**EPITHELIAL-TO-MESENCHYMAL TRANSITION INDUCED DRUG RESISTANCE IN HEPATOCELLULAR CARCINOMA DERIVED CANCER STEM CELLS**

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Lay Abstract

The global burden of Hepatocellular carcinoma (HCC) is increasing partly due to the limited treatment options available for this disease and recurrence due to therapy resistance. The mechanism of therapy resistance in many cancers including HCC is implicated to be through specialized cell populations known as cancer stem cells (CSCs) whose emergence is dependent on a phenomenon called epithelial-to-mesenchymal transition (EMT). The aim of this study to characterize these CSCs and examine the relationship between CSCs and EMT. We enriched CSCs and examined various characteristics that suggests these CSCs are drug resistant and aggressive cell populations that should be targeted. We found that CSCs are more resistant to drugs and possess EMT traits making them motile and aggressive. Thus, our study highlights the role of EMT in emergence of drug resistant CSCs in HCC and that these CSCs can be used as targets for treating HCC.

Scientific Abstract

Epithelial-to-mesenchymal transition (EMT) responsible for emergence of cancer stem cells (CSCs) is implicated as the mechanism of therapy resistance in cancers including HCC. The aim of this study is to enrich CSCs from human and murine HCC cell lines and to examine the relationship between therapy resistance and EMT properties of CSCs. A modified serum-free culture system was used to enrich stem-like cells from human and mouse HCC cell lines which were assessed by CSC markers and high resolution multiphoton fluorescence lifetime imaging (MPM-FLIM). The effect of Doxorubicn was assessed by MTS assay and EMT traits were assessed via expression of EMT markers E-cadherin, Vimentin, Snail and Slug. Motility assay was used to examine the relationship between drug resistant CSCs and EMT. The clinical relevance of our findings were examined using HCC patient datasets available in bioinformatics platform SurvExpress. Three-dimensional spheres that expressed enhanced stemness markers *CD133* and *CD44* were enriched. CSCs showed significantly higher cell viability to Doxorubicin. The role of EMT in emergence of CSCs is shown by downregulation of *E-Cadherin* and upregulation of *Vimentin, Snail* and *Slug* in CSCs along with higher motility of CSCs. The HCC patient datasets showed that expression of EMT markers resulted in worse overall survival and recurrence free survival further supporting the link of EMT to therapy resistance in HCC. Our study highlights the role of EMT as a key driver for drug resistance in HCC. CSCs that exhibit distinctive EMT markers can be utilized as biomarkers and druggable targets for HCC.