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Short communication

Laminin expression during bone marrow mononuclear cell transplantation in hepatectomized rats

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Abstract

The adult bone marrow retains two populations of stem cells with emerging importance for the treatment of diverse liver diseases: hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). However, the mechanisms that control liver regeneration after bone marrow cell transplantation are still controversial. Liver regeneration after partial hepatectomy is a complex process that requires the proliferation of all hepatic cells. Growth factors, cytokines and extracellular matrix molecules are key elements in this process. Laminins are a family of extracellular matrix proteins with adhesive and chemotactic functions, expressed in the portal and centrolobular veins of the normal liver. The aim of this study was to investigate laminin expression during liver regeneration induced by partial hepatectomy followed by bone marrow mononuclear cell (BMMNC) transplantation. Rat BMMNCs were isolated by Ficoll-gradient centrifugation, stained with DAPI and injected into recently hepatectomyzed rats via the portal vein. Liver sections obtained 15 min, 1 day and 3 days after the surgery were immunolabeled with anti-rat CD34 and/or laminin primary antibodies and observed under a laser scanning confocal microscope. Results showed that 15 min after partial hepatectomy, a transplanted $CD34^{+}$ HSC was found in contact with laminin, which was localized in the portal and centrolobular veins of rat livers. Furthermore, 1 and 3 days after hepatectomy, transplanted BMMNCs were found in the hepatic sinusoids expressing laminin. These results strongly suggest that laminin might be an important extracellular matrix component for bone marrow cell attachment and migration in the injured liver.

Keywords: Bone marrow mononuclear cells; Hematopoietic stem cells; Hepatectomy; Laminin

1. Introduction

Bone marrow cell therapy has emerged as a promising approach to treat many diseases which lack efficient pharmacological treatment, and to avoid the complications of organ transplantation. In this respect, the mononuclear fraction of the bone marrow contains two populations of stem cells – hematopoietic stem cells (HSC) and mesenchymal stem cells (MSCs) – that are thought to participate in regenerative processes (Abdel Aziz et al., 2007; Thorgeirsson and Grisham, 2006).

Many studies have shown the application of bone marrow cells, or purified bone marrow stem cells of hematopoietic or mesenchymal origin, in animal models of liver diseases such as fibrosis, cirrhosis and metabolic alterations, has for liver function. However, the exact mechanism of how these cells contribute to hepatic regeneration has been discussed. It is well established that, under certain conditions, MSCs can differentiate into functional hepatocytes, although this

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phenomenon is considered rare and limited for HSCs (Abdel Aziz et al., 2007; Yu et al., 2007; Kuhlmann and Peschke, 2006; Thorgeirsson and Grisham, 2006). The loss of hepatic tissue due to resection, or partial hepatectomy, triggers a mitotic wave in all hepatic cells, and the liver can regenerate its original mass without any detectable morphological or functional alteration.

Laminins are a family of heterotrimeric extracellular matrix proteins with adhesive and chemotactic functions through recruitment of integrins and other cell surface receptors, implicated in important biological processes such as cell migration and differentiation (Sasaki et al., 2004). In the normal liver, laminin is expressed around the portal vessels and centrolobular veins; however, 3 days after hepatectomy, laminin

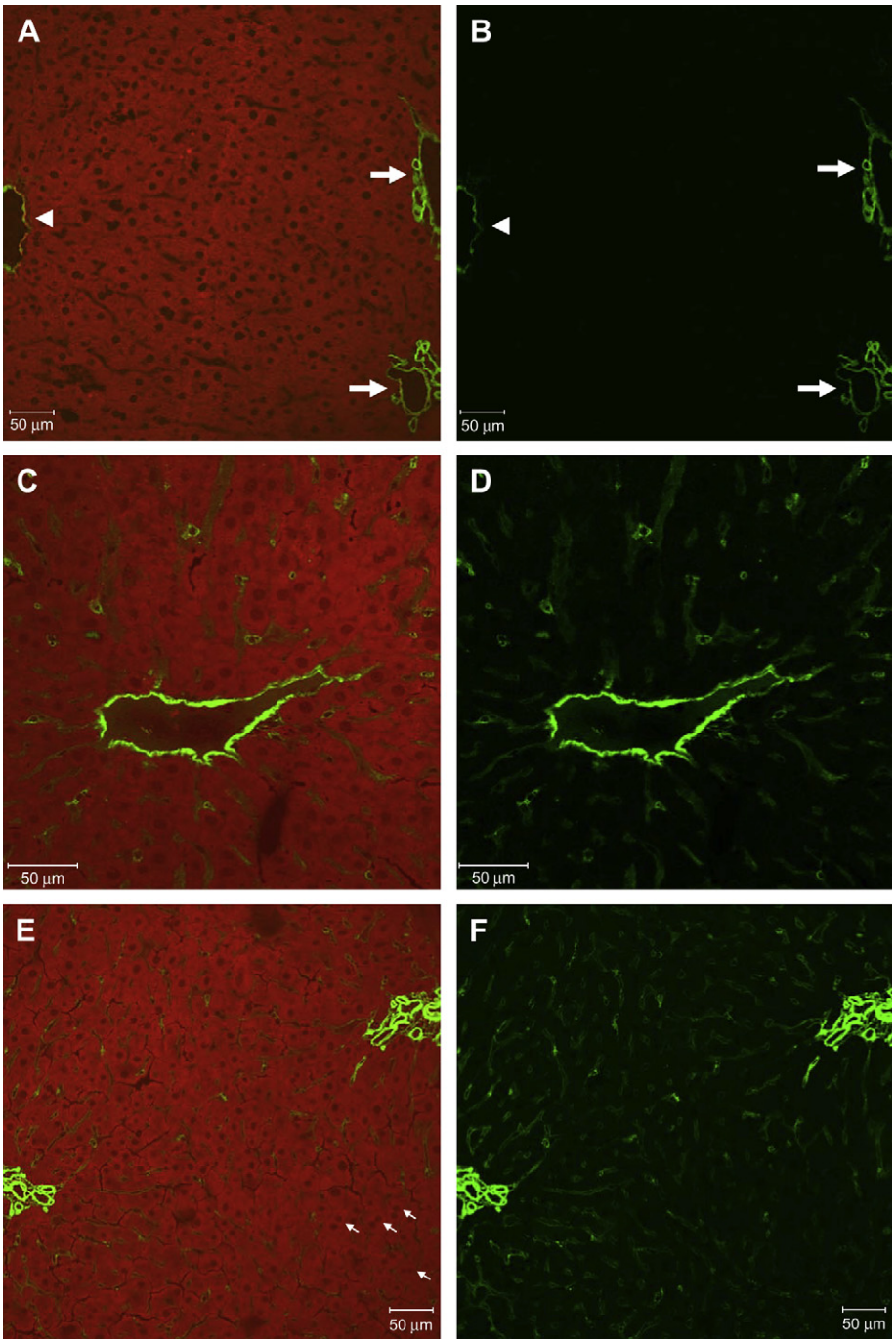

Fig. 1. Confocal laser scanning microscopy of liver sections obtained after partial hepatectomy in rats. Immunofluorescence using anti-laminin primary antibody and FITC-conjugated secondary antibody, followed by Evans blue staining. (A, C, E) Overlay of FITC (laminin) and Evans blue images. (B, D, F) Laminin immunolabeling. (A, B) Fifteen minutes after hepatectomy, portal spaces (arrows) and centrolobular veins (arrowheads) present a marked laminin expression, which is negative in the hepatic parenchyma (40×, bar = 50 µm). (C, D) One day after hepatectomy, laminin expression is detectable in the sinusoids of the hepatic parenchyma, besides the labeling in centrolobular veins (63×, bar = 20 µm). (E, F) Three days after hepatectomy, laminin expression is present in the hepatic sinusoids and in portal spaces. The arrows indicate proliferating binucleated hepatocytes (40×, bar = 50 µm).