Liver Organogenesis from Murine Embryonic Stem Cells

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Our purpose in the present study was to establish a system for *in vitro* hepatic morphogenesis, consisting of not only hepatocytes but also cell lineages supporting hepatic differentiation, such as cardiomyocytes and endothelial cells, from murine ES cells. We succeeded in establishing a novel system of hepatic morphogenesis from murine ES cells based on naturally occurring embryological events.

Introduction

The pluripotency of embryonic stem (ES) cells has been applied to obtain a variety of cell lineages in regenerative medical studies. However, the targets of these systems have always been limited to only a single lineage, which was isolated from other differentiated cell populations. For examples, there have been a few reports of the differentiation of murine ES cells to hepatocyte-like or albumin-producing cells. These studies focused only on hepatocytes as a single cell lineage, and did not refer to liver organogenesis. The liver is composed of not only parenchymal hepatocytes but also non-parenchymal cells, such as sinusoid endothelial cells, Kupffer cells, and cholangiocytes, to fulfill the many liver-specific functions. We succeeded to establish an *in vitro* system of liver organogenesis from murine ES cells, to obtain the innate functions corresponding to this organ[1)].

Materials and Methods

Differentiation of ES cells to hepatic tissue

E14-1 embryonic stem (ES) cells were grown on mitomycin C-treated mouse embryonic fibroblast feeder layers to maintain them in the undifferentiated state. The cells were dissociated with 0.05% trypsin - 1% chicken serum - 1 mM EDTA in PBS, and re-suspended in Iscove's modified Dulbecco's medium containing 20% FBS, 1 mM sodium pyruvate, 100 µM non-essential amino acids, 100 µM 2-mercaptoethanol without LIF, and embryoid bodies (EBs) were formed into a hanging drop at a concentration of 1000 cells per 50 µl in each drop.

Immunohistochemical analysis

EB outgrowths were fixed with 4% paraformaldehyde/PBS, and then permeabilized with 0.1% Triton X. The fixed samples were incubated in blocking buffer consisting of 4% donkey serum, and were incubated with the primary antibody and with the secondary antibody for one hour in a humidified chamber. The following antibodies were used: rabbit IgG against mouse albumin, goat IgG against mouse PECAM-1, TRITC-conjugated Swine Anti-Rabbit immunoglobulin, and Fluorescein (FITC)-conjugated donkey anti-goat IgG. The samples were mounted in DAKO fluorescent mounting medium. These samples were observed under a fluorescence microscope and a confocal laser microscope.

Results and Discussion

The liver develops from the ventral foregut in vertebrates, receiving multiple stimuli of growth factors, cytokines, and hormonal factors, as well as intercellular and matrix cellular interactions. Especially, the pre-cardiac mesoderm produces factors that trigger hepatic development[2)], *i.e.*, cardiomyocytes support liver organogenesis. Therefore, we considered that the emergence of cardiomyocytes in an *in vitro* differentiation system using ES cells is necessary for liver organogenesis. We, first, established a system for spontaneous differentiation to contracting cardiomyocytes with a high frequency of emergence. Contracting cardiomyocytes emerged in the central area of EB outgrowth.

As the hepatic precursor cells migrate into the septum transversum to form a liver bud[3)], endothelial progenitor cells arise there simultaneously, in liver organogenesis. The contribution of the non-parenchymal hepatic cell population is necessary for hepatic *in vitro* morphogenesis from ES cells. Interestingly, the CD31/PECAM-1-positive cells were seen to be migrating in the albumin-positive areas of the EB outgrowths at A10 and made contact with the juxtapositions of the albumin-positive cells, similar to liver bud formation[3)]. The number of albumin-producing cells increased cumulatively in the expanding vascular network area during differentiation to the late stage. It was established that *in vitro* differentiation of murine ES cells within EBs led to complex structures that can mimic the liver organogenesis in the body formation process, as shown in figure 1. Our liver morphogenesis system is not a simple co-culture of ES cells with endothelial cells prepared from liver sinusoids or blood vessels, but is a novel system for the differentiation of ES cells to cardiomyocytes to support the subsequent differentiation and to endothelial cells and hepatocytes as liver tissues due to pluripotency in ES cells. This *in vitro* system for the construction of liver morphogenesis from ES cells was close to the innate events of liver development occurring *in vivo*.

References

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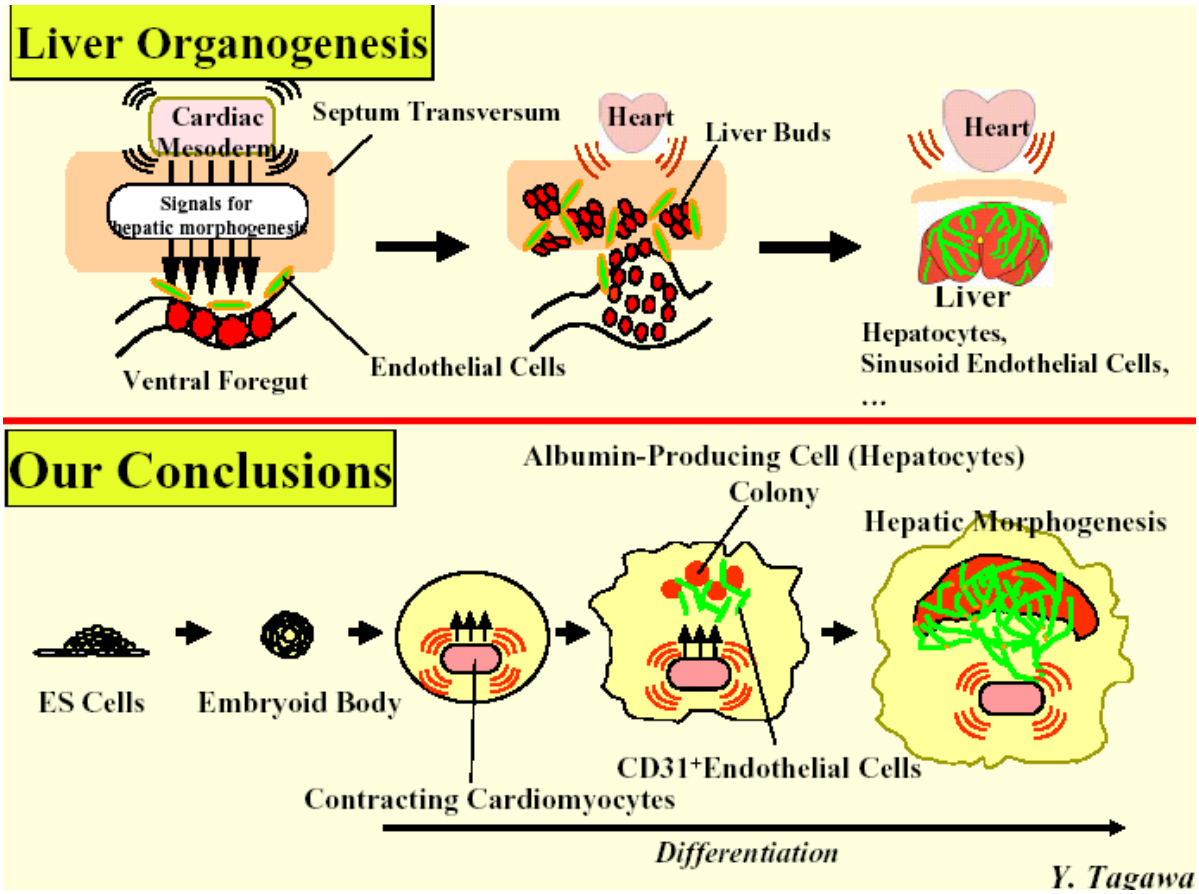

Figure 1. Illustrations of in vivo liver organogenesis and our in vitro hepatic morphogenesis using murine ES cells (conclusion).