
SECTION 2: BASIC SCIENCE

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Development and characterization of novel cholangiocarcinoma cell sublines resistant to cisplatin or 5-fluorouracil

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Introduction: The efficacy of pharmacological treatment for cholangiocarcinoma (CCA) is very limited due to the presence of effective chemoresistance mechanisms. Therefore, there is an urgent need to improve therapeutic options, which requires the identification of new molecular targets. The development of novel 2D and 3D cellular models sensitive and resistant to chemotherapy enables progress in understanding the resistome and contributes to the search for new therapeutic tools.

Aim: To generate and characterize the resistome of chemo-resistant sublines derived from human CCA cells (EGI-1) resistant to cisplatin or 5-fluorouracil (5-FU), for their use in 2D and 3D cultures and the identification of new therapeutic targets.

Methods: Chemoresistant cells were generated by prolonged exposure to increasing concentrations of the drugs. Revertant cells were obtained by withdrawal of drug pressure. Cross-resistance was assessed by viability assays (MTT). Proliferation, migration, colony formation, and cell cycle distribution were analyzed using holographic microscopy and flow cytometry. Tumorigenic capacity was evaluated in immunodeficient athymic (nu/nu) mice. Resistome

gene expression was determined by RNA-seq. Tumor-derived organoids were generated from xenograft tumors.

Results: Two resistant sublines were established against cisplatin (EGI-1 CR) and 5-FU (EGI-1 FR), along with their corresponding revertant sublines. EGI-1 CR cells exhibited cross-resistance to oxaliplatin, whereas EGI-1 FR cells were markedly resistant to gemcitabine and irinotecan. Both resistant sublines showed reduced proliferation, migration, and colony-forming ability, as well as alterations in cell cycle progression, while retaining tumorigenic capacity in vivo. Upon drug withdrawal, revertant cells recovered proliferation and migration, while partially maintaining resistance, suggesting phenotypic reversibility. RNA-seq analysis identified approximately 200 differentially expressed genes among the groups. In addition, organoids were generated from tumors derived from EGI-1 WT and resistant cells, providing complementary preclinical models, and some of the changes observed in the RNA-seq analysis were validated.

Conclusion: Two in vitro models of CCA cells in 2D and 3D cultures resistant to cisplatin or 5-FU have been developed and characterized. The partial reversibility of resistance after drug withdrawal suggests dynamic adaptive mechanisms. These models represent valuable tools for studying the resistance and for the preclinical evaluation of new therapeutic strategies in CCA.