Optimization for Tissue Factor Detection in a Mouse Model of Carbon Tetrachloride-Induced Liver Injury

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Evidence suggests activation of the blood clotting cascade drives pathogenesis of chronic liver injury; however, mechanisms driving coagulation activation in this setting are unclear. Our prior findings suggest that coagulation activation following challenge with the hepatotoxicant carbon tetrachloride (CCl_4) is mediated by hepatic non-parenchymal cell tissue factor (TF). The purpose of this study was to optimize in-situ hybridization (ISH) and immunohistochemistry (IHC) assays to detect cellular expression of TF mRNA and protein in the injured liver. Wild-type mice were challenged with CCl_4 or Corn Oil (1mL/kg) for 24 h and formalin-fixed paraffin-embedded liver sections were labeled using a commercially available RNAScope kit for ISH and a goat anti-mouse TF (polyclonal) antibody for IHC. In the ISH, positive and negative control probes were used on liver sections. The positive control showed abundant red staining and negative showed no staining. Mouse *F3* probes showed red staining in livers from mice that express mouse TF (mTF+/+) and no staining in mice that do not express *F3* (mTF-/-). In IHC, heart samples were used as controls as they express high levels of TF, and positive staining was detected using the TF antibody and no staining on sample not treated with antibody. However, when labeling liver samples from mTF+/+ mice and mTF-/- mice, an identical staining pattern was observed. The optimized RNAScope protocol demonstrated specificity, allowing for accurate detection of TF (*F3*) mRNA. The IHC method exhibited non-specific staining, rendering it unreliable for TF protein detection in this model. Supported by: NIH R25ES020721-14 and the ASPET SURF program