INTRODUCTION

Bile canaliculi (BC) are tubular structures that form the most proximal channels of the biliary tree and carry bile secreted by hepatocytes. BC are formed by apical membranes of adjacent hepatocytes, and are around 1 \( \mu \)m in diameter. It has been thought to be difficult that cultured hepatocytes can form functionally active BC. Small hepatocytes (SHs), which are known to be hepatic progenitor cells, can be isolated from the adult rat liver [1]. They can rapidly proliferate with maintaining hepatic functions such as albumin secretion and ammonia metabolism, and form a monolayer colony consisting of 20 to 30 cells within 10 days. About 10 days after plating, many colonies are surrounded by hepatic nonparenchymal cells (NPCs) such as liver epithelial cells and stellate cells, and several days later, piled-up cells appear in some of the colonies. In these colonies, translucent belts can be distinguished between the piled-up cells under phase-contrast microscopy. With time in culture, they develop into anastomosing networks, which may be BC. In this study, we investigated the motility behavior of these BC-like structures using phase-contrast microscope equipped with a time-lapse system. We also investigated the effect of cytochalasin B (CB) on the functions of BC.

MATERIALS AND METHODS

Isolation and Culture of SHs

Hepatic cells, including SHs, were isolated from male Sprague-Dawley rats using the two-step liver perfusion method. The cells were inoculated on culture dishes coated with rat tail collagen and cultured with Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 20 mM HEPES, 25 mM NaHCO\(_3\), 30 mg/L L-proline, 0.5 mg/L insulin, 10^(-7) mol/L dexamethasone, 10% fetal bovine serum, 10 mM nicotinamide, 1 mM ascorbic acid 2-phosphate, 10 ng/ml epidermal growth factor, and antibiotics. The cells were placed in a humidified, 5% CO\(_2\)/95% air incubator at 37°C and the medium was subsequently changed every other day. From day 4 (96 hrs after plating), 1% dimethyl sulfoxide (DMSO) was added to the culture medium.

Time-lapse Microscopy

The cells, that reconstructed BC-like structures, were placed in a humidified, 5% CO\(_2\)/95% air chamber on the stage. Images of BC-like structures were recorded at 5-sec intervals for 3 hrs using a phase-contrast microscope equipped with a time-lapse system. The data were analyzed using the software of MetaMorph (Universal Imaging Corp.). Thresholding to phase-contrast micrographs were applied to clarify the diameter of BC. We then set regions on the image (#1-4 in Fig. 1A), and measured thresholding area in each region.

CB Treatment

To investigate the effect of CB on the behavior of BC-like structures, SH colonies containing well-developed BC networks were treated with CB (Fig. 2A). Sequential photographs were taken soon after an addition of the medium with CB. The CB solution was prepared from stock solution containing 25 mg/ml of CB dissolved in DMSO. The cells were treated with the final concentration of 10 \( \mu \)g/ml CB in DMEM. After taking images using time-lapse system, the medium was changed to a fresh medium without CB, and then the cells were kept in an incubator. The same cells were photographed after overnight incubation.

RESULTS AND DISCUSSION

Reconstruction of BC-like Structures

SHs began to proliferate 2-3 days after inoculation, and then formed colonies. With time in culture, the colonies surrounded by NPCs changed in shape from flat to piled-up, interacting with NPCs such as liver epithelial cells and stellate cells. BC-like structures were reconstructed between piled-up hepatocytes in these colonies, and developed into anastomosing networks (Figs. 1A and 2A). In the structures, BC proteins such as ectoATPase, 5'-nucleotidase, and multidrug-resistance associated protein 2 were expressed in the membranes of the BC-like structures (data not shown), and these results showed that differentiated SHs could possess a membrane polarity.
Coordination of BC contraction

Fig. 1A shows a phase-contrast image of the piled-up cells, in which the anastomosing BC network developed well. We applied thresholding to the image to clarify the BC, and then measured the thresholding areas in each region set on the image (#1-4 in Fig. 1A). Normalized ratio was calculated and the results were graphically shown in Fig. 1B. It indicated a big contraction of BC that developed during the experimental period. Although the BC-like structures in regions #1, #2, and #3 contracted synchronously, the structure in region #4 showed no contraction within the period (Fig. 1B). These results suggest that BC networks do not contract randomly, but in a coordinated manner to enable them to transport the substances. In particular, BC contraction may not be the behavior of an individual cell but regional. The colony in Fig. 1 had a tendency to develop toward the tip of the colony. The BC in region #1 was reconstructed earlier than the BC in region #4. There may be a functional difference between the root and the tip of the colony.

This may be the first experiment that demonstrates tubular structures of BC in vitro that have motility for bile transport. SHs merely proliferate and provide hepatic functions at the beginning of the culture. With time in culture, however, they reconstruct their assembly to function as a tissue not obtained by individual cells. The coordination of BC contraction is an integrated model of the cell system, and is therefore a topic of “Physiome”. This culture system is useful to investigate how biological systems work, in particular the bile transport system in the liver.

Effect of CB on BC-like structures

The hepatocytes treated with CB dramatically changed their morphology. BC-like structures started to dilate soon after addition of CB. Remarkable dilatations of the BC-like structures were observed at 20 min after addition of CB (Fig 2B). BC-like structures continued to dilate throughout the experimental period, and therefore canalicular structures changed into cystic structures (Fig 2C). Interestingly, when the cells were re-incubated with a medium without CB, they could recover their canalicular structures within a day (Fig 2D).

It is well known that actin filaments are present in the hepatocytes and especially rich in the region of BC. Actin filaments around BC are believed to be playing an important role in BC contractions using couplets that are pairs of hepatocytes [2-4]. In our culture system, actin filaments were also rich in the reconstructed BC-like structures. CB is known to be a reagent that inhibits actin polymerization and it therefore inhibits actin filament-mediated cell motility functions. In the present study, inhibition of BC contractions and progressive dilatation of BC-like structures are observed. The results help to understand the mechanism of contractions of BC-like structures.

References