

Hepatic Stellate Cell-mediated Increase in CCL5 Chemokine Expression Following X-ray Irradiation Determined In Vitro and In Vivo

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SUPPLEMENTARY INFORMATION

SUPPLEMENTARY MATERIALS AND METHODS

Quantification of C-C motif chemokine receptor 5 (Ccr5) gene expression in the liver

Frozen OCT-embedded livers were prepared from male mice 1 week after irradiation with 0, 1.9, and 3.8 Gy X-rays. Then, RNA was extracted from the frozen OCT-embedded livers with BioMasher II (Nippi, Tokyo, Japan), TRIzol Reagent (Thermo Fisher Scientific) and Direct-zol RNA MiniPrep (Zymo Research). As described in Section 2.8, real-time PCR analysis for *Ccr5* gene expression was performed using the primer set: forward, 5'-GTCTACTTTCTCTTCTGGACTCC-3' and reverse, 5'-CCAAGAGTCTCTGTTGCCTGCA-3'.

SUPPLEMENTARY FIGURE LEGENDS

FIG. S1. Quantification of interface hepatitis in liver tissue sections 1 week after X-ray irradiation in 1-week-old mice. The vertical axis represents the percentage of the Glisson's sheaths (that represent interface hepatitis). The numbers in parentheses indicate the number of Glisson's sheaths on hematoxylin and eosin-stained liver tissue sections. Data were obtained from three mice for each radiation dose. Differences in percentages were evaluated using Fisher's exact test with Bonferroni correction (0 Gy vs. 1.9 Gy: $P = 0.018$).

FIG. S2. Number of isolated HSCs per mouse. The vertical axis represents the mean number of isolated HSCs per mouse. The horizontal axis shows the mean weight per mouse. Each plot was obtained from one isolation experiment using approximately 10 female and male mice from the same litter. Isolation experiments were performed 33 times ($N = 33$). Pearson's correlation value (P) = 0.407, $P = 0.019$. HSCs, hepatic stellate cells.

FIG. S3. Representative images for secreted IL-1 β , IL-6, and TNF- α protein levels in cultured hepatic stellate cells 7-days post in vitro X-ray irradiation. The images were obtained from the same cytokine array as Figure 3C. The top and bottom images indicate 0 and 3.8 Gy samples, respectively. Two spots surrounded by squares indicate each protein secretion.

FIG. S4. Analyses of *Ccr5* gene expression in the liver after in vivo irradiation. Real-time PCR assessment of *Ccr5* gene expression levels in the liver from male mice 1 week after irradiation with 0, 1.9, and 3.8 Gy X-rays. The vertical axis represents the relative expression of *Ccr5* gene per *Gapdh* gene. All values are represented as mean \pm SD. Data were obtained from three mice ($N = 3$) for each radiation dose. *Ccr5*, C-C motif chemokine receptor 5; *Gapdh*, glyceraldehyde-3-phosphate dehydrogenase.