IW-04
A Quantitative Estimation System for Fibrosis in Non-alcoholic Steatohepatitis by Using Transgenic Collagen Promoter/Luciferase Reporter Mouse
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Keywords: Liver fibrosis, Non-alcoholic steatohepatitis, Methionine-choline deficient diet
Background & Aims: Non-alcoholic fatty liver diseases including its progressive form, non-alcoholic steatohepatitis (NASH), are often associated with metabolic syndrome. Early detection and treatment of NASH are very important for preventing its progression to liver fibrosis and cirrhosis. In order to seek pharmaceutical agents that suppress fibrogenesis at an early stage of NASH, here we report a quantitative estimation system to detect activation of type I collagen promoter in a murine NASH model.
Methods: Transgenic mice harboring collagen gene (COL1A2) enhancer/promoter sequences linked to a firefly luciferase gene were fed with methionine-choline deficient diet (MCDD). Mice were sacrificed 2 or 4 weeks later to collect serum and liver tissue. Histopathological changes were evaluated by hematoxylin-eosin and Mallory-Azan staining, and activation of hepatic stellate cells, the major source of collagen in the fibrotic liver, was detected by α-smooth muscle actin (SMA) immunostaining. COL1A2 promoter activities in liver were determined by luciferase assays of tissue homogenates.
Results: The mean levels of serum alanine aminotransferase in mice fed with control diet and in those with MCDD are 25 U/L and 160 U/L after 2 weeks, and 41 U/L and 193 U/L after 4 weeks, respectively. Neutrophil infiltration and lipid droplets were observed in the liver parenchyma after 2 weeks of MCDD feeding. Although those histopathological findings became more evident, α-SMA expression and accumulation of collagen fibers were still limited after 4 weeks. On the other hand, luciferase activity in liver tissue was significantly increased up to 217% in mice fed with MCDD for 4 weeks as compared with control animals.
Conclusions: By using transgenic reporter mice, COL1A2 promoter activation can be detected sensitively and quantitatively at an early stage of liver fibrosis associated with experimental NASH, which can be a good system to evaluate anti-fibrotic agents for the treatment of NASH.

IW-05
Versican expression is transient during wound healing but continues at high levels in keloid: Role of versican in keloid formation in a new mouse model
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Keywords: Versican, Keloids, Wound healing
Background & Aims: Keloids are a refractory disease characterized by excessive deposition of extracellular matrices (ECMs). We previously found that increased expression of versican, a large chondroitin sulfate proteoglycan, was one of the key features that characterize keloid tissues as well as cultured keloid cells (KL cells). In this study, factors regulating versican expression were investigated with KL cells and normal dermal fibroblasts using RT-PCR and luciferase assay. KL cells showed two-fold higher transcription level. Screening of Wnt, β catenin, TGF-β, androgen, IL-1β and PI3K showed the latter two signals were involved in versican gene regulation.
Versican expression in normal skin wound healing process was also studied using C57BL mice. Immunohistochemistry showed transient versican expression which reached the maximum at 5 days post-wounding. To trace the destination of versican-expressing cells, we generated transgenic mice expressing versican promoter-Cre recombinase/rosa26. The number of LacZ positive cells reached the maximum at 5 days post-wounding, thereafter decreased and disappeared within 14 days. We hypothesized from these observations that persistent survival and proliferation of these versican-expressing cells might be involved in the keloid pathogenesis.
In order to establish the experimental model of keloids, we implanted KL cells with collagen sponge scaffolds in nude mice. Sponges with KL cells (KL sponges) appeared thicker and more opaque and weighed significantly heavier than those with normal fibroblasts (normal fb sponges) after 4 weeks. KL sponges deposited more versican than normal fb sponges did, which suggested that this model reflected the ECMs-producing characteristics of keloids. Administration of IL-1β and chondroitinase ABC to KL sponges for 4 weeks successfully suppressed the deposition of glycosaminoglycans as well as the increase in weight. Thus, this in vivo model could provide a valuable tool for the evaluation of therapeutic reagents targeted for keloids.