Research Article

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Use of mouse liver mesothelial cells to prevent postoperative adhesion and promote liver regeneration after hepatectomy

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Background & Aims: Repeated hepatectomy is widely accepted as one of the most effective curative treatment for recurrent hepatocellular carcinoma or liver metastasis from colorectal cancer. It has, however, two critical issues; postoperative adhesion and decrease of liver regenerative capacity. Postoperative adhesion makes surgical operations technically more demanding, leading to increased mortality and morbidity rates. Although the liver has a remarkable regenerative ability, volume and functional restoration after multiple repeated hepatectomy is not generally complete. So a new procedure that overcomes these two issues is required. We examined if a fetal liver mesothelial cells (FL-MCs) sheet could solve these two clinical issues simultaneously.

Methods: We established a novel mouse hepatectomy model that reproduces postoperative adhesion on the resected liver surface. We isolated FL-MCs from mouse fetal liver and prepared a cell sheet. The FL-MCs sheet was then transplanted to the resected liver surface.

Results: The FL-MCs sheet effectively prevented postoperative adhesion by expressing PCLP1, one of the transmembrane sialomucin family proteins and by activating the fibrinolytic system. Furthermore, the FL-MCs sheet facilitated liver regeneration by providing growth factors for hepatocytes, allowing quick recovery of liver weight and function. Additionally, we showed that an allogeneic FL-MCs sheet was as effective as a syngeneic cell sheet.

Conclusions: We demonstrate that the FL-MCs sheet is able to not only prevent postoperative adhesion but also promote liver regeneration in both syngeneic and allogeneic transplantation, and hence FL-MCs may serve as a potentially useful cell source for regenerative medicine after hepatectomy.

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Abbreviations: FL-MCs, fetal liver mesothelial cells; P-MCs, peritoneal mesothelial cells; HCC, hepatocellular carcinoma; PHx, partial hepatectomy; PPHx, practical partial hepatectomy; AST, aspartate aminotransferase; ALT, alanine aminotransferase; POD, postoperative day; GFP, green fluorescent protein; PCLP1, podocalyxin-like protein 1; Msln, mesothelin; tPA, tissue-type plasminogen activator; PAI-1, plasminogen activator inhibitor-1; HGF, hepatocyte growth factor; IL-6, interleukin-6; HB-EGF, heparin-binding EGF-like growth factor; Mdk, midkine; Ptn, pleiotrophin; n.s., not significant.

Introduction

Liver malignant neoplasms, including hepatocellular carcinoma (HCC) and liver metastasis from colorectal cancer, are one of the top three causes of cancer deaths globally [1]. There are various treatment options for liver malignant neoplasms, such as surgery, chemotherapy, percutaneous embolization therapy, and radiotherapy. Among them, hepatectomy has been generally approved as potentially curative for HCC and liver metastasis [2,3]. Since liver malignant neoplasms recur frequently, repeated hepatectomy is widely accepted as one of the most curative treatments and long-term survival non-inferior to that of primary resection [4–7]. However, repeated hepatectomy has two intrinsic problems. The first is postoperative adhesion; postoperative adhesion after hepatectomy is an unavoidable wound healing process that results in a higher risk of increased intraoperative bleeding and longer operation time compared to those of a primary hepatectomy [8,9]. This makes the operation much more technically demanding and leads to increased mortality and morbidity rates [9–11]. Anti-adhesive polymeric materials have been developed and are used in clinical practice to avoid postoperative adhesion, but their use in hepatectomy is controversial [12,13]. The second issue for repeated hepatectomy is that the remnant liver has a limited regenerating ability. Although the liver has a remarkable potential to regenerate, volume restoration is not complete [14] and its regenerating and functional activities decline after repeated hepatectomy [15,16], leading to liver failure that limits further operations. Therefore, a new procedure that overcomes these two issues is required. In order to solve the two critical issues after hepatectomy, we focused on the fetal liver mesothelial cells (FL-MCs).

Mesothelial cells (MCs) cover all the internal organ surface and body wall, and play a crucial role in providing a protective barrier and frictionless gliding of intraperitoneal organs. Moreover, FL-MCs were shown to express various growth factors for fetal liver progenitor cells (hepatoblasts) such as Midkine (Mdk), pleiotrophin (Ptn), HGF and EGF, and contribute to the liver organogenesis by stimulating hepatoblasts proliferation [17]. Because of such unique features, we reasoned that these cells may be useful to develop a therapy to prevent postoperative adhesion and enhance regeneration after partial hepatectomy. In this report, we show that a cell sheet made of FL-MCs can effectively inhibit postoperative adhesion and promote liver regeneration simultaneously.

Materials and methods

Detailed materials and methods used in this study are provided in the Supplementary materials and methods.

Practical partial hepatectomy (PPHx)

All experimental procedures in this study were approved by the institutional animal care and use committee of the University of Tokyo. After laparotomy, the median lobe was removed. The next step was the key procedure for PPHx. After cauterizing the liver capsule circumferentially in the middle of the left lobe (1.1 cm from the top of the left lobe) using an electric scalpel, the left portal veins and left hepatic veins were ligated en bloc and the half of the left lobe was transected. The epididymal adipose tissue was lifted up to the upper part of the abdomen and was placed on the transected surface of the left lobe for reproducibility of postoperative adhesion. Furthermore, we sutured the epididymal adipose tissue onto the abdominal wall with one stitch to keep it from slipping.

Transplantation of a tissue engineered cell sheet into PPHx mice model

1.5×10^5 primary cultured cells were re-plated on temperature-responsive cell cultureware. Four days after culture at 37 °C, the temperature was lowered to detach cells from the cultureware, as a cell sheet. The cell sheet was then transplanted to the resected surface of the mice during the PPHx operation.

Results

Mouse model of postoperative adhesion

The rodent liver consists of multiple lobes. The best-studied model of liver regeneration is a 70% partial hepatectomy (PHx) that removes the left lateral and median lobes by tying the pedicle with threads and ablating the tissue [18,19]. This procedure leaves the remaining liver tissue intact, allowing the liver to regenerate without exposing resected surfaces (Fig. 1A left). However, this PHx situation is quite different from the actual surgical procedures for patients, because the human liver is a large organ with unseparated lobes. Hepatectomy of patients' livers inevitably exposes resected surfaces, leading to adhesion. At present there is no rodent model of PHx that reproduces actual surgical procedures. First, we established a mouse model of liver resection and named it a practical partial hepatectomy (PPHx). Briefly, the middle of the left lateral lobe is resected using an electric scalpel after removal of the median lobe by tying the pedicle of the median lobe with a thread and cutting them off (Fig. 1A right), thus exposing the liver transected surface (Fig. 1B). We investigated the degree of liver damage from both PPHx and 70% PHx by measuring serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels (Supplementary Fig. 1). AST levels increased on postoperative day 1 (POD 1) and decreased on POD 2 in both models. However, ALT levels peaked on POD 1, declined gradually to a normal level in PPHx mice, while ALT levels declined drastically at 2 days after PHx. The decrease in ALT levels in PPHx mimics those observed in human hepatectomy. Next, we examined whether PPHx is appropriate as an adhesion model in mice. On POD 10 severe adhesions of adipose tissues were observed over the wide area of the hepatic resected surface (Fig. 1C). Hematoxylin and eosin (H&E) stained sections showed the morphologic feature of adhesion observed in human; tight tissue adhesions consisted of fibroblasts were observed between hepatocytes and adipocyte in all mice after PPHx (Fig. 1D). Then, we quantified the ratio of adhesion formation; i.e., the longest diameter of surface adhered/the longest diameter of total transected surface, and found that it was almost 100% in all C57BL/6J mice tested on POD 10 (Fig. 1E). The adhesion ratio was reduced on POD 30 and it remained at a similar degree even on POD 548 (about 1.5 years) (Fig. 1E). These results supported that PPHx reflects the healing process as well as the hepatectomy procedure in humans. In addition, we examined liver regeneration by quantifying the ratio of Ki67 positive hepatocytes. There were no significant differences between the resected left and right lobes (Fig. 1F). These results indicate that PPHx induce hepatocyte proliferation equally in all lobes.

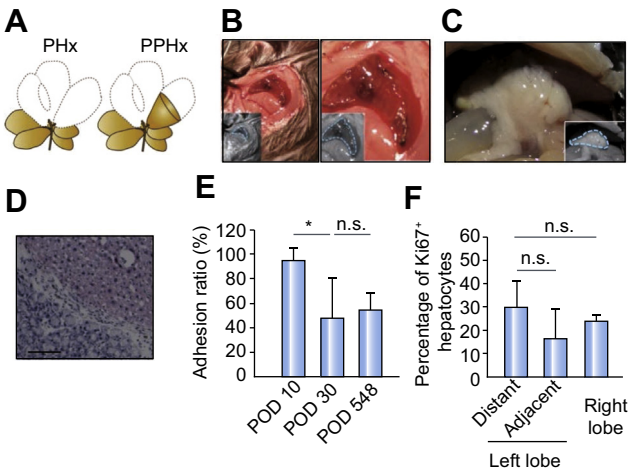

Fig. 1. Mouse model of postoperative adhesion. (A) Schema of partial hepatectomy (PHx) and practical partial hepatectomy (PPHx) in mouse liver. A resected surface was created in the PPHx. (B) The hepatic resected surface of C57BL/6J mouse with PPHx operation. The right panel is a higher-magnification image of the left panel. The hepatic transected surface is shown by the dotted lines in the insets. (C) The extent of postoperative adhesion formation on the hepatic resected surface on POD 10. The resected surface is indicated by dotted lines in the inset. (D) Histology of the adhesion site. Liver section on POD 10 stained with H&E. Scale bar, 100 μm. (E) Quantification of postoperative adhesion formation ratio on POD 10, 30, and 548 (mean ± SD; n = 5 on POD 10 and 30, n = 3 on POD 548. *p <0.05). (F) Liver regeneration on POD 3 after PPHx. Liver section of left lobe and right lobe on POD 3 were immunostained with Ki67, phalloidin and Hoechst33342 and then quantified Ki67-positive hepatocytes by imaging cytometry.

Prevention of postoperative adhesion using fetal liver mesothelial cells sheet after PPHx

To solve two critical issues after hepatectomy, we attempted to use the FL-MCs. Initially, we investigated whether FL-MCs were

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