wendler, O.; Giedl, J.; Daifalla, K.; Gaisa, N.T.; Richter, G.; Campean, V.; Burger, M.; Wullich, B.; et al. P53 codon 72 polymorphism and risk for squamous cell carcinoma of the penis: A Caucasian case-control study. *J. Cancer* **2018**, *9*, 4234–4241. [CrossRef]
12. Lopes, A.; Bezerra AL, R.; Pinto CA, L.; Serrano, S.V.; de Mello, C.A.; Villa, L.L. p53 as a new prognostic factor for lymph node metastasis in penile carcinoma: Analysis of 82 patients treated with amputation and bilateral lymphadenectomy. *J. Urol.* **2002**, *168*, 81–86. [CrossRef]
13. Ficarra, V.; Akduman, B.; Bouchot, O.; Palou, J.; Tobias- Machado, M. Prognostic factors in penile cancer. *Urology* **2010**, *76*, S66–S73. [CrossRef]
14. Horenblas, S.; van Tinteren, H. Squamous cell carcinoma of the penis. IV. Prognostic factors of survival: Analysis of tumor, nodes and metastasis classification system. *J. Urol.* **1994**, *151*, 1239–1243. [CrossRef]
15. Rocha, R.M.; Ignácio, J.A.; Jordán, J.; Carraro, D.M.; Lisboa, B.; Lopes, A.; Carvalho, K.C.; Da Cunha, I.W.; Cubilla, A.; Guimarães, G.C.; et al. A clinical, pathologic, and molecular study of p53 and murine double minute 2 in penile carcinogenesis and its relation to prognosis. *Hum. Pathol* **2012**, *43*, 481–488. [CrossRef]
16. Gunia, S.; Kakies, C.; Erbersdobler, A.; Hakenberg, O.W.; Koch, S.; May, M. Expression of p53, p21 and cyclin D1 in penile cancer: p53 predicts poor prognosis. *J. Clin. Pathol.* **2012**, *65*, 232–236. [CrossRef]
17. Prapiska, F.F.; Warli, S.M. P53 and survival rate in penile cancer. *Open Access Maced. J. Med. Sci.* **2019**, *7*, 1170–1173. [CrossRef]
18. Yanagawa, N.; Osakabe, M.; Hayashi, M.; Tamura, G.; Motoyama, T. Detection of HPV-DNA, p53 alterations, and methylation in penile squamous cell carcinoma in Japanese men. *Pathol. Int.* **2008**, *58*, 477–482. [CrossRef]
19. del Pino, M.; Rodriguez-Carunchio, L.; Ordi, J. Pathways of vulvar intraepithelial neoplasia and squamous cell carcinoma. *Histopathology* **2013**, *62*, 161–175. [CrossRef]
20. Rakislova, N.; Alemany, L.; Clavero, O.; Saco, A.; Torné, A.; Del Pino, M.; Munmany, M.; Rodrigo-calvo, M.T.; Guerrero, J.; Marimon, L.; et al. P53 immunohistochemical patterns in HPV- independent squamous cell carcinomas of the vulva and the associated skin lesions: A study of 779 cases. *Int. J. Mol. Sci.* **2020**, *21*, 8091. [CrossRef]
21. Tessier-Cloutier, B.; Kortekaas, K.E.; Thompson, E.; Pors, J.; Chen, J.; Ho, J.; Prentice, L.M.; McConechy, M.K.; Chow, C.; Proctor, L.; et al. Major p53 immunohistochemical patterns in in situ and invasive squamous cell carcinomas of the vulva and correlation with TP53 mutation status. *Mod. Pathol.* **2020**, *33*, 1595–1605. [CrossRef]
22. Kortekaas, K.E.; Bastiaannet, E.; van Doorn, H.C.; van Steenwijk, P.J.D.V.; Ewing-Graham, P.C.; Creutzberg, C.L.; Akdeniz, K.; Nooij, L.S.; van der Burg, S.H.; Bosse, T.; et al. Vulvar cancer subclassification by HPV and p53 status results in three clinically distinct subtypes. *Gynecol. Oncol.* **2020**, *159*, 649–656. [CrossRef]
23. Darragh, T.M.; Colgan, T.J.; Cox, J.T.; Heller, D.S.; Henry, M.R.; Luff, R.D.; McCalmont, T.; Nayar, R.; Palefsky, J.M.; Stoler, M.H.; et al. The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions: Background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. *Arch. Pathol. Lab. Med.* **2012**, *36*, 1266–1297. [CrossRef]

24. Manzotti, C.; Chulo, L.; López del Campo, R.; Trias, I.; del Pino, M.; Saúde, O.; Basílio, I.; Tchamo, N.; Lovane, L.; Lorenzoni, C.; et al. Penile Squamous Cell Carcinomas in Sub-Saharan Africa and Europe: Differential Etiopathogenesis. *Cancers* **2022**, *14*, 5284. [CrossRef]
25. Barlow, E.L.; Lambie, N.; Donoghoe, M.W.; Naing, Z.; Hacker, N.F. The Clinical Relevance of p16 and p53 Status in Patients with Squamous Cell Carcinoma of the Vulva. *J. Oncol.* **2020**, *2020*, 3739075. [CrossRef]
26. Kortekaas, K.E.; Solleveld-Westerink, N.; Tessier-Cloutier, B.; Rutten, T.A.; van Poelgeest, M.I.E.; Gilks, C.B.; Hoang, L.N.; Bosse, T. Performance of the Pattern Based Interpretation of p53 Immunohistochemistry as a Surrogate for TP53 Mutations in Vulvar Squamous Cell Carcinoma. *Histopathology* **2020**, *77*, 92–99. [CrossRef]
27. McKenna, A.; Hanna, M.; Banks, E.; Sivachenko, A.; Cibulskis, K.; Kernytsky, A.; Garimella, K.; Altshuler, D.; Gabriel, S.; Daly, M.; et al. The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* **2010**, *20*, 1297–1303. [CrossRef]
28. Boeva, V.; Popova, T.; Bleakley, K.; Chiche, P.; Cappo, J.; Schleiermacher, G.; Janoueix-Lerosey, I.; Delattre, O.; Barillot, E. Control-FREEC: A tool for assessing copy number and allelic content using next-generation sequencing data. *Bioinformatics* **2012**, *28*, 423–425. [CrossRef]
29. Chahoud, J.; Gleber-Netto, F.O.; McCormick, B.Z.; Rao, P.; Lu, X.; Guo, M.; Morgan, M.B.; Chu, R.A.; Martinez-Ferrer, M.; Eterovic, A.K.; et al. Whole-exome sequencing in penile squamous cell carcinoma uncovers novel prognostic categorization and drug targets similar to head and neck squamous cell carcinoma A C. *Clin. Cancer Res.* **2021**, *27*, 2560–2570. [CrossRef]
30. Liu, Y.; Chen, C.; Xu, Z.; Scuoppo, C.; Rillahan, C.D.; Gao, J.; Spitzer, B.; Bosbach, B.; Kastenhuber, E.R.; Baslan, T.; et al. Deletions linked to TP53 loss drive cancer through p53-independent mechanisms. *Nature* **2016**, *531*, 471–475. [CrossRef]
31. ClinVar National Center for Biotechnology Information: ClinVar entry NM_000546.6(TP53):c.637C>T (p.Arg213Ter). Available online: https://www.ncbi.nlm.nih.gov/clinvar/variation/43590/?new_evidence=true (accessed on 16 February 2023).
32. Landis, J.R.; Koch, G.G. The measurement of observer agreement for categorical data. *Biometrics* **1977**, *33*, 159–174. [CrossRef]
33. Liu, J.-Y.; Li, Y.-H.; Zhang, Z.-L.; Yao, K.; Ye, Y.-L.; Xie, D.; Han, H.; Liu, Z.-W.; Qin, Z.-K.; Zhou, F.-J. The risk factors for the presence of pelvic lymph node metastasis in penile squamous cell carcinoma patients with inguinal lymph node dissection. *World J. Urol.* **2013**, *31*, 2519–2524. [CrossRef]
34. Zargar-Shoshtari, K.; Spiess, P.E.; Berglund, A.E.; Sharma, P.; Powsang, J.M.; Giuliano, A.; Magliocco, A.M.; Dhillon, J. Clinical Significance of p53 and $p16^{ink4a}$ Status in a Contemporary North American Penile Carcinoma Cohort. *Clin. Genitourin. Cancer* **2016**, *14*, 346–351. [CrossRef] [PubMed]
35. Watkins, J.C.; Yang, E.; Crum, C.P.; Herfs, M.; Gheit, T.; Tommasino, M.; Nucci, M.R. Classic Vulvar Intraepithelial Neoplasia with Superimposed Lichen Simplex Chronicus: A Unique Variant Mimicking Differentiated Vulvar Intraepithelial Neoplasia. *Int. J. Gynecol. Pathol.* **2019**, *38*, 175–182. [CrossRef] [PubMed]
36. Jeffreys, M.; Jeffus, S.K.; Herfs, M.; Quick, C.M. Accentuated p53 staining in usual type vulvar dysplasia-A potential diagnostic pitfall. *Pathol. Res. Pract.* **2018**, *214*, 76–79. [CrossRef]
37. Hwang, H.J.; Nam, S.K.; Park, H.; Park, Y.; Koh, J.; Na, H.Y.; Kwak, Y.; Kim, W.H.; Lee, H.S. Prediction of TP53 mutations by p53 immunohistochemistry and their prognostic significance in gastric cancer. *J. Pathol. Transl. Med.* **2020**, *54*, 378–386. [CrossRef]
38. Berg, S.; D'Andrea, D.; Vetterlein, M.W.; Cole, A.P.; Fletcher, S.A.; Krimphove, M.J.; Marchese, M.; Lipsitz, S.R.; Sonpavde, G.; Noldus, J.; et al. Impact of adjuvant chemotherapy in patients with adverse features and variant histology at radical cystectomy for muscle-invasive carcinoma of the bladder: Does histologic subtype matter? *Cancer* **2019**, *125*, 1449–1458. [CrossRef] [PubMed]
39. Singh, N.; Leen, S.L.; Han, G.; Faruqi, A.; Kokka, F.; Rosenthal, A.; Jiang, X.R.; Kim, R.; McAlpine, J.N.; Gilks, C.B. Expanding the morphologic spectrum of differentiated VIN (dVIN) through detailed mapping of cases with p53 loss. *Am. J. Surg. Pathol.* **2015**, *39*, 52–60. [CrossRef]
40. Ribera-Cortada, I.; Guerrero-Pineda, J.; Trias, I.; Veloza, L.; Garcia, A.; Marimon, L.; Diaz-mercedes, S.; Alamo, J.R.; Rodrigo-calvo, M.T.; Vega, N.; et al. Pathogenesis of penile squamous cell carcinoma: Molecular update and systematic review. *Int. J. Mol. Sci.* **2022**, *23*, 251. [CrossRef]
41. Zehir, A.; Benayed, R.; Shah, R.H.; Syed, A.; Middha, S.; Kim, H.R.; Srinivasan, P.; Gao, J.; Chakravarty, D.; Devlin, S.M.; et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat. Med.* **2017**, *23*, 703–713. [CrossRef]

5.2. Segon estudi

ARTICLE NUMERO 2

Autors: **Isabel Trias,** Ferran Algaba, Inés de Torres, Adela Saco, Lorena Marimon, Nuria Peñuelas, Laia Diez-Ahijado, Lia Sisuashvili, Katarzyna Darecka Alba Morató, Marta del Pino, Carla Ferrándiz-Pulido, María José Ribal, Tarek Ajami, Juan Manuel Corral, Josep Maria Gaya, Oscar Reig, Oriol Ordi, Inmaculada Ribera-Cortada, Adriana García -Herrera, Natalia Rakislova .

Títol**: p53 Immunohistochemistry Defines a Subset of Human Papillomavirus-Independent Penile Squamous Cell Carcinomas with Adverse Prognosis**

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Resum

Objectius: Valorar l'impacte pronòstic del VPH i la p53 en els CEP en una sèrie amplia de 122 pacients. La tinció de p53 es va valorar seguint un sistema de patrons d'expressió immunohistoquímics descrits recentment les quals inclouen dos patrons d'expressió normals (dispers i suprabasal) i quatre d'anormals (difús, sobreexpressió basal, citoplasmàtic i nul).

Mètodes: Es van incloure 122 pacients diagnosticats i tractats de CEP en tres hospitals de Barcelona, Espanya. Basant-se en l'expressió de p16 i p53 IHC, els tumors es van classificar en tres tipus moleculars: associats al VPH, independents del VPH amb expressió normal de p53 i independents del VPH amb expressió anormal de p53. Es va analitzar la mortalitat i la supervivència lliure de malaltia.

Resultats: 36 tumors (29%) estan associats al VPH, 35 (29%) son independents del VPH amb expressió normal de p53, i 51 (42%) independents del VPH amb expressió anormal de p53. El grup independent per VPH amb p53 anòmala mostra la màxima mortalitat (14/51, 27%) amb 7/14 pacients que presenten patrons d'expressió de p53 infreqüents (basal, citoplasmàtic i nul); l'independent de VPH amb p53 normal mostra una mortalitat del 0% i finalment els casos associats a VPH un 8% ($p<0,001$). En l'anàlisi multivariant, tant els tumors anormals independents del VPH amb patró immunohistoquímic mutat de p53 com l'estadi avançat s'associen amb la mortalitat més alta ($p = 0,002$ i $p = 0,004$, respectivament).

Conclusions: Es reconeixen grups pronòstics diferents en el CEP basats en el VPH (p16) i l'expressió immunohistoquímica de p53 basada en patrons (expressió difusa, basal, citoplasmàtica o nul·la per els patrons mutats i dispersa o suprabasal per els no mutats). El grup de pitjor pronòstic és el independent de VPH amb expressió anòmala de p53.

Article

p53 Immunohistochemistry Defines a Subset of Human Papillomavirus-Independent Penile Squamous Cell Carcinomas with Adverse Prognosis

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Simple Summary: Penile cancer is currently classified on the basis of its relationship or not with human papilloma virus (HPV) infection. HPV-associated tumors have better prognosis than HPV-independent tumors. In this study we show that p53 immunohistochemistry, a simple technique that shows good correlation with the mutational status of the *TP53* gene, allows subclassifying HPV-independent penile carcinomas in two groups: tumors with normal (wild type) p53, with good prognosis similar to the HPV-associated tumors, and tumors with abnormal (mutated) p53, aggressive carcinomas with poor prognosis.

Abstract: Background: Penile squamous cell carcinoma (PSCC) is classified into two prognostically distinct types: human papillomavirus (HPV)-associated and HPV-independent. Conversely, the impact of p53 status on prognosis remains controversial. We correlated HPV and p53 status with prognosis in a large series of PSCC patients. p53 was analysed according to a recently described immunohistochemical (IHC) pattern-based framework that includes two normal (scattered, mid-epithelial) and four abnormal patterns (diffuse, basal overexpression, cytoplasmic and null) and closely correlates with *TP53* mutational status. Methods: A total of 122 patients with surgically treated PSCC in three hospitals in Barcelona, Spain, were included. Based on HPV in situ hybridization and p16 and p53 IHC expression, the tumors were classified into three molecular types: HPV-associated, HPV-independent/p53 normal, and HPV-independent/p53 abnormal. All patients were followed for at least 22 months (median 56.9 months), and disease-specific survival (DSS) was analysed. Results: Thirty-six tumors (29%) were HPV-associated, 35 (29%) were HPV-independent/p53 normal, and 51 (42%) were HPV-independent/p53 abnormal. Disease-related deaths were observed in 3/36 (8%), 0/35 (0%) and 14/51 (27%) of the patients, respectively ($p<0.001$). A total of 7/14 deaths in the latter group were patients with tumors showing p53 patterns not recognized in

the classical p53 IHC interpretation (basal, null, cytoplasmic). According to our multivariate analysis, HPV-independent/p53 abnormal tumors and advanced stage were associated with impaired DSS (hazard ratio=23.4, 95% confidence interval [CI]= 2.7-3095.3; p=0.001 and 16.3, 95% CI=1.8-2151.5; p=0.008, respectively). CONCLUSION: Compared with patients with HPV-associated and HPV-independent/p53-normal PSCC, patients with HPV-independent/p53 abnormal PSCC have worse clinical outcomes. p53 IHC results define two prognostic categories in HPV-independent PSCC: HPV-independent/p53-normal tumors as low-risk tumors, whereas HPV-independent/p53-abnormal tumors as aggressive neoplasms.

Keywords: penile cancer; penile squamous cell carcinoma; HPV; p53; prognosis; disease-specific survival

1. Introduction

Penile squamous cell carcinoma (PSCC) is an uncommon neoplasm. Its incidence has marked geographic variability [1], and it is particularly high (2.5/100,000 inhabitants) in Spain [2]. Phimosis, absence of circumcision, chronic inflammation, lichen sclerosus, smoking, and human papil-lomavirus (HPV) infection have been described as risk factors for the development of the disease [3]. According to the World Health Organization (WHO), PSCCs should be classified based on their association with HPV. For this reason, immunohistochemical (IHC) staining for p16, a well-known surrogate of transforming HPV infection, is strongly recommended by the WHO in its last classification of urological tumors [3].

Genomic instability secondary to the overexpression of the oncoproteins E6 and E7, leading to an uncontrolled progression of the cell cycle is considered a key molecular mechanism involved in HPV-associated PSCC [4,5]. Conversely, the molecular background of HPV-independent PSCC is less well understood, with several recent series reporting that *TP53* mutations are highly recurrent in this subset of tumors [6–9]. Remarkably, a few studies have suggested that the *TP53* mutational status is associated with nodal metastases and thus with a worse prognosis in PSCC patients [4,6,8,10–13].

In a recent study [14], we showed that a p53 IHC pattern-based framework described initially for vulvar tumors [15] can also be applied to PSCC and strongly correlates with *TP53* mutational status, suggesting that, if carefully evaluated, a simple p53 IHC technique, available in most pathology laboratories, could be considered a reliable surrogate of *TP53* mutational status. In this scheme, two p53 IHC patterns (scattered and mid-epithelial), defined as "normal", indicate a wild-type *TP53* protein, whereas four well-defined patterns (diffuse overexpression, basal over-expression, cytoplasmic and null), defined as "abnormal", strongly correlate with a mutated *TP53*. Remarkably, three of the mutant patterns in this scheme, namely, basal overexpression, cytoplasmic and null, have been classified as "normal" according to conventional evaluations in previous studies on PSCC [14,16,17].

We aimed to evaluate the HPV and *TP53* statuses in a large series of PSCC from three public institutions in Spain by classifying the tumors based on HPV in situ hybridization and p16 and p53 IHC analysis into three molecular categories: HPV-associated, HPV-independent/p53 normal and HPV-independent/p53 abnormal. We analysed the prognostic implications of this molecular classification system.

2. Methods

2.1. Patients

We retrospectively identified all patients surgically treated for PSCC in two tertiary general hospitals (Hospital Clinic de Barcelona, Hospital Vall d'Hebron) and a monographic

urological center (Fundació Puigvert) in Barcelona, Spain, from January 2000 to December 2020. All patients fulfilled the following inclusion criteria: 1) had a primary diagnosis of PSCC, 2) had a follow-up of at least 22 months or until death, and 3) had sufficient available tumor tissue for ancillary IHC studies. All patients were treated following the guidelines of the European Association of Urology (EAU) [18] depending on the clinical staging, which was determined on the basis of physical examination plus imaging techniques (ultrasound scan, computed tomography scan and/or positron emission tomography, etc.) when required. All local excisions aimed at organ sparing and reconstructive techniques were used when necessary to minimize the functional impact. Inguinal lymph node evaluation was performed if required. Guided sentinel node biopsy was the first option. Endoscopic inguinal modified lymphadenectomy was performed when sentinel node biopsy was not available. In all patients with positive sentinel node a radical inguinal lymphadenectomy was performed.

The following clinical and pathological variables were retrieved from the electronic files: age at diagnosis, tumor location, type and date/s of treatment/s, margin status, vascular invasion, perineural invasion, stage at diagnosis, date of first cancer recurrence, and patient status at follow-up.

The study was approved by the Healthcare Ethics Committee of the Hospital Clinic of Barcelona, Hospital Vall d'Hebron and Fundació Puigvert (HCB/2020/1207, PR(AG)578/2021, FP2021/05c, respectively). Informed written consent was obtained from all the patients included in the study.

2.2. p16 IHC

IHC for p16 was performed for all samples using the CINtec Histology Kit (clone E6H4; Roche, Heidelberg, Germany). Tumors with strong and diffuse block-type staining were considered positive, whereas patchy or completely negative p16 staining was considered p16 negative [3,19,20]. In each run a p16-positive squamous carcinoma of the vulva was used as positive control. All patients were independently evaluated by two pathologists with expertise in urological pathology and interpretation of p16 staining (I.T. and N.R.).

2.3. ISH

RNA in situ hybridization (ISH) was performed for all samples using the automated Leica Biosystems BOND-III and RNAscope ISH Probe High Risk HPV. The assay qualitatively detects E6 mRNA in 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82 high-risk HPV types. In each run a carcinoma of the uterine cervix with known HPV16 positivity was used as control.

2.4. p53 IHC

p53 IHC was performed in all patients with a monoclonal antibody (clone DO-7; Roche) according to the manufacturer's protocol. All the IHC staining procedures (for p16 and p53) were performed on an automated staining system (Ventana Benchmark ULTRA, Ventana Medical Systems, Tucson, AZ, USA) on whole slide sections. IHC staining was evaluated for invasive tumors following the recently described p53 pattern-based interpretation framework described for squamous cell carcinomas of the vulva [21]and confirmed in PSCC [14]; this method includes two major categories: "normal", which correlates with wild-type *TP53*, and "abnormal staining", which correlates with mutated *TP53*". The "normal" category included two patterns: 1) occasional positive nuclei in the basal and/or parabasal layer (scattered pattern) and 2) moderate to strong nuclear p53 IHC staining in the parabasal layers with absence of expression in the basal cells (mid-epithelial pattern). The "abnormal" category included four p53 IHC patterns: 1) continuous, strong nuclear staining of the basal layer (basal overexpression pattern); 2) continuous and strong nuclear basal staining with suprabasal extension of the

positive cells (diffuse overexpression pattern); 3) cytoplasmic staining with or without nuclear positivity (cytoplasmic pattern); and 4) complete absence of staining in the tumor, with evidence of intrinsic positive control in the adjacent skin, stromal or inflammatory cells (null pattern). All patients were independently evaluated by two pathologists with expertise in urological pathology and interpretation of p53 staining (I.T. and N.R.). All discrepancies were discussed in a consensus meeting, and a final evaluation was achieved. In each run a normal tonsil showing scattered positive staining and a serous carcinoma of the ovary with known *TP53* mutation and diffuse p53 IHC overexpression were used as controls. Thirty-three patients were previously included in a recent study focused on the validation of this pattern-based p53 interpretation framework against *TP53* mutational analysis, in which 95% concordance was observed [14].

2.5. The Criteria for PSCC Classification into Three Groups

All the study cases were classified into three main categories based on HPV ISH and p16 IHC results and the pattern of p53 IHC expression. The categories included the following: 1) HPV-associated PSCC (positive for HPV ISH and p16 IHC, independent of the p53 IHC pattern); 2) HPV-independent/p53 normal PSCC (negative for HPV ISH and p16 IHC and a scattered or mid-epithelial p53 IHC pattern); and 3) HPV-independent/p53 abnormal PSCC (negative for HPV ISH and p16 IHC and diffuse, basal overexpression, cytoplasmic or null patterns of p53 IHC).

2.6. Statistical Analysis

The statistical analyses were conducted using R Statistical Software (v4.3.2; R Core Team 2021). The chi-square test and Fisher's exact test were employed for categorical data, while the Wilcoxon rank-sum test was utilized for numerical data, enabling the comparison of clinical and histopathological data.

The endpoints for prognosis were recurrence-free survival (RFS) and disease-specific survival (DSS), which were calculated from the date of treatment (primary surgery) to the date of first recurrence or progression or to death due to the disease, respectively. Cumulative incidences were depicted through plotted curves, and differences between the curves were assessed using Gray's test. Univariate and adjusted (multivariate) models were obtained using the Cox proportional hazards model. For the multivariate analysis, two models were built, one including the molecular type and the second including the p53 IHC status, due to the collinearity of these two variables. Two-sided tests were used, and a p value less than 0.05 indicated statistical significance.

3. Results

3.1. Clinical Pathological Features of the Overall Series

One hundred twenty-two patients were included in the study. Of these, 43 were from the Hospital Clinic de Barcelona, 35 were from the Hospital Vall d'Hebron and 44 were from the Fundació Puigvert. The mean age at diagnosis was 68.6 years (range 40-96). Fifty-eight patients (47.5%) were stage I at diagnosis, 40 (32.8%) were stage II, 16 (13.1%) were stage III, and 8 (6.6%) were stage IV tumors. The median follow-up period was 56.9 months (range 22-60 months). Sixty-five patients (53.3%) underwent penectomy (partial or radical), 48 (39.3%) glansectomy and in 9 patients (7.4%) circumcision was performed. Metastatic involvement of the lymph nodes was identified in 24 patients (19.7%).

3.2. HPV ISH and p16 and p53 IHC Results and Tumor Classification

HPV ISH was positive in 36/122 tumors (29.5%), and in all of them, p16 IHC was positive. These tumors were classified accordingly as HPV-associated tumors. Thirty-two of the 36 HPV-associated tumors had a normal pattern of p53 IHC expression (88.9%), 31 had a scattered pattern, and one had a mid-epithelial pattern of p53 IHC. Only four (11.1%) HPV-associated tumors presented an abnormal p53: two had diffuse over-expression, one had basal overexpression, and one had a null pattern.

Eighty-six out of the 122 tumors (70.5%) were negative for HPV ISH and p16 IHC and were classified as HPV-independent. Among the 86 tumors, 35 (28.7% of the overall series) showed a normal pattern of p53 IHC expression and were classified as HPV-independent/p53 normal, all of which exhibited a scattered pattern. Fifty-one tumors in this HPV-independent category (41.8% of the overall series) had an abnormal p53 IHC staining pattern and were classified as HPV-independent/p53 abnormal. The most common abnormal p53 IHC pattern in this cohort was diffuse overexpression (27/51, 53.0%), followed by a null pattern (14 patients, 27.4%), basal overexpression (8 patients, 15.7%) and cytoplasmic expression (2/51, 3.9%).

3.3. Characteristics of the Three Molecular Types of PSCC

Table 1 summarizes the clinical and pathological features of the patients and the three molecular categories defined in this study. Patients with HPV-independent/p53 normal tumors were older (mean age of 72 years) than were those with the other two categories (67 years for both HPV-associated and HPV-independent/p53 abnormal tumors [p=0.040]). Patients with HPV-independent/p53 abnormal tumors had a greater risk of lymph node metastases than patients with HPV-independent/p53-normal tumors (p=0.012). The histological variants of the HPV-associated tumors significantly differed from the variants identified in the HPV-independent molecular types. Moreover, there were no differences in terms of histological variants between HPV-independent/p53 normal and HPV-independent/p53 abnormal tumors. There were no differences in terms of anatomical location, vascular or perineural invasion, margin status, or stage at diagnosis. Figure 1 shows a representative example of each of the three tumor categories, including hematoxylin and eosin staining features as well as HPV ISH and p16 and p53 staining.

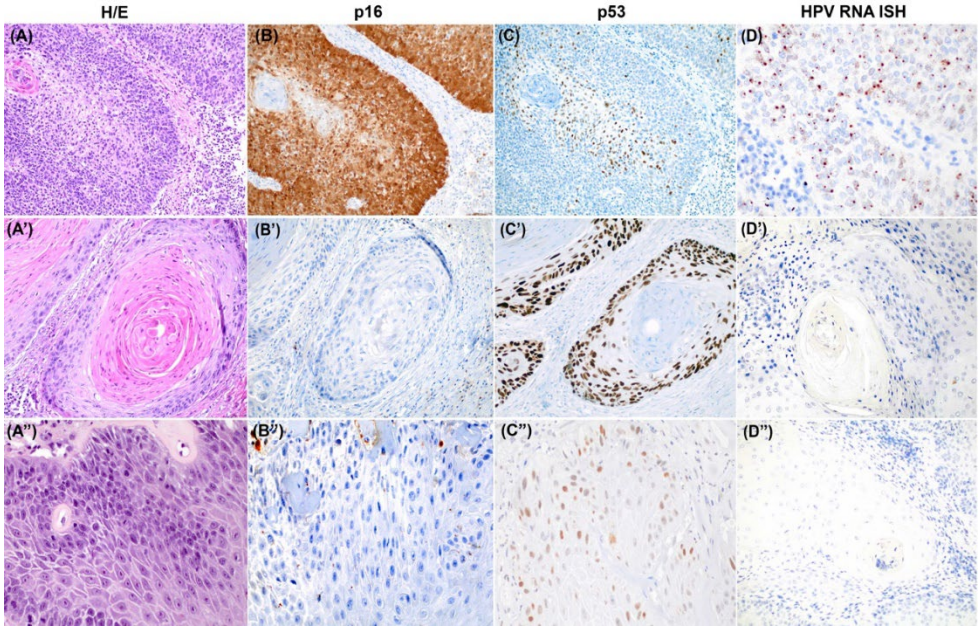

Figure 1. Histological (hematoxylin and eosin), p16 and p53 immunohistochemical (IHC) expression and human papillomavirus (HPV) *in situ* hybridization of a typical example of HPV-associated PSCC (first row), HPV-independent penile squamous cell carcinoma (PSCC) with abnormal p53 (second row) and HPV-independent PSCC with normal p53 (third row) A, A' and

A'', hematoxylin and eosin; B, B' and B'' p16 IHC; C, C' and C'', p53 IHC; D, D', D'' HPV RNA *in situ* hybridization.

Table 1. Pathological features, staging and follow-up results in the three types of penile squamous cell carcinoma: human papillomavirus (HPV)-associated, HPV-independent with normal p53 expression and HPV-independent with abnormal p53 expression.

	HPV-associated	**HPV-independent**		
	(n=36)	**p53 normal (n=35)**	**p53 abnormal (n=51)**	**p**
Age	67.1 (45.0, 94.0)	72.5 (46.0, 96.0)	67.1 (40.0, 87.0)	0.223
Anatomical location				0.195
Glans	21 (58.3%)	25 (71.4%)	41 (80.4%)	
Foreskin, coronal sulcus, body	11 (30.6%)	8 (22.9%)	9 (17.6%)	
Not recorded	4 (11.1%)	2 (5.7%)	1 (2.0%)	
Histological type				**<0.001**
Usual	7 (19.4%)	27 (77.1%)	39 (76.5%)	
Verrucous	0 (0.0%)	5 (14.3%)	3 (5.9%)	
Basaloid	16 (44.4%)	0 (0.0%)	4 (7.8%)	
Warty	4 (11.1%)	1 (2.9%)	0 (0.0%)	
Mixed	7 (19.4%)	1 (2.9%)	1 (2.0%)	
Sarcomatoid	1 (2.8%)	0 (0.0%)	4 (7.8%)	
Lymphoepitelioma-like	1 (2.8%)	0 (0.0%)	0 (0.0%)	
Cuniculatum	0 (0.0%)	1 (2.9%)	0 (0.0%)	
Vascular invasion				0.992
No	33 (91.7%)	32 (91.4%)	47 (92.2%)	
Yes	3 (8.3%)	3 (8.6%)	4 (7.8%)	
Perineural invasion				0.340
No	33 (91.7%)	34 (97.1%)	47 (92.2%)	
Yes	3 (8.3%)	1 (2.9%)	7 (13.7%)	
Lymph node metastases				**0.040**
No	28 (77.8%)	33 (94.3%)	37 (72.5%)	
Yes	8 (22.2%)	2 (5.7%)	14 (27.5%)	
Stage				0.124
I	13 (36.1%)	22 (62.9%)	23 (45.0%)	
II	15 (41.7%)	11 (31.4%)	14 (27.5%)	
III	6 (16.7%)	1 (2.9%)	9 (17.6%)	
IV	2 (5.6%)	1 (2.9%)	5 (9.8%)	
Surgical margins (invasive tumor)				1.000
Free	31 (86.1%)	31 (88.6%)	44 (86.3%)	
Affected	5 (13.9%)	4 (11.4%)	7 (13.7%)	
Chemotherapy				0.219
No	31 (86.1%)	34 (97.1%)	45 (88.2%)	
Yes	5 (13.9%)	1 (2.9%)	6 (11.8%)	
Radiotherapy				0.118
No	31 (86.1%)	33 (94.3%)	40 (78.4%)	
Yes	5 (13.9%)	2 (5.7%)	11 (21.6%)	
Recurrence				0.067
No	30 (83.3%)	29 (82.9%)	33 (64.7%)	

Yes	6 (16.7%)	6 (17.1%)	18 (35.3%)	
Death of disease				**<0.001**
No	33 (91.7%)	35 (100%)	37 (72.5%)	
Yes	3 (8.3%)	0 (0.0%)	14 (27.5%)	

3.4. Survival Analysis

Thirty patients (24.6%) experienced disease recurrence during follow-up, but no differences were observed among the three molecular types in terms of recurrence rate (p=0.067). Seventeen patients (13.9%) died due to PSCC, and 13 (10.6%) died due to other causes. Disease-related death was observed in 3/36 (8.3%) patients with HPV-associated PSCC and 0/35 (0.0%) patients with HPV-independent/p53 normal PSCC, with the highest number of events occurring in patients with HPV-independent/p53 abnormal PSCC (14/51 (27.5%) (p<0.001)). The patterns of p53 IHC expression in the HPV-independent tumors of the patients who died due to the tumor were diffuse overexpression (7/27; 25.9%), null pattern (5/14; 35.7%), basal overexpression (1/8; 12.5%) and cytoplasmic expression (1/2; 50%).

Figure 2 shows the cumulative incidence curves for RFS and DSS for the three molecular categories. No differences in RFS were observed between the three molecular types (p=0.083); however, significant differences in DSS were detected (p=0.001), with patients with HPV-independent/p53 abnormal PSCC having the worst prognosis. In terms of tumor staging, no differences in RFS were observed between patients with early-stage tumors and patients with advanced-stage tumors (p=0.073); however, significant differences in DSS were identified (p<0.001). Remarkably, 10/14 (71.4%) HPV-independent/p53 abnormal PSCC patients diagnosed with stage III or IV died from the disease compared to only 2/8 (25.0%) of the patients with HPV-associated tumors in stages III or IV and 0/2 (0.0%) of the HPV-independent/p53 normal patients in stages III or IV (p=0.048).

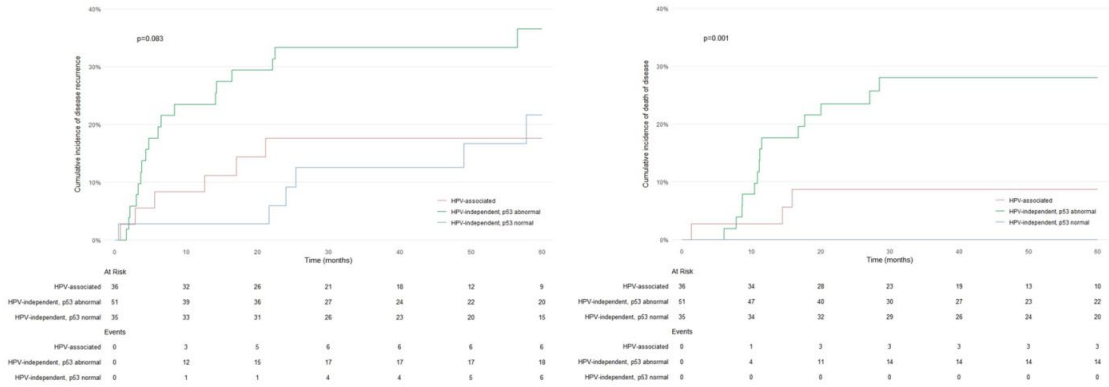

Figure 2. Cumulative incidence graphs for disease recurrence and death from one of the three types of penile squamous cell carcinoma: human papillomavirus (HPV)-associated, HPV-independent with normal p53 expression and HPV-independent with abnormal p53 expression.

Table 2 shows the Cox regression analysis for RFS. Vascular invasion, perineural invasion, lymph node metastasis and advanced disease stage were associated with impaired RFS in the univariate analysis. According to the multivariate analysis, only vascular invasion reached statistical significance. The results of the Cox regression analysis for DSS are shown in Table 3. The molecular type (HPV-independent/p53 abnormal), p53 IHC abnormal pattern, vascular invasion, perineural invasion, lymph node metastases and advanced stages were associated with impaired DSS in the univariate analysis. According to the two multivariate models, HPV-independent/p53 abnormal molecular type (p=0.001) or p53 IHC expression (p=0.001), in addition to vascular and perineural invasion, lymph node metastases, and advanced stage, were significantly associated with impaired DSS.

Table 2. Univariate and multivariate Cox model for recurrence-free survival.

Variable	Univariate model			Multivariate model with molecular type			Multivariate model with p53		
	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p
Age	1	0.9-1	0.415						
Molecular type*									
HPV-independent/p53 normal	1			1			-	-	
HPV-associated	1.1	0.4-3.3	0.865	1.3	0.4-4.2	0.672	-	-	-
HPV-independent/p53 abnormal	2.2	0.9-5.9	0.062	2.3	0.9-6.5	0.065	-	-	-
p53 immunohistochemistry									
Normal	1			-	-		1		
Abnormal	1.9	0.9-4.1	0.062	-	-	-	1.2	0.9-4.2	0.070
Vascular invasion									
No	1			1			1		
Yes	8.0	3.2-18.0	**<0.001**	3.9	1.1-12.7	**0.031**	3.6	1.1-11.7	**0.037**
Perineural invasion									
No	1			1			1		
Yes	6.8	2.9-14.6	**<0.001**	2.3	0.7-6.4	0.150	2.3	0.7-6.5	0.148
Lymph node metastases									
No	1			1			1		
Yes	4.4	2.1-9.1	**<0.001**	2.3	0.9-5.9	0.079	2.4	0.9-6.4	0.063
Staging									
Early (I)	1			1			1		
Advanced (II-IV)	2.2	1.1-4.8	**0.033**	1.2	0.5-3.1	0.712	1.2	0.4-2.9	0.737

HR: hazard ratio; CI: confidence interval; HPV: human papillomavirus.

Table 3. Univariate and multivariate Cox model for disease-specific mortality.

Variable	Univariate model			Multivariate model with molecular type			Multivariate model with p53		
	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p
Age	1	0.9-1	0.808						
Molecular type									
HPV-independent/p53 normal	1			1			-	-	
HPV-associated	7.3	0.7-986.3	0.102	6.7	0.6-929.2	0.138	-	-	-
HPV-independent/p53 abnormal	21.9	2.9-2804.6	**<0.001**	23.4	2.7-3095.3	**0.001**	-	-	-
p53 immunohistochemistry									
Normal	1			-	-		1		
Abnormal	5.42	1.9-20.9	**0.001**	-	-	-	5.9	1.9-23.7	**0.001**
Vascular invasion									
No	1			1			1		
Yes	10.3	3.7-26.7	**<0.001**	3.5	1.1-10.9	**0.035**	2.9	0.9-9.2	0.071
Perineural invasion									
No	1			1			1		
Yes	9.7	3.6-24.8	**<0.001**	3.0	1.0-8.7	**0.049**	2.8	0.9-8.2	0.065
Lymph node metastases									
No	1			1			1		
Yes	13.5	5.1-40.5	**<0.001**	2.8	1.0-8.8	**0.045**	3.2	1.1-9.9	**0.028**
Staging									

Early (I)	1			1			1		
Advanced (II-IV)	36.9	5.0-4706.3	**<0.001**	16.3	1.8-2151.5	**0.008**	16.0	1.8-2103.0	**0.008**

HR: hazard ratio; CI: confidence interval; HPV: human papillomavirus.

4. Discussion

The most remarkable finding of our study, which included a large series of patients with PSCC treated at three different institutions in Barcelona, Spain, was the difference in prognosis observed between the three molecular types of PSCC defined according to their association with HPV and p53 IHC: HPV-associated, HPV-independent with normal p53, and HPV-independent with abnormal p53 PSCC. Remarkably, the classification of the tumors based on HPV status and p53 IHC patterns had a stronger impact on DSS in the multivariate analysis than did the staging system, suggesting that not only HPV status but also p53 IHC should be routinely evaluated in all PSCC patients.

The good prognosis of patients with HPV-associated tumors (over 90% DSS at 5 years) strongly supports the current 2022 WHO classification of PSCC, which separates tumors based on HPV status [22]. Studies evaluating the prognostic impact of HPV status in PSCC patients have shown controversial results, with some reporting no differences in DSS [23], whereas others showing longer DSS in HPV-associated PSCC [9,13], similar to what occurs in HPV-associated carcinomas in other anatomical sites, such as the head and neck [24] and the lower female genital tract [25]. The good DSS of patients with HPV-associated tumors in our study is remarkable, especially considering that these patients were frequently diagnosed in advanced stages and had metastatic involvement of the lymph nodes in almost 25% of the patients. These results suggest that, as shown in HPV-associated tumors from other anatomical areas, HPV-associated PSCC is highly sensitive to radiation and chemotherapy [26]. In accordance with the findings of other European series [27], HPV-associated tumors represented a small percentage (29.5%) of all PSCC, which is in contrast with the findings of studies from sub-Saharan Africa or the Caribbean, which showed rates of HPV-associated PSCC as high as 80% [28]. As previously reported, p16 IHC results have shown excellent correlation with HPV ISH [29], reinforcing the validity of the recent WHO 2022 recommendation of using p16 IHC as a surrogate for the presence of high-risk HPV [22]. Our study revealed that the second type of PSCC defined by the WHO, HPV-independent tumors, includes at least two categories with different clinical and pathological features and, most importantly, a different prognosis. In the first category, HPV-independent PSCC with normal p53 expression is associated with several specific clinical pathological features. These tumors arise in older men, have a very low rate of lymph node metastases and are rarely diagnosed at stage III or IV. Remarkably, although these patients had similar rates of recurrence compared to those in the other two groups, they had excellent DSS, with no tumor-related deaths. This category of HPV-independent tumors with normal p53 IHC has previously been described in the vulva [30,31], where they show similar behavior to that observed in our series of PSCC, with frequent recurrences but extremely good DSS [32].

The most frequent category of PSCC in our study (60% of all HPV-independent tumors and 40% of all tumors) was HPV-independent/p53 abnormal PSCC. This percentage of abnormal p53 IHC results is similar to the percentage reported by other studies in HPV-independent PSCC [6,9]. In contrast with the favorable DSS of patients with HPV-associated tumors and HPV-independent/p53 normal tumors, the prognosis of patients with HPV-independent/p53 abnormal PSCC is poor, with a 27% 5-year mortality. Importantly, the impaired DSS of this subgroup was confirmed via multivariate analysis. Our study confirmed that the pattern-based framework of p53 IHC interpretation, previously described as having an excellent correlation with the *TP53* mutational status in PSCC [14], significantly improved the conventional evaluation of p53 IHC. In addition to the diffuse overexpression classically accepted by previous researchers [33], this framework recognizes additional patterns (null, cytoplasmic and basal overexpression) usually neglected in previous studies [34]. As shown in this study, these

patterns correlated with an adverse prognosis, as 7/14 deaths in the HPV-independent/p53 abnormal group were related to tumors showing p53 patterns not recognized via classical p53 IHC. These differences in p53 IHC results may explain the differences observed in previous studies regarding the prognostic impact of p53 IHC [35].Four out of the 36 HPV-associated PSCC (11%) in our series had abnormal p53 IHC (one null, one basal and two diffuse overexpression).

Although the vast majority of HPV-associated PSCC showed a normal pattern of p53 expression [14], a small percentage of patients in our series (11%) exhibited an abnormal p53 IHC pattern. This finding has been previously described [17]. Moreover, *TP53* mutations have been detected in small percentages of HPV-associated PSCC [36], in HPV-associated tumors of the head and neck [37] and in the vulva [38,39], indicating that abnormal *TP53* (or abnormal p53 IHC staining) is not an exclusive finding of HPV-independent carcinomas. Interestingly, none of the patients with HPV-associated PSCC with an abnormal p53 IHC expression pattern died due to the disease, suggesting that *TP53* mutation does not impair prognosis in this molecular category, although further studies including a greater number of HPV-associated tumors with abnormal p53 IHC staining are needed to reach strong conclusions. Finally, it should be emphasized that although in this study the correlation between p16 IHC overexpression and HPV detection was 100%, a percentage of around 10% of discrepant results has been reported in other studies focused on head and neck and vulvar tumors [32], and that this phenomenon should also be expected as probably occurring in PSCC.

Interestingly, the presence of vascular invasion was the only factor associated with disease recurrence according to the multivariate analysis. This association is not surprising considering the previously reported association between vascular invasion and lymph node metastases in PSCC [40].

Our study has several limitations. Due to the large time frame of inclusion, a significant number of patients, mainly from the initial period of inclusion, did not undergo inguinal staging using sentinel lymph node analysis; thus, some inguinal lymph node microscopic metastases could have been missed. In addition, the results could not be corrected for the different treatments due to the small number of patients requiring adjuvant therapies. Finally, the small number of disease-related deaths in the series might have affected the strength of the statistical estimations.

5. Conclusions

Our study showed that patients with HPV-independent/p53 abnormal PSCC have adverse clinical outcomes than patients with HPV-associated and HPV-independent/p53 normal PSCC. p53 IHC defines two prognostic categories in HPV-independent PSCC: HPV-independent/p53 normal PSCC are low-risk tumors, whereas HPV-independent/p53 abnormal tumors can be considered aggressive neoplasms. Our study suggests that PSCC be stratified into three molecular types with distinct clinicopathological features and behaviors based on p16 (as a surrogate of HPV status) and p53 IHC (as a surrogate of *TP53* mutational status) status. If these results are confirmed in prospective studies, they could help to refine the staging work-up, treatment schemes and follow-up strategies for patients with PSCC.

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patients, clinical follow-up, writing and editing; J.M.G.: recruitment of patients, clinical follow-up, writing and editing; O.R.: recruitment of patients, clinical follow-up, writing and editing; O.O.: data analysis; writing and editing; I.R-C.: study design, investigation, methodology, writing of the first draft, writing and editing; A.G-H.: study design, investigation, methodology, writing of the first draft, writing and editing; N.R. study design, investigation, methodology, writing of the first draft, writing and editing, project management. All authors have read and agreed to the published version of the manuscript.

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References

1. Emmanuel, A.; Nettleton, J.; Watkin, N.; Berney, D.M. The Molecular Pathogenesis of Penile Carcinoma—Current Developments and Understanding. *Virchows Archiv* **2019**, *475*, 397–405, doi:10.1007/s00428-019-02607-8.
2. Borque-Fernando, Á.; Gaya, J.M.; Esteban-Escaño, L.M.; Gómez-Rivas, J.; García-Baquero, R.; Agreda-Castañeda, F.; Gallioli, A.; Verri, P.; Ortiz-Vico, F.J.; Amir-Nicolau, B.F.; et al. Epidemiology, Diagnosis and Management of Penile Cancer: Results from the Spanish National Registry of Penile Cancer. *Cancers (Basel)* **2023**, *15*, 1–12, doi:10.3390/cancers15030616.
3. Amin M.B., Berney D.M., Comperat, E.M., Hartmann, A., Menon S., Netto G.J., Raspollini, M. R., Rubin, M. A., Tickoo, S. and Tujaric, S. *Who Classification of Tumours. 5th Edition. Urinary and Male Genital Tumours*; Board, W.C. of T.E., Ed.; 5th ed.; IARC: Lyon (France), 2022; ISBN 9789283245124(pbk).
4. Chaux, A.; Velazquez, E.; Algaba, F.; Ayala, G.; Cubilla, A. Developments in the Pathology of Penile Squamous Cell Carcinomas. *Urology* **2010**, *76*, doi:10.1016/J.UROLOGY.2010.02.038.
5. Gross G; Pfister H Role of Human Papillomavirus in Penile Cancer, Penile Intraepithelial Squamous Cell Neoplasias and in Genital Warts. *Med Microbiol Immunol* **2004**, *193*, 35–44.
6. Kashofer, K.; Winter, E.; Halbwedl, I.; Thueringer, A.; Kreiner, M.; Sauer, S.; Regauer, S. HPV-Negative Penile Squamous Cell Carcinoma: Disruptive Mutations in the TP53 Gene Are Common. *Modern Pathology* **2017**, *30*, 1013–1020, doi:10.1038/modpathol.2017.26.
7. Feber, A.; Worth, D.C.; Chakravarthy, A.; de Winter, P.; Shah, K.; Arya, M.; Saqib, M.; Nigam, R.; Malone, P.R.; Tan, W.S.; et al. CSN1 Somatic Mutations in Penile Squamous Cell Carcinoma. *Cancer Res* **2016**, *76*, 4720–4727, doi:10.1158/0008-5472.CAN-15-3134.
8. Wang, Y.; Wang, K.; Chen, Y.; Zhou, J.; Liang, Y.; Yang, X.; Li, X.; Cao, Y.; Wang, D.; Luo, L.; et al. Mutational Landscape of Penile Squamous Cell Carcinoma in a Chinese Population. *Int J Cancer* **2019**, *145*, 1280–1289, doi:10.1002/ijc.32373.
9. McDaniel, A.S.; Hovelson, D.H.; Cani, A.K.; Liu, C.-J.; Zhai, Y.; Zhang, Y.; Weizer, A.Z.; Mehra, R.; Feng, F.Y.; Alva, A.S.; et al. Genomic Profiling of Penile Squamous Cell Carcinoma Reveals New Opportunities for Targeted Therapy. *Cancer Res* **2015**, *75*, 5219–5227, doi:10.1158/0008-5472.CAN-15-1004.
10. Stoehr, R.; Weisser, R.; Wendler, O.; Giedl, J.; Daifalla, K.; Gaisa, N.T.; Richter, G.; Campean, V.; Burger, M.; Wullich, B.; et al. P53 Codon 72 Polymorphism and Risk for Squamous Cell Carcinoma of the Penis: A Caucasian Case-Control Study. *J Cancer* **2018**, *9*, 4234–4241, doi:10.7150/jca.26050.

11. Lopes A; Bezerra AL; Pinto CA; Serrano SV; de Mello CA; Villa LL. P53 as a New Prognostic Factor for Lymph Node Metastasis in Penile Carcinoma: Analysis of 82 Patients Treated with Amputation and Bilateral Lymphadenectomy. *J Urol.* **2002**, *168*, 81–86.
12. Jacob, J.; Ferry, E.; Gay, L.; Elvin, J.A.; Vergilio, J.A.; Ramkissoon, S.; Severson, E.; Necchi, A.; Killian, J.K.; Ali, S.M.; et al. Comparative Genomic Profiling of Refractory and Metastatic Penile and Nonpenile Cutaneous Squamous Cell Carcinoma: Implications for Selection of Systemic Therapy. *J Urol* **2019**, *201*, 541–548, doi:10.1016/J.JURO.2018.09.056.
13. Ferrandiz-Pulido, C.; Masferrer, E.; Toll, A.; Hernandez-Losa, J.; Mojal, S.; Pujol, R.; Ramon y Cajal, S.; de Torres, I.; Garcia-Patos, V. MTOR Signaling Pathway in Penile Squamous Cell Carcinoma: PmTOR and PeIF4E over Expression Correlate with Aggressive Tumor Behavior. *J Urol* **2013**, *190*, 2288–2295, doi:10.1016/J.JURO.2013.06.015.
14. Trias, I.; Saco, A.; Marimon, L.; López del Campo, R.; Manzotti, C.; Ordi, O.; del Pino, M.; Pérez, F.M.; Vega, N.; Alós, S.; et al. P53 in Penile Squamous Cell Carcinoma: A Pattern-Based Immunohistochemical Framework with Molecular Correlation. *Cancers (Basel)* **2023**, *15*, doi:10.3390/cancers15102719.
15. Kortekaas KE, Solleveld-Westerink N, Tessier-Cloutier B, Rutten TA, Poelgeest MIE, Gilks CB, Hoang LN, B.T. Performance of the Pattern-Based Interpretation of P53 Immunohistochemistry as a Surrogate for TP53 Mutations in Vulvar Squamous Cell Carcinoma. *Histopathology* **2020**, *77*, 92–99, doi:10.1111/his.14109.
16. Liu, J.Y.; Li, Y.H.; Zhang, Z.L.; Yao, K.; Ye, Y.L.; Xie, D.; Han, H.; Liu, Z.W.; Qin, Z.K.; Zhou, F.J. The Risk Factors for the Presence of Pelvic Lymph Node Metastasis in Penile Squamous Cell Carcinoma Patients with Inguinal Lymph Node Dissection. *World J Urol* **2013**, *31*, 1519–1524, doi:10.1007/s00345-013-1024-4.
17. Zargar-Shoshtari, K.; Spiess, P.E.; Berglund, A.E.; Sharma, P.; Powsang, J.M.; Giuliano, A.; Magliocco, A.M.; Dhillon, J. Clinical Significance of P53 and P16ink4a Status in a Contemporary North American Penile Carcinoma Cohort. *Clin Genitourin Cancer* **2016**, *14*, 346–351, doi:10.1016/j.clgc.2015.12.019.
18. Brouwer, O.R.; Albersen, M.; Parnham, A.; Protzel, C.; Pettaway, C.A.; Ayres, B.; Antunes-Lopes, T.; Barreto, L.; Campi, R.; Crook, J.; et al. European Association of Urology-American Society of Clinical Oncology Collaborative Guideline on Penile Cancer: 2023 Update. *Eur Urol* **2023**, *83*, 548–560, doi:10.1016/J.EURURO.2023.02.027.
19. Cheng, A.S.; Karnezis, A.N.; Jordan, S.; Singh, N.; McAlpine, J.N.; Gilks, C.B. P16 Immunostaining Allows for Accurate Subclassification of Vulvar Squamous Cell Carcinoma Into HPV-Associated and HPV-Independent Cases. *International Journal of Gynecological Pathology* **2016**, *35*, 385–393, doi:10.1097/PGP.0000000000000263.
20. Santos, M.; Landolfi, S.; Olivella, A.; Lloveras, B.; Klaustermeier, J.; Suárez, H.; Alòs, L.; Puig-Tintoré, L.M.; Campo, E.; Ordi, J. P16 Overexpression Identifies HPV-Positive Vulvar Squamous Cell Carcinomas. *Am J Surg Pathol* **2006**, *30*, 1347–1356.
21. Tessier-Cloutier, B.; Kortekaas, K.E.; Thompson, E.; Pors, J.; Chen, J.; Ho, J.; Prentice, L.M.; McConechy, M.K.; Chow, C.; Proctor, L.; et al. Major P53 Immunohistochemical Patterns in in Situ and Invasive Squamous Cell Carcinomas of the Vulva and Correlation with TP53 Mutation Status. *Modern Pathology* **2020**, *33*, 1595–1605, doi:10.1038/s41379-020-0524-1.
22. Menon, S.; Moch, H.; Berney, D.M.; Cree, I.A.; Srigley, J.R.; Tsuzuki, T.; Compérat, E.; Hartmann, A.; Netto, G.; Rubin, M.A.; et al. WHO 2022 Classification of Penile and Scrotal Cancers: Updates and Evolution. *Histopathology* 2023, *82*, 508–520.
23. Kidd, L.C.; Chaing, S.; Chipollini, J.; Giuliano, A.R.; Spiess, P.E.; Sharma, P. Relationship between Human Papillomavirus and Penile Cancer-Implications for Prevention and Treatment. *Transl Androl Urol* **2017**, *6*, 791–802, doi:10.21037/tau.2017.06.27.
24. Zhou, X.; Wang, X. Radioimmunotherapy in HPV-Associated Head and Neck Squamous Cell Carcinoma. *Biomedicines* **2022**, *10*, doi:10.3390/BIOMEDICINES10081990.
25. Rakislova, N.; Saco, A.; Sierra, A.; Del Pino, M.; Ordi, J. Role of Human Papillomavirus in Vulvar Cancer. *Adv Anat Pathol* **2017**, *24*, 201–214, doi:10.1097/PAP.0000000000000155.

26. Szymonowicz, K.A.; Chen, J. Biological and Clinical Aspects of HPV-Related Cancers. *Cancer Biol Med* **2020**, *17*, 864–878, doi:10.20892/J.ISSN.2095-3941.2020.0370.
27. Visser, O.; Adolfsson, J.; Rossi, S.; Verne, J.; Gatta, G.; Maffezzini, M.; Franks, K.N.; Zielonk, N.; Van Eycken, E.; Sundseth, H.; et al. Incidence and Survival of Rare Urogenital Cancers in Europe. *Eur J Cancer* **2012**, *48*, 456–464, doi:10.1016/J.EJCA.2011.10.031.
28. Manzotti, C.; Chulo, L.; López del Campo, R.; Trias, I.; del Pino, M.; Saúde, O.; Basílio, I.; Tchamo, N.; Lovane, L.; Lorenzoni, C.; et al. Penile Squamous Cell Carcinomas in Sub-Saharan Africa and Europe: Differential Etiopathogenesis. *Cancers (Basel)* **2022**, *14*, doi:10.3390/cancers14215284.
29. Mohanty, S.K.; Mishra, S.K.; Bhardwaj, N.; Sardana, R.; Jaiswal, S.; Pattnaik, N.; Pradhan, D.; Sharma, S.; Kaushal, S.; Baisakh, M.R.; et al. P53 and P16ink4a As Predictive and Prognostic Biomarkers for Nodal Metastasis and Survival in A Contemporary Cohort of Penile Squamous Cell Carcinoma. *Clin Genitourin Cancer* **2021**, *19*, 510–520, doi:10.1016/j.clgc.2021.07.001.
30. Nooij, L.S.; Ter Haar, N.T.; Ruano, D.; Rakislova, N.; van Wezel, T.; Smit, V.T.H.B.M.; Trimbos, B.J.B.M.Z.; Ordi, J.; van Poelgeest, M.I.E.; Bosse, T. Genomic Characterization of Vulvar (Pre)Cancers Identifies Distinct Molecular Subtypes with Prognostic Significance. *Clin Cancer Res* **2017**, *23*, 6781–6789, doi:10.1158/1078-0432.CCR-17-1302.
31. Kortekaas, K.E.; Bastiaannet, E.; van Doorn, H.C.; de Vos van Steenwijk, P.J.; Ewing-Graham, P.C.; Creutzberg, C.L.; Akdeniz, K.; Nooij, L.S.; van der Burg, S.H.; Bosse, T.; et al. Vulvar Cancer Subclassification by HPV and P53 Status Results in Three Clinically Distinct Subtypes. *Gynecol Oncol* **2020**, *159*, 649–656, doi:10.1016/j.ygyno.2020.09.024.
32. Carreras-Dieguez, N.; Saco, A.; del Pino, M.; Marimon, L.; López del Campo, R.; Manzotti, C.; Fusté, P.; Pumarola, C.; Torné, A.; Garcia, A.; et al. Human Papillomavirus and P53 Status Define Three Types of Vulvar Squamous Cell Carcinomas with Distinct Clinical, Pathological, and Prognostic Features. *Histopathology* **2023**, 17–30, doi:10.1111/his.14925.
33. Martins, A.C.P.; Faria, S.M.; Cologna, A.J.; Suaid, H.J.; Tucci, S. Immunoexpression of P53 Protein and Proliferating Cell Nuclear Antigen in Penile Carcinoma. *Journal of Urology* **2002**, *167*, 89–93, doi:10.1016/s0022-5347(05)65389-x.
34. Bowie, J.; Singh, S.; O'hanlon, C.; Shiatis, V.; Brunckhorst, O.; Muneer, A.; Ahmed, K. A Systematic Review of Non-HPV Prognostic Biomarkers Used in Penile Squamous Cell Carcinoma. *Turk J Urol* **2021**, *47*, 358–365, doi:10.5152/TUD.2021.21199.
35. Yanagawa, N.; Osakabe, M.; Hayashi, M.; Tamura, G.; Motoyama, T. Detection of HPV-DNA, P53 Alterations, and Methylation in Penile Squamous Cell Carcinoma in Japanese Men. *Pathol Int* **2008**, *58*, 477–482, doi:10.1111/j.1440-1827.2008.02259.x.
36. Ermakov, M.S.; Kashofer, K.; Regauer, S. Different Mutational Landscapes in Human Papillomavirus–Induced and Human Papillomavirus–Independent Invasive Penile Squamous Cell Cancers. *Modern Pathology* **2023**, *36*, 100250, doi:10.1016/J.MODPAT.2023.100250.
37. Westra, W.H.; Taube, J.M.; Poeta, M.L.; Begum, S.; Sidransky, D.; Koch, W.M. Inverse Relationship between Human Papillomavirus-16 Infection and Disruptive P53 Gene Mutations in Squamous Cell Carcinoma of the Head and Neck. *Clinical Cancer Research* **2008**, *14*, 366–369, doi:10.1158/1078-0432.CCR-07-1402.
38. Zięba, S.; Kowalik, A.; Zalewski, K.; Rusetska, N.; Goryca, K.; Piaścik, A.; Misiek, M.; Bakuła-Zalewska, E.; Kopczyński, J.; Kowalski, K.; et al. Somatic Mutation Profiling of Vulvar Cancer: Exploring Therapeutic Targets. *Gynecol Oncol* **2018**, *150*, 552–561, doi:10.1016/J.YGYNO.2018.06.026.
39. Yang, H.; Almadani, N.; Thompson, E.F.; Tessier-Cloutier, B.; Chen, J.; Ho, J.; Senz, J.; McConechy, M.K.; Chow, C.; Ta, M.; et al. Classification of Vulvar Squamous Cell Carcinoma and Precursor Lesions by P16 and P53 Immunohistochemistry: Considerations, Caveats, and an Algorithmic Approach. *Modern Pathology* **2023**, *36*, 100145, doi:10.1016/j.modpat.2023.100145.
40. Slaton, J.W.; Morgenstern, N.; Levy, D.A.; Santos, M.W.; Tamboli, P.; Ro, J.Y.; Ayala, A.G.; Pettaway, C.A. Tumor Stage, Vascular Invasion and the Percentage of Poorly Differentiated Cancer: Independent Prognosticators for Inguinal Lymph Node Metastasis in Penile Squamous Cancer. *J Urol* **2001**, *165*, 1138–1142, doi:10.1016/S0022-5347(05)66450-6.

5.3. Tercer estudi

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Resum

Objectius: Molts dels CEP independents del VPH es relacionen amb una lesió precursora coneguda com NIP diferenciada, caracteritzada per una atípia limitada a la capa basal amb marcada maduració superficial molt diferent de les NIP basaloides i d'alt grau associades a HPV. Estudis previs en càncer vulvar, que té una etiopatogènia dual similar, han demostrat que al voltant d'una cinquena part de les lesions precursores independents del VPH són morfològicament indistingibles de les lesions precursores associades al VPH. No obstant això, aquestes lesions no han estat descrites en CEP.

Mètodes: Del 2000 al 2021 es van identificar 55 mostres quirúrgiques de CEP. En tots els casos es va realitzar una avaluació morfològica exhaustiva, es va procedir a la detecció de l'ADN del VPH i es va fer tinció immunohistoquímica de p16, p53 i Ki-67. Aquells casos negatius per p16 juntament amb absència de ADN víric van ser classificats com CEP independents de VPH.

Resultats: Trenta-sis dels 55 CEP (65%) van ser independents del VPH. Es va identificar NIP en 26/36 casos (72%). Cinc d'ells (19%) tenien característiques basaloides morfològicament indistingibles de la NIP associada al VPH. L'edat mitjana dels 5 pacients va ser de 74 anys (rang: 67 a 83 anys). Els 5 casos van ser p16 i ADN VPH negatius. Immunohistoquímicament, 3 casos van mostrar un patró anormal de p53, i 2 van mostrar tinció de p53 de tipus nadiu. El carcinoma invasiu associat va ser basaloide en 4 casos i del tipus habitual en 1.

Conclusions: En conclusió, una petita proporció dels CEP independents del VPH mostren lesions intraepitelials adjacents morfològicament idèntiques als descrits com associats al VPH. Aquest patró histològic inusual no s'ha caracteritzat prèviament en detall en el CEP. Tant p16 com p53 son molt valuoses en la caracterització d'aquestes lesions.

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HPV-negative Penile Intraepithelial Neoplasia (PeIN) With Basaloid Features

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Abstract: Most human papillomavirus (HPV)-independent penile squamous cell carcinomas (PSCCs) originate from an intraepithelial precursor called differentiated penile intraepithelial neoplasia, characterized by atypia limited to the basal layer with marked superficial maturation. Previous studies in vulvar cancer, which has a similar dual etiopathogenesis, have shown that about one fifth of HPV-independent precursors are morphologically indistinguishable from high-grade squamous intraepithelial lesions (HSILs), the precursor of HPV-asssociated carcinomas. However, such lesions have not been described in PSCC. From 2000 to 2021, 55 surgical specimens of PSCC were identified. In all cases, thorough morphologic evaluation, HPV DNA detection, and p16, p53, and Ki-67 immunohistochemical (IHC) staining was performed. HPV-independent status was assigned based on both negative results for p16 IHC and HPV DNA. Thirty-six of the 55 PSCC (65%) were HPV-independent. An intraepithelial precursor was identified in 26/36 cases (72%). Five of them (19%) had basaloid features, morphologically indistinguishable from HPV-associated HSIL. The median age of the 5 patients was 74 years (range: 67 to 83 y). All 5 cases were p16 and DNA HPV-negative. Immunohistochemically, 3 cases showed an abnormal p53 pattern, and 2 showed wild-type p53 staining. The associated invasive carcinoma was basaloid in 4 cases and the usual (keratinizing) type in 1. In conclusion, a small proportion of HPV-independent PSCC may arise on adjacent intraepithelial lesions morphologically identical to HPV-associated HSIL. This unusual histologic pattern has not been previously characterized in detail in PSCC. p16 IHC is a valuable tool to identify these lesions and differentiate them from HPV-associated HSIL.

Key Words: penile cancer, differentiated penile intraepithelial neoplasia, p53, p16, HPV

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Penile cancer is a rare malignancy,[1] and penile squamous cell carcinomas (PSCCs) represent >90% of the malignancies of this organ. Classically, PSCC had been subclassified based on its histologic features.[2,3] Growing evidence showing that PSCC may arise following 2 distinct etiopathogenic pathways, one associated with human papillomavirus (HPV) and a second independent of HPV[4] has led to a significant switch in the classification of these tumors, which in the last revision of the World Health Organization (WHO) are primarily subclassified according to their association or not with HPV.[5] Significant epidemiological differences have been identified between these 2 main types of PSCC, with HPV-associated neoplasms usually arising in men with a history of smoking habit and multiple sexual partners,[6] while the HPV-independent tumors are frequently associated with chronic inflammatory conditions, lichen sclerosus, and phimosis.[1] However, unlike other anatomic sites with HPV-associated

and HPV-independent cancers, in which the former have consistently shown to have a better prognosis than the latter,[7] it is still unclear whether this prognostic implication also applies to PSCC.[8,9] Recently, a few studies have shown a trend towards longer disease-free survival in patients with HPV-associated PSCC.[10,11]

Similar to vulvar squamous cell carcinomas,[12] the majority of PSCC develop from premalignant lesions generically named penile intraepithelial neoplasia (PeIN).[13] Remarkably, each etiopathogenic type is associated with a type of PeIN lesion with specific morphologic features. The PeIN lesion on which HPV-associated PSCC develop was previously known as Bowenoid or undifferentiated PeIN, and after the launch in 2012 of the lower anogenital squamous terminology is also referred to as penile high-grade squamous intraepithelial lesion (HSIL).[14] Penile HSILs are etiologically and morphologically identical to the HSIL of the vulva, uterine cervix, or anus and typically show basaloid or warty features, with obvious architectural and cytologic disarray involving the whole thickness of the epithelium.[13] Characteristically, penile HSIL and HPV-associated PSCC show p16 overexpression[15] resulting in "block-type" immunohistochemical (IHC) staining and wild-type pattern of p53 IHC expression. The PeIN lesion on which HPV-independent PSCC originate is called simplex or differentiated penile intraepithelial neoplasia (dPeIN), a lesion that has marked similarities with differentiated vulvar intraepithelial neoplasia (dVIN), the precursor of HPV-independent vulvar squamous cell carcinoma.[16] Both dPeIN and dVIN are characterized by acanthosis, prominent intercellular bridges, large keratinocytes, and atypia limited to the basal layer with marked maturation in the superficial layers of the epithelium.[16,17] Presence of inflammatory conditions, such as lichen sclerosus and lichen simplex chronicus, is also frequently associated with dPeIN.[17] dPeIN is typically negative for p16, and about half of them show abnormal p53 IHC expression,[18] similarly to dVIN.[19]

Our group described in 2009 an unusual morphologic variant of dVIN that mimicked HSIL.[20] Recent studies have shown that this variant accounts for about one fifth of the precursors of HPV-independent vulvar squamous cell carcinomas.[21] In the penis, a few studies have occasionally briefly mentioned PeIN lesions showing mixed differentiated and basaloid or differentiated and warty features.[17,22,23] However, this HSIL-like pattern of dPeIN has not been thoroughly characterized in terms of HPV testing and p16 and p53 IHC staining patterns. In this study, we aimed to describe, in a series of well-characterized HPV-independent PSCC (negative for HPV DNA and p16), the prevalence and the histologic features of dPeIN with HSIL-like morphology, a lesion that can be easily misdiagnosed as HPV-associated penile HSIL.

MATERIALS AND METHODS

Case Selection and Histologic Evaluation of the Invasive Carcinoma

A computer-based search was conducted on the pathology database of the Hospital Clinic of Barcelona to retrieve all patients surgically treated of PSCC from January 1, 2000, through December 31, 2021. Two expert pathologists (I.R.-C. and I.T.) and 1 trainee (J.G.) reviewed all the available slides. Five to 34 slides were available per case for revision (mean: 12 slides). Morphologic evaluation was performed on hematoxylin and eosin glass slides. The histologic subtype of PSCC was established following the WHO 2016 classification.[24] Histologic evaluation was blind to IHC and HPV DNA detection results.

Clinical variables, including treatment and outcome, were collected from the medical records. The TNM staging was performed according to the eighth edition of the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) staging manual.[25] All samples and data were used in accordance with the ethical standards of 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was approved by the Institutional Review Board of the Hospital Clínic of Barcelona (Protocol code HCB.2020.1207).

Histologic Evaluation of the Adjacent Skin

The skin adjacent to the invasive carcinoma was carefully evaluated in search of (1) associated premalignant (PeIN) and (2) inflammatory lesions. One to 15 slides with adjacent skin were available per case for revision (median: 5 slides). Following the criteria of WHO 2016 classification,[24] PeIN were further classified based on pure morphologic criteria as dPeIN (HPV-independent PeIN) and HSIL (HPV-associated PeIN). A distance of at least 1 cm away from the invasive carcinoma was required to categorize a case as PeIN.

The diagnosis of dPeIN (HPV-independent PeIN) was based on the presence of moderate to marked basal atypia, dyskeratosis, and elongated and anastomosing rete ridges, along with preserved maturation of the upper layers of the epithelium.[17] HSIL (HPV-associated PeIN) was diagnosed on the basis of evident cytologic and architectural atypia involving the whole thickness of the epithelium with the absence of maturation and basaloid looking or koilocytic-like (warty) features.

Inflammatory lesions such as lichen simplex chronicus and lichen sclerosus were also recorded if present. Lichen simplex chronicus was diagnosed in the presence of acanthosis, spongiosis, and hyperkeratosis, along with papillary dermal fibrosis and interstitial inflammation with histiocytes and lymphocytes.[26] Lichen sclerosus was identified by a thickened basement membrane, as well as subepidermal edema and dense fibrosis.[26]

Immunohistochemistry

A representative paraffin block was selected from each case for p16 and p53 IHC staining. The selection was based on the presence in the block of invasive PSCC, as well as adjacent skin, including, if present, premalignant and/or inflammatory lesions. The automated BenchMark ULTRA platform (Ventana, Tucson, AZ) was used for the IHC techniques, following the manufacturer's protocol. For each biomarker, the evaluation was performed

separately in the invasive carcinoma and in the adjacent premalignant lesion, the inflammatory lesion (if present), and the normal epithelium.

p16 staining (clone E6H4; Roche) was performed in all cases in the invasive tumor as well as in the adjacent skin lesions (PeIN and inflammatory lesions). Only diffuse and continuous cytoplasmic and nuclear staining in a group of contiguous cells at the basal and parabasal layers (block staining) was considered positive for p16. The absence of staining or patchy staining were considered as a negative result for p16.

Staining for p53 (clone DO-7; Roche) was conducted in all cases in the invasive tumor and in the premalignant and/or inflammatory lesions (if present). The p53 6-pattern framework recently introduced in vulvar squamous cell carcinoma was used to evaluate p53 staining.[27,28] Two staining patterns were classified as "normal," indicative of wild-type protein: (1) scattered and (2) mid-epithelial. The scattered pattern was defined as weak or moderate heterogenous nuclear staining in isolated cells, while the mid-epithelial pattern consisted of strong staining in mid-epithelial cells, with sparing of the basal and lower parabasal cells. Four patterns were considered as "abnormal" indicative of mutated p53 protein: (1) basal overexpression (at least 80% of the cells in the basal layer), (2) parabasal/diffuse overexpression (positive staining in at least 80% of basal cells with extension to cells in the superficial layers), (3) null pattern (complete absence of staining), and (4) cytoplasmic expression (with or without nuclear staining).[21] Normal adjacent skin, stromal, or inflammatory cells served as an inner staining control.

Ki-67 IHC analysis was performed with the monoclonal antibody (clone 30-9; Roche).

HPV Analysis

In all cases, the same block of formalin-fixed, paraffin-embedded tissue used for IHC including both the PSCC and the adjacent skin (with a premalignant and inflammatory lesion, if present), was selected. No microdissection was conducted. DNA extraction was performed on two 10 µm whole tissue sections. Paraffin sections without tissue were cut before and after each carcinoma sample to avoid contamination. The microtome blade was replaced after each case. The DNA was extracted after 1-hour incubation in 20 µL of proteinase K solution (1 mg/mL) at 56°C. Subsequently, proteinase K was heat-inactivated at 95°C for 1 hour. The DNA was isolated using a commercial kit (QIAamp Tissue Kit; Qiagen, Hilden, Germany) according to the manufacturing instructions.

A volume of 10 µL of isolated DNA was used for PCR amplification, using the SPF10-LiPA system (Fujirebio, Gent, Belgium). HPV genotyping was performed using INNO-LiPA HPV Genotyping Extra II kit (Fujirebio). This system allows the amplification and genotyping of high-risk HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68; of the probable high-risk HPV 26, 53, 66, 70, 73, and 82; and of low-risk HPVs 6, 11, 40, 42, 43, 44, 54, 61, 62, 67, 81, 83, and 89. Quality in each run was ensured with both positive and negative controls for DNA isolation, amplification, hybridization, and genotyping.

RESULTS

A total of 55 PSCC were evaluated; 36 (55%) were HPV DNA-negative and p16-negative and thus were classified as HPV-independent PSCC. Among these 36 HPV-independent PSCC, PeIN was identified in the adjacent skin in 27 cases (75%), 5 cases (14%) showed only inflammatory lesions with no atypia (4 lichen simplex chronicus and 1 lichen sclerosus and lichen simplex), while 4 cases (11%) showed normal skin. Twenty-two of 27 PeIN (82%) showed classic differentiated features with marked basal atypia and thus were classified as dPeIN. The remaining 5 of the 27 PeIN lesions (18%) were deemed unusual due to clear and extensive basaloid traits,

TABLE 1. Clinical and Pathologic Characteristics of the 5 Unusual, HSIL-like dPeIN Lesions

			Histologic Subtype					IHC Results				
Case No.	Age (y)	Location of PeIN	PeIN	Invasive Carcinoma	Associated Lesions	Surgery	HPV DNA	p16	Ki-67 (%)	p53 IHC Pattern	Size (Invasive Tumor) (mm)	TNM Stage
1	70	Glans and foreskin	Basaloid	Usual type (keratinizing)								
2	72	Foreskin	Basaloid	Basaloid	—	Partial penectomy	Negative	Negative	20	Mutated-type (null pattern)	23	pT1aN0M0
3	67	Foreskin	Basaloid	Warty-basaloid	—	Shaving	Negative	Negative	20	Mutated-type (basal overexpression)	11	pT1aN0M0
4	76	Glans	Basaloid	Basaloid	LSC	Glandectomy	Negative	Negative	30	Wild-type (scattered staining)	5	pT1aN0M0
5	83	Glans and foreskin	Basaloid	Basaloid	—	Partial penectomy	Negative	Negative	10	Wild-type (mid-epithelial staining)	20	pT1aNoM0

LSC indicates lichen simplex chronicus.

morphologically indistinguishable from HPV-associated PeIN (HSIL). Table 1 outlines the clinical and pathologic characteristics of the 5 unusual, HSIL-like dPeIN lesions. The procedure for the selection of cases for this study is shown in Figure 1.

Clinical Features

The median age at diagnosis of the 5 patients with unusual HSIL-like dPEIN was 74 years (range, 67 to 83 y). Three patients were consulted for phimosis (cases 1, 2, and 4). The average size of the invasive carcinoma was 17 mm (range: 5 to 28 mm). Two lesions (40%) were located in the inner foreskin, 1 (20%) in the glans, and the remaining 2 (40%) compromised both inner foreskin and the glans. At presentation, 4 patients were at clinical stage I and 1 at stage IIIa.

In 2 cases, the limits between dPeIN and invasive carcinoma were clinically distinguishable (cases 2 and 4). In both cases, PeIN was described as a persistent erythematous lesion with superficial ulceration, unifocal (case 2), or multifocal (case 4). Patient 2 had unsuccessfully been treated with imiquimod. In 3 cases (cases 1, 3, and 5), the PeIN lesion had not been identified clinically. In case 3, the entire lesion was described as a giant exophytic lesion reminiscent of giant condyloma, and no distinction between PeIN and invasive carcinoma was made. The lesion had been treated with topical imiquimod without clinical response.

Only 1 of the patients (case 3) had a documented history of cigarette smoking and alcohol consumption. None of the patients referred multiple sex partners. No synchronous or metachronous anogenital tumors were observed in any of the 5 patients.

Histologic Characteristics

Figure 2 shows the typical histologic characteristics of the HSIL-like HPV-independent precursor. All 5 unusual dPeIN displayed evident HSIL-like basaloid morphologic traits. The epidermis was entirely occupied by undifferentiated, basaloid-like keratinocytes with an increased nucleus-to-cytoplasmic ratio. Loss of maturation ("wind-blown" pattern) and moderate to severe cellular atypia were evident throughout the epithelium. Prominent mitoses were identified in all 5 cases (Fig. 2). In 1 of the cases (case 4), the adjacent skin additionally showed the presence of lichen simplex chronicus (case 4). None of the patients had adjacent lichen sclerosus.

In 1 patient (case 1), the adjacent invasive carcinoma of usual keratinizing type, 3 patients (cases 2, 3, and 5) showed basaloid type carcinoma, and 1 (case 4) mixed basaloid-warty carcinoma. Areas of associated dPeIN with typical features (prominent intercellular bridges, large keratinocytes, and abnormal maturation with basal atypia) were identified in case 1.

IHC Findings

The characteristic IHC features of HPV-independent lesions mimicking HSIL are shown in Figure 3. p16 IHC was completely negative in the 5 intraepithelial lesions, as well as in the adjacent invasive PSCC. Three cases showed abnormal p53 IHC staining (suggestive of mutation): 2 of them showed diffuse overexpression (cases 1 and 3), and 1 null pattern (case 2). Two lesions (cases 4 and 5) showed wild-type p53 staining. Ki-67 staining ranged from 5% to 30% of the cells in the HSIL-like lesion. All 5 lesions showed a low index of proliferation, with a mean of 17% (range: 5% to 30%).

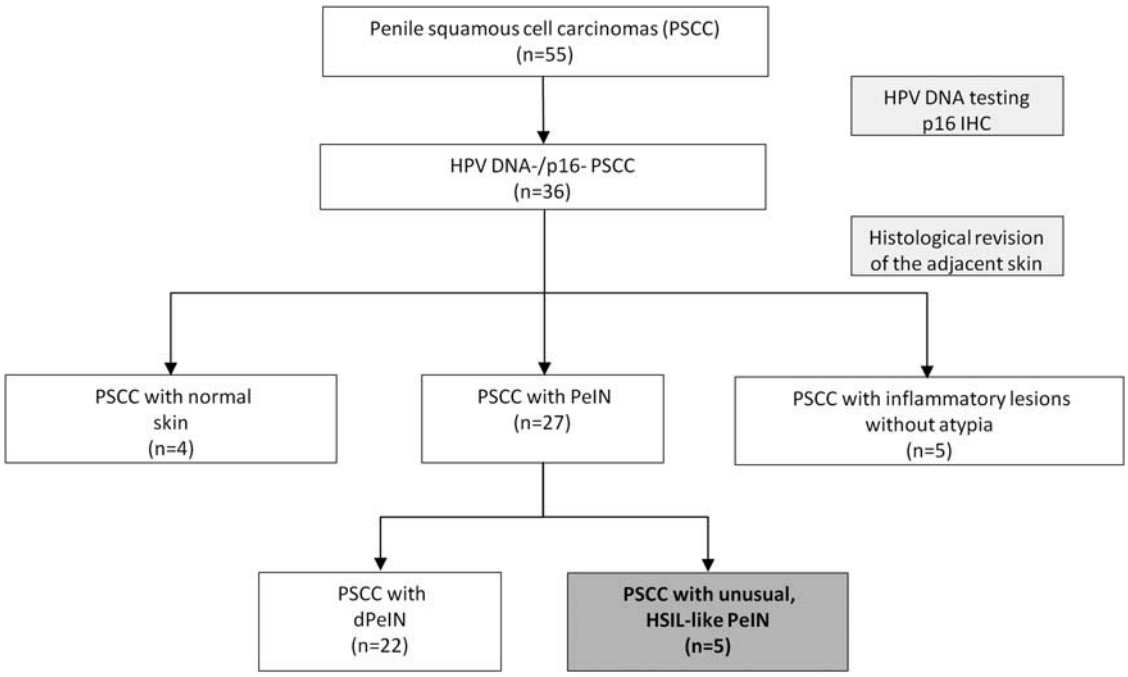

FIGURE 1. Study algorithm.

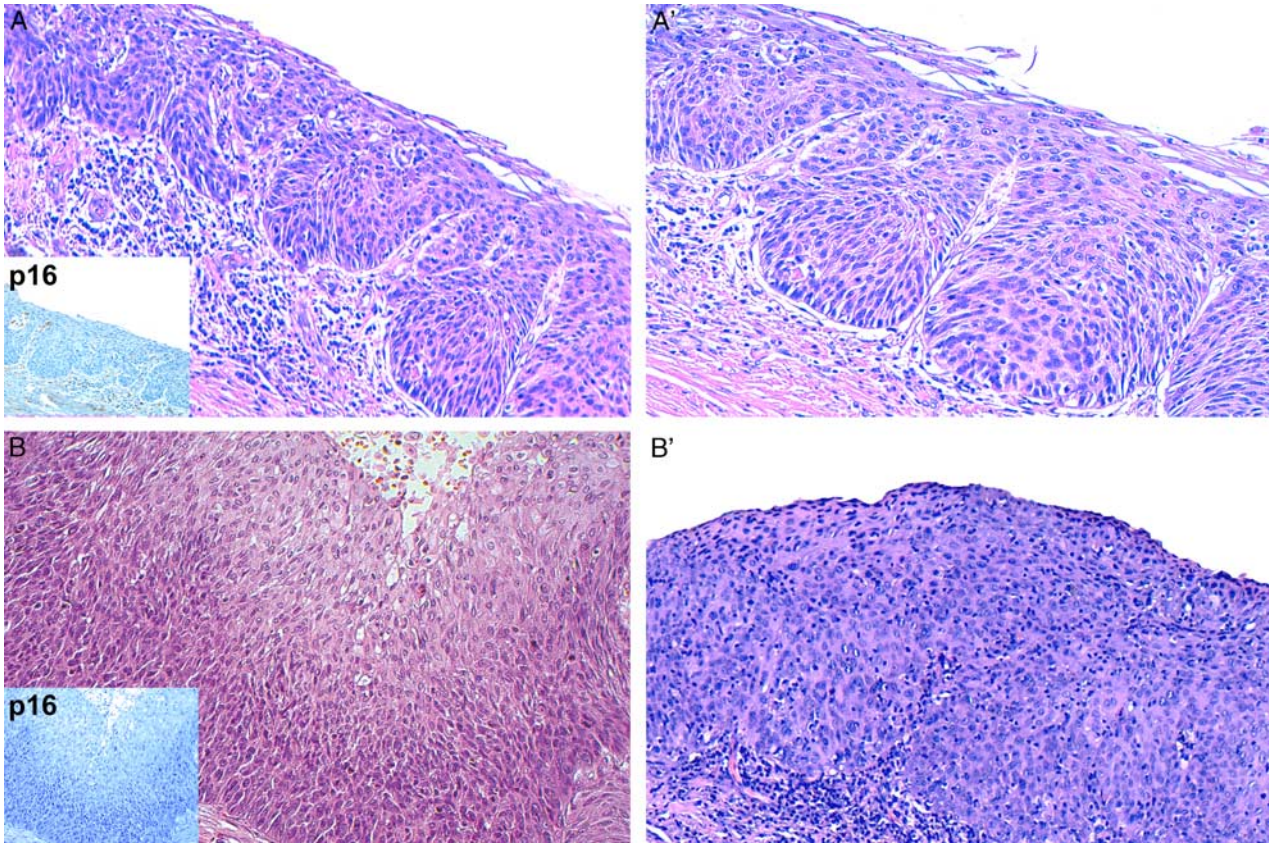

FIGURE 2. Two cases (cases 1 and 3) with penile HPV-independent PeIN simulating HPV-associated PeIN (HSIL). Case 1 (A, A′), The epidermis is markedly disorganized, showing the "wind-blown" appearance, abundant mitotic features and atypical, slightly enlarged keratinocytes. Only minimal maturation is observed in the superficial layer. p16 staining is negative. Case 3 (B, B′), The epidermis is thickened and entirely replaced by small, undifferentiated, basaloid-like keratinocytes disposed in a disorganized manner. Mitotic figures are evident throughout the epithelium. p16 is negative in the lesion.

HPV DNA Testing

All the 5 unusual PeIN as well as their associated invasive carcinoma were negative for both low-risk and high-risk HPV types (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, and 74).

DISCUSSION

In this study, we describe a subset of well-characterized, HPV-independent dPeIN lesions (HPV-negative and p16-negative) with histologic features indistinguishable from typical HPV-associated PeIN (HSIL). All 5 lesions displayed unequivocal traits typical of HPV-associated lesions (HSIL) such as architectural disarray, loss of maturation, and altered nucleus-to-cytoplasmic ratio evident throughout all levels of the epithelium. Negativity for both HPV DNA and p16 in the invasive PSCC as well as in the adjacent PeIN allowed ruling out any role of HPV in the pathogenesis of these tumors. Interestingly, all 5 lesions showed low proliferative index with Ki-67 staining, which is in keeping with the findings reported in HPV-associated precursors throughout the anogenital tract.[29,30] To the best of our knowledge, this unusual histologic pattern of HPV-negative PeIN has not been reported in detail in PSCC, although a few dPeIN lesions with mixed basaloid and differentiated features have occasionally been mentioned in a few reports.[17,22,23]

IHC stains were supportive of an HPV-independent oncogenic pathway involved in these cases. Expression of p16 was consistently negative in all 5 cases, both in the basaloid dPeIN and the associated PSCC. The staining of p53 showed an abnormal (suggestive of mutation) pattern in 3 cases. Considering that *TP53* mutations are far more common in HPV-independent PSCC,[31] the unusual lesions described in this study probably comprise a morphologic spectrum of dPeIN.

A few cases of HPV-associated invasive PSCC with p16-negative IHC result have been previously identified by Chahoud et al.[32] These tumors had nonsense *CDKN2A* mutations, which could explain the p16 IHC-negative result. In our series, the negative result for HPV DNA, in addition to the negative staining for p16, strongly favors the absence of any carcinogenic role of HPV in these cases.

Interestingly, similar HPV-independent lesions with HSIL-like, basaloid pattern have been described.[20,21] Indeed, we described in 2009 for the first time a subset of 4 HPV DNA-negative (and p16-negative) cases of vulvar intraepithelial

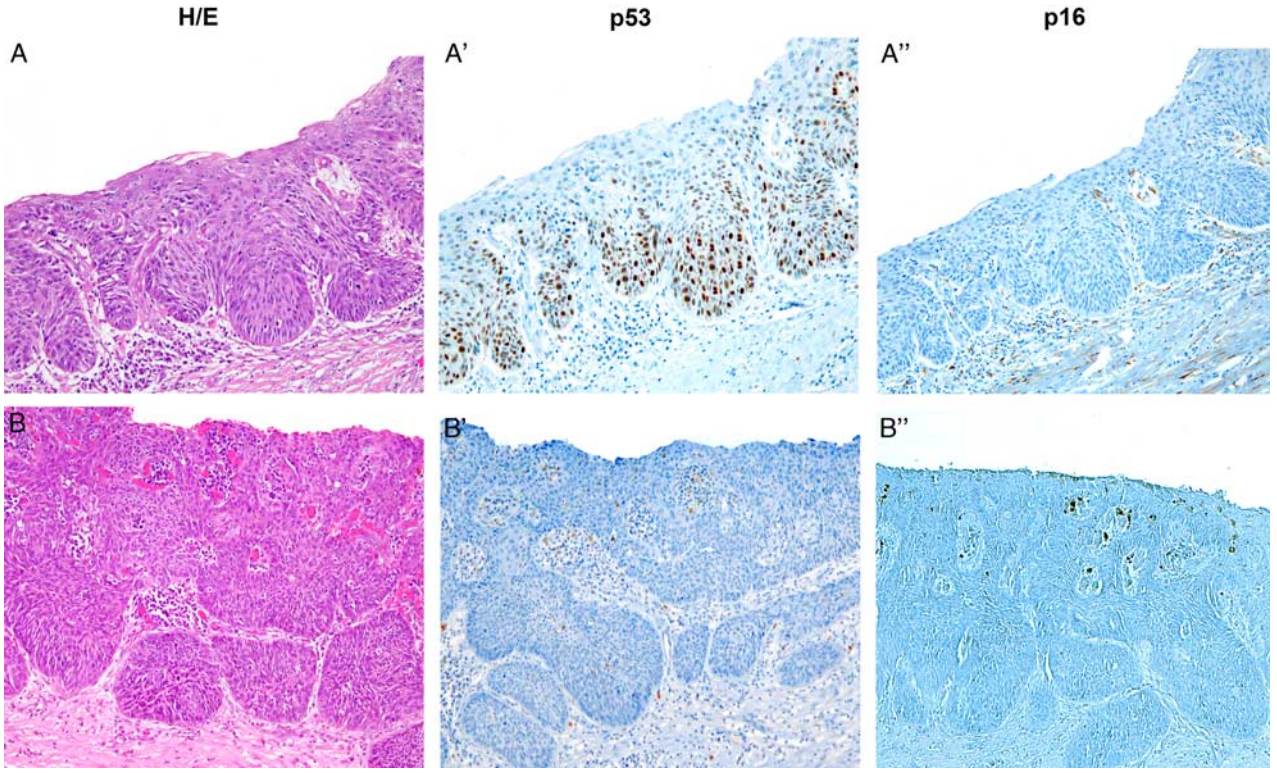

FIGURE 3. Histologic and IHC features of HPV-independent lesions mimicking HSIL. Case 1. A, The epidermis shows marked architectural and cellular atypia. A′, p53 stain showing suprabasal overexpression. A″, Negative p16 stain. Case 2. B, Acanthotic epidermis filled with small, basaloid-like cells. B′, p53 stain showing absence of expression (null pattern), with conserved staining in the inflammatory cells. B″, Negative p16 stain. H/E indicates hematoxylin and eosin.

neoplasia, morphologically indistinguishable from HSIL.[20] In 2020, we further characterized these unusual precursors of vulvar cancer in a larger international multicenter cohort study[21] showing that 6% of all dVIN showed definite basaloid and/or warty features. The basaloid histology of these unusual vulvar lesions was identical to the 5 lesions identified in this series. Remarkably, vulvar squamous carcinoma is a rare neoplasm with dual etiopathogenesis (HPV-associated and HPV-independent) and a highly similar genomic landscape (frequent *TP53*, *CDKN2A*, and *NOTCH-1* mutations).[33,34] Thus, it is plausible that both types of carcinomas also share unusual HPV-negative precursors, mimicking HPV-associated PeIN.

Several clinical characteristics of the 5 cases presented in this study might be considered as additional evidence of a carcinogenic origin independent of HPV. First, most of the patients did not have lifestyle risk factors for HPV-associated neoplasm, such as multiple sex partners, smoking, and alcohol consumption history. Second, the absence of any response to imiquimod, a treatment that has shown to be effective in HPV-associated lesions.[28] Third, the foreskin was compromised in most of the cases, in accordance with the evidence accumulated for dPeIN,[17] and contrarily to HPV-associated HSIL, which mostly affects the glans.[35] Unfortunately, the clinical behavior of these lesions is unknown. However, the appropriate classification of these lesions as HPV-independent might be clinically relevant in similarity with HPV-independent squamous premalignant lesions of the vulva which have proven to be more aggressive in the vulva.[36] Thus, the patients with HSIL-like HPV-independent lesions of the penis will probably have to undergo a stricter follow-up than patients with penile HSIL.

In summary, our study shows that HPV-independent precursors may have a broader morphologic spectrum than originally thought, featuring occasionally a basaloid morphology identical to HPV-associated HSIL/PeIN. Immunostaining for p16 is a reliable tool in differential diagnosis.

REFERENCES

1. Thomas A, Necchi A, Muneer A, et al. Penile cancer. *Nat Rev Dis Primers*. 2021;7:12.
2. Chaux A, Velazquez E, Algaba F. Developments in the pathology of penile squamous cell carcinomas. *Urology*. 2010;76:S7–S14.
3. Chaux A, Reuter V, Lezcano C, et al. Comparison of morphologic features and outcome of resected recurrent and nonrecurrent squamous cell carcinoma of the penis: a study of 81 cases. *Am J Surg Pathol*. 2009;33:1299–1306.
4. Mannweiler S, Sygulla S, Winter E, et al. Two major pathways of penile carcinogenesis: HPV-induced penile cancers overexpress p16ink4a, HPV-negative cancers associated with dermatoses express p53, but lack p16ink4a overexpression. *J Am Acad Dermatol*. 2013;69:73–81.
5. Cubilla AL, Velazquez EF, Amin MB, et al. The World Health Organisation 2016 classification of penile carcinomas: a review and

update from the International Society of Urological Pathology expert-driven recommendations. *Histopathology*. 2018;72:893–904.
6. Vieira CB, Feitoza L, Pinho J, et al. Profile of patients with penile cancer in the region with the highest worldwide incidence. *Sci Rep*. 2020;10:2965.
7. Kühn JP, Schmid W, Körner S, et al. HPV status as prognostic biomarker in head and neck cancer—which method fits the best for outcome prediction? *Cancers (Basel)*. 2021;13:4730.
8. Kidd LC, Chaing S, Chipollini J, et al. Relationship between human papillomavirus and penile cancer-implications for prevention and treatment. *Transl Androl Urol*. 2017;6:791–802.
9. Chipollini J, Pollock G, Hsu C, et al. National trends and survival outcomes of penile squamous cell carcinoma based on human papillomavirus status. *Cancer Med*. 2021;10:7466–7474.
10. Lont AP, Kroon BK, Horenblas S, et al. Presence of high-risk human papillomavirus DNA in penile carcinoma predicts favorable outcome in survival. *Int J Cancer*. 2006;119:1078–1081.
11. Ashley S, Shanks JH, Oliveira P, et al. Human papilloma virus (HPV) status may impact treatment outcomes in patients with pre-cancerous penile lesions (an eUROGEN Study. *Int J Impot Res*. 2021;33:620–626.
12. del Pino M, Rodriguez-Carunchio L, Ordi J. Pathways of vulvar intraepithelial neoplasia and squamous cell carcinoma. *Histopathology*. 2013;62:161–175.
13. Velazquez EF, Chaux A, Cubilla AL. Histologic classification of penile intraepithelial neoplasia. *Semin Diagn Pathol*. 2012;29:96–102.
14. Darragh TM, Colgan TJ, Thomas Cox J, et al. The Lower Anogenital Squamous Terminology Standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. *Int J Gynecol Pathol*. 2013;32:76–115.
15. Cubilla AL, Lloveras B, Alejo M, et al. Value of p16(INK)4 389 (a) in the pathology of invasive penile squamous cell carcinomas: a report of 202 cases. *Am J Surg Pathol*. 2011;35:253–261.
16. Reutter JC, Walters RA, Selim MA. Differentiated vulvar intraepithelial neoplasia : what criteria do we use in practice ? *J Low Genit Tract Dis*. 2016;20:261–266.
17. Chaux A, Velazquez EF, Amin A, et al. Distribution and characterization of subtypes of penile intraepithelial neoplasia and their association with invasive carcinomas: a pathological study of 139 lesions in 121 patients. *Hum Pathol*. 2012;43:1020–1027.
18. Chaux A, Pfannl R, Lloveras B, et al. Distinctive association of p16INK4a overexpression with penile intraepithelial neoplasia depicting warty and/or basaloid features: a study of 141 cases evaluating a new nomenclature. *Am J Surg Pathol*. 2010;34:385–392.
19. Pinto AP, Miron A, Yassin Y, et al. Differentiated vulvar intraepithelial neoplasia contains Tp53 mutations and is genetically linked to vulvar squamous cell carcinoma. *Mod Pathol*. 2010;23:404–412.
20. Ordi J, Alejo M, Fusté V, et al. HPV-negative vulvar intraepithelial neoplasia (VIN) with basaloid histologic pattern: an unrecognized variant of simplex (differentiated) VIN. *Am J Surg Pathol*. 2009;33:1659–1665.
21. Rakislova N, Alemany L, Clavero O, et al. HPV-independent precursors mimicking high-grade squamous intraepithelial lesions (HSIL) of the vulva. *Am J Surg Pathol*. 2020;44:1506–1514.
22. Alvarado-Cabrero I, Sanchez DF, Piedras D, et al. The variable morphological spectrum of penile basaloid carcinomas: differential diagnosis, prognostic factors and outcome report in 27 cases classified as classic and mixed variants. *Appl Cancer Res*. 2017;37:3.
23. Oertell J, Caballero C, Iglesias M, et al. Differentiated precursor lesions and low-grade variants of squamous cell carcinomas are frequent findings in foreskins of patients from a region of high penile cancer incidence. *Histopathology*. 2011;58:925–933.
24. Moch H, Cubilla AL, Humphrey PA, et al. The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs—Part A: Renal, Penile, and Testicular Tumours. *Eur Urol*. 2016;70:93–105.
25. Sanchez DiF, Fernandez-Nestosa MJ, Canete-Portillo S, et al. What is new in the pathologic staging of penile carcinoma in the 8th Edition of AJCC TNM Model: rationale for changes with practical stage-by-stage category diagnostic considerations. *Adv Anat Pathol*. 2021;28:209–227.
26. Chan MP, Zimarowski MJ. Vulvar dermatoses: a histopathologic review and classification of 183 cases. *J Cutan Pathol*. 2015;42:510–518.
27. Kortekaas KE, Solleveld-Westerink N, Tessier-Cloutier B, et al. Performance of the pattern based interpretation of p53 immunohistochemistry as a surrogate for tp53 mutations in vulvar squamous cell carcinoma. *Histopathology*. 2020;77:92–99.
28. Tessier-Cloutier B, Kortekaas KE, Thompson E, et al. Major p53 immunohistochemical patterns in in situ and invasive squamous cell carcinomas of the vulva and correlation with TP53 mutation status. *Mod Pathol*. 2020;33:1595–1605.
29. Simionescu C, Mărgăritescu CL, Stepan A, et al. The utility of p16, E-cadherin and Ki67 in cervical squamous intraepithelial lesions diagnosis. *Rom J Morphol Embryol*. 2010;51:621–626.
30. Walts AE, Lechago J, Bose S. P16 and Ki67 immunostaining is a useful adjunct in the assessment of biopsies for HPV-associated anal intraepithelial neoplasia. *Am J Surg Pathol*. 2006;30:795–801.
31. Kashofer K, Winter E, Halbwedl I, et al. HPV-negative penile squamous cell carcinoma: disruptive mutations in the TP53 gene are common. *Mod Pathol*. 2017;30:1013–1020.
32. Chahoud J, Gleber-Netto FO, McCormick BZ, et al. Whole-exome sequencing in penile squamous cell carcinoma uncovers novel prognostic categorization and drug targets similar to head and neck squamous cell carcinoma. *Clin Cancer Res*. 2021;27:2560–2570.
33. Salama AM, Momeni-Boroujeni A, Vanderbilt C, et al. Molecular landscape of vulvovaginal squamous cell carcinoma: new insights into molecular mechanisms of HPV-associated and HPV-independent squamous cell carcinoma. *Mod Pathol*. 2022;35:274–282.
34. Carreras-Dieguez N, Guerrero J, Rodrigo-Calvo M, et al. Molecular landscape of vulvar squamous cell carcinoma. *Int J Mol Sci*. 2021;22:7069.
35. Gregoire L, Cubilla AL, Reuter VE, et al. Preferential association of human papillomavirus with high-grade histologic variants of penile-invasive squamous cell carcinoma. *J Natl Cancer Inst*. 1995;87:1705–1709.
36. Voss FO, Thuijs NB, Vermeulen RFM, et al. The vulvar cancer risk in differentiated vulvar intraepithelial neoplasia: a systematic review. *Cancers (Basel)*. 2021;13:6170.

6. Discussió

Els estudis que integren aquesta tesi doctoral estan dirigits a relacionar la presencia de la mutació de *TP53* i la seva expressió immunohistoquímica en els CEP classificats en funció de la seva relació amb VPH i la seva aplicació en la rutina assistencial, tant des del punt de vista diagnòstic com pronòstic.

La utilització d'aquest sistema de patrons de tinció immunohistoquímica per p53 afecta tant els casos de CEP com al diagnòstic de les NIP. En les lesions precursores, l'aplicació d'aquest sistema pot ser especialment útil en aquells que es presenten amb morfologies no concordants.

Un dels objectius principal és demostrar que el sistema de patrons de tinció immunohistoquímica de p53 descrits prèviament a vulva, té una bona relació amb l'estat mutacional de *TP53* en els CEP. Alhora s'ha estudiat la distribució d'aquests patrons en funció de la tinció de p16 utilitzada com tinció subrogada de VPH.

Per tal de poder utilitzar la tinció de p53 calia investigar si els patrons descrits es reconeixen també al penis i si es relacionen amb la presència de mutació amb *TP53.* Amb aquest objectiu, s'ha estudiat la correlació entre l'expressió immunohistoquímica de p53 i les mutacions de *TP53* en el CEP, utilitzant per primera vegada el sistema d'avaluació basat en patrons immunohistoquímics de p53 descrit recentment en tumors vulvars i que consisteixen en sis patrons que s'agrupen en dues grans categories: normals i anormals.

La categoria "normal", que és consistent amb la proteïna de tipus nadiu, inclou dos patrons: 1) nuclis positius ocasionals a la capa basal i/o parabasal (patró dispers), i 2) presència de tinció nuclear moderada a forta de p53 IHC a les capes para basals amb absència d'expressió a les cèl·lules basals (patró suprabasal).

La categoria "anormal", que es correlaciona amb la proteïna mutant, inclou quatre patrons de tinció immunohistoquímica de p53: 1) tinció contínua i forta dels nuclis a la capa basal (patró de sobre expressió basal), 2) tinció contínua

i forta dels nuclis a la capa basal amb extensió suprabasal de les cèl·lules positives (patró de sobreexpressió difusa), 3) tinció citoplasmàtica amb o sense expressió nuclear (patró citoplasmàtic), i 4) absència completa de tinció en el tumor, amb evidència de control positiu intrínsec a la pell adjacent, cèl·lules estromals o inflamatòries .(80).

Diversos estudis han demostrat que l'estat mutacional de *TP53* és clínicament rellevant en pacients amb CEP perquè les mutacions s'associen amb un major risc de metàstasi dels ganglis limfàtics i un pitjor pronòstic (25,78,87–89), no obstant això, la seqüenciació de *TP53* és tècnicament difícil d'implementar de manera rutinària. Alguns investigadors han proposat utilitzar la tinció immunohistoquímica de p53 com a substitut de l'estudi molecular de *TP53* (90), amb resultats contradictoris en la correlació entre ambdues tècniques (25,91) Aquesta mala correlació es pot atribuir a diversos factors, però el motiu principal és una manca d'estandardització en l'avaluació de la tinció de p53 que habitualment es basa en el percentatge de nuclis positius a les capes basal i parabasal (90), sense que el llindar de positivitat relacionat amb la presencia de mutació s'hagi establert de manera definitiva. Alguns estudis consideren una tinció anormal una positivitat en almenys el 20% dels nuclis (77) i altres investigadors han utilitzat una combinació d'intensitat i extensió de la positivitat (24). En el nostre estudi, la sensibilitat i la precisió del sistema basat en patrons per detectar la mutació de *TP53* varen ser de 95,5% i 92,5%, respectivament. Aquests resultats son clarament superiors a la sensibilitat i precisió obtingudes utilitzant el criteri clàssic de tinció (positivitat difusa) (9) de 54,5% i 70,0%, respectivament.

La correlació entre la tinció immunohistoquímica de p53 considerada com normal utilitzant el sistema basat en patrons i un *TP53* nadiu va ser excel·lent en el nostre estudi (16/17; 94,1%). Cal destacar que els dos patrons de tinció descrits com a normals en carcinomes vulvars (patró dispers i suprabasal) (80) també es van identificar en la nostra sèrie. El patró suprabasal és de particular interès ja que aquest tipus de tinció reflecteix probablement la senescència de les cèl·lules neoplàsiques infectades pel

VPH d'alt risc, i pot transformar-se en un escull diagnòstic ja que la sobre expressió suprabasal de p53 pot ser mal interpretada com a positiva (92).

La correlació entre els patrons immunohistoquímics de p53 anormals i la presencia de mutació de *TP53* va ser excel·lent (21/23; 91,3%). El patró immunohistoquímic anormal més freqüent va ser la sobreexpressió difusa (61%), l'únic patró considerat prèviament com a anormal en estudis previs sobre CEP (25). També és el patró més freqüent en tumors vulvars (80, 93-94), d'estómac (95) i d'ovari (96), generalment associats a una mutació de *TP53* tipus *missense* que és el tipus de mutació somàtica més freqüent en la nostra sèrie tot i que també hem detectat pèrdues numèriques de *TP53*.

A més, en un terç dels CEP independents del VPH es van identificar dos patrons anormals addicionals (nul i citoplasmàtic) habitualment no reportats en el CEP. Finalment, la sobre expressió basal va ser el patró més infreqüent de la nostra sèrie, observat en un sol tumor. La majoria dels casos amb els patrons anormals més inusuals (nul, citoplasmàtic i basal) es van associar amb presencia de mutació *TP53.*

Per altra banda, el 75% dels CEP independents de VPH i el 8,3% dels associats al VPH de la nostra sèrie presentaven alteracions de *TP53* (p < 0,001) lo qual és similar a les dades reportades prèviament (81,97). Aquestes diferències també es van observar en l'expressió immunohistoquímica de p53, amb un 75% de patrons anormals presents en els CEP independents del VPH i en el 16,6% dels associats al VPH amb un resultat estad´siticament significatiu (p = 0,005).

A més, donat que la valoració per patrons demana que es tinguin en compte tincions inusuals (especialment la citoplasmàtica i la basal) i per tant es poden generar problemes d'interpretació, es va fer també un estudi de reproductibilitat interobservador entre tres observadors. Es va aconseguir un acord moderat en un observador i substancial en els altres dos.

Després de fer una reunió de consens l'acord va millorar de manera substancial arribant a ser gairebé excel·lent (k=0.85), lo qual confirma que l'ús d'aquest sistema és factible i molt útil com marcador subrogat de la presència de mutació de *TP53* en el CEP.

Un cop demostrat que el sistema de patrons descrit a vulva també es reconeix al penis i es relaciona bé amb l'estat mutacional de *TP53,* s'estudia si l'expressió immunohistoquímica de p53 basada en el sistema de patrons té impacte pronòstic en una sèrie de 122 casos de CEP classificats segons la seva relació amb el VPH i amb un seguiment suficient.

Si el sistema funciona i detecta de manera indirecte la presència de mutació els resultats de la tinció immunohistopquímica hauria de tenir un impacte en el pronòstic ja que és coneguda la relació entre mutació de *TP53* i metàstasis ganglionar en els casos de CEP (78). En el nostre estudi es demostra que la presència de patrons anormals de tinció immunohistoquímica de p53 en els casos independents de VPH té un impacte pronòstic important.

Per tal de valorar l'impacte en el pronòstic es van fer estudis immunohistoquímics per p16 i p53 sempre interpretat segons els sistema de patrons, sobre una sèrie de 122 casos de CEP inicials procedents de tres centres de Barcelona (Hospital Clínic, Hospital de la Vall d'Hebron i Fundació Puigvert) amb un seguiment suficient per fer una valoració pronòstica. Tots ells varen ser classificats en tres grups en funció de la seva relació amb el VPH i l'expressió de p53, seguint el model proposat per el carcinoma de vulva i que es resumeixen en: CEP associats al VPH, CEP independents del VPH amb p53 normal, i CEP independents del VPH amb p53 anormals (79).

De manera semblant a d'altres sèries europees (98), els tumors associats al VPH representen un petit percentatge (29,5%) de tots els CEP de la nostra sèrie, essent la majoria (70,5%) CEP independents de VPH.

Pel que fa al pronòstic del grup de CEP associat a VPH, la literatura mostra

resultats controvertits, amb estudis que no reporten diferències en la supervivència entre els casos associats o independents de VPH (99), mentre que altres, igual que passa amb els carcinomes de cap i coll (100) i del tracte genital femení inferior (101), demostren un millor pronòstic (81, 102). En el nostre estudi hem trobat una bona supervivència en aquest grup, inclús en estadis avançats. Un 11% d'aquests pacients (xifra semblant a la reportada en la literatura) (25,81) mostren una expressió anòmala de p53, no obstant cap d'ells ha mort per malaltia. Aquesta dada podria indicar un impacte pronòstic diferent de les alteracions de *TP53* en aquest grup.

En quan als CEP independents del VPH, hem identificat dues categories una amb expressió anòmala de p53 (60%) i l'altre amb expressió normal (40%) Aquest percentatge de resultats anormals per p53 és similar al reportat per altres estudis en CEP independent del VPH (25,81). Entre els dos grups hi ha diferències clíniques i patològiques i un pronòstic diferent. El pronòstic dels pacients amb CEP independent del VPH amb expressió anòmala de p53 és pobre, amb un 27% de mortalitat a 5 anys lo qual hem confirmat mitjançant una anàlisi multivariant. En canvi els pacients amb CEP independents de HPV i amb expressió normal de p53 tenen una taxa molt baixa de metàstasi ganglionars. Una de les dades més rellevants és que rarament es diagnostiquen en estadis III o IV.

Sorprenentment, tot i que aquests pacients tenen taxes de recurrència similars en comparació amb els dels altres dos grups, la seva supervivència és excel·lent, sense morts relacionades amb el tumor. Aquesta categoria de CEP independents del VPH amb expressió immunohistoquímica de p53 normal, s'ha descrit prèviament a la vulva (79,80), on es mostra un comportament similar, amb recidives freqüents però amb supervivències extremadament bones (103).

El nostre estudi confirma que la interpretació immunohistoquímica de p53 utilitzant el sistema basat en patrons (sobre expressió difusa, nul, citoplasmàtica i basal) millora significativament l'avaluació convencional

(104,105) i reconeix patrons addicionals generalment no observats en publicacions prèvies (106).

Aquesta manera de classificar els CEP en funció del VPH i expressió de p53, mostra un gran impacte en les dades de supervivència específica de malaltia en l'anàlisi multivariant en la nostre sèrie, superant a l'estadificació, el que suggereix que en tots els pacients amb CEP s'hauria d'avaluar la p53 de manera concomitant a la p16 per el VPH.

En la nostra experiència 7/14 morts en el grup de CEP independent del VPH amb expressió anormal de p53, mostraven algun dels patrons més infreqüents lo qual pot explicar les diferències observades en la literatura sobre l'impacte pronòstic de les tincions immunohistoquímiques de p53 (91).

En el cas de les lesions precursores, que també es valoren en funció de la seva relació amb el VPH, habitualment presenten característiques similars al component invasor al qual sovint acompanyen. No obstant ocasionalment les NIP es presenten aïllades, sense neoplàsia infiltrant associada. Tal i com s'ha esmentat previament, les NIP associades a VPH son morfològicament diferents de les VPH independents(10). Normalment les formes associades a VPH presenten un patró basaloide, d'aspecte més indiferenciat i son relativament més fàcils de identificar que les independents del VPH (10), no obstant, de manera ocasional, aquesta morfologia no es relaciona amb la presència de VPH, fenòmen que anomenem morfologia no concordant (20, 107). Es en aquesta circumstància on la correcta interpretació de les tincions immunohistoquímiques per p53 té també una vessant pràctica i molt útil. Efectivament, detectar la presència de p53 mutada en el els NIP morfològicament no concordants, evita errors de classificació, lo qual millora la precisió diagnòstica.

En aquest cas l'estudi es va centrar en un subconjunt de NIP ben caracteritzades i independents del VPH, amb característiques histològiques indistingibles de la NIP associada al VPH. En una revisió de 55 CEP dels

quals 36 eren VPH independents, es van identificar un total de 5 casos amb els trets morfològics inequívocs descrits en les lesions associades al VPH, i totalment negatives per a l'ADN del VPH i per a p16 tant en la NIP com en el CEP adjacent. A més, les característiques clíniques dels 5 casos no presentaven conductes de risc per infecció de VPH i la localització de totes les lesions va ser en el prepuci tal i com s'espera en les NIP diferenciades independents de VPH.

Aquest patró histològic inusual de NIP independent del VPH no ha estat reportat en detall en el CEP, tot i que s'han publicat ocasionals casos amb característiques mixtes de NIP basaloide i diferenciat (20,107).

La tinció de p53 va mostrar un patró anormal (suggestiu de mutació) en 3 casos. Tenint en compte que les mutacions de *TP53* són molt més freqüents en el CEP independent del VPH les lesions inusuals descrites en aquest estudi probablement formen part d'un espectre morfològic de NIP diferenciat poc reconegut.

L'any 2009 el nostre grup va descriure per primera vegada un subconjunt de 4 casos de lesions precursores vulvars negatives tant per ADN del VPH com per p16, que eren morfològicament indistingibles de les lesions precursores d'alt grau relacionades amb VPH (29). El 2020, es varen caracteritzar encara més aquests precursors inusuals del càncer vulvar en un estudi de cohort multi cèntric internacional ampliat que va mostrar que el 6% de tots els NIVd mostraven característiques basaloides i / o berrugoses (28). La histologia basaloide d'aquestes lesions vulvars inusuals va ser idèntica a les 5 lesions identificades en aquesta sèrie de lesions penianes, suggerint que la similitud observada entre les lesions neoplàsiques de vulva i de penis es troben també en les seves lesions precursores.

Tant el CEP com el carcinoma escamós vulvar comparteixen una etiopatogènia dual (associada al VPH i independent del VPH) i un paisatge genòmic molt similar (mutacions freqüents de *TP53, CDKN2A* i *NOTCH-1*)

(108,109). Per tant, és plausible que ambdós tipus de carcinomes també comparteixin les mateixes variacions en les lesions precursores incloent les formes inusuals. Això afecta especialment als casos de NIP independents del VPH que es presenten amb una morfologia similar al NIP basaloide associat al VPH.

A vulva aquest tipus de lesions ha demostrat ser més agressiu (110) per tant, és molt recomanable fer una classificació adequada d'aquestes lesions ja que també al penis podrien ser clínicament rellevants, lo qual pot implicar el disseny d'un seguiment i maneig diferent si realment els NIP amb morfologia basaloide independents de VPH, tenen un comportament més agressiu. Una de les variants morfològiques de CEP més agressiva és la basaloide (33) que amb freqüencia s'associa a NIP adjacents també basaloides i acostuma a estar relacionada amb el VPH. A més, els NIP associats a VPH tenen un índex de progressió a neoplàsia infiltrant més alta que els NIP independents del VPH (10). És doncs raonable, estudiar si els NIP de patró basaloide independents de VPH (morfologia discordant) arrosseguen també el mateix comportament agressiu que els seus homòlegs associats a VPH (12,19). Malauradament, fins ara, no s'ha estudiat en profunditat el comportament clínic dels NIP amb morfologia discordant ja que probablement la majoria dels NIP basaloides s'han considerat com associats a VPH. Tampoc s'ha pogut estudiar en profunditat si la presència de mutacions de *TP53* en aquestes lesions té algun impacte en el pronòstic, tot i que 3 dels nostres 5 casos mostraven patrons immunohistoquímics de p53 mutats. Tenint en compte que els CEP infiltrants independents de VPH amb patró immunohistoquímic de p53 mutat tenen pitjor pronòstic, és possible que això s'extengui també a la lesió precursora.

En resum, aquest estudi mostra que les lesions precursores independents del VPH poden tenir un espectre morfològic més ampli del que es pensava originalment, presentant ocasionalment una morfologia basaloide idèntica a la NIP associada al VPH. Aquesta morfologia discordant pot generar equivocacions diagnòstiques si el patòleg desconeix la seva existència. La

valoració de la tinció conjunta de p16 i p53 pot ser de molta ajuda en el diagnòstic especialment en els casos de NIP aïllats.

En conjunt l'estudi d'aquesta tesi confirma que la interpretació de les tincions immunohistoquímiques amb el sistema de patrons descrita en els carcinomes de vulva (80), s'identifiquen també en els CEP. A més s'ha demostrat que el sistema és reproduïble, d'aplicació fàcil en la rutina diària i assequible a la gran majora de laboratoris d'anatomia patològica. Tot i que els sis patrons estan molt ben definits cal remarcar que dels dos patrons considerats normals, n'hi ha un (tinció suprabasal) que pot ser confós amb una tinció positiva si no es coneix la seva existència. En el cas dels patrons mutats també n'hi ha un (tinció basal) que pot passar desapercebut i ser interpretat com patró normal. Afortunadament, igual com passa a vulva, aquests dos patrons son el més infreqüents en la nostra sèrie (93).

La principal fortalesa de la tesi consisteix en haver pogut demostrar una bona correlació entre els patrons de tinció immunohistoquímic de p53 i la presència de mutacions de *TP53* lo qual és especialment útil ja que ens permet utilitzar aquest sistema de valoració com marcador subrogat de la presència o absència de mutació de *TP53,* tant en els CEP com en les seves lesions precursores.

El seu ús com factor pronòstic s'ha revelat amb probable impacte clínic en el grup dels CEP independents del VPH on crida l'atenció el bon pronòstic dels casos de CEP independents del virus i que no s'associen a alteracions en la tinció de p53. També hem trobat rellevant la seva aplicació en el diagnòstic de NIP on la precisió diagnòstica millora substancialment.

En quan a les limitacions, cal esmentar que l'escassa representació de pacients amb CEP associats a VPH fa que sigui difícil arribar a conclusions concretes en aquest subgrup de pacients. Les dades indiquen que la presència de mutació de *TP53* és molt infreqüent en els casos de CEP associats a VPH, no obstant en totes les sèries (també en la nostra) hi ha un

petit grup de malalts que son portadors de mutació (52). En l'estudi actual la presència de mutació no ha suposat cap impacte en la supervivència d'aquests pacients. Això pot estar indicant que l'impacte de la mutació de *TP53* en els casos associats a VPH no té el mateix valor perquè els mecanismes oncogènics dels dos tipus de CEP (associat i independent a VPH) son també diferents (12). Malgrat tot cal tenir en compte que l'escassa representació d'aquest subgrup de pacients (malalts amb CEP associats a VPH i amb patró immunohistoquímic de p53 mutat) no ens permet arribar a conclusions en aquest sentit.

Arribats a aquets punt seria recomanable un estudi prospectiu d'una sèrie exhaustiva de malalts (en la que s'incloguin més casos de CEP associats a VPH), amb valoració conjunta de les dues tincions immunohistoquímiques (p16 i p53) i utilitzant el sistema de patrons com mètode subrogat de detecció de la mutació de *TP53*. Això permetria confirmar l'impacte pronòstic de les mutacions de *TP53* i valorar si cal fer modificacions en el maneig dels malalts en funció de la presència o no de *TP53,* sempre tenint en compte la relació amb VPH ja que l'impacte de la presència de patrons immunohistoquímics alterats de p53 sembla no ser el mateix en els CEP associats a VPH i en les CEP independents de VPH.

Finalment en el cas dels NIP amb morfologia no concordant és recomanable un estudi exhaustiu que inclogui els patrons immunohistoquímics de p53 i p16 per classificar-los amb precisió i determinar si els casos de morfologia discordant amb patró basaloide independents del VPH tenen comportaments semblants als dels seus homòlegs associats a VPH.

7. Conclusions

1. L'avaluació per patrons de tinció immunohistoquímica de p53, descrits prèviament en el carcinoma de vulva, amb dos patrons considerats com a normals (cèl·lules aïllades i suprabasals) i quatre patrons considerats com a anormals (sobreexpressió difusa, sobreexpressió basal, patró citoplasmàtic i nul) pot ser aplicat també al càncer de penis, on tots sis patrons han estat identificats.

2. La concordança entre l'expressió immunohistoquímica de p53 avaluada seguint el sistema de patrons descrit a la vulva i la mutació del gen TP53 és excel·lent (valor de l'estadístic kappa de 0.85, casi perfecte).

3. L'avaluació immunohistoquímica per patrons demostra una millor correlació amb la mutació del gen que el mètode d'avaluació clàssic , amb uns valors de sensibilitat i especificitat per al diagnòstic de mutació per sobre del 90%, molt superiors al 54% i 70%, respectivament, del mètode clàssic.

4. El càncer associat a la infecció per virus del papil·loma humà (VPH) representa a la nostra àrea geogràfica només una tercera part dels càncers de penis, mentre que les dues terceres parts restants corresponen a càncers VPH-independents. Dintre d'aquest segon grup, la majoria dels tumors presenten mutació de TP53.

5. L'estudi immunohistoquímic per a p53 permet definir dues categories pronòstiques dins del grup de carcinomes de penis VPH-independents: els carcinomes VPH-independents amb p53 normal amb baixa agressivitat i els carcinomes VPH-independents amb p53 anormal, altament malignes. Per contra, p53 sembla no tenir implicacions en els pacients amb tumors associats a VPH ..

6. La mortalitat associada a carcinoma de penis es va associar fonamentalment amb els càncers VPH-independents amb mutació de TP53. Aquests tumors

7. van ser responsables de més del 80% de les morts i van demostrar una clara relació la mortalitat en l'anàlisi multivariada, essent l'únic paràmetre independent, a més de l'estadi, amb una correlació amb el pronòstic.

8. La meitat de les morts observades en el grup de pacients amb tumors VPH- independents amb mutació de TP53, es van produir en tumors amb patrons anormals d'expressió de p53 no reconeguts en els sistemes d'avaluació clàssics, posant de manifest la rellevància d'utilitzar l'avaluació per patrons en la valoració d'aquest marcador ..

9. Aproximadament una cinquena part de les lesions precursores intraepitelials independents del VPH de penis presenten característiques morfològiques indistingibles de les lesions precursores associades a VPH. El diagnòstic d'aquestes lesions, no descrites prèviament, és impossible amb criteris purament morfològics i requereix l'ús de tincions immunohistoquímiques per a p16 o p53 i/o la negativitat de les tècniques moleculars de detecció del VPH.

10. La tinció immunohistoquímica per a p16 i p53 s'hauria d'introduir de manera rutinària tant en el diagnòstic dels carcinomes escamosos de penis com en les lesions precursores del càncer de penis, ja que permet classificar-los en diferents grups pronòstics en el primer cas i identificar lesions amb característiques histològiques paradoxals en el segon.

8. Bibliografia

1. Thomas A, Necchi A, Muneer A, Tobias-Machado M, Tran ATH, Van Rompuy A-S, et al. Penile cancer. Nat Rev Dis Prim 2021 11;7(1):11-35.

2. Douglawi A, Masterson TA. Penile cancer epidemiology and risk factors: a contemporary review. Curr Opin Urol 2019;29(2):145-149.

3. Prigge ES, von Knebel Doeberitz M, Reuschenbach M. Clinical relevance and implications of HPV-induced neoplasia in different anatomical locations. Mutat Res Rev Mutat Res. 2017;772:51–66.

4. Fu L, Tian T, Yao K, Chen XF, Luo G, Gao Y, Lin YF,et al. Global Pattern and Trends in Penile Cancer Incidence: Population-Based Study. JMIR Public Heal Surveill. 2022;8(7):e34874.

5. Arya, M.; Li, R.; Pegler, K.; Sangar, V.; Kelly, J.D.; Minhas, S.; Muneer, A.; et al. Long-term trends in incidence, survival and mortality of primary penile cancer in England. Cancer Causes Control. 2013;24(12):2169–76.

6. Borque-Fernando Á, Gaya JM, Esteban-Escaño LM, Gómez-Rivas J, García-Baquero R, Agreda-Castañeda F, et al. Epidemiology, Diagnosis and Management of Penile Cancer: Results from the Spanish National Registry of Penile Cancer. Cancers (Basel). 2023;15(3):616.

7. Olesen TB, Sand FL, Rasmussen CL, Albieri V, Toft BG, Norrild B, et al. Prevalence of human papillomavirus DNA and p16INK4a in penile cancer and penile intraepithelial neoplasia: a systematic review and meta-analysis. Lancet Oncol. 2019;20(1):145–58.

8. Alemany L, Cubilla A, Halec G, Kasamatsu E, Quiros B, Masferrer E, et al. Role of Human Papillomavirus in Penile Carcinomas Worldwide. Eur Urol. 2016;69(5):953–61.

9. Manzotti C, Chulo L, López Del Campo R, Trias I, Del Pino M, Saúde O, et al. Penile Squamous Cell Carcinomas in Sub-Saharan Africa and Europe: Differential Etiopathogenesis. Cancers (Basel). 2022;14(21):5284.

10. Amin MB, Berney DM, Comperat, EM, Hartmann, A, Menon S, Netto GJ,et al. Who Classification of Tumours 5th Edition. Urinary and Male Genital Tumours. International Agency for Research on Cancer. WHO editor. Lyon: IARC publications; 2022. 361p.

11. Watanabe R, Lokuhetty D, White V. Who Classification of T umours 5th Edition. Female Genital Tumours. International Agency for Research on Cancer. WHO editor. Lyon: IARC publications; 2020. 632 p.

12. Emmanuel A, Nettleton J, Watkin N, Berney DM. The molecular pathogenesis of penile carcinoma—current developments and understanding. Virchows Arch. 2019;475(4):397–405.

13. Van Poppel H, Watkin NA, Osanto S, Moonen L, Horwich A, Kataja V. Penile cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2013;24(Suppl 6):vi115-124..

14. Brouwer OR, Rumble RB, Ayres B, Sánchez Martínez DF, Oliveira P, Spiess PE, et al. Penile Cancer: EAU-ASCO Collaborative Guidelines Update Q and A. JCO Oncol Pract. 2024;20(1):33-37

15. Menon S, Moch H, Berney DM, Cree IA, Srigley JR, Tsuzuki T, et al. WHO 2022 classification of penile and scrotal cancers: updates and evolution. Histopathology. 2023;82(4):508–20.

16. Darragh TM, Colgan TJ, Thomas Cox J, Heller DS, Henry MR, Luff RD, et al. The Lower Anogenital Squamous Terminology Standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. Int J Gynecol Pathol. 2013;32(1):76–115

17. Moguelet P, Compérat EM. Penile intraepithelial neoplasia. Ann Pathol. 2022;42(1):15–9.

18. Spiess PE, Dhillon J, Baumgarten AS, Johnstone PA, Giuliano AR. Pathophysiological basis of human papillomavirus in penile cancer: Key to prevention and delivery of more effective therapies. CA Cancer J Clin. 2016;66(6):481–95.

19. Hoekstra RJ, Trip EJ, Ten Kate FJ, Horenblas S, Lock MT. Penile intraepithelial neoplasia: Nomenclature, incidence and progression to malignancy in the Netherlands. Int J Urol. 2019;26(3):353–7.

20. Chaux A, Velazquez EF, Amin A, Soskin A, Pfannl R, Rodríguez IM, et al.

Distribution and characterization of subtypes of penile intraepithelial neoplasia and their association with invasive carcinomas: a pathological study of 139 lesions in 121 patients. Hum Pathol. 2012;43(7):1020–7.

21. Barbagli G, Palminteri E, Mirri F, Guazzoni G, Turini D, Lazzeri M. Penile carcinoma in patients with genital lichen sclerosus: a multicenter survey. J Urol. 2006;175(4):1359–63.

22. Canete-Portillo S, Velazquez EF, Kristiansen G, Egevad L, Grignon D, Chaux A, et al. Consultation Conference on Molecular Pathology of Urogenital Cancers V: Recommendations on the Use of Immunohistochemical and Molecular Biomarkers in Penile Cancer. Am J Surg Pathol. 2020;44(7):e80–6.

23. Canete-Portillo S, Sanchez DF, Cubilla AL. Pathology of Invasive and Intraepithelial Penile Neoplasia. Eur Urol Focus. 2019;5(5):713–7.

24. Zargar-Shoshtari K, Spiess PE, Berglund AE, Sharma P, Powsang JM, Giuliano A, et al. Clinical Significance of p53 and p16(ink4a) Status in a Contemporary North American Penile Carcinoma Cohort. Clin Genitourin Cancer. 2016;14:346–51.

25. Kashofer K, Winter E, Halbwedl I, Thueringer A, Kreiner M, Sauer S, et al. HPV-negative penile squamous cell carcinoma: Disruptive mutations in the TP53 gene are common. Mod Pathol. 2017;30(7):1013–20.

26. Hatano Y. The pathology according to p53 pathway. Pathobiology. 2023;14:1–14.

27. Guerrero J, Trias I, Veloza L, Del Pino M, Garcia A, Marimon L,et al. HPV-negative Penile Intraepithelial Neoplasia (PeIN) With Basaloid Features. Am J Surg Pathol. 2022;46(8):1071–7.

28. Rakislova N, Alemany L, Clavero O, Del Pino M, Saco A, Marimon L, et al. HPV-independent Precursors Mimicking High-grade Squamous Intraepithelial Lesions (HSIL) of the Vulva. Am J Surg Pathol. 2020;44(11):1506–14.

29. Ordi J, Alejo M, Fusté V, Lloveras B, Del Pino M, Alonso I, et al. HPV-negative vulvar intraepithelial neoplasia (VIN) with basaloid histologic

pattern: an unrecognized variant of simplex (differentiated) VIN. Am J Surg Pathol. 2009;33(11):1659–65.

30. Cubilla A.L, Amin M.B., Ayala A. , Ayala G., Chaux A., Corbishley C., et al Who Classification of Tumours 4th Edition. Tumours of the Urinary System and Male Genital Organs. International Agency for Research on Cancer. WHO editor. Lyon: IARC publications 2016. 279p.

31. Rosendo-Chalma P, Antonio-Véjar V, Ortiz Tejedor JG, Ortiz Segarra J, Vega Crespo B, Bigoni-Ordóñez GD. The Hallmarks of Cervical Cancer: Molecular Mechanisms Induced by Human Papillomavirus. Biology (Basel). 2024;13(2):77.

32. Chaux A, Sanchez DF, Fernández-Nestosa MJ, Cañete-Portillo S, Rodríguez IM, Giannico GA,et al. The dual pathogenesis of penile neoplasia: The heterogeneous morphology of human papillomavirus-related tumors. Asian J Urol. 2022;9(4):349–58.

33. Cubilla AL, Reuter VE, Gregoire L, Ayala G, Ocampos S, Lancaster WD, et al. Basaloid squamous cell carcinoma: a distinctive human papilloma virus-related penile neoplasm: a report of 20 cases. Am J Surg Pathol. 1998;22(6):755–61.

34. Cubilla AL, Lloveras B, Alemany L, Alejo M, Vidal A, Kasamatsu E, et al. Basaloid squamous cell carcinoma of the penis with papillary features: a clinicopathologic study of 12 cases. Am J Surg Pathol. 2012;36(6):869–75.

35. Chaux A, Tamboli P, Ayala A, Soares F, Rodríguez I, Barreto J. et al. Warty-basaloid carcinoma: clinicopathological features of a distinctive penile neoplasm. Report of 45 cases. Mod Pathol. 2010;23(6):896–904.

36. Liegl B, Regauer S. Penile clear cell carcinoma: a report of 5 cases of a distinct entity. Am J Surg Pathol. 2004;28(11):1513–7.

37. Mentrikoski MJ, Frierson HF Jr, Stelow EB, Cathro H. Lymphoepithelioma-like carcinoma of the penis: association with human papilloma virus infection. Histopathology. 2014;64(2):312–5.

38. Guimarães GC, Cunha IW, Soares FA, Lopes A, Torres J, Chaux A, et al.

Penile squamous cell carcinoma clinicopathological features, nodal metastasis and outcome in 333 cases. J Urol. 2009;182(2):528–34.

39. Kayes O, Ahmed HU, Arya M, Minhas S. Molecular and genetic pathways in penile cancer. Lancet Oncol. 2007; 8(5):420–9.

40. Persson B, Sjödin JG, Holmberg L, Windhal TS. Committee of the National Penile Cancer Register in Sweden. The National Penile Cancer Register in Sweden 2000-2003. Scand J Urol Nephrol. 2007;41(4):278– 82.

41. Moch H, Amin MB, Berney DM, Compérat EM, Gill AJ, Hartmann A, et al. The 2022 World Health Organization Classification of Tumours of the Urinary System and Male Genital Organs-Part A: Renal, Penile, and Testicular Tumours. Eur Urol. 2022;82(5):458–68.

42. Ackerman LV. Verrucous carcinoma of the oral cavity. Surgery. 1948;23(4):670–8.

43. Chaux A, Soares F, Rodríguez I, Barreto J, Lezcano C, Torres J, et al. Papillary squamous cell carcinoma, not otherwise specified (NOS) of the penis: clinicopathologic features, differential diagnosis, and outcome of 35 case. Am J Surg Pathol. 2010;34(2):223–30.

44. Velazquez EF, Melamed J, Barreto JE, Aguero F, Cubilla AL. Sarcomatoid carcinoma of the penis: a clinicopathologic study of 15 cases. Am J Surg Pathol. 2005; 29(9):1152–8.

45. Yugawa, T. Kiyono T. Molecular mechanisms of cervical carcinogènesis by high- risk human papillomaviruses: novel functions of E6 and E7 oncoproteins. Rev Med Virol. 2009;19(97–113).

46. Boyer SN, Wazer DE, Band V. E7 protein of human papil loma virus-16 induces degradation of retinoblastoma protein through the ubiquitin-proteasome pathway. Cancer Res. 1996;56(20):4620–4.

47. Stankiewicz E, Prowse DM, Ktori E, Cuzick J AL, Zhang X, Kudahetti S, et al. The retinoblastoma protein/p16 INK4A pathway but not p53 is disrupted by human papillomavirus in penile squamous cell carcinoma. Histopathology. 2011;58(3):433–9.

48. Ferreux E, Lont AP, Horenblas S, Gallee MP, Raaphorst FM, von Knebel

et al. Evidence for at least three alternative mechanisms targeting the p16INK4A/cyclin D/Rb pathway in penile carcinoma, one of which is mediated by high-risk human papillomavirus. J Pathol. 2003;201(1):109–18.

49. Ferrándiz-Pulido C, Masferrer E, de Torres I, Lloveras B, Hernandez-Losa J, Mojal S, et al. Identification and genotyping of human papillomavirus in a Spanish cohort of penile squamous cell carcinomas: correlation with pathologic subtypes, p16(INK4a) expression, and prognosis. J Am Acad Dermatol. 2013;68(1):73–82.

50. Gewin L, Myers H, KiyonoT, Galloway DA. Identification of a novel telomerase repressor that interacts with the human papillomavirus type-16 E6/ E6-AP complex. Genes Dev. 2004;18(2269–2282).

51. Bleeker MC, Heideman DA, Snijders PJ, Horenblas, S DillnerJ, Meijer CJLM. Penile cancer: epidemiology, pathogenesis and prevention. World J Urol. 2009;27(2):141-50.

52. Ermakov MS, Kashofer K, Regauer S. Different Mutational Landscapes in Human Papillomavirus-Induced and Human Papillomavirus-Independent Invasive Penile Squamous Cell Cancers. Mod Pathol. 2023;36(10):100250.

53. De Paula AA, Motta ED, Alencar Rde C, Saddi VA, da Silva RC, Caixeta GN, et al. The impact of cyclooxygenase-2 and vascular endothelial growth factor C immunoexpression on the prognosis of penile carcinoma. J Urol. 2012;187(1):134–40.

54. Afonso LA, Carestiato FN, Ornellas AA, Ornellas P, Rocha WM, Cordeiro TI, et al. Human papillomavirus, Epstein-Barr virus, and methylation status of p16ink4a in penile cancer. J Med Virol. 2017;89(10):1837–43.

55. Adimonye A, Stankiewicz E, Kudahetti S, Trevisan G TB, Corbishley C, Lu YJ, et al. Analysis of the PI3K-AKT-mTOR pathway in penile cancer: evaluation of a thera peutically targetable pathway. Oncotarget. 2018;9(22):16074–16086.

56. Ferrándiz-Pulido C, Hernández-Losa J, Masferrer E, Vivancos A, Somoza

R, Marés R, et al. Identification of somatic gene mutations in penile squamous cell carcinoma. Genes Chromosom Cancer. 2015;54(10):629–37.

57. Gou HF, LiX, Qiu M, ChengK, LiLH, DongH, et al. Epidermal growth factor receptor (EGFR)-RAS signaling pathway in penile squamous cell carcinoma. PLoS One. 2013;8(4):e62175.

58. Stankiewicz E, Prowse DM, Mansum Ng, CuzickJ, MesherD H, Lu YJ, et al. Alternative HER/PTEN/Akt pathway activation in HPV positive and negative penile carcinomas. PLoS One. 2011;6(3):e17517.

59. Brierley JD, Gospodarowicz MK, Wittekind C. TNM Classification of Malignant Tumorurs 8th Edition. Uinion For International Cancer Control.. Oxford: Wiley Blackwell; 2017. 188p

60. Mink JN, Khalmurzaev O, Pryalukhin A, Geppert CI, Lohse S, Bende K, Lobo J, et al. Evaluation of Prognostic Parameters to Identify Aggressive Penile Carcinomas. Cancers (Basel). 2023;15(19):4748.

61. Sanchez D, Fernandez-Nestosa MJ, Canete-Portillo S, Rodriguez I, Cubilla A.L. What Is New in the Pathologic Staging of Penile Carcinoma in the 8th Edition of AJCC TNM Model: Rationale for Changes with Practical Stage-by-Stage Category Diagnostic Considerations. Adv. Anat. Pathol. 2021;28:209–227

62. Fankhauser CD, de Vries HM, Roussel E, Jakobsen JK, Issa A, Lee EWC, et al. Lymphovascular and perineural invasion are risk factors for inguinal lymph node metastases in men with T1G2 penile cancer. J Cancer Res Clin Oncol. 2022;148(9):2231-34.

63. Alvarado-Cabrero I, Fernández-Nestosa MJ, Valencia-Cedillo R, Urizar C, Cañete-Portillo S, Sánchez DF, Cubilla AL. Gradual and synergistic correlation of tumor thickness and histological grade in penile invasive carcinomas. Hum Pathol. 2024;144:77-82

64. Sali AP, Menon S, Murthy V, Prakash G, Bakshi G, Joshi A, Desai SB. A Modified Histopathologic Staging in Penile Squamous Cell Carcinoma Predicts Nodal Metastasis and Outcome Better Than the Current AJCC

Staging. Am J Surg Pathol. 2020;44(8):1112-111

65. Chahoud J, Zacharias NM, Pham R, Qiao W, Guo M, Lu X, et al. Prognostic Significance of P16 and Its Relationship with Human Papillomavirus Status in Patients with Penile Squamous Cell Carcinoma: Results of 5 Years Follow-Up. Cancers 2022;14(24):6024.

66. Kobayashi K, Hisamatsu K, Suzui N, Hara A, Tomita H, Miyazaki T, et al. A Review of HPV-Related Head and Neck Cancer. J. Clin. Med. 2018;7(9):241

67. Moen CA, Falkenthal TE, Thorkelsen TK, Hopland A, Rio OE, Honoré A, et al.. Penile Cancers Attributed to Human Papillomavirus Are Associated with Improved Survival for Node-positive Patients. Findings from a Norwegian Cohort Study Spanning 50 Years. Eur Urol Oncol. 2023;8:S2588-931.

68. Chipollini J, Pollock G, Hsu CH, Batai K, Recio-Boiles A, Lee BR. National trends and survival outcomes of penile squamous cell carcinoma based on human papillomavirus status. Cancer Med. 2021;10(21):7466-7474

69. Eldar A, Rozenberg H, Diskin-Posner Y, Rohs R, Shakked Z. Structural studies of p53 inactivation by DNA-contact mutations and its rescue by suppressor mutations via alternative protein-DNA interactions. Nucleic Acids Res. 2013;41:8748–8759

70. Joerger AC, Fersht AR- Structural biology of the tumor suppressor p53 and cancer-associated mutants. Adv. Cancer Res. 2007;97:1–23

71. Brown CJ, Cheok CF, Verma CS, Lane DP. Reactivation of p53: From peptides to small molecules. Trends Pharmacol. Sci. 2011;32:53–62.

72. Cho Y, Gorina S, Jeffrey PD, Pavletich NP. Crystal structure of a p53 tumor suppressor-DNA complex: Understanding tumorigenic mutations. Science 1994;265:346–355.

73. Rivlin N, Brosh R, Oren M, Rotter V. Mutations in the p53 Tumor Suppressor Gene: Important Milestones at the Various Steps of Tumorigenesis. Genes Cancer. 2011;2(4):466-74.

74. Herrero AB, Rojas EA, Misiewicz-Krzeminska I, Krzeminski P, Gutiérrez

NC. Molecular Mechanisms of p53 Deregulation in Cancer: An Overview in Multiple Myeloma. Int J Mol Sci. 2016;17(12):2003.

75. Chen J, Yao K, Li Z, Deng C, Wang L, Yu X, et al. Establishment and characterization of a penile cancer cell line, penl1, with a deleterious TP53 mutation as a paradigm of HPV-negative penile carcinogenesis. Oncotarget. 2016;7:51687-51698.

76. Rocha RM, Ignacio JA, Jordan J, Carraro DM, Lisboa B, Lopes A, et al. A clinical, pathologic, and molecular study of p53 and murine double minute 2 in penile carcinogènesis and its relation to prognosis. Hum Pathol. 2012;43:481-8

77. Liu JY, Li YH, Zhang ZL, Yao K, Ye YL, Xie D, et al. The risk factors for the presence of pelvic lymph node metastasis in penile squamous cell carcinoma patients with inguinal lymph node dissection. World J Urol. 2013;31:2519–24.

78. Lopes A, Bezerra AL, Pinto CA, Serrano SV, de MellO CA, Villa LL. p53 as a new prognostic factor for lymph node metastasis in penile carcinoma: analysis of 82 patients treated with amputation and bilateral lymphadenectomy. J Urol. 2002;168(1):81–6.

79. Kortekaas KE, Bastiaannet E, van Doorn HC, de Vos van Steenwijk PJ, Ewing-Graham PC, Creutzberg CL, et al. Vulvar cancer subclassification by HPV and p53 status results in three clinically distinct subtypes. Gynecol Oncol. 2020;159(3):649–56.

80. Tessier-Cloutier B, Kortekaas KE, Thompson E, Pors J, Chen J, Ho J, et al. Major p53 immunohistochemical patterns in in situ and invasive squamous cell carcinomas of the vulva and correlation with TP53 mutation status. Mod Pathol. 2020;33(8):1595–605.

81. McDaniel AS, Hovelson DH, Cani AK, Liu CJ, Zhai Y, Zhang Y, et al. Genomic profiling of penile squamous cell carcinoma reveals new opportunities for targeted therapy. Cancer Res. 2015;75(24):5219–27.

82. Ottenhof SR, Djajadiningrat RS, Thygesen HH, Jakobs PJ, Józwiak K, Heeren AM, et al. The prognostic value of immune factors in the tumor

microenvironment of penile squamous cell carcinoma. Front Immunol.2018;9:1253.

83. Djajadiningrat RS, Graafland NM, van Werkhoven E, Meinhardt W, Bex A, van der Poel HG, et al. Contemporary management of regional nodes in penile cancer-improvement of survival? J Urol. 2014;191:68–73.

84. Bandini M, Pederzoli F, Necchi A. Neoadjuvant chemotherapy for lymph node-positive penile cancer: current evidence and knowledge. Curr Opin Urol. 2020;30(2):218-22

85. Paner GP, Stadler WM, Hansel DE, Montironi R, Lin DW, Amin MB. Updates in the Eighth Edition of the Tumor-Node-Metastasis Staging Classification for Urologic Cancers. Eur Urol. 2018;73(4):560-569

86. Brouwer OR, Albersen M, Parnham A, Protzel C, Pettaway CA, Ayres B, et al. European Association of Urology-American Society of Clinical Oncology Collaborative Guideline on Penile Cancer: 2023 Update. Eur Urol. 2023;83(6):548–60.

87. Wang Y, Wang K, Chen Y, Zhou J, Liang Y, Yang X, et al. Mutational landscape of penile squamous cell carcinoma in a Chinese population. Int J Cancer. 2019;145(5):1280–9.

88. Stoehr R, Weisser R, Wendler O, Giedl J, Daifalla K, Gaisa NT, et al. P53 codon 72 polymorphism and risk for squamous cell carcinoma of the penis: A Caucasian case-control study. J Cancer. 2018;9(22):4234–41.

89. Ficarra V, Akduman B, Bouchot O, Palou J, Tobias- Machado M. Prognostic factors in penile cancer. Urology. 2010;76:S66–73.

90. Horenblas S, van Tinteren H. Squamous cell carcinoma of the penis. IV. Prognostic factors of survival: analysis of tumor, nodes and metastasis classification system. J Urol. 1994;151:1239–43.

91. Yanagawa N, Osakabe M, Hayashi M, Tamura G, Motoyama T. Detection of HPV-DNA, p53 alterations, and methylation in penile squamous cell carcinoma in Japanese men. Pathol Int. 2008;58:477–82

92. Jeffreys M, Jeffus SK, Herfs M, Quick CM. Accentuated p53 staining in usual type vulvar dysplasia-A potential diagnostic pitfall. Pathol Res Pract.

2018;214(1):76–9.

93. Rakislova N, Alemany L, Clavero O, Saco A, Torné A, Del Pino M, et al. P53 immunohistochemical patterns in HPV- independent squamous cell carcinomas of the vulva and the associated skin lesions: A study of 779 cases. Int J Mol Sci. 2020;21(21):1–15.

94. Kortekaas KE, Solleveld-Westerink N, Tessier-Cloutier B, Rutten TA, van Poelgeest MIE, Gilks CB, et al. Performance of the Pattern Based Interpretation of p53 Immunohistochemistry as a Surrogate for TP53 Mutations in Vulvar Squamous Cell Carcinoma. Histopathology. 2020;77(1):92–9.

95. Hwang HJ, Nam SK, Park H, Park Y, Koh J, Na HY, et al. Prediction of TP53 mutations by p53 immunohistochemistry and their prognostic significance in gastric cancer. J Pathol Transl Med. 2020;54(5):378–86.

96. Cole AJ, Dwight T, Gill AJ, Dickson KA, Zhu Y, Clarkson A, et al. Assessing mutant p53 in primary high-grade serous ovarian cancer using immunohistochemistry and massively parallel sequencing. Sci Rep. 2016;18(6):26191.

97. Chahoud J, Gleber-Netto FO, McCormick BZ, Rao P, Lu X, Guo M, et al. Whole-exome sequencing in penile squamous cell carcinoma uncovers novel prognostic categorization and drug targets similar to head and neck squamous cell carcinoma. Clin Cancer Res. 2021;27(9):2560–70.

98. Visser O, Adolfsson J, Rossi S, Verne J, Gatta G, Maffezzini M, et al. Incidence and survival of rare urogenital cancers in Europe. Eur J Cancer. 2012;48(4):456–64.

99. Kidd LC, Chaing S, Chipollini J, Giuliano AR, Spiess PE, Sharma P. Relationship between human papillomavirus and penile cancer-implications for prevention and treatment. Transl Androl Urol. 2017;6(5):791–802.

100. Zhou X WX. Radioimmunotherapy in HPV-Associated Head and Neck Squamous Cell Carcinoma. Biomedicines. 2022;10(8):1990.

101. Rakislova N, Saco A, Sierra A, Del Pino M, Ordi J. Role of Human

Papillomavirus in Vulvar Cancer. Adv Anat Pathol. 2017;24(4):201-214

102. Ferrandiz-Pulido C, Masferrer E, Toll A, Hernandez-Losa J, Mojal S, Pujol RM, et al. mTOR signaling pathway in penile squamous cell carcinoma: pmTOR and peIF4E over expression correlate with aggressive tumor behavior. J Urol. 2013;190(6):2288–95.

103. Carreras-Dieguez N, Saco A, Del Pino M, Marimon L, López Del Campo R, Manzotti C, et al. Human papillomavirus and p53 status define three types of vulvar squamous cell arcinomas with distinct clinical,pathological, and prognostic features. Histopathology. 2023;83(1):17-30.

104. Trias I, Saco A, Marimon L, López Del Campo R, Manzotti C, Ordi O, et al. P53 in Penile Squamous Cell Carcinoma: A Pattern-Based Immunohistochemical Framework with Molecular Correlation. Cancers (Basel). 2023;15(10):2719.

105. Martins AC, Faria SM, Cologna AJ, Suaid HJ, Tucci SJ. Immunoexpression of p53 protein and proliferating cell nuclear antigen in penile carcinoma. J Urol. 2002;167(1):89–92.

106. Bowie J, Singh S, O'Hanlon C, Shiatis V, Brunckhorst O, Muneer A AK. A systematic review of non-HPV prognostic biomarkers used in penile squamous cell carcinoma. Turk J Urol. 2021;47(5):358–65.

107. Oertell J, Caballero C, Iglesias M, Chaux A, Amat L, Ayala E, et al. Differentiated precursor lesions and low-grade variants of squamous cell carcinomas are frequent findings in foreskins of patients from a region of high penile cancer incidence. Histopathology. 2011;58(6):925–33

108. Salama AM, Momeni-Boroujeni A, Vanderbilt C, Ladanyi M SR. Molecular landscape of vulvovaginal squamous cell carcinoma: new insights into molecular mechanisms of HPV-associated and HPV-independent squamous cell carcinoma. Mod Pathol. 2022;35(2):274–28.

109. Carreras-Dieguez N, Guerrero J, Rodrigo-Calvo MT, Ribera-Cortada I, Trias I, Jares P, et al. Molecular Landscape of Vulvar Squamous Cell Carcinoma. Int J Mol Sci. 2021;22(13):7069.

110. Voss FO, Thuijs NB, Vermeulen RFM, Wilthagen EA, van Beurden M,

Bleeker M. The Vulvar Cancer Risk in Differentiated Vulvar Intraepithelial Neoplasia: A Systematic Review. Cancers (Basel). 2021;13(24):6170.