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Little Contribution of Epithelial-to-mesenchymal Transition of Biliary Epithelial Cells to the Progression of Experimental Biliary Fibrosis

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Background & Aims: It has recently been reported that portal fibroblasts play a central role in the progression of biliary fibrosis, and that epithelial-to-mesenchymal transition (EMT) of biliary epithelial cells might be a source of those collagen-producing cells. Here we examined possible contribution of EMT to the development of experimental biliary fibrosis by using transgenic collagen promoter reporter mice.

Methods: Transgenic mice harboring tissue-specific enhancer/promoter sequences of alpha 2(I) collagen gene (COL1A2) linked to either firefly luciferase or enhanced green fluorescent protein (EGFP) gene underwent ligation of the common bile duct (BDL) to introduce biliary fibrosis. Activation of COL1A2 promoter was already activated 3-fold on day 0 to day 14 after BDL. The localization of EGFP-positive cells was determined by a laser-scanning confocal microscopic examination.

Results: A number of alpha smooth muscle actin (SMA)-positive myofibroblasts appeared around the dilated bile ducts on day 2 following BDL, where accumulation of collagen fibrils was observed. Prior to those histopathological changes, COL1A2 promoter was already activated 3-fold on day 1, and further increased thereafter. EGFP-positive cells were detected in the fibrous tissue underneath the dilated biliary epithelial cells as early as 2 days after BDL. Most of them were positive for alpha SMA. Biliary epithelial cells did not express EGFP, nor were they stained positive for alpha SMA throughout the observation period.

Conclusions: By using transgenic reporter mice which detect COL1A2 promoter activation with high sensitivity and specificity, we exclude collagen production by biliary epithelial cells, which indicates a limited role of EMT in the development of biliary fibrosis.

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Autophagy eliminates misfolded procollagen aggregates in the endoplasmic reticulum for cell survival

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Objectives: Type I collagen is a major component of the extracellular matrix, and mutations in the collagen cause several matrix-associated diseases. These mutant procollagens are misfolded and often aggregated in the endoplasmic reticulum (ER). Although the misfolded procollagens are potentially toxic to the cell, little is known about how these misfolded procollagens are eliminated from the ER.

Methods: We examined two collagen degradation pathways, ERAD and autophagy, using two models: one is Hsp47-null chaperone-deficient cells and the other is Mov13 cell lines, which produce disease-causing collagen mutants. Furthermore, we analyzed the role of autophagy for cell survival against the cytotoxicity of ER-accumulated misfolded collagen by RNAi-mediated knockdown of autophagy proteins.

Results: Procollagen trimers aggregated in the ER are eliminated by an autophagy-lysosome pathway, but not by ERAD. Inhibition of autophagy by specific inhibitors and RNAi-mediated knockdown significantly stimulated accumulation of aggregated procollagen trimers, and treatment with an autophagy activator resulted in reduced amount of aggregates. In contrast, monomer procollagen mutant, which is deficient in trimer formation, is degraded by ERAD. The autophagic elimination of aggregated procollagen occurs independent of ERAD system. Moreover, we found that autophagy plays an essential role in cell survival against toxicity of the ERAD-inefficient procollagen aggregates.

Conclusions: Our study demonstrates that autophagic degradation of misfold procollagen aggregates in the ER is strictly dictated according to their conformation, and autophagic activity is essential for the cell survival by eliminating the ERAD-untreatable procollagen aggregates.