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Involvement of smad2 and Erk/Akt cascade in TGF-β1-induced apoptosis in human gingival epithelial cells

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ABSTRACT

Periodontitis is the most prevalent infectious disease caused by periodontopathic bacteria and is also a chronic inflammatory disease. Gingival crevicular fluid (GCF) is an inflammatory exudate that seeps into the gingival crevices or periodontal pockets around teeth with inflamed gingiva, and contains various materials including leukocytes and cytokines. Since gingival epithelial cells, which form a barrier against bacterial challenges, are affected by GCF, cytokines or other materials contained within GCF are engaged in the maintenance and disruption of the epithelial barrier. Accordingly, its compositional pattern has been employed as a reliable objective index of local inflammation. Transforming growth factor β1 (TGF-β1) levels in GCF were previously shown to be markedly higher in patients with periodontitis than in healthy subject. However, it currently remains unclear how TGF-β1 affects gingival epithelial cell growth or apoptosis; therefore, elucidating the mechanism responsible may lead to a deeper understanding of pathogenic periodontitis. In the present study, the human gingival epithelial cell line, OBA9 cells were stimulated with recombinant TGF-β1. Apoptosis-related protein levels were determined by Western blotting. Caspase-3/7 activity was measured with a Caspase-Glo assay kit. Surviving and apoptotic cells were detected using an MTS assay and TUNEL staining, respectively. TGF-βRI siRNA and smad2 siRNA were transfected into cells using the lipofectamine RNAiMAX reagent. TGF-β1 elevated caspase-3 activity and the number of TUNEL-positive apoptotic cells in OBA9 cells. Furthermore, while the levels of the pro-apoptotic proteins Bax, Bak, Bim, and Bad were increased in OBA9 cells stimulated with TGF-β1, the TGF-β1 treatment also decreased the levels of anti-apoptotic proteins such as Bcl-2 and Bcl-xL in a time-dependent manner. Additionally, TGF-β1 up-regulated the protein levels of cleaved caspase-9. These results indicated that TGF-β1-induced apoptosis was involved in a mitochondria-related intrinsic pathway. TGF-β1 phosphorylated smad2 in OBA9 cells and this phosphorylation was clearly reduced by SB431542 (a TGF-β type I receptor inhibitor). Consistent with this result, SB431542 or smad2 siRNA-induced reductions in smad2 protein expression levels attenuated TGF-β1-induced apoptosis. On the other hand, the ligation of TGF-β1 on its receptor also stimulated the phosphorylation of Erk and Akt, which are smad2-independent pathways. However, the inhibition of Erk/Akt signaling pathways by U0126, a MEK-Erk inhibitor and LY294002, a PI3Kinase-Akt inhibitor, augmented TGF-β1-induced apoptosis in OBA9 cells. Taken together, the results of present study demonstrated that TGF-β1 activated both the smad2 and Erk/Akt cascades via its receptor on gingival epithelial cells, even though these two pathways have opposite roles in cell death and survival, and the culmination of these signaling events induced mitochondria-dependent apoptosis in gingival epithelial cells. Based on the results of the present study, we herein proposed for the first time, that TGF-β1 is a novel target cytokine for monitoring the progression of periodontal disease.

1. Introduction

Periodontitis is the most prevalent infectious disease caused by periodontopathic bacteria and is also a chronic inflammatory disease. Gingival crevicular fluid (GCF), which is an inflammatory

Abbreviations: GCF, gingival crevicular fluids; TGF-β, transforming growth factor-β; MAPKs, mitogen activated protein kinases.

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exudate that seeps into the gingival crevices or periodontal pockets around teeth with inflamed gingiva, contains various materials including leukocytes (mainly neutrophils), antibodies, complement proteins, enzymes, and cytokines. These materials in the GCF originate from periodontal tissue consisting of gingival epithelial cells, gingival fibroblasts, and a wide variety of immune cells, and promote periodontal tissue destruction in coordinated manner. Accordingly, its compositional pattern has been employed as a reliable objective index of local inflammation [1,2].

The gingival junctional epithelium located at the strategically important interface at the bottom of gingival sulcus is an early line of defense against microbial assaults. This barrier line is maintained by gingival epithelial cells. The turnover of the gingival junctional epithelium is rapid, thereby maintaining homeostasis. This turnover is sustained by the proliferation of progenitor cells and apoptosis of superficial cells. Apoptosis in superficial cells in the junctional epithelium is regulated in order to eliminate aged, damaged, or infected cells from tissues. However, previous studies reported that periodontopathic bacteria and virulence factors increased the number of apoptotic gingival epithelial cells, and also that many apoptotic cells were detected in the gingival epithelium of patients with periodontitis [3,4]. Thus, the induction of apoptosis in the gingival junctional epithelium by periodontopathic bacteria may be involved in the onset and progression of periodontitis [5,6]. Since gingival epithelial cells are known to be affected by GCF, cytokines or other materials contained within GCF are engaged in disrupting the epithelial barrier.

Transforming growth factor β1 (TGF-β1), which is produced by inflammatory immune cells, vascular endothelial cells, gingival fibroblasts, and epithelial cells, is a pleiotropic cytokine that regulates a broad spectrum of cellular processes including cell growth and apoptosis. Previous studies showed that TGF-β1 levels in GCF were markedly higher in patients with gingivitis and moderate periodontitis than in healthy subjects, suggesting its involvement in the progression of periodontal disease [1,7,8]. Moreover, TGF-β1 has been shown to induce apoptosis in epithelial cells [9,10]. Based on these findings, we can predict whether TGF-β1 in GCF induces apoptosis in gingival epithelial cells.

TGF-β1 exerts its biological effects by binding to a cell surface receptor complex of type I and type II receptors. Upon its ligation, the type II receptor phosphorylates the type I receptor (TGF-βRI). The activated receptors then stimulate multiple downstream signaling cascades involving smad2-dependent and smad2-independent pathways, as typified by mitogen-activated protein kinases (MAPKs) and Akt, respectively [11,12]. As a consequence, the culmination of these signaling events regulates cell growth or apoptosis.

Therefore, according to these accumulating lines of evidence, we hypothesized that TGF-β1 in GCF induced apoptosis in gingival epithelial cells, thereby contributing to the progression of periodontal disease. However, it currently remains unclear whether TGF-β1 induces cell growth or apoptosis in gingival epithelial cells. Therefore, we herein examined the effects of TGF-β1 on gingival epithelial cell growth or apoptosis, as well as the molecular mechanisms underlying these TGF-β1-mediated events.

2. Materials and methods

2.1. Chemicals

Recombinant human TGF-β1 (10 ng/ml) was obtained from R&D systems (Minneapolis, MN, USA). The following chemicals were used as inhibitors of cell signaling molecules: SB431542 (a TGF-β

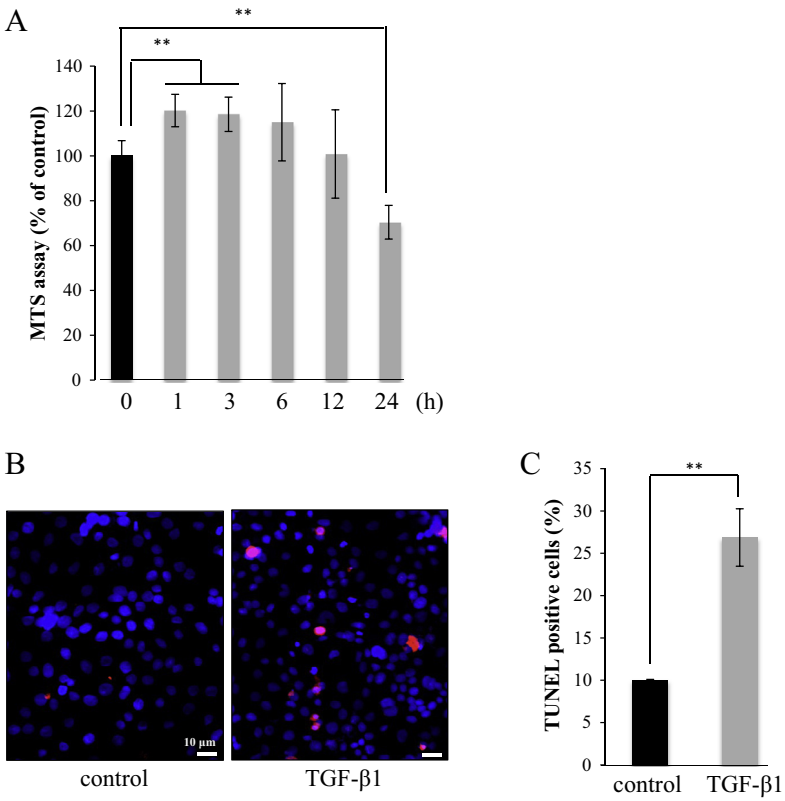

Fig. 1. Effect of TGF-β1 on proliferation and apoptosis in OBA9 cells. (A) OBA9 cells were exposed to TGF-β1 (10 ng/ml) for the indicated times. Cell proliferation was measured with MTS assay. Values represent means ± SD of five wells. (B and C) OBA9 cells were treated with or without TGF-β1 and incubated for 24 h. TUNEL-positive apoptotic cells (red) are shown under each set of conditions (B), and the graph shows the percentage of TUNEL-positive cells. Values represent means ± SD of three wells (C). Similar results were obtained from three experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)