SUMMARY OF Ph.D. DISSERTATION

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Title

Reconstruction of hepatic organoids with functional bile canaliculi using rat small hepatocytes

Abstract

Development of bioartificial livers is an ongoing study for the future therapy of liver diseases. Despite their regenerative capability in vivo, functional hepatocytes are difficult to retain in vitro. However, recent studies have revealed that hepatic stem/progenitor cells have capabilities to regenerate hepatic organoids in vitro. This dissertation focuses on the reconstruction of hepatic organoids using small hepatocytes (SHs) i.e., hepatic progenitor cells. Hepatic cells, including SHs, were isolated from adult rat livers and cultured. For the hepatic organoid formation, individual cells were integrated into tissues in culture. SHs proliferated and formed colonies within 10 days. Some SHs differentiated into mature hepatocytes interacting with hepatic nonparenchymal cells, such as stellate cells and liver epithelial cells. Bile canaliculi (BC) like structures were formed by the differentiated cells. In terms of organoid formation, the BC formations can be considered as the first step in tissue organization because BC are formed between adjacent hepatocytes and pairs of the cells are integrated through the BC formation. The investigations studied how BC developed with the maturation of SHs and whether the tubular structures were functionally active as BC. The results revealed that differentiated SHs possessed membrane polarity and excreted metabolites into the BC-like structures. This suggests that SHs can reconstruct functional BC that are similar to those in vivo. Analyses of the BC concentrated on their tissue-level functions. In the liver, hepatocytes secrete bile into BC and the secreted bile is transported into bile ducts through the BC. Investigations were conducted to determine whether the groups of the differentiated SHs moved in a coordinated manner to enable to the BC to transport their contents. Time-lapse microscopy was carried out and reveled that the BC contracted and dilated spontaneously and the movements occurred in a coordinated manner. These results indicate that groups of the cells that form the BC act in a coordinated manner and possess a highly differentiated function that cannot be achieved by individual cells. To expand these organoids, a 3D-culture method was developed by stacking up the 2D tissues of SHs. The studies investigated whether the stacked cells were organized into orchestrated tissues. The results revealed that the SHs of the upper and lower layers adhered to one another, and that BC formed between them. Furthermore, SHs within the structures exhibited the mRNA transcription of hepatic-differentiation markers and retained a relatively high level of albumin secretion. This indicates that differentiated 3D tissues, including functional BC, can be reconstructed by stacking up SH layers. In conclusion, the combination of SHs and 3D stacked-up culture can contribute to successful reconstruction of tissue-engineered livers.