Oncology 2025
The Cancer Control Challenge:
Better Understanding It to Better Face It

GENETIC AND MORPHOLOGICAL CHARACTERIZATION OF A CELL LINE OF ADENOID CYSTIC CARCINOMA OF THE SALIVARY GLAND

Alessandra Santos dos Santos[1], Fernanda Jardim da Silva[1], Thaís Ferreira da Silva[1], Juscelino Carvalho de Azevedo Junior[1], Danielle Queiroz Calcagno[1].

[1]Center for Oncology Research, Federal University of Pará (UFPA)

Introduction: Adenoid cystic carcinoma (ACC) is a rare tumor of the salivary glands, with slow growth but high potential for perineural invasion and metastasis. Genetic alterations, such as *MYB* gene rearrangements and DNA copy number variations (CNAs), are common in these tumors. Although surgery is the standard treatment, its efficacy is limited in metastatic cases, and the scarcity of in vitro models hinders progress in translational research. **Objectives:** To characterize morphologically and genetically a cell line derived from ACC. **Methods:** The study was approved by the Research Ethics Committee of the Federal University of Pará (CAAE: 09239518.9.0000.0018). A tumor fragment located in the trigone of the salivary gland was obtained from a 59-year-old female patient treated at Hospital Ophir Loyola. After washing and mechanical dissociation, a portion of the sample was used for DNA extraction and the other was cultured in D-MEM medium, incubated at 37°C with 5% CO_2. Cell proliferation kinetics were analyzed at passage 45, and the growth curve was generated using GraphPad Prism. A total of 3.17×10^3 cells/well were seeded and counted at 24, 48, 72, and 96 hours. DNA was extracted from tumor tissue and the 59th cell passage using the QiAmp DNA Isolation Kit, followed by quantification with Nanodrop and validation via agarose gel electrophoresis. Morphological analysis was performed by histopathology. Immunophenotyping was conducted by immunofluorescence using antibodies against vimentin, cytokeratin AE1/AE3, cytokeratin 19 (CK19), and alpha-smooth muscle actin (α-SMA). Cytogenetic analysis was performed using array comparative genomic hybridization (aCGH) with the SurePrint G3 CGH+SNP platform (Agilent), considering significant alterations those with logRatio > 0.25 or < −0.25. **Results:** Histopathological analysis of the tumor revealed basaloid myoepithelial cells, tubular growth pattern, perineural invasion, and muscle infiltration. The derived NAT cell line exhibited an adherent phenotype and reached stable growth after the 30th passage, without the need for genetic manipulation for immortalization. The growth curve showed exponential proliferation over the 96-h observation period, with no stationary phase and a doubling time of approximately 29.4 h. Immunofluorescence revealed high expression of vimentin, low expression of AE1/AE3, and moderate expression of CK19 and α-SMA, suggesting a predominantly mesenchymal and myoepithelial phenotype. aCGH analysis showed shared alterations between the tumor and the cell line, including amplifications at 8p11.22 (*ADAM9*) and 22q11.22 (*MAPK1*), and a deletion at 19p13.2 affecting the *CDKN2D*, *KEAP1* and *SMARCA1* genes. **Conclusion:** According to the proposal of

this work, it was possible to characterize morphologically and genetically the cell line originating from ACC. The genomic alterations identified highlight its value as a representative model for the investigation of molecular mechanisms, for the development of biomarkers and targeted therapies.

Keywords: Adenoid cystic carcinoma; Oral cancer; Molecular profiling; Targeted therapy.