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Molecular genetic heterogeneity in undifferentiated endometrial carcinomas

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Abstract

Undifferentiated and dedifferentiated endometrial carcinomas are rare and highly aggressive subtypes of uterine cancer, not well characterized at a molecular level. To investigate whether dedifferentiated carcinomas carry molecular genetic alterations similar to those of pure undifferentiated carcinomas, and to gain insight into the pathogenesis of these tumours, we selected a cohort of 18 undifferentiated endometrial carcinomas, 8 of them with a well differentiated endometrioid carcinoma component (dedifferentiated endometrioid carcinomas), and studied them by immunohistochemistry and massive parallel and Sanger sequencing. Whole exome sequencing of the endometrioid and undifferentiated components as well as normal myometrium, was also carried out in one case. According to The Cancer Genome Atlas classification, we distributed 95% of the undifferentiated carcinomas in this series as follows: a) hypermutated tumours with loss of any mismatch repair protein expression and microsatellite instability (eight cases, 45%); b) ultramutated carcinomas carrying mutations in the exonuclease

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domain of *POLE* (two cases, 11%); c) high copy number alterations (copy-number high) tumours group exhibiting only *TP53* mutations and high number of alterations detected by FISH (two cases, 11%) ; and d) low copy number alterations (copy-number low) tumours with molecular alterations typical of endometrioid endometrial carcinomas (five cases, 28%). Two of the latter cases, however, also had *TP53* mutations and higher number of alterations detected by FISH and could have progressed to a copy-number high phenotype. Most dedifferentiated carcinomas belonged to the hypermutated group whereas pure undifferentiated carcinomas shared molecular genetic alterations with copy-number low or copy-number high tumours. These results indicate that undifferentiated and dedifferentiated endometrial carcinomas are molecularly heterogeneous tumours, which may have prognostic value.

Introduction

Undifferentiated endometrial carcinoma is a rare but highly aggressive subtype of endometrial carcinoma [1], representing approximately 9% of endometrial carcinomas [2]. It is often misdiagnosed as grade 3 endometrioid carcinoma. Defining features include: a) monotonous medium or large-sized cells; b) diffuse pattern of growth; and c) a lack of appreciable glandular, papillary, squamous, or neuroendocrine differentiation [1, 3, 4]. Undifferentiated endometrial carcinoma lacks expression of epithelial markers, such as keratins, E-cadherin, and mir-200 but express ZEB-1, a well-known repressor of E-cadherin [5]. In addition, undifferentiated endometrial carcinoma is frequently associated with mismatch repair deficiency [5].

Occasionally, undifferentiated endometrial carcinoma is associated with a low-grade endometrioid carcinoma component, hence it is referred to as “dedifferentiated endometrioid endometrial carcinoma” [2]. In a recent study [6], a common origin of both components was proposed since all driver mutations present in the endometrioid component were also found in the undifferentiated (dedifferentiated) component. In that study, most mutations were found in genes such as *PTEN* (40%), *PIK3CA* (50%), *CTNNB1* (30%), and *TP53* (30%), which are frequently involved in the development of endometrioid carcinomas [6].

However, it is still unclear whether pure undifferentiated carcinomas differ molecularly from so-called dedifferentiated carcinomas.

Recently, The Cancer Genome Atlas (TCGA) Research Network reported an integrated molecular analysis of 373 endometrial carcinomas which were evaluated at the genomic, exomic, transcriptomic, epigenomic, and protein levels. This analysis showed that endometrioid and serous carcinomas can be classified into four distinct molecular categories: hypermutated microsatellite unstable, *POLE* ultramutated, copy-number low/ microsatellite stable and copy-number high/”serous-like”. The hypermutated, ultramutated and copy-number low subgroups were represented predominantly by endometrioid tumours whereas the copy-number high/”serous-like” subgroup was composed of both serous and high-grade endometrioid subtypes. Mixed endometrial carcinomas were assigned to the copy-number low (endometrioid) and the copy-number high (“serous-like”) tumours, indicating the molecular genetic diversity of these neoplasms. However, no mixed undifferentiated/well differentiated (dedifferentiated) carcinomas were included in the

TCGA study. Finally, a less sophisticated and practical way of assigning endometrial carcinomas to the TCGA categories, based on immunohistochemical analysis of mismatch repair proteins and *TP53* and *POLE* mutational analyses, has been proposed [7].

In the current study, we investigated whether undifferentiated and dedifferentiated carcinomas share molecular genetic alterations and or they represent a molecularly homogenous group of tumours. To this end, we selected a cohort of 18 undifferentiated endometrial carcinomas, eight of them with an endometrioid carcinoma component, to be massively sequenced using Haloplex Cancer Research Panel enrichment kit and Ion Torrent sequencing platform. In addition, one case was subjected to whole exome sequencing for comparing the genetic alterations of the endometrioid and the undifferentiated elements.

Materials and Methods

Case selection

This study includes a total of 22 endometrial carcinomas, diagnosed initially as undifferentiated carcinomas, from the files of the Departments of Pathology of Hospital Universitario Ramón y Cajal, Madrid; Massachusetts General Hospital, Boston; Memorial Sloan Kettering Cancer Center, New York; Hospital de la Santa Creu i Sant Pau, Barcelona; and Hospital Universitari Arnau de Vilanova, Lleida). Clinicopathological features are presented in Suppl. Table 1. The pathological and immunohistochemical features of 10 cases have been previously reported in part [5]. The diagnosis of undifferentiated carcinoma was based not only on conventional morphological features but also on the characteristic immunohistochemical pattern reported previously; i.e., absence of E-cadherin expression together with ZEB1 nuclear immunoreaction (see Figures 1 and 2) [8]. Two of the 22 tumours were reclassified as grade 3 endometrioid carcinomas containing areas of well-differentiated carcinoma. In these two cases, the solid component, interpreted initially as undifferentiated carcinoma, expressed membranous E-cadherin and lacked the nuclear ZEB1 immunoreaction. Two other cases were reclassified as carcinosarcomas with areas of undifferentiated carcinoma. The study was approved by the Institutional Review Board of the Hospital Ramón y Cajal and by the other participating medical institutions.

Immuhistochemistry

The following antibodies were used: E-cadherin (cat #IR059; DAKO, Glostrup, Denmark; ready to use) and ZEB1 (cat #ab87280; abcam, Cambridge, UK; dilution 1:300), p53: (cat #IR616; DAKO, Glostrup, Denmark; ready to use), MLH1 (cat #M3640; DAKO, Glostrup, Denmark; ready to use), PMS2 (cat #IR087; DAKO, Glostrup, Denmark; ready to use), MSH2 (cat #M3639; DAKO, Glostrup, Denmark; ready to use), MSH6 (cat #M3646; DAKO, Glostrup, Denmark; ready to use), β-catenina (cat #0001109QD; Master Diagnóstica, Granada, Spain; ready to use), ARID1A (cat #HPA005456; Sigma, St Louis, USA; dilution 1:500) and SMARCB1 (BD Biosciences, Franklin Lakes, NJ USA; dilution: 1/100). Immunostaining was performed using the EnVision detection system (K5007, Dako).

Massive parallel sequencing

Haloplex Cancer Research Panel was used as enrichment kit. This panel targeted 1205 hotspots in 199 regions from 47 genes, including most frequently mutated genes in endometrioid endometrial carcinoma (*PTEN*, *PIK3CA*, *PIK3R1*, *KRAS*, *CTNNB1*, *FGFR2* or *TP53*), with the exception of *ARID1A*. In order to know whether targeted regions overlapped with mutations found in TCGA study [9], we calculated the percentage of variants located across covered regions and across hotspot regions plus/minus 30 bp. Sequencing of libraries was performed on Ion Torrent sequencing platform in four chips class 316 following standard procedures, expecting an average of 25 Mb per sample (around 17 Mb per sample were recommended by Haloplex Protocol).

To analyze sequencing results and to avoid an unacceptable false negative rate, a bioinformatics analysis pipeline was developed using TMAP (https://github.com/iontorrent/TS/tree/master/Analysis/TMAP) as aligner and VarScan [10] as variant-caller, with no filters. Variant annotation was performed using the VEP from Ensembl with version 74 of the human reference genome (http://www.ensembl.org/info/docs/tools/vep/index.html). Variants were then filtered using the functional information (selecting only deleterious variants), the variant allele frequency (> 0.15) and the strand-bias from both the variant and the reference allele. In the case of having normal tissue available, those variants present in the normal component were ruled out. Taking into account the information from Sanger sequencing, visual inspection of variants was performed using IGV browser [11] as the final selection step.

FISH analyses

In 11 tumours, interphase fluorescence in situ hybridization (FISH) analysis was performed using commercial probes delineating several loci on different chromosomal regions (see Supp. Table 2). In dedifferentiated tumours, FISH analysis was limited to the undifferentiated component. Pre-treatment of slides, hybridization, post-hybridization processing, and signal detection were performed as reported elsewhere [12]. Only samples showing sufficient FISH efficiency (> 90% nuclei with signals) were evaluated. Signals were scored in at least 50 no overlapping, intact nuclei. Non-neoplastic cells present in the section were used as a control. Results were interpreted as follows: a) gain: when the ratio between gene and control probe signals was between 1 and 2.5 (both excluded); b) amplification: when the ratio between gene and control probe signals was ≥ 2.5; c) deletion: when the ratio between gene and control probe signals was < 1; d) aneuploidy: the presence of only one gene and centromeric probes in more than 50% of cells evaluated was considered monosomy while presence of ≥ 3 gene and centromeric probes was considered polisomy. For *EGFR* gene evaluation the presence of three and four centromeric probes was considered trisomy and tetrasomy respectively and the presence of ≥ 5 centromeric and gene probes was considered polisomy.

Whole exome analysis

To find out whether or not the mutational profile of the undifferentiated component of the mixed carcinomas differed from that of the differentiated component within an individual tumour, we performed whole exome sequencing of both components, together with normal

myometrial tissue (UEC-14), following technical specifications from Sistemas Genómicos S.L., on an Illumina Hiseq 2000 platform. The enrichment method was Agilent SureSelect Exome V4, targeting a total of 51 Mb. Bioinformatics analysis was performed as described above, with the exception of using Novoalign (http://www.novocraft.com/products/novoalign/) instead of TMAP in the alignment step. Somatic mutations were considered those present in the tumour but not in normal tissue. CONTRA package [13] was used to analyse the genome instability using the normal component data as control.

Sanger sequencing

To validate massive parallel sequencing data, primers were designed to target different mutations in *PIK3CA*, *PIK3R1*, *ATM*, and *SMARCB1* (Supp. Table 3). Since Haloplex Cancer Research Panel lacks coverage of some *PTEN* mutations, we also designed 12 primer-pairs (Supp. Table 3) to amplify the nine *PTEN*'s exons by PCR and perform Sanger sequencing for all cases. To further classify tumours within the TCGA ultramutated group [9], we designed two pairs of primers (Supp. Table 3) to sequence the two usually mutated exons (Exon 9 and Exon 13) containing the protein catalytic domain of *POLE* in all cases.

Results

Pathological and immunohistochemical features

Of the 18 undifferentiated endometrial carcinomas, seven were pure and the remaining 11 cases exhibited an endometrioid component (a grade 1–2 endometrioid carcinoma in 10, and a grade 3 endometrioid carcinoma in 1).

The most relevant clinical, pathological, inmunohistochemical, and molecular genetic features of all 22 tumours are presented in Supp. Table 4. Here we describe the immunohistochemical and molecular features of the 18 undifferentiated/dedifferentiated endometrial carcinomas.

Every immunohistochemical essay was performed in the 7 pure undifferentiated cases, in the undifferentiated component of the 11 dedifferentiated cases and in the differentiated component of 7 dedifferentiated cases. In the differentiated component of 4 dedifferentiated cases, IHQ essays were not possible to perform due to the lack of dedifferentiated tissue in the preparations under study.

Diagnosis for undifferentiated status was confirmed as stated in material and methods: undifferentiated component showed lack of E-cadherin staining and nuclear staining of ZEB1 whereas differentiated component showed normal membrane staining of E-cadherin and absence of ZEB1 staining. Only in case UEC-5 this pattern was not confirmed due to the lack of material.

Loss of ARID1A expression was observed in 10 out of the 18 cases (56 %); being the loss total in eight and focal in two cases respectively. Noteworthy, the two cases with focal loss were dedifferentiated carcinomas and the loss of expression was limited almost exclusively to the undifferentiated component (in one case, a few areas of the differentiated

endometrioid component had loss of ARID1A expression, see Supp. Figure 1). Both cases also exhibited loss of MLH1/PMS2 expression across the entire tumour.

Loss of expression of at least one mismatch repair protein was found in eight tumours (45%); six showed loss of MLH1/PMS2, one loss of PMS2 only, and one loss of MSH2/MSH6.

p53 overexpression (at least in 75% of tumour cells) was found in three cases (17%). Noteworthy, in the dedifferentiated carcinoma, p53 overexpression was restricted only to the undifferentiated component.

Nuclear β-catenin immunoreaction was seen only in one case (6%) and was also confined to the undifferentiated component of a dedifferentiated carcinoma (Supp. Figure 2).

All tumours were checked for SMARCB1 (INI1, BAF47) expression by immunohistochemistry. Normal nuclear SMARCB1 staining was found in all cases.

Mutation analysis

We first compared the mutations covered by Haloplex Cancer Panel in *PTEN*, *PIK3CA*, *PIK3R1*, *KRAS*, *CTNNB1*, *FGFR2*, and *TP53* with those previously reported for endometrioid endometrial carcinomas, mainly by TCGA [9]. We found coverage greater than 90% for all genes except for PTEN in which coverage reached 50% only. (Supp. Table 5). Accordingly, we performed Sanger sequencing for all 9 PTEN exons.

For the 16 undifferentiated endometrial carcinomas subjected to massive parallel sequencing (Supp. Table 6), the mean number of bases obtained per sample was 25,711,256, which allowed a median depth per sample of 40. However, the distribution of the depth was not homogeneous along the target regions and most of the depth was limited to the hotspot regions (median depth per sample of 236). We observed that was due to the fact that larger amplicons could not be sequenced in most of the FFPE samples as several regions were not covered at all since designed amplicons of suitable-size were not available for Ion Torrent protocol (data not shown).

Taking into account Haloplex and Sanger sequencing results, a total of 43 different pathogenic mutations were identified in the set of 16 undifferentiated carcinomas (Supp. Table 4). Ten out of 43 (23%) mutations were found in *PTEN*, and four of them were only detected by Sanger sequencing. Mutations were also detected in other genes commonly mutated in different types of endometrial carcinomas, such as *TP53* (4 out of 43, 9%), *PIK3R1* (3 out of 43, 7%) and *PIK3CA* (3 out of 43, 7%), *KRAS* (2 out of 43, 5%) and *CTNNB1* (1 out of 43, 2%) (Supp. Table 4). *POLE* mutational study showed that two tumours carried variants previously described as pathogenic and associated with ultramutated phenotype (Supp. Table 4) [9, 14–16].

Regarding phenotype-genotype correlations, only one pure undifferentiated carcinoma had mismatch repair deficiency in contrast to seven out of 11 dedifferentiated tumours. The other six pure undifferentiated carcinomas carried *PTEN* or *TP53* mutations as their main molecular alterations (Figure 3).

Chromosomal alteration detected by FISH

A total of seven chromosomes and 11 genes were checked for alterations in 11 cases (six pure undifferentiated and five dedifferentiated cases) (Supp. Table 7). Pure undifferentiated cases with *TP53* mutations showed higher number of alterations (5.75 in average) compared to *TP53* wild type cases (1 in average), which is consistent with previous results [9]. Noteworthy, one case with no identified molecular alteration showed the highest number of aberrations (3) among *TP53* wild type cases.

TCGA based molecular classification of undifferentiated/dedifferentiated carcinomas

According to the molecular classification scheme proposed by TCGA and following the simplified approach proposed in a recent study [7], we classified 17 out of the18 undifferentiated carcinomas in this series as follows: eight cases (45%) showing loss of any mismatch repair protein expression into the microsatellite instability hypermutated category; two cases (11%) carrying pathogenic mutations in the exonuclease domain of *POLE* into the ultramutated group; two cases (11%) showing only *TP53* mutations and high number of alterations detected by FISH into the copy-number high group; and five cases (28%) with molecular alterations typical of copy-number low endometrioid endometrial carcinomas. However, two of the latter cases showed additional mutations in *TP53* and higher number of alterations detected by FISH; therefore, these tumours could have progressed to a copy-number high phenotype (Figure 3B). In the remaining case, different chromosomal alterations were identified by FISH but no molecular alteration that allowed its classification was found.

Exome sequencing

Whole exome sequencing allowed the identification of somatic variants across most exonic regions in the genome of UEC-14. The final median depth across target regions was 38, 33 and 36 with 0.20%, 0.17% and 0.25% of regions not covered at all (>90% of bases not covered for at least one read) for normal tissue, endometrioid, and undifferentiated carcinoma samples respectively. A total of 123 somatic variants (40 indels and 83 substitutions) were identified for both tumour components (see genes affected in Supp. Table 8). In addition, 44 somatic variants (19 indels and 25 substitutions) were identified only in the undifferentiated component, and three somatic variants (two indels and one substitution) were exclusively found in the endometrioid component. Mutations identified by massive parallel sequencing were also identified by whole exome sequencing. In agreement with the loss of expression of ARID1A in this case, two pathogenic mutations (Y1233* and 788delC) were detected in both endometrioid and undifferentiated components.

Genome instability study (see Figure 1K) showed a flat pattern for the differentiated component with no apparent alteration, whereas the pattern for the dedifferentiated component (see Figure 1K) showed trisomy of chromosome 8, partial loss of 1p36.33 and uncertain monosomy of chromosomes 15 and 16. Chromosome 8 trisomy was confirmed by FISH (see Figure 1J).

UEC-14 showed rhabdoid features in the undifferentiated component. Since we found a heterozygous deleterious mutation in *SMARCB1* gene, we analysed all 18 tumours for

SMARCB1 (INI1, BAF47) expression by immunohistochemistry. As stated before, normal nuclear *SMARCB1* expression was found in all cases.

Discussion

The results of this study suggest that undifferentiated carcinomas may develop through any of the four molecular pathways described by TCGA for endometrial carcinomas; i.e., hypermutated (mismatch repair deficiency), ultramutated (*POLE* mutated), copy-number low, and copy-number high (*TP53* mutated) [9]. Nearly half (45%) of the undifferentiated carcinomas in this series belong to the hypermutated group, which contrasts with the frequency of microsatellite instability reported in most series of sporadic endometrial cancer (15%–30%) [17]. However, the frequency of mismatch repair deficiency seems to be higher in high-grade endometrioid carcinomas (45%–63%) [5, 18] suggesting an intrinsic potential for tumour progression of carcinomas from the hypermutated group.

A remarkable finding in this study was that two undifferentiated carcinomas carried *POLE* mutations that affect the activity of the catalytic subunit, involved in nuclear DNA replication and repair as described by TCGA [9, 14, 15]. Endometrioid carcinomas with *POLE* exonuclease domain mutations have been recently described to be frequently of high histologic grade exhibiting morphologic heterogeneity and ambiguity, and tumour-infiltrating lymphocytes and/or peri-tumour lymphocytes. [19]. One of the two ultramutated tumours in this series was a dedifferentiated carcinoma rich in tumour-infiltrating lymphocytes and the other was a pure undifferentiated carcinoma. The former tumour also had a *TP53* mutation confined to the undifferentiated component (Figure 2) similarly to the TCGA ultramutated carcinomas. A dedifferentiated carcinoma carrying *POLE* exonuclease domain mutations has recently been reported [16]. In this case, aberrant p53 immunoreaction was also confined to the undifferentiated component.

One third of undifferentiated carcinomas in our series derive from copy-number low endometrial carcinomas. In this group, the most frequent driver alteration was *PTEN* mutation, often associated with mutations in other genes of the same pathway (*PIK3CA, PIK3R1*), in endometrioid endometrial carcinomas [9]. However, two cases (11%) that lacked the characteristic molecular genetic alterations of endometrioid carcinomas, had *TP53* mutations, suggesting that at least some undifferentiated carcinomas might develop through the “serous-like” (copy-number high) pathway.

Regarding genotype-phenotype correlation and based on a relatively small number of cases, this study suggests that dedifferentiated carcinomas occur mainly in the setting of endometrioid carcinomas with microsatellite instability or *POLE* mutations, whereas most pure undifferentiated carcinomas develop from the copy-number low or “serous-like” copy-number high tumours.

Another purpose of this study was to investigate the molecular mechanisms that allow “dedifferentiation” of endometrioid carcinomas to undifferentiated carcinomas. The role of *CTNNB1, PPP2R1A*, and *TP53* in this phenomenon has been suggested by a previous report [6]; however, that report lacked information regarding mismatch repair proteins, ARID1A

expression, and *POLE* mutation analysis. Our results suggest that different pathways may be followed depending on the molecular subtype (Figure 3). Thus, tumours with mismatch repair deficiency (hypermutated) seem to acquire a dedifferentiated phenotype through accumulation of mutations in genes that are regularly altered in endometrioid carcinomas and not *TP53* mutations. Furthermore, our results suggest that *ARID1A* plays a role in the progression of endometrial carcinomas with mismatch repair deficiency [20]. We found that 70% of tumours with loss of ARID1A had microsatellite instability and that loss of ARID1A occurred mainly in the undifferentiated component of two dedifferentiated carcinomas with mismatch repair deficiency in both components. [21]. Similarly, in the case of one dedifferentiated carcinoma with microsatellite instability, we found *CTNNB1* mutations exclusively in the undifferentiated component (Supp. Table 4).

On the other hand, tumours with *PTEN* mutations as main driver (copy-number low carcinomas) probably might acquire undifferentiated phenotype through mutations in *TP53*, as such mutations were encountered in 2 out of 5 cases with *PTEN* mutations lacking mismatch repair deficiency or *POLE* mutations.

In our series, the ultramutated tumours seemed to have progressed to undifferentiated carcinomas by accumulating mutations in genes involved in the development of endometrioid carcinomas and/or *TP53* mutations. The dedifferentiated tumour with *POLE* mutation showed loss of ARID1A in both components but p53 overexpression and *TP53* mutation were found exclusively in the poorly differentiated/undifferentiated elements [16]. In contrast, the pure undifferentiated tumour had ARID1A loss and *PTEN* mutations. Our results are consistent with those of the TCGA which reported mutations in *PTEN* (94%), *FBXW7* (82%), *ARID1A* (76%), *PIK3CA* (71%), and *TP53* (35%) in the *POLE*-mutated tumours [19]. Regarding prognosis, although the size of our series is not large enough to extract conclusions, we observed that the 4 patients classified as copy-number high or copy-number low to copy-number high had deceased whereas the two patients classified as copy-number low were alive at the time of this study (no data was available for the third patient classified as copy-number low).

In our series, the study of the complete exome in one dedifferentiated carcinoma with rhabdoid-like features revealed the presence of a heterozygous mutation in *SMARCB1* limited to the undifferentiated component. This led us to analyze SMARCB1 (INI1) expression in the entire series; however, no tumour showed loss of SMARCB1expression. Thus, it appears that inactivation of one copy of *SMARCB1* is not enough for the development of rhabdoid features in undifferentiated carcinomas. However, other studies have demonstrated that expression of *SMARCB1* and more frequently of *SMARCA4* can be lost in the undifferentiated component of dedifferentiated carcinomas [22, 23]. These findings, together with our observation of frequent ARID1A loss, suggest a role of SWI/SNF complex alterations in the pathogenesis of an undifferentiated phenotype in endometrial carcinomas.

In summary, this study shows that undifferentiated endometrial carcinomas may share molecular genetic alterations with any of the four molecular subgroups of endometrial cancer described by TCGA. Most undifferentiated carcinomas with a differentiated

component (dedifferentiated carcinomas) occurred in the setting of mismatch repair deficiency (hypermutated tumours) with accumulation of molecular genetic alterations characteristic of endometrioid carcinomas, such as *ARID1A*, *PIK3CA* or *CTNNB1* mutations in the undifferentiated component. *TP53* mutation may act as the initial driver for some undifferentiated carcinomas developing through a "serous-like" pathway or it may be involved in the progression of endometrioid tumours without microsatellite instability but exhibiting *PTEN* mutations. Lastly, undifferentiated carcinomas carrying *POLE* mutations can evolve through endometrioid and/or "serous-like" pathways.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Tafe LJ, Garg K, Chew I, Tornos C, Soslow RA. Endometrial and ovarian carcinomas with undifferentiated components: clinically aggressive and frequently underrecognized neoplasms. Mod Pathol. 2010; 23:781–789. [PubMed: 20305618]
2. Silva EG, Deavers MT, Malpica A. Undifferentiated carcinoma of the endometrium: a review. Pathology. 2007; 39:134–138. [PubMed: 17365829]
3. Altrabulsi B, Malpica A, Deavers MT, Bodurka DC, Broaddus R, Silva EG. Undifferentiated carcinoma of the endometrium. Am J Surg Pathol. 2005; 29:1316–1321. [PubMed: 16160474]
4. Silva EG, Deavers MT, Bodurka DC, Malpica A. Association of low-grade endometrioid carcinoma of the uterus and ovary with undifferentiated carcinoma: a new type of dedifferentiated carcinoma. Int J Gynecol Pathol. 2006; 25:52–58. [PubMed: 16306785]
5. Romero-Perez L, Garcia-Sanz P, Mota A, Leskela S, Hergueta-Redondo M, Diaz-Martin J, Lopez-Garcia MA, Castilla MA, Martinez-Ramirez A, Soslow RA, et al. A role for the transducer of the Hippo pathway, TAZ, in the development of aggressive types of endometrial cancer. Mod Pathol. 2015; 28:1492–1503. [PubMed: 26381823]
6. Kuhn E, Ayhan A, Bahadirli-Talbott A, Zhao C, Shih Ie M. Molecular characterization of undifferentiated carcinoma associated with endometrioid carcinoma. Am J Surg Pathol. 2014; 38:660–665. [PubMed: 24451280]
7. Talhouk A, McConechy MK, Leung S, Li-Chang HH, Kwon JS, Melnyk N, Yang W, Senz J, Boyd N, Karnezis AN, et al. A clinically applicable molecular-based classification for endometrial cancers. Br J Cancer. 2015; 113:299–310. [PubMed: 26172027]
8. Romero-Perez L, Lopez-Garcia MA, Diaz-Martin J, Biscuola M, Castilla MA, Tafe LJ, Garg K, Oliva E, Matias-Guiu X, Soslow RA, et al. ZEB1 overexpression associated with E-cadherin and microRNA-200 downregulation is characteristic of undifferentiated endometrial carcinoma. Mod Pathol. 2013; 26:1514–1524. [PubMed: 23743934]
9. Cancer Genome Atlas Research N. Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, Robertson AG, Pashtan I, Shen R, et al. Integrated genomic characterization of endometrial carcinoma. Nature. 2013; 497:67–73. [PubMed: 23636398]
10. Koboldt DC, Zhang Q, Larson DE, Shen D, McLellan MD, Lin L, Miller CA, Mardis ER, Ding L, Wilson RK. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res. 2012; 22:568–576. [PubMed: 22300766]

11. Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov JP. Integrative genomics viewer. Nat Biotechnol. 2011; 29:24–26. [PubMed: 21221095]
12. Bubendorf L, Kononen J, Koivisto P, Schraml P, Moch H, Gasser TC, Willi N, Mihatsch MJ, Sauter G, Kallioniemi OP. Survey of gene amplifications during prostate cancer progression by high-throughout fluorescence in situ hybridization on tissue microarrays. Cancer Res. 1999; 59:803–806. [PubMed: 10029066]
13. Li J, Lupat R, Amarasinghe KC, Thompson ER, Doyle MA, Ryland GL, Tothill RW, Halgamuge SK, Campbell IG, Gorringe KL. CONTRA: copy number analysis for targeted resequencing. Bioinformatics. 2012; 28:1307–1313. [PubMed: 22474122]
14. Meng B, Hoang LN, McIntyre JB, Duggan MA, Nelson GS, Lee CH, Kobel M. POLE exonuclease domain mutation predicts long progression-free survival in grade 3 endometrioid carcinoma of the endometrium. Gynecol Oncol. 2014; 134:15–19. [PubMed: 24844595]
15. Church DN, Briggs SE, Palles C, Domingo E, Kearsey SJ, Grimes JM, Gorman M, Martin L, Howarth KM, Hodgson SV, et al. DNA polymerase epsilon and delta exonuclease domain mutations in endometrial cancer. Hum Mol Genet. 2013; 22:2820–2828. [PubMed: 23528559]
16. Espinosa I, D'Angelo E, Palacios J, Prat J. Mixed and Ambiguous Endometrial Carcinomas: A Heterogenous Group of Tumors With Different Clinicopathologic and Molecular Genetic Features. Am J Surg Pathol. 2016
17. López-García, MA., Vieites, B., Castilla, MA., Romero-Pérez, L., Díaz-Martín, J., Biscuola, M., Palacios, J., Genetics of Endometrial Carcinoma. Cancer Genomics: Molecular Classification, Prognosis and Response Prediction. Pfeffer, U., editor. Springer; 2013. p. 349-390.
18. Nelson GS, Pink A, Lee S, Han G, Morris D, Ogilvie T, Duggan MA, Kobel M. MMR deficiency is common in high-grade endometrioid carcinomas and is associated with an unfavorable outcome. Gynecol Oncol. 2013; 131:309–314. [PubMed: 23938375]
19. Hussein YR, Weigelt B, Levine DA, Schoolmeester JK, Dao LN, Balzer BL, Liles G, Karlan B, Kobel M, Lee CH, et al. Clinicopathological analysis of endometrial carcinomas harboring somatic POLE exonuclease domain mutations. Mod Pathol. 2015; 28:505–514. [PubMed: 25394778]
20. Allo G, Bernardini MQ, Wu RC, Shih Ie M, Kalloger S, Pollett A, Gilks CB, Clarke BA. ARID1A loss correlates with mismatch repair deficiency and intac p53 expression in high-grade endometrial carcinomas. Mod Pathol. 2014; 27:255–261. [PubMed: 23887303]
21. Wang K, Kan J, Yuen ST, Shi ST, Chu KM, Law S, Chan TL, Kan Z, Chan AS, Tsui WY, et al. Exome sequencing identifies frequent mutation of ARID1A in molecular subtypes of gastric cancer. Nat Genet. 2011; 43:1219–1223. [PubMed: 22037554]
22. Karnezis AN, Hoang LN, Coatham M, Ravn S, Almadani N, Tessier-Cloutier B, Irving J, Meng B, Li X, Chow C, et al. Loss of switch/sucrose non-fermenting complex protein expression is associated with dedifferentiation in endometrial carcinomas. Mod Pathol. 2016; 29:302–314. [PubMed: 26743474]
23. Ramalingam P. Morphologic, Immunophenotypic, and Molecular Features of Epithelial Ovarian Cancer. Oncology (Williston Park). 2016; 30:166–176. [PubMed: 26892153]

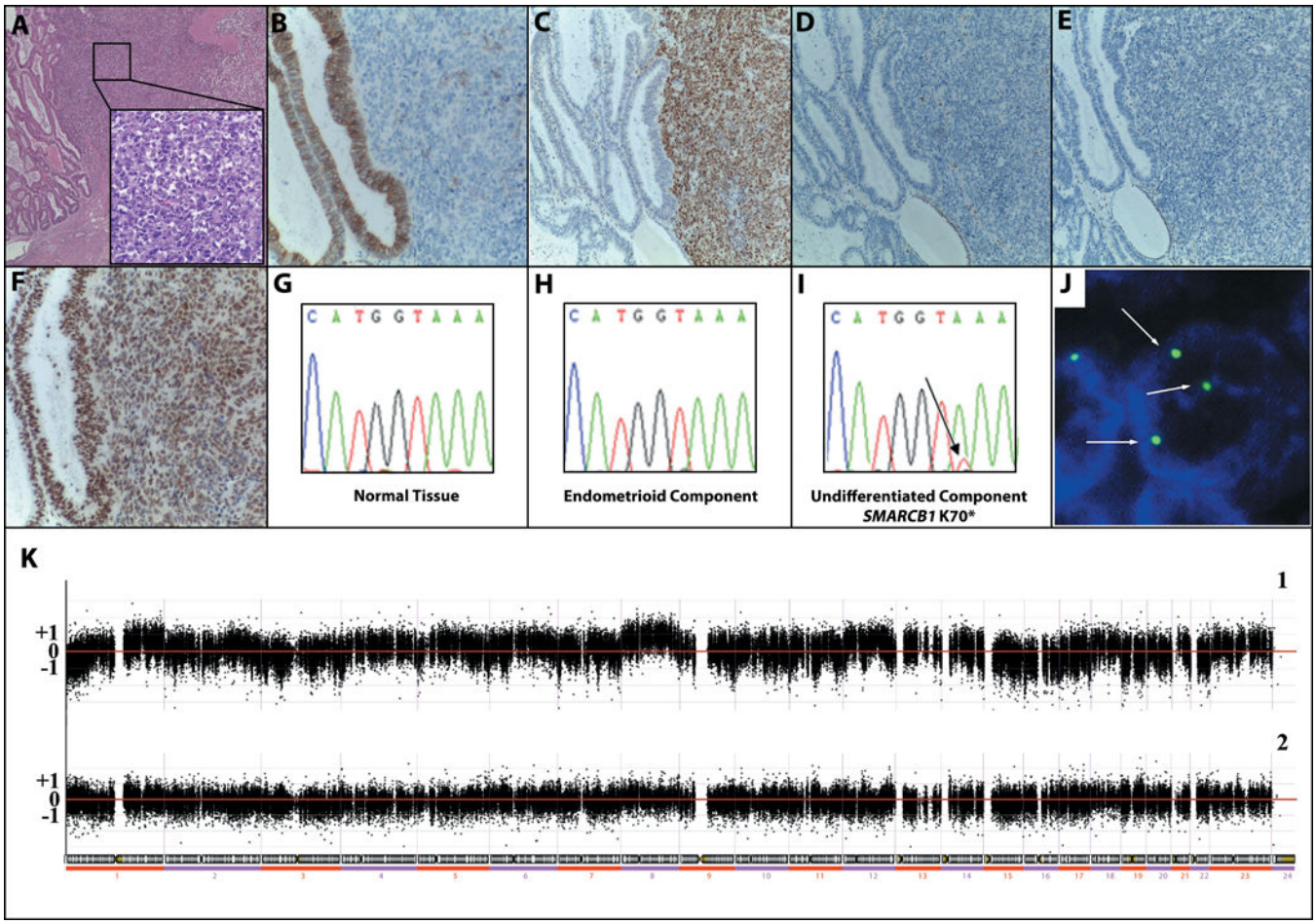

Figure 1.

Molecular characteristics of UEC-14, a dedifferentiated endometrioid carcinoma with mismatch repair and ARID1A deficiency. A) Hematoxylin-Eosin staining showing a well-differentiated component besides an undifferentiated component. Magnified image showed the rhabdoid features of the undifferentiated component. B) E-cadherin staining positive for differentiated component, negative for undifferentiated component. C) ZEB-1 staining positive for undifferentiated component, negative for differentiated component. D) Negative MLH-1 staining. E) Negative ARID1A staining. F) Positive SMARCB1 staining. G) Wild-type *SMARCB1* (chr22:24134051-24134059) sequence obtained for UEC-14 normal component. H) Wild-type *SMARCB1* sequence obtained for UEC-14 endometrioid component. I) *SMARCB1* variant (chr22:24134057 A->T) found in UEC-14 undifferentiated component identified by exome sequencing. Observed low variant allele frequency agreed with NGS results (~18%). I) Confirmation of chr8 trisomy constrained to undifferentiated component by FISH using a chr8-centromere probe.

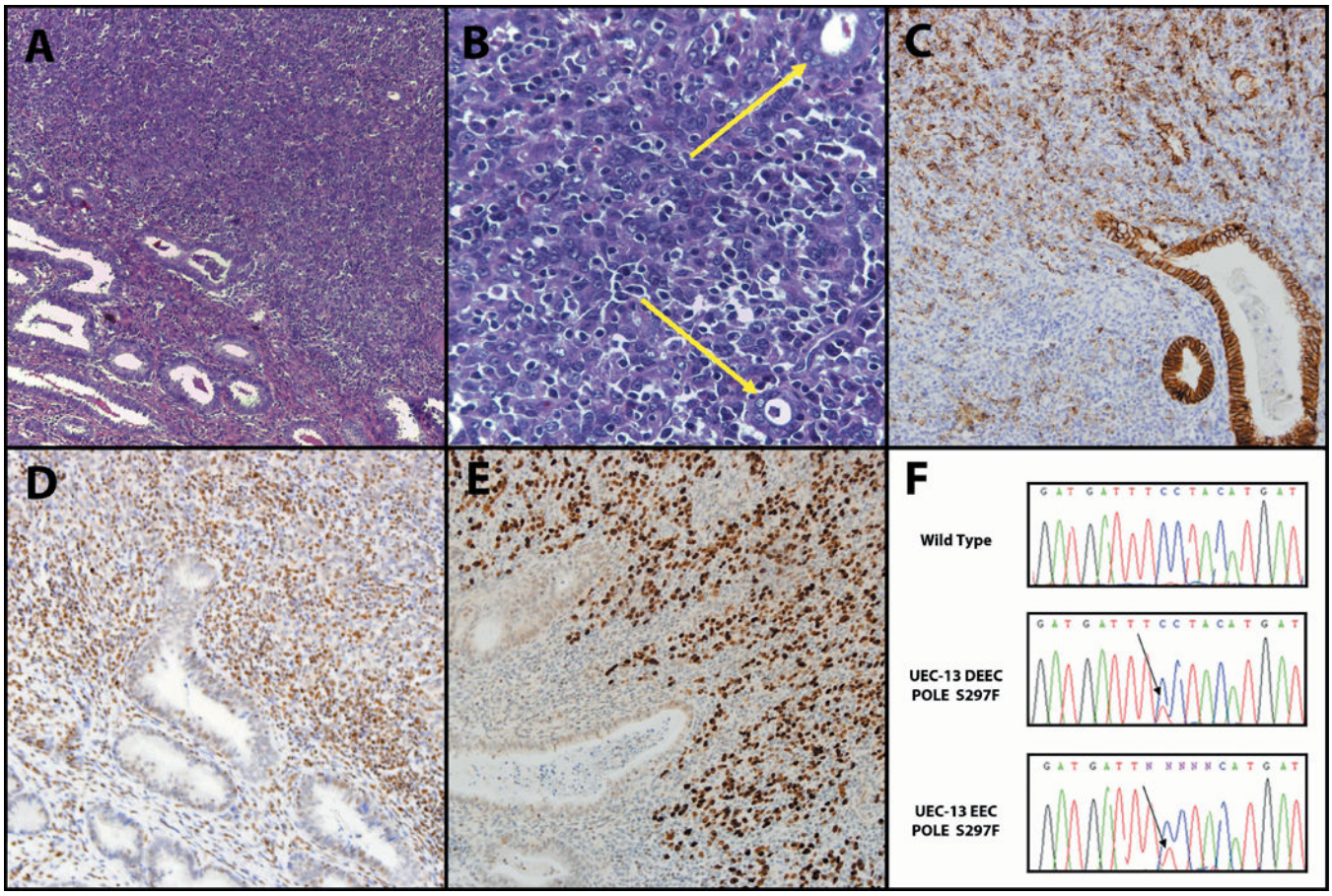

Figure 2.

Molecular characteristics of UEC-13, a dedifferentiated endometrioid carcinoma with *POLE* and *TP53* mutations. A) Hematoxylin-Eosin staining showing a well-differentiated component beside undifferentiated component. B) Hematoxylin-Eosin staining showing a field within the undifferentiated component with two glands (yellow arrows). C) Progressive loss of E-cadherin expression throughout undifferentiated component. D) Focal gain of ZEB-1 expression throughout undifferentiated component. E) TP53 staining positive for undifferentiated component, negative for differentiated component. F) Chr12:133253143-133253160 sequence. From up to bottom: wild-type sequence, *POLE* S297F mutation identified in UEC-13 undifferentiated component and *POLE* S297F mutation identified in UEC-13 endometrioid component.

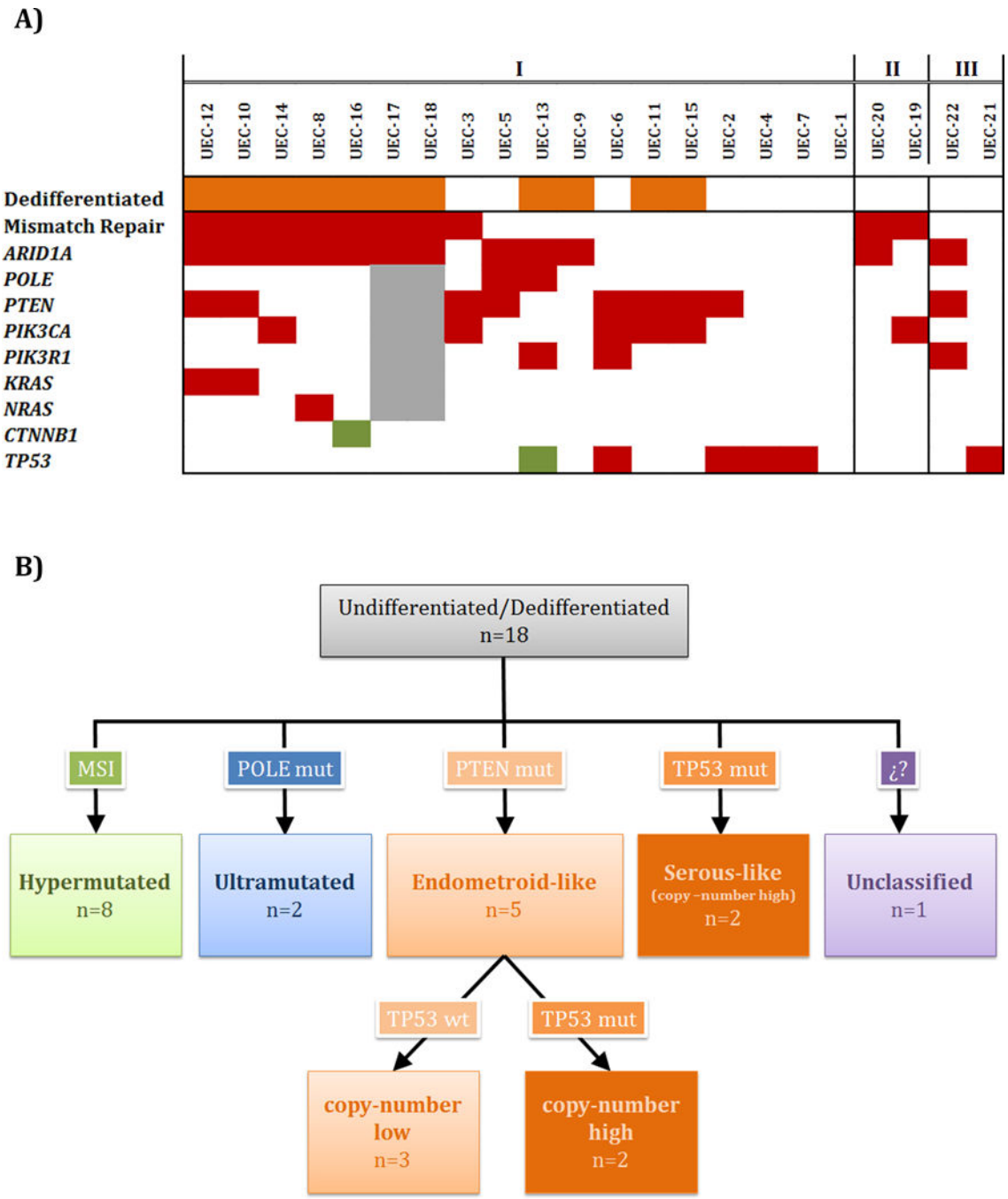

Figure 3.

A) Summary of the molecular characteristics observed in dedifferentiated, pure undifferentiated (I) and high-grade endometrioid carcinomas (II); and carcinosarcomas (III). Alteration for a gene (mutation or loss of expression) is indicated in red, no identified alteration for a gene (wild-type sequence or regular expression) in white and gene-not-analyzed for a sample in grey. B) Schematic classification of the 18 undifferentiated/dedifferentiated tumours in this series according to TCGA molecular classification.