ECTO-NUCLEOTIDASE EXPRESSION AND ACTIVITY IN ENDOMETRIOID-TYPE ENDOMETRIAL CARCINOMA CELL LINES

Aitor Rodríguez-Martínez[1,2], Xavier Matias-Guiu[2,3], Mireia Martín-Satué[1,2]

[1]Departament de Patologia i Terapèutica Experimental, Facultat de Medicina i Ciències de la Salut, Campus Bellvitge, Universitat de Barcelona, Spain
[2]Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Spain
[3]Servei d'Anatomia Patològica, Hospital de Bellvitge, Barcelona, Spain

Introduction: ATP and adenosine are known for their role in promoting an immunosuppressive environment at the tumor site. It is known that some ecto-nucleotidases, proteins that handle the hydrolysis of tri-, di- and monophosphate nucleotides, are overexpressed in endometrial tumor tissues although the mechanisms underlying these processes remain controversial. In the present study we characterized, with cytochemistry and i mmunofluorescence, the ecto-nucleotidase profiles in endometrioid-type endometrial carcinoma cell lines. Furthermore, we have developed a new approach capable of simultaneously detecting both alkaline phosphatase expression and activity.

Materials and methods: *Cell lines*: 3 endometrioid endometrial carcinoma cell lines (Ishikawa, HEC-1B and ECC-1) were used for cytochemistry and immunofluorescence experiments. *In situ ecto-nucleotidase enzyme activities:* ATPase, ADPase, and AMPase activity experiments were performed in all cell lines, based on the Wachstein/Meisel technique. *Immunofluorescence experiments:* cell immunolabeling was performed using primary antibodies against different members of the ecto-nucleotidases: anti-ectonucleoside triphosphate diphosphohydrolase 2 (E-NTPDase2), anti-E-NTPDase3, anti-CD73, and anti-placental-like alkaline phosphatase (PLAP). *Alkaline phosphatase immunoactivity assay:* for the detection of both activity and al kaline phosphatase expression we developed a combinatorial assay in which enzyme activity can be co-visualized with protein expression. Immunofluorescence followed by an activity assay was performed on AP-expressing cells.

Results: Cytochemistry enzyme assays showed moderate ATPase activity in both Ishikawa and ECC-1 cell lines. Moderate ADPase activity was found in ECC-1 cells and high AMPase activity was detected in HEC-1B cells. In immunolabeling experiments we found NTPDase2 protein expression in all three cell lines, with NTPDase3 expression restricted to ECC-1 cells. CD73 was detected in all three cell lines. Moderate alkaline phosphatase activity and protein expression were found in Ishikawa and ECC-1 cells.
A wide differential range of activities and ecto-nucleotidase expression among the studied cell lines was observed. NTPDase2 and NTPDase3 expression correlated with ATPase activity. CD73 protein expression coincided with the high AMPase activity shown with HEC-1B.

Conclusions: Ishikawa, HEC-1B, and ECC-1 cell lines represent a useful cell model for the study of ecto-nucleotidases in the context of endometrioid-type endometrial carcinoma.

Acknowledgements: This work was supported by a grant from the *Instituto de Salud Carlos III* (*FIS PI15/00036*), co-funded by FEDER funds/European Regional Development Fund (ERDF)- "a Way to Build Europe"- // *FONDOS FEDER "una manera de hacer Europa",* and a grant from the *Fundación Merck Salud* (*Ayuda Merck de Investigación 2016-Fertilidad*). ARM was awarded a fellowship from the *Asociación Española Contra el Cáncer* (*AECC*). We are grateful for the technical support of Serveis Científics i Tecnològics, Campus Bellvitge, Universitat de Barcelona.