

Poster 27

Tumor aggregates from ovarian cancer patients ascitic fluid present low caspase-3 expression

C. Batista-Pinto^{1,2,*}, **A. D. Resende**^{1,2}, **R. Andrade**¹, **F. Garcez**^{1,3}, **V. Ferreira**⁴,
C. Lobo⁴, **P. Monteiro**⁴, **M. H. Abreu**^{5,6}, **C. Bartosch**^{4,6,7} and **S. Ricardo**^{1,2,8,9}

¹ IUCS, Instituto Universitário de Ciências da Saúde, CESPU, CRL, 485-116 Gandra, Portugal

² TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal

³ UNIPRO – Oral Pathology and Rehabilitation Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal

⁴ IPOPOP – Instituto Português de Oncologia do Porto, Departamento de Patologia, 4200-162 Porto, Portugal

⁵ IPOPOP – Instituto Português de Oncologia do Porto, Serviço de Oncologia Médica, 4200-162 Porto, Portugal

⁶ Porto Comprehensive Cancer Center Raquel Seruca, 4200-162 Porto, Portugal

⁷ Grupo Biologia do Cancro & Epigenética, Centro de Investigação do IPOPOP, 4200-162 Porto, Portugal

⁸ i3S – Institute for Research and Innovation in Health, University of Porto, Differentiation and Cancer Group, 420-135 Porto, Portugal

⁹ FMUP – Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal

* Correspondence: carlam.pinto@iucs.cespu.pt

Abstract

Background: Ascites is observed in ovarian cancer advanced stages because of the inflammatory process caused by tumor cells invasion of the peritoneal cavity [1,2]. In the ascitic fluid microenvironment, tumor cells can be found isolated or forming aggregates, being key mediators of transceolomic metastization [1,2]. *In vitro* multicellular spheroids show anoikis resistance presenting high survival levels when compared to isolated tumor cells [3]. The viability of these tumor cells is crucial for the establishment of patient-derived organoids (PDOs) that constitute a valuable preclinical platform for drug testing [3]. **Objective:** This study aims to evaluate the tumor cells apoptotic levels (single cells and aggregates) in the ascitic fluid of ovarian cancer patients. **Methods:** We evaluated 23 cytologic samples from ovarian cancer patients with ascites admitted at IPOPOP under a project approved by IPOPOP ethics committee (CES.092R1/019). Standard histologic processing was performed on the formalin-fixed and Histogel™ embedded ascitic fluid. For the apoptotic cell detection an immunohistochemistry technique with anti-caspase-3 antibody was applied and evaluated by microscopy. **Results:** During standardization, the ideal primary antibody concentration and incubation time were set, as also the antigenic retrieval procedure was optimized. We included a positive control to validate the technique in each run. Our results show that, in most of the samples, cellular aggregates were negative for caspase-3 expression (>75% of the cells) but some positivity was observed in isolated tumor cells. **Conclusions:** The evaluation of caspase-3 expression by immunohistochemistry proved to be a reliable methodology to evaluate the apoptotic levels in cytology samples. In general, tumor cells within aggregates showed high viability levels, whereas some isolated tumor cells presented caspase-3 expression, which indicate they are undergoing an apoptotic process. The tumor aggregates high viability in these samples is a good indicator that the establishment of PDOs from these tumor cells will be successful.

Keywords: ascites; tumor aggregates; apoptosis; immunohistochemistry; patient-derived organoids

Acknowledgments

This work was developed in association with i3S/IPATIMUP, an Associate Laboratory of the Ministério da Ciência, Tecnologia e Ensino Superior (MCTES), and partially supported by Fundação para a Ciência e Tecnologia (FCT)/MCTES. This research was supported by CESPU, grant number “OVCARTEST_GI2-CESPU_2022” and TOXRUN.

References

1. Nunes, D.; Ricardo, S. Ovarian Cancer Ascites as a Liquid Tumor Microenvironment. In *Ovarian Cancer* [Internet]; Lele S., editor; Brisbane (AU): Exon Publications, 2022; Chapter 3.
2. Al Habyan, S.; Kalos, C.; Szymborski, J.; McCaffrey, L. Multicellular detachment generates metastatic spheroids during intra-abdominal dissemination in epithelial ovarian cancer. *Oncogene* **2018**, *37*(37), 5127–5135.
3. Nunes, M.; Ricardo, S. Chemoresistance in Ovarian Cancer: The Role of Malignant Ascites. In *Ovarian Cancer* [Internet]; Lele S., editor; Brisbane (AU): Exon Publications, 2022; Chapter 2.



In *Scientific Letters*, works are published under a CC-BY license (Creative Commons Attribution 4.0 International License at <https://creativecommons.org/licenses/by/4.0/>), the most open license available. The users can share (copy and redistribute the material in any medium or format) and adapt (remix, transform, and build upon the material for any purpose, even commercially), as long as they give appropriate credit, provide a link to the license, and indicate if changes were made (read the full text of the license terms and conditions of use at <https://creativecommons.org/licenses/by/4.0/legalcode>).