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ANNEXIN A3 IS A THERAPEUTIC TARGET FOR CD133+ LIVER CANCER STEM CELLS

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Frequent tumor relapse in hepatocellular carcinoma (HCC) has been commonly attributed to the failure to completely eradicate cancer stem cells (CSCs) in the tumor residues by conventional treatments. We have previously reported that the tumor growth of HCC is fuelled, at least in part, by a small subset of CSCs marked by the CD133 surface phenotype. Our present study aims 1) to delineate the molecular mechanism by which CD133+ liver CSCs mediate HCC tumor formation and progression; and 2) to develop a novel diagnostic / prognostic biomarker and targeted therapy for HCC detection and treatment. RNA-Seq profiling was employed to compare the gene expression profiles between sorted CD133+ and CD133- subsets isolated from HCC cell lines Huh7 and PLC8024. Annexin A3 (ANXA3) was identified as the most significantly up-regulated secretory protein in the CD133+ subset. Validation in additional HCC cell lines and clinical samples likewise showed ANXA3 to be preferentially expressed in the CD133+ subset. Up-regulation of ANXA3 in both endogenous and secretory forms was tightly associated with advanced tumor stages. In addition, quantification of serum ANXA3 provides a novel biomarker with high specificity and sensitivity for HCC diagnosis. Functional studies involving lentiviral-based knockdown and overexpression approaches found ANXA3 to regulate cancer and stem cell-like properties including tumor initiation, migration, invasion, angiogenesis, self-renewal and resistance to chemotherapy and apoptosis. Subsequent gene expression profiling by cDNA microarray found ANXA3 to be functionally involved in driving CD133+ CSCs via a deregulated JNK/AP-1 pathway. In light of the functional significance and clinical relevance of ANXA3 in HCC, we subsequently developed a novel neutralizing monoclonal antibody specific against ANXA3 and tested for its application as a therapeutic treatment against HCC. In vitro functional studies showed that ANXA3 neutralizing antibody treatment dramatically reduced cell proliferation, sphere formation, migration, invasion, angiogenesis, and sensitized cells to apoptosis and cisplatin treatment in vitro. This observation was further confirmed in vivo where neutralizing antibody treatment attenuated tumor growth, concomitant with a reduced CD133+ subset in the residual xenografts. Mechanistically, treatment of HCC cells with ANXA3 neutralizing antibody in vitro and in vivo, similarly led to a suppressed JNK pathway. Taken together, ANXA3 confers both cancer and stem cell-like properties in CD133+ liver CSCs via JNK pathway. ANXA3 neutralizing antibody possesses therapeutic effect in HCC by eliminating CD133+ liver CSCs. We believe that ANXA3 could serve as a potential therapeutic target to attain complete eradication of HCC.