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The correlation between human adipose-derived stem cells differentiation and cell adhesion mechanism

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ABSTRACT

In recent years, research in the areas of stem cells has dramatically increased, including studies of cellular adhesion to a substrate. We sought to determine the adhesive properties of human adipose-derived stem cells (hASCs) for extracellular matrix proteins. The adhesion of hASCs to collagens and laminin was completely inhibited by a monoclonal antibody, Mab 2253, which binds to the β1 integrin subunit. These data indicate that hASC adhesion to collagens and laminin was exclusively mediated by an integrin. Cell adhesion on fibronectin (Fn) was inhibited by the heparin-binding peptide (HBP) in the presence of Mab 2253, but not by either Mab 2253 or HBP alone. These results indicate that both the β1 subunit and the heparan sulfate proteoglycan participated in the cell adhesion to Fn. Microscopic views showed extensive spreading of hASCs cultured on Fn, whereas the cells maintained a round shape when cultured on a heparin-binding domain (HBD) substrate. hASCs differentiated into adipocytes, which stained positive for lipid vacuoles by Oil Red-O analysis, more readily on HBD substrate than on FN substrate. These results suggest that hASCs have an adhesion mechanism for the HBD of Fn and hASC morphology is controlled by the adhesion mechanism and strongly correlated with adipogenic differentiation.

1. Introduction

Human adipose-derived stem cells (hASCs) are isolated from adipose tissue by liposuction and differentiated into a variety of mesenchymal lineage cells, such as osteoblasts, adipocytes, and chondrocytes [1,2]. hASCs are differentiated into endothelial lineage cells *in vitro* and have proangiogenic action in an ischemia model [3,4]. Thus, hASCs are regarded as an attractive cell source in regenerative medicine, including cell therapies and tissue engineering, as adipose tissue is available in much larger quantities than cord blood or bone marrow.

It is well known that some ECM components, such as fibronectin (Fn), the collagens, and laminin, are cell adhesion molecules that can facilitate cell attachment in culture. The use of components taken from a cell's native environment will aid in the regulation of cells growth and differentiation *in vitro*. Significant progress has also been made in the use of naturally derived ECM components or synthetic molecules as matrices for cellular adhesion and tissue development [5]. Synthetic biomimetic materials have been designed as artificial ECMs (art-ECMs) to stimulate cell adhesion and particular cellular functions and the resulting field has been termed "matrix engineering" [6]. With the rise of interest in stem cells and tissue engineering, the research focused on understanding cellular adhesion to a substrate has also heightened. For example, ex vivo expansion of BM-derived MSCs on a denatured collagen type I matrix resulted in the retention of adipogenic differentiation potential [7,8]. MSCs were adhered and highly proliferated on titanium surface having osteogenic differentiation potential [9].

Fibronectin is a multifunctional cell adhesive glycoprotein present in the ECM and plasma that is composed of a variety of binding domains for different bioactive molecules such as heparin, collagen, fibrin, DNA, and various integrin receptors [10]. Extensive research has shown that Fn plays a major role in cellular morphology and function through cell–Fn interactions [11]. An RGD peptide motif acts as the minimal essential sequence of Fn for attachment of various cell types. Attachment to the RGD motif of Fn is mediated primarily by the β1 subunit of integrin. Integrins, which are composed of an alpha and beta subunit, are a family of cell adhesion receptors linking the ECM to intracellular signaling

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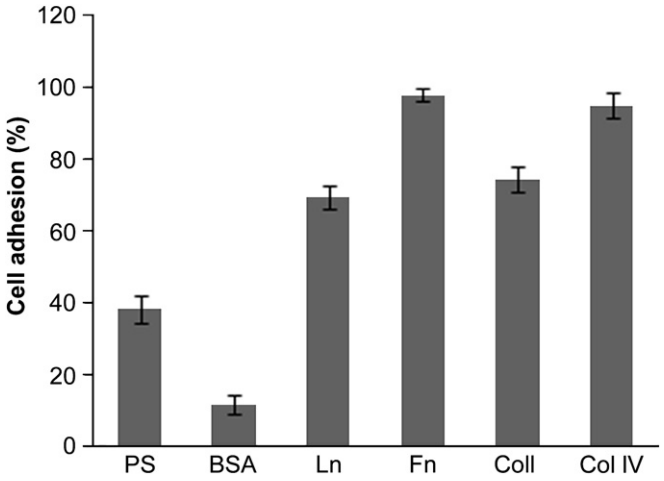

Fig. 1. Adhesion of hASCs to ECM proteins. Human ASCs were allowed to adhere for 30 min at 37 °C to PS Plate coated with BSA, Col I, Col IV, Ln, and Fn. The percentage of cell adhesion was determined as described under "Materials and methods".

molecules and the cytoskeleton network. Signaling pathways are often initiated through cell–matrix interactions and are directly linked to cell survival, proliferation, differentiation, migration, and morphogenesis [12–14]. Studies indicate that hASCs are rich in the cell surface β1 subunit [15,16]. Recent investigations found that blocking the β1 subunit played an important role in the onset of chondrogenesis in ASCs [17]. β1 subunit expression was up-regulated by low level laser irradiation, which triggered the enhancement of cell viability and proliferation, in the primary culture of ASCs [18]. In addition to the RGD motif, other domains of Fn can promote the attachment of specific cells. The type III connecting segment and adjacent heparin-binding region promotes the attachment of melanoma cells [19]. Heparan sulfate proteoglycans are involved in cell adhesion via the heparin-binding region of Fn and modulate the osteogenic differentiation of MSCs in the bone morphogenetic protein signaling pathways [20,21].

Our study focused on the adhesive characteristics of primary cultured hASCs on various substrates to determine uses for hASC cell sources in stem cell therapies or tissue engineering applications. Here, we sought to demonstrate the mechanism of hASC adhesion to Fn and to regulate cell function through the control of the adhesion mechanism by an art-ECM.

2. Materials and methods

2.1. Materials

Type I and IV collagens were obtained from Nitta Gelatin (Osaka, Japan). Fibronectin and Laminin were supplied by Sigma Chemical Co. (St. Louis, Mo). Mouse monoclonal anti-β1, CD34, and HLA-DR were purchased from Chemicon International Inc. (Temecula, CA). CD45, CD90, and CD105 were supplied by Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Rabbit anti-β actin was supplied by Cell signaling (Danvers, MA). Fluorescent secondary antibodies were obtained from Molecular probe (Carlsbad, CA). Dulbecco's modified Eagle's medium F-12 (DMEM/F12) and fetal bovine serum (FBS) were supplied by Welgene (Daegu, Korea). Heparin-binding peptide (fibronectin fragment 1977–1991) was obtained from Sigma–Aldrich Co. (St. Louis, Mo). All other chemical reagents were purchased from Sigma unless otherwise described.

2.2. Isolation and maintenance of human adipose-derived stem cell

Human subcutaneous adipose tissue samples were obtained from the abdomen of 7 different female donors at the ages between 52 and 58. A procedure was processed to

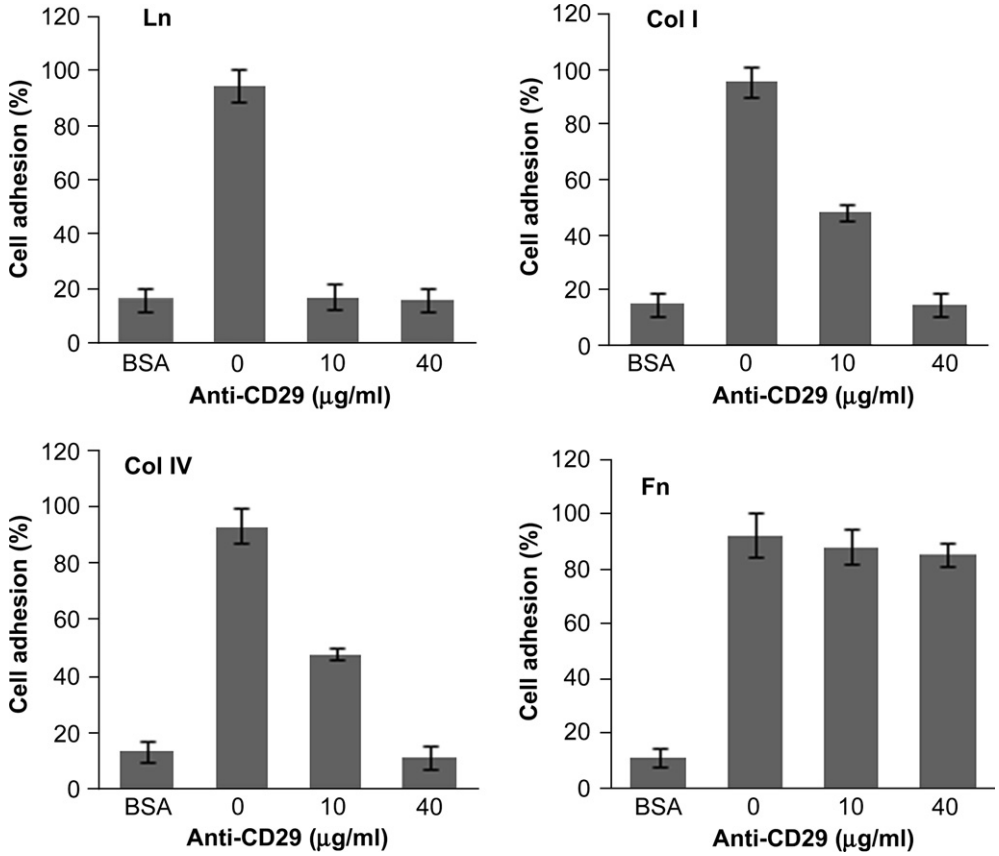

Fig. 2. Effect of β1 subunit blocking antibody on hASCs adhesion to ECM proteins. Suspended hASCs were incubated with 10 μg/ml or 40 μg/ml Mab 2253 for 10 min at 25 °C and allowed to adhere for 30 min on Col I, Col IV, Ln, and Fn substrates.