Cancer-Associated Fibroblasts Regulate Tumor-Initiating Cell Plasticity in Hepatocellular Carcinoma through c-Met/FRA1/HEY1 Signaling

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Background: Hepatocellular carcinoma (HCC) is one of the deadliest malignancies worldwide. Increasing evidence showed that tumor-initiating cells (T-ICs) are responsible for treatment failure and tumor relapse of HCC. Like normal stem cells, T-ICs are regulated extrinsically within the tumor microenvironment. Because HCC develops primarily in the context of cirrhosis, in which there is an enrichment of activated fibroblasts, we hypothesized that cancer-associated fibroblasts (CAFs) would regulate liver T-ICs. **Methods:** The clinico-pathological relevance of α-SMA(+) CAF was evaluated in a large cohort of HCC clinical samples by immuno-histochemistry (IHC). CAFs from fresh HCC samples were isolated, and the conditioned medium (CM) of CAFs was collected to examine its effect on functional properties of liver T-ICs. By cytokine profiling, we identified the cytokine preferentially secreted by CAFs that was critical for regulating liver T-ICs. The molecular pathway mediating the regulation was identified by RNA-sequencing analysis and verified by functional experiments. **Results:** IHC analysis revealed that the presence of α-SMA(+) CAFs was correlated with poor clinical outcome in HCC patients. By collecting the CM of CAFs, we found that CAFs enriched the population of liver T-IC through paracrine secretion as evidenced by the enhanced tumorigenicity, spheroid forming ability, expression of liver T-IC markers, tumor invasiveness, and chemoresistance. Cytokine profiling identified hepatocyte growth factor (HGF) to be the most potent CAF-derived cytokine in promoting liver T-IC properties, and this finding was further confirmed by blocking HGF/c-Met pathway activation with HGF neutralizing antibody and c-Met kinase inhibitor. To delineate the downstream mechanism by which HGF/c-Met signaling regulated liver T-ICs, a cDNA microarray analysis was employed and we identified FOS-like antigen 1 (FRA1) to be significantly upregulated upon HGF treatment. Stable knockdown of FRA1 abolished HGF-induced T-IC properties, which confirmed the functional importance of FRA1 in HGF/c-Met-mediated T-IC phenotypes. Further investigation by RNA-sequencing analysis identified HEY1 as a direct downstream effector of FRA1, which was confirmed by subsequent rescue

experiments and promoter activation analysis. Using the STAM non-alcoholic steatohepatitis (NASH)-HCC mouse model, we found that HGF-induced FRA1 activation was associated with fibrosis-dependent HCC development. **Conclusions:** CAFs regulate liver T-ICs through the HGF-mediated c-Met/FRA1/HEY1 cascade, which represents a potential therapeutic target for the treatment of HCC.

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