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Crossregulation of β-catenin/Tcf pathway by NF-κB is mediated by lzts2 in human adipose tissue-derived mesenchymal stem cells

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Abstract

β-catenin/Tcf and NF-κB signaling pathways play an important role in biological functions and crosstalk between these pathways has been reported. We found that the modulation of NF-κB activity showed a direct correlation with β-catein/Tcf pathway in human adipose tissue (hASCs) and bone marrow (hBMSCs)-derived mesenchymal stem cells. Expression of lzts2, which inhibits nuclear translocation of β-catenin and its transactivation activity, was regulated by NF-κB activity. Downregulation of lzts2 by RNA interference increased the nuclear translocation of β-catenin and NF-κB activity in hASCs. NF-κB activation by the downregulation of lzts2 was accompanied by the increase of β-TrCP1 expression and the decrease of IκB level. Downregulation of lzts2 increased the proliferation of hASCs and hBMSC, and blocked the NF-κB inhibitor-induced inhibitory effect on their proliferation and Tcf promoter activation. These findings provide the first evidence that the reciprocal crosstalk between β-catenin/Tcf pathway and NF-κB signaling in hMSCs is mediated through the regulation of lzts2 expression.
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Keywords: hASCs; β-Catenin/Tcf; NF-κB; lzts2; β-TrCP

1. Introduction

Postnatal human bone marrow stromal cells (hBMSCs) or mesenchymal stem cells (hMSCs) have the capacity to regenerate a hematopoietic-supportive bone marrow organ and associated bone trabecular, when transplanted into immunocompromised mice [1,2]. Recent studies have also reported that MSCs are more plastic than first realized, by virtue of their ability to develop into diverse cell lineages such as myelosupportive stroma, osteoblasts, chondrocytes, adipocytes, myoblasts, hepatocytes, cardiomyocytes, and neural cells [3–5]. These developments have prompted investigations into the possible use of ex vivo expanded MSC populations for a wide range of tissue engineering and gene therapy applications [6]. Therefore, it is important to have a thorough understanding of the specific signals dictating cellular behavior and the specific cues that induce or inhibit differentiation, and/or promote the maintenance of these cells. Human adipose tissues have been known to possess multipotential adult stem cells, capable of differentiating into a variety of cell types such as osteoblasts, chondrocytes, adipocytes, muscle cells, and neural cells [5,7]. We and other investigators have shown that culture expanded human adipose tissue-derived cells have a characteristic of MSCs and show similar biologic properties as hBMSCs in vitro and in vivo [8–12], although freshly isolated cells from human adipose tissues contain heterogenous cells and show different surface marker expression with hBMSCs [13–17].

The Wnt family of secretory glycoproteins plays an important role in embryonic development, the induction of cell polarity, and in the determination of cell fate. Deregulation of Wnt signaling disrupts axis formation in embryos [18–20] and is associated with multiple human malignancies [21]. Wnt signaling also plays an important role in the proliferation

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and differentiation of stem cells including hMSCs [22,23]. The NF-κB signaling pathway is another important signal transduction pathway that plays a critical role in activating the expression of genes involved in the immune and inflammatory response and in regulating cellular apoptosis [24–27]. NF-κB is an important mediator for the production of cytokine and chemokines in hMSCs [28].

Deng and colleagues [29] first suggested that β-catenin interacted with NF-κB and inhibited its activity in human colon and breast cancer cells. They found that β-catenin could physically complex with NF-κB, resulting in a reduction of NF-κB DNA binding, transactivation activity, and target gene expression. Subsequently, several studies have demonstrated that the activation of the β-catenin/Tcf pathway negatively regulates the NF-κB pathway in colon and breast cancer cells [30] and in response to bacterial stimulation in intestinal epithelial cells [31]. On the other hand, stimulation of the Wnt cascade through the upregulation of either Wnt or degradation-resistant β-catenin significantly enhances both baseline and TNF-α-induced NF-κB activity, which is mediated through the E3 ligase TrCP1, in vascular smooth muscles [32]. These findings highlight the complex interactions between the β-catenin/Tcf and NF-κB signaling pathways and further emphasize the importance of characterizing their interactive role in biological functions. However, crosstalk between these two pathways has not been examined in hMSCs. The aim of this study was to determine the effect of the modulation of NF-κB activity on β-catenin/Tcf signaling and the molecular mechanisms underlying this activity in hMSCs.

2. Materials and methods

2.1. Cell culture

Human adipose tissue-derived mesenchymal stem cells (hASCs) and human bone marrow stromal cells (hBMSCs) were cultured as described by Lee et al. [9]. All protocols involving human subjects were approved by the Institutional Review Board of Pusan National University. We obtained the adipose tissues of 3 different patients with informed consent who performed abdominoplasty (41-year-old male patient, 52-year-old female patient and 58-year-old female patient). To isolate hASCs, adipose tissue samples were washed with phosphate-buffered saline (PBS) and digested at 37 °C for 30 min with 0.075% type I collagenase. Enzyme activity was neutralized with α-modified Eagle's medium (α-MEM) containing 10% fetal bovine serum (FBS). The samples were centrifuged at 1200×*g* for 10 min and the pellet was incubated overnight at 37 °C under 5% CO_2 in a control medium (α-MEM, 10% FBS, 100 U/ml of penicillin and 100 μg/ml of streptomycin). Following incubation, the tissue culture plates were washed to remove any residual nonadherent cells and then maintained at 37 °C under 5% CO_2 in the control medium. Bone marrow samples were obtained from the proximal femur of two patients (52-year-old male patient and 54-year-old female patient) who performed total hip replacement surgery. Mononuclear cells from bone marrow were separated by centrifugation in a Ficoll-Hypaque gradient (density = 1.077 g/cm^3; Sigma, USA), suspended in α-MEM containing 10% FBS, 100 U/ml of penicillin and 100 μg/ml of streptomycin and seeded at a concentration of 1×10^6 cells/cm^2. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. When the monolayer of adherent cells reached 70% confluence, they were trypsinized

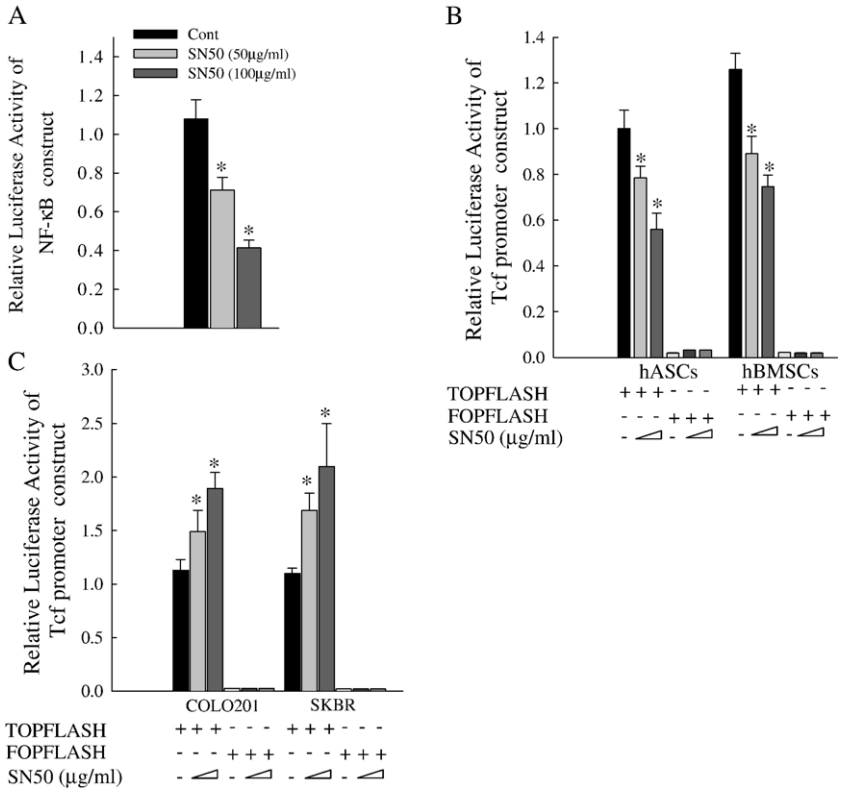

Fig. 1. Effect of NF-κB inhibitors on NF-κB and β-catenin/Tcf signaling pathways in hASCs and hBMSCs. (A) hASCs were cotransfected with NF-κB reporter constructs and β-Gal vector. (B and C) hASCs and hBMSCs (B) and cancer cell lines (C) were cotransfected with either TOPFLASH or FOPFLASH reporter constructs and β-Gal vector. Transfected cells were treated without or with SN50 (50 and 100 μg/ml) for 48 h. Luciferase activity was normalized by β-galactosidase activity. Data represent mean±S.E.M. of four different experiments. *$p<0.05$ compared with data in the absence of SN50.

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