A03-5 The Origin and Early Dynamics of Collagen-Producing Cells in Cholestatic Liver Fibrosis

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**Background & Aims:** Portal (myo) fibroblasts are closely related with cholestatic liver fibrosis, and epithelial-to-mesenchymal transition (EMT) of cholangiocytes is implicated in the fibrogenic process. However, recent fate mapping studies have denied contribution of EMT, and the origin of collagen-producing cells has not been fully identified yet. Here we reassessed the origin and early dynamics of collagen-producing cells by using sensitive and specific type I collagen reporter mice.

**Methods:** Transgenic mice harboring tissue-specific enhancer/promoter sequences of alpha2(I) collagen gene linked to an enhanced green fluorescent protein (EGFP) gene (COL/EGFP Tg) were subjected to bile duct ligation (BDL), and co-localization of EGFP and specific cell markers were examined by confocal laser-scanning microscopy. A non-parenchymal cell fraction including hepatic stellate cells (HSC) was isolated from normal or fibrotic liver, and analyzed by immunofluorescent staining and fluorescence-activated cell-sorter scanner (FACS).

**Results:** While only a few EGFP-positive cells were observed in portal areas and hepatic parenchyma of untreated COL/EGFP Tg mice, EGFP-positive cells appeared in the subepithelial areas of bile ducts at as early as day 1 after BDL. Most of them became positive for alpha-smooth muscle actin at day 3. None of the bile epithelial cells expressed EGFP. In FACS analyses of non-parenchymal cells obtained from untreated mice, the majority was EGFP-negative irrespective of their GFAP expression, a representative marker of quiescent HSC. On the other hand, the numbers of both EGFP+GFAP⁻ and EGFP+GFAP+ cells increased at day 2 after BDL.

**Conclusion:** In addition to portal fibroblasts around bile duct, a significant proportion of HSC are involved in collagen production in the onset and progression of cholestatic liver fibrosis.