SESSIONE: ***Advances in cancer research and therapeutics***

EXPLORING THE COOPERATIVE EFFECT OF OSILODROSTAT AND MITOTANE IN 3D SPHEROID MODELS OF ADRENOCORTICAL CARCINOMA

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Adrenocortical carcinoma (ACC) is a rare and aggressive neoplasm, often associated with poor prognosis, due to the limited therapeutic options currently available. Mitotane is the only adrenolytic therapy approved for the treatment of this tumor. Hypercortisolism is a frequent clinical manifestation in patients with ACC [1]. Osilodrostat, a steroidogenesis inhibitor used in the treatment of Cushing's syndrome, may also be effective for controlling hypercortisolism in patients with ACC [2]. However, despite the clinical use of these two drugs, there is a lack of experimental data clarifying their direct interaction at the cellular level in adrenal tumor cells. In this study, the steroid-secreting cell line NCI-H295R was used as a model of adrenal carcinoma, cultured in three-dimensional (3D) systems capable of more realistically mimicking the tumor *in vivo* microenvironment [3]. The cytotoxicity of osilodrostat and mitotane was evaluated in both 3D cultures (spheroids) and traditional monolayer cultures (2D). Osilodrostat did not show a direct cytotoxic effect, confirming its predominantly functional role in steroidogenesis. In contrast, mitotane induced a dose-dependent reduction in cell viability, with an IC_{50} value in spheroids of the same order of magnitude as the therapeutic window observed in patients. The interaction of the two drugs in combination was then evaluated in 3D models using a dose-response matrix and a pharmacological analysis based on the Highest Single Agent (HSA) model, which supports the presence of a cooperative interaction at the functional concentrations of the two compounds. From a morphological point of view, the combination of the two drugs resulted in a significant reduction of spheroid size, greater than that observed after single adrenolytic treatment with mitotane. Fluorescence-based viability staining and direct viable cell counting confirmed these observations. *In silico* molecular docking analyses suggested that osilodrostat and mitotane interact with CYP11B1 through distinct, non-simultaneous binding interactions in the enzyme's heme-containing pocket, which could enable simultaneous mitotane binding at another site, generating a cooperative effect. Overall, these results validate the value of three-dimensional ACC models for studying drug interactions, lay the groundwork for further investigation into the mechanisms underlying the cooperativity of the two drugs, and provide preclinical justification for combined therapeutic strategies that combine steroidogenesis inhibition and adrenolytic treatment.

References

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