Purpose/Objective(s): Hepatitis B virus (HBV) reactivation is one unique pathogenesis in Asian carriers with liver toxicity after radiotherapy for hepatobiliary malignancies. In vitro radiation (RT)-induced HBV replication is mediated through the bystander effect of irradiated endothelial cells on releasing interleukin-6 (IL-6). This study attempts to delineate the in vivo effect and mechanism of IL-6 on RT-induced HBV reactivation.

Materials/Methods: Sixteen HBV transgenic mice with no immune-competence on HBV were treated with whole liver RT (4 Gy qd for 5 days) with or without pre-RT and concomitant treatment of IL-6 (400 MU bid for 15 days). Serum HBV DNA was measured by real-time quantitative PCR, while IL-6 concentration was by ELISA. Immunohistochemical staining of mouse liver with HBV core protein and phosphorylated signal transducer and activator of transcription (STAT)3 was qualitatively analyzed. HepG2.2.15 cells (a human hepatoblastoma cell line transfected with HBV DNA) were used to investigate the molecular mechanism.

Results: Radiation induced HBV reactivation was in mice treated with combined IL-6/RT (3.77±1.84 fold) but not with RT (1.35±0.66 fold, p=0.0004), IL-6 (1.06±0.17 fold, p=0.0004), or sham groups (1.00±0.09 fold, p=0.0003). The average IL-6 concentrations from week 2 to week 8 were 9.2±0.3 pg/ml in sham, 18.3±10.8 pg/ml in IL-6, 18.2±2.2 pg/ml in RT, and 27.5±6.7 pg/ml in IL-6+RT groups, respectively. Serum IL-6 concentration in IL-6+RT group was significantly higher than either sham (p=0.00001) or RT group (p=0.004). The difference in serum IL-6 concentration was borderline significant between IL-6+RT and IL-6 groups (p=0.08), but was not statistically different between IL-6 and RT groups (p=0.97). HBV core protein staining of mouse liver confirmed the intrahepatic HBV replication. IL-6/RT induced HBV DNA replication in HepG2.2.15 cells, which was suppressed by STAT3 inhibitor AG490. Dominant-negative STAT3 plasmid transfection showed its inhibition in IL-6/RT reactivated HBV. The strongest staining of phosphorylated STAT3 was revealed in liver treated with IL-6/RT. This reactivation was through the interaction of phospho-STAT3/ hepatocyte nuclear factor-3 complex and HBV enhancer 1 in mobility shift assay. HepG2.2.15 cells treated with wild-type footprint V, but not mutant-type footprint V decoy oligodeoxynucleotides before IL-6/RT treatment, showed significant inhibition in HBV DNA (from 4.1±0.5 fold increase in IL-6/RT group to 1.6±0.4 fold in decoy group, p=0.0003).

Conclusions: Radiation induced HBV reactivation in liver is from the non-immune bystander synergistic effect of IL-6 and RT through STAT3 signal transduction pathway. IL-6 might be a potential biomarker to quantify RT-induced liver damage.

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