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Transforming growth factor (TGF)-β in conjunction with H-ras activation promotes malignant progression of MCF10A breast epithelial cells

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Abstract

To address how transforming growth factor (TGF)-β and oncogenic H-ras signal transduction pathways interact with each other in the malignant progression of breast epithelial cells, we investigated the role of TGF-β signaling pathway in invasive and migrative properties of H-ras-transformed MCF10A human breast epithelial cells in this study. Here we show that TGF-β treatment significantly enhanced invasion and migration of H-ras MCF10A cells. H-ras-mediated activation of p38 MAPK and ERK-1/2 was stimulated by TGF-β. TGF-β increased expression of matrix metalloproteinase (MMP)-2 through transcriptional activation while TGF-β-stimulated MMP-9 up-regulation did not occur at transcription level. Activation of p38 MAPK pathway was required for TGF-β-induced cell migration, invasion and MMP-2/-9 up-regulation, indicating a critical role of p38 MAPK signaling in TGF-β-promoted tumor progression of H-ras-activated cells. ERKs signaling was also crucial for TGF-β-enhanced invasive and migrative phenotypes but the up-regulation of MMP-2/-9 was not dependent on ERKs activity. Taken together, we show that TGF-β promotes H-ras-mediated cell migration and invasive phenotypes in which p38 MAPK and ERKs signaling pathways are involved. Our findings revealing how H-ras and TGF-β signal pathways interact with each other in MCF10A human breast cells may provide an insight into molecular mechanisms for contribution of TGF-β to a malignant progression of breast cancer in collaboration with activated H-ras.

Keywords: TGF-β; H-ras; Breast epithelial cells; Invasion; Migration; MMP; MAPK

1. Introduction

The cytokine transforming growth factor (TGF)-β exerts diverse effects on a wide array of cellular processes ranging from proliferation, differentiation and apoptosis [1,2]. TGF-β has been identified as a potent inhibitor of the growth of normal epithelial cells while it acts as a stimulator of tumor invasion in advanced cancer cells. It has emerged as a potent inhibitor of the progression of normal epithelial cells and endothelial cells by growth arrest in the cell cycle [3,4]. On the other hand, TGF-β can exacerbate the malignant phenotype at later stages of tumorigenesis [5,6] by inducing epithelial-to-mesenchymal transition (EMT), cell invasion and migration of epithelial tumor cells [7,8]. TGF-β stimulates type IV collagenases, 72 kDa matrix metalloproteinase (MMP)-2 and/or 92 kDa MMP-9 [9], which can degrade type IV collagen, the major structural collagen of the basement membrane and thus play a critical role in tumor invasion and metastasis formation [10,11].

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Signaling of TGF-β is mediated by a heteromeric complex of two types of transmembrane serine/threonine kinase receptors. Binding of TGF-β to the receptor complex leads the type II receptor kinase to phosphorylate and thereby activate the type I receptor kinase. The activated type I receptor then phosphorylates receptor-activated Smads (R-Smads), Smad2 and Smad3 [1]. After phosphorylation by the type I receptor kinase, the R-Smads bind to Smad4 and move into the nucleus. In the nucleus, this Smad complex associates with other transcription factors to activate transcription of target genes [12,13]. In addition to the Smad-mediated TGF-β signaling pathway, recent evidences suggest that TGF-β may signal through other pathways, e.g. mitogen-activated protein kinases (MAPKs) including c-Jun-N-terminal protein kinase (JNK) [14], extracellular signal-regulated kinase (ERK) [15] and p38 MAPK [16].

Ras proteins are activated by multiple extracellular stimuli and are involved in regulatory biological processes from the outside of the cell to its interior through a complex array of downstream effectors, thereby controlling a variety of cellular responses such as proliferation, apoptosis, adhesion, and cytokine/matrix production [17–19]. Ras expression has been suggested as a marker for tumor aggressiveness of breast cancer [20–22]. It has been demonstrated that TGF-β collaborates with oncogenic Ras and brings about metastatic and invasive phenotypic changes in Ras-transformed mammary epithelial cells [23]. This process requires cooperation of Ras-MAPK and TGF-β signaling pathways contributing to tumor invasion [24,25].

We have previously shown that active mutant of H-ras$^{G12\rightarrow D12}$, but not N-ras$^{G12\rightarrow D12}$, induces invasive and migrative phenotypes in MCF10A human breast epithelial cells [26,27]. Our previous study also showed that TGF-β treatment induced migrative and invasive phenotypes, important phenotypic conversion during tumor progression, in preneoplastic MCF10A cells [28]. To address how H-ras and TGF-β signal transduction pathways interact with each other in malignant breast cell behavior, we investigated the TGF-β signaling pathway and its role in invasive and migrative properties of H-ras MCF10A cells. Here, we show that TGF-β stimulates H-ras-mediated cell migration and invasive phenotypes, which involves activation of p38 MAPK and ERKs pathways. We also provide evidence that TGF-β-enhanced MMP-2 and MMP-9 expression depends only on p38 MAPK signaling but is independent of ERKs activity.

2. Results

2.1. TGF-β stimulates invasion and migration of H-ras MCF10A cells

To investigate if TGF-β enhanced malignant cell behavior of highly invasive H-ras MCF10A cells, we examined invasive and migrative phenotypes of these cells. TGF-β significantly increased the number of invaded (Fig. 1A) and migrated (Fig. 1B) cells of H-ras MCF10A, suggesting that TGF-β contributes to tumor progression by converting H-ras-transformed MCF10A to a more malignant breast cancer cell line.

2.2. TGF-β stimulates H-ras-mediated activation of ERK-1/2 and p38 MAPK

Our previous study revealed that H-ras induced activation of p38 MAPK and ERK-1/2 but not that of JNK in MCF10A cells [27]. We examined the effect of TGF-β on the H-ras-induced activation of MAPK family members. H-ras MCF10A cells were treated with various concentrations of TGF-β for 1 h and the activation of JNK-1, ERK-1/2 and p38 MAPK was determined by immunoblot analysis using antibodies specific for the phosphorylated forms of these MAPKs. As shown in Fig. 2A, TGF-β enhanced the activation of ERKs and p38 MAPK in a concentration-dependent manner with 0.1 ng/ml being sufficient to increase phosphorylation of

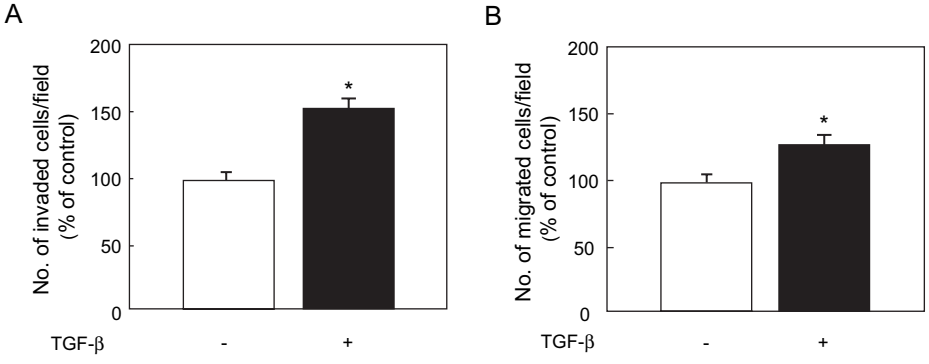

Fig. 1. TGF-β enhances invasive and migrative phenotypes of H-ras MCF10A cells. Cells were treated with TGF-β (10 ng/ml) and subjected to in vitro invasion assay (A) and in vitro migration assay (B). The number of invaded/migrated cells per field was counted (×400) in 13 fields. The results represent mean ± SE of triplicates. *Statistically different from control at $p < 0.05$.