Research Interest in Hepatitis C Viruses

We are enthusiastic to work on Hepatitis C virus (HCV), a known causative agent of chronic hepatitis, fibrosis, cirrhosis, and eventually hepatocellular carcinoma. It has been shown that Osteopontin (OPN), a secreted phosphoprotein, originally characterized in malignant-transformed epithelial cells which is associated with tumor metastasis of several tumors. Importantly, OPN is overexpressed in hepatocellular carcinoma (HCC) tissue involving HCC invasion and metastasis. It is also significantly up-regulated in liver injury, inflammation, and hepatitis C virus (HCV)-associated HCC. However, the underlying mechanisms of OPN activation and its role in HCV-mediated liver disease pathogenesis are not known.

Hepatitis C virus (HCV) replication and assembly occur at the specialized site of endoplasmic reticulum (ER) membranes and lipid droplets (LDs), respectively. Recently, we demonstrated the important relationship among osteopontin (OPN), the ER, and LDs. Our current studies show a very significant reduction in HCV replication, assembly, and infectivity in presence of small interfering RNA (siRNA) against OPN, αVβ3, and CD44. We also observed the association of endogenous OPN with HCV proteins (NS3, NS5A, NS4A/B, NS5B, and core). Confocal microscopy revealed the colocalization of OPN with HCV NS5A and core in the ER and LDs, indicating a possible role for OPN in HCV replication and assembly. Interestingly, the secreted OPN activated HCV replication, infectivity, and assembly through binding to αVβ3 and CD44. Our studies uncovered the importance of OPN in HCV replication and assembly. These observations provide evidence that HCV-induced endogenous and secreted OPN play pivotal roles in HCV replication and assembly in HCV-infected cells (Fig. 1). Taken together, our findings clearly demonstrate that targeting OPN may provide opportunities for therapeutic intervention of HCV pathogenesis.
**Research Interest in Herpes Viruses**

We are also interested to work on Herpes viruses and host innate response. Previously, our lab and others have shown that IFI16 (gamma-interferon-inducible protein 16), a predominantly nuclear protein involved in transcriptional regulation, also functions as an innate immune response DNA sensor and induces the IL-1β and antiviral type-1 interferon-β (IFN-β) cytokines. We have shown that IFI16, in association with BRCA1, functions as a sequence independent nuclear sensor of episomal dsDNA genomes of KSHV, EBV and HSV-1. Recognition of these herpesvirus genomes resulted in IFI16 acetylation, BRCA1-IFI16-ASC-procaspase-1 inflammasome formation, cytoplasmic translocation, and IL-1β generation. Acetylated IFI16 also interacted with cytoplasmic STING and induced IFN-β. However, the identity of IFI16 associated nuclear proteins involved in STING activation and the mechanism is not known. Mass spectrometry of proteins precipitated by anti-IFI16 antibodies from uninfected endothelial cell nuclear lysate revealed that histone H2B interacts with IFI16. Single and double proximity ligation microscopy, immunoprecipitation, EdU-genome labeled virus infection, and chromatin immunoprecipitation studies demonstrated that H2B is associated with IFI16 and BRCA1 in the nucleus in physiological conditions. De novo KSHV and HSV-1 infection as well as latent KSHV and EBV infection induces the cytoplasmic distribution of H2B-IFI16, H2B-BRCA1 and IFI16-ASC complexes. Vaccinia virus (dsDNA) cytoplasmic replication didn't induce the redistribution of nuclear H2B-IFI16 or H2B into the cytoplasm. H2B is critical in KSHV and HSV-1 genome recognition by IFI16 during de novo infection. Viral genome sensing by IFI16-H2B-BRCA1 leads to BRCA1 dependent recruitment of p300, and acetylation of H2B and IFI16. BRCA1 knockdown or inhibition of p300 abrogated the acetylation of H2B-IFI16 or H2B. Ran-GTP protein mediated the translocation of acetylated H2B and IFI16 to the cytoplasm along with BRCA1 that is independent of IFI16-ASC inflammasome. ASC knockdown didn't affect the acetylation of H2B, its cytoplasmic transportation, and the association of STING with IFI16 and H2B during KSHV infection. Absence of H2B didn't affect IFI16-ASC association and cytoplasmic distribution and thus demonstrating that IFI16-H2B complex is independent of IFI16-ASC-procaspase-1-inflammasome complex formed during infection. The H2B-IFI16-BRCA1 complex interacted with cGAS and STING in the cytoplasm leading to TBK1 and IRF3 phosphorylation, nuclear translocation of pIRF3 and IFN-β production. Silencing of H2B, cGAS and STING inhibited IFN-β induction but not IL-1β secretion, and cGAMP activity is significantly reduced by H2B and IFI16 knockdown during infection. Silencing of ASC inhibited IL-1β secretion but not IFN-β secretion during de novo KSHV and HSV-1 infection. These studies identify H2B as an innate nuclear sensor mediating a novel extra chromosomal function, and reveal that two IFI16 complexes mediate KSHV and HSV-1 genome recognition responses, with recognition by the IFI16-BRCA1-H2B complex resulting in IFN-β responses and recognition by IFI16-BRCA1 resulting in inflammasome responses (Fig. 2). We are interested to discover that how viruses evade antiviral defense mechanism to stay longer in the host cells for further consequences by manipulating immune system.
Fig. 2