ROLE OF CD36 AND FREE FATTY ACID UPTAKE IN EPITHELIAL-MESENCHYMAL TRANSITION OF HEPATOCELLULAR CARCINOMA CELLS

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Hepatocellular carcinoma (HCC) is the third-leading cause of cancer-related death worldwide. The liver is the main site of free fatty acid (FFA) metabolism; epidemiological studies link HCC tumorigenesis and elevated mortality rates to obesity, which manifests as increased FFA. Previous studies investigated the cytotoxic and pro-inflammatory effects of FFA, but did not focus on its influence on HCC progression. In this study, we hypothesized that elevated FFAs induce the epithelial-mesenchymal transition (EMT) program, facilitating a metastatic, invasive HCC phenotype.

We investigated the association between obesity, EMT progression and alterations in FFA-uptake proteins in TCGA HCC gene expression data and validated the results in protein samples from human HCC tumors. In order to scrutinize the phenotypic effects of elevated FFA, we assessed the migratory and invasive ability of HCC cell lines treated with various FFAs, and further verified the expression of EMT markers using qPCR, confocal microscopy and flow cytometry.

Bioinformatic analysis of TCGA data revealed that obese patients have higher levels of CD36, a trans-membrane protein that facilitates FFA transport into the cell. CD36 expression levels were strongly correlated with an EMT gene signature. However, the degree of EMT itself was not associated with the body mass index (BMI) of the patients. These results were corroborated in protein measurements from human HCC tumor samples. Additionally, we observed that saturated and monounsaturated FFA-treated HCC cell lines exhibited increased migration, invasion, dissociation, and development of the characteristic EMT morphology. Next, we confirmed the expression of EMT markers using qPCR and confocal imaging, demonstrating that chemical inhibition of CD36 reversed the FFA-induced EMT phenotype. Given the known cytotoxic effects of elevated FFAs on hepatocytes, we tested a population-distribution hypothesis using flow cytometry. We found that although some cells succumbed to the cytotoxic effects of high FFA, a distinct population not only evaded cell death, but also acquired EMT. We further utilized PCR arrays to determine that Wnt and TGF-beta signaling pathways, potential drivers of the EMT program, were activated upon FFA treatment and showed that repression of these pathways prevented migration and invasion in FFA treated cells.

Our research demonstrates the role of CD36 and FFAs in activating the EMT program via induction of Wnt and TGF-beta signaling, and provides the first direct evidence that elevated FFA uptake promotes progression of HCC through EMT. Further elucidation of this program may provide insights for management of advanced HCC