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Transient expression of laminin α1 chain in regenerating murine liver: Restricted localization of laminin chains and nidogen-1

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Abstract

Most interstitia between epithelial and endothelial cells contain basal laminae (BLs), as defined by electron microscopy. However, in liver, the sinusoidal interstitium (called space of Disse) between hepatocytes and sinusoidal endothelial cells (SECs) lacks BLs. Because laminins are major components of BLs throughout the body, whether laminins exist in sinusoids has been a controversial issue. Despite recent advances, the distribution and expression of laminin chains have not been well defined in mammalian liver. Here, using a panel of antibodies, we examined laminins in normal and regenerating mouse livers. Of α chains, α5 was widely observed in all BLs except for sinusoids, while the other α chains were variously expressed in Glisson's sheath and central veins. Laminin γ1 was also distributed to all BLs except for sinusoids. Although the β2 chain was observed in all BLs and sinusoids, the expression of β1 chain was restricted to Glisson's sheath. Detailed analysis of regenerating liver revealed that α1 and γ1 chains appeared in sinusoids and were produced by stellate cells. The staining of α1 and γ1 chains reached its maximum intensity at 6 days after two-thirds partial hepatectomy (PHx). Moreover, in vitro studies showed that α1-containing laminin promoted spreading of sinusoidal endothelial cells (SECs) isolated from normal liver, but not other hepatic cells. In addition, SECs isolated from regenerating liver elongated pseudopodia on α1-containing laminin more so than did cells from normal liver. The transient expression of laminin α1 may promote formation of sinusoids after PHx.

Keywords: Basal lamina; Laminin; Hepatic regeneration

Introduction

Liver plays a central role in metabolic homeostasis and is the major detoxifying organ in the body. After toxic or surgical injury, the liver exhibits unique properties of regeneration. Two-thirds partial hepatectomy (PHx) is a model that most clearly demonstrates the regenerative capacity of the liver. After PHx, the liver is capable of complete regeneration, restoring original size, architecture, and functions. Hepatic regeneration has multiple steps and involves many factors such as cytokines, growth factors, and extracellular matrix (ECM) [1–3]. Of multiple factors, ECM plays a potentially important role in maintaining and remodeling hepatic architecture.

The basal lamina (BL) is an extracellular matrix structure underlying many cell types, including epithelia, endothelia, muscle, fat, and peripheral nerve. BLs (also-called basement membranes) are defined by electron microscopy. Hepatic BLs are observed in bile ducts, arteries, and veins [1]. Interestingly, the sinusoidal interstitium (called space of Disse) between hepatocytes and SECs lacks BLs. BLs are formed by the complex interactions of its major compo-

Abbreviations: BL, basal lamina; PHx, two-thirds partial hepatectomy; SECs, sinusoidal endothelial cells; BECs, biliary epithelial cells; DMEM, Dulbecco's modified Eagle medium; PBS (−), Ca^{2+} and Mg^{2+}-free phosphate-buffered saline; BSA, bovine serum albumin; FBS, fetal bovine serum; EM, electron microscopy; PECAM, platelet endothelial cell adhesion molecule; CK19, cytokeratin 19.

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nents: laminins, collagen IV, perlecan, and nidogens [4]. Of these components, it is well known that laminins regulate various cellular functions such as adhesion, motility, growth, differentiation, and apoptosis through interactions with specific cell surface receptors [5,6]. All laminins are composed of three subunits, designated α, β, and γ chains. Laminin was initially isolated from tumor cells as a heterotrimer of A, B1, and B2 subunits [7,8], later renamed α1, β1, and γ1 [9]. Until now, five α, four β, and three γ chains have been identified. The existence of multiple chains provides a means to generate not only structural but also functional diversity within a common structural framework [10]. To date, 15 different laminin heterotrimers have been found to be synthesized and secreted by various cells [9,11,12]. More trimeric molecules are theoretically possible. Despite recent advances, limited information is available about the distribution of the laminin chains in a liver.

To investigate the roles of laminins in normal and regenerating livers, their spatiotemporal depositions were characterized by immunohistochemistry. In the present study, we found that laminin chains were variously distributed in Glisson's sheath, sinusoids, central veins, and mesothelium. They were expressed in overlapping but distinct patterns in liver, as observed in other tissues. Of laminin chains, we focused on laminin α1 that is transiently expressed in sinusoids during hepatic regeneration. In vitro studies also showed that the adhesion of SECs was promoted by α1-containing laminin. The SECs isolated from regenerating liver revealed active movement on α1-containing laminin more so than on fibronectin. Our results suggest that transient expression of laminin α1 is associated with reorganization of liver lobules.

Materials and methods

Animals and surgery

All animal studies were performed according to Sapporo Medical University guidelines, and the animals used in the experiments received humane care. ICR mice (20–24 weeks old) and Sprague–Dawley rats (8–10 weeks old) were purchased from Nihon SLC (Hamamatsu, Japan) and Nihon Charls River (Yokohama, Japan), respectively. For PHx, mice and rats were subjected to conventional PHx under ethyl ether anesthesia [13]. According to the standard method of Higgins and Anderson [13], two-thirds partial hepatectomy (PHx) was performed in adult mouse liver. At different time after PHx, the mice and rats were anesthetized with ethyl ether, and livers were processed for histology and cell isolation.

Proteins and antibodies

Mouse laminin-1 (α1-containing laminin) and human fibronectin were purchased from BD Biosciences (Bedford, MA). Human laminin-8 (α4-containing laminin) [14] was a gift from Dr. Kiyotoshi Sekiguchi (Osaka University, Osaka, Japan). Rat monoclonal antibody 8B3 to laminin α1 [15] was a gift from Dr. Dale Abrahamson (University of Kansas Medical Center, Kansas City, KS). Polyclonal antibodies against the following mouse laminins were gifts from Dr. Takako Sasaki (Max-Planck Institute, Martinsried, Germany): domain VI of laminin α1, domain IIIa of laminin α3, domain IIIa of mouse laminin α4, domain IV of laminin β1, domain IV of laminin β2, domain IV of laminin β3, and LE module 4–6 of laminin γ2 [16–19]. Polyclonal antibody against the globular (G) domain of laminin α2 [20] was a gift from Dr. Peter D. Yurchenco (Robert Wood Johnson Medical School, Piscataway, NJ). Rabbit antibody against domain IIIb/IVa of mouse laminin α5 was produced and characterized as described [11]. Rat monoclonal antibodies MAB1914 to laminin γ1 and MAB1946 to entactin/nidogen-1 were purchased from Chemicon International Inc. (Temecula, CA). Rat monoclonal antibodies MEC 13.3 to platelet endothelial cell adhesion molecule (PECAM) and CI:A3-1 to F4/80 antigen were purchased from BD Biosciences and Serotec (Oxford, UK), respectively. Mouse monoclonal antibodies D33 to desmin and RPN1165 to cytokeratin 19 (CK19) were purchased from Dako (Glostrup, Denmark) and Amersham Biosciences (Piscataway, NJ), respectively. Mouse monoclonal antibodies ED1 to CD68 and SE-1 to SECs were purchased from Serotec (Oxford, UK) and IBL (Fujinaka, Japan), respectively. Rabbit antibody to rat albumin was purchased from Cappel (Aurora, OH).

Immunohistochemistry

For immunohistochemistry, mouse livers were frozen in OCT compound and quick-frozen in 2-methylbutane cooled in a dry ice-ethanol bath. Sections were cut at 7 μm in a cryostat and air-dried. For staining, sections were blocked in 10% normal goat serum and then incubated with primary antibody. All antibody incubations were in PBS (−) containing 1% bovine serum albumin (BSA), and all washes were in PBS (−). Secondary antibodies were conjugated to Alexa488 or 594 (Invitrogen, Carlsbad, CA). After several washes, sections were mounted in 90% glycerol containing 0.1 × PBS (−) and 1 mg/ml *p*-phenylenediamine. Images captured using a Zeiss LSM510 confocal microscope (Carl Zeiss, Oberkochen, Germany) were imported into Adobe Photoshop CS and Adobe Illustrator CS (San Jose, CA) for processing and layout.

Transmission electron microscopy

The liver at 7 days after PHx was perfused with fixation buffer containing 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) followed by Hanks's balanced solution. The liver was postfixed in 2% osmium tetroxide