Identification of the Subcellular Localization of Alternatively Spliced Tissue Factor in Hepatocytes in Health and in Disease

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Introduction: According to the American Cancer Society, more than 800,000 people are diagnosed with liver cancer each year while more than 700,000 deaths per year are associated with liver cancer. In recent years, the clinical use of biomarkers has exploded and it is believed that alternatively spliced tissue factor (asTF) could be a possible biomarker for liver diseases due to its unique biochemical structure and functional characteristics. Unpublished observations in the Bogdanov lab revealed that asTF is expressed at high levels in hepatocytes of tissue samples obtained from patients suffering from chronic liver disease. Interestingly, the pattern of asTF protein expression that we observe in diseased human and murine hepatocytes suggests that it is stored in specific granular (sub)compartments. With this in mind, the aim of this project will be to perform in- detail examination of the subcellular localization of asTF in healthy and diseased human and murine liver specimens.

Methods: All experiments were performed utilizing the Huh7 hepatic cell line cultured in medium 199 under conditions of Serum Free medium, 2% Fetal Bovine Serum (FBS), and 10% FBS, as well as under normoxia and hypoxia conditions. Immunofluorescence staining was the performed to visualize the golgi apparatus, endoplasmic reticulum, nuclei, exocytotic vesicles, as well as asTF. PDI, Calnexin, DAPI, and EXOC2 were utilized to visualize the golgi apparatus, endoplasmic reticulum, nuclei, exocytotic vesicles, respectively. The samples were then mounted to a coverslip and imaged utilizing super-resolution microscopy. Colocalization analysis was performed.

Results: Under hypoxic conditions, asTF appears to display perinuclear localization for Serum Free, 2% FBS, and 10% FBS mediums. Additionally, asTF appears to colocalize with exocytotic vesicles. Further imaging and development of the ImageJ pipeline needs to be accomplished before statistical analysis can be performed.

Conclusions: Huh7 cells under hypoxic conditions appear to display perinuclear asTF localization suggesting involvement of the endoplasmic reticulum. asTF also appears to colocalize with exocytotic vesicles. Further analysis needs to be completed in order to perform statistical analysis.

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