The Intercellular junctions as a modulator of the TGF-β signaling pathway in epithelial cells

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ABSTRACT

In this study we show that polarity establishment is used by polarized epithelial cells to repress TGF-β signaling through the restriction of TGF-β receptors localization to the basolateral cell domain, while restricting the TGF-β ligands in the apical side of the epithelium.

INTRODUCTION

TGF-β (Tumor Gowth Factor β) is a pleiotropic cytokine involved in several physiological and pathological processes such as: cell proliferation, cancer and immunity. The TGF-β pathway is activated when the TGF-β ligand binds to its membrane receptors (TBRI, TBRII and TBRIII), which induces phosphorylation of the cytoplasmic proteins called SMADs. Phosphorylated Smads undergo nuclear translocation and act as transcriptional cofactors and induce the target genes of TGF-β. This cytokine is secreted by several cell types including epithelial cells. At high cell density, epithelial cell lines such as EpH4 and MDCK, form a polarized epithelium of cells whose apical domain differs from the basolateral domain in its composition in lipids and proteins. The separation between these two areas is ensured and maintained through the establishment of intercellular junctions, allowing them to perform different functions. However, other epithelial cell lines like HaCat form non polarized epithelia. It has been shown that in polarized epithelial cells, TGF-β is secreted exclusively at the apical domain.

Problématique → La polarisation des cellules épithéliales permet elle d'inhiber la signalisation……. À formuler

Only polarized epithelium block the transit of TGF-β from the apical to the basolateral domain

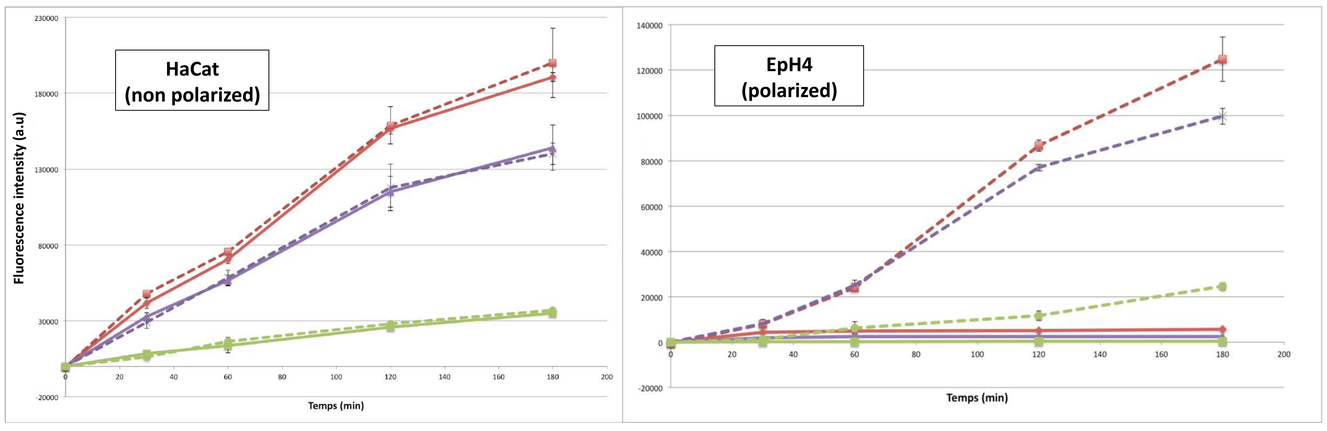
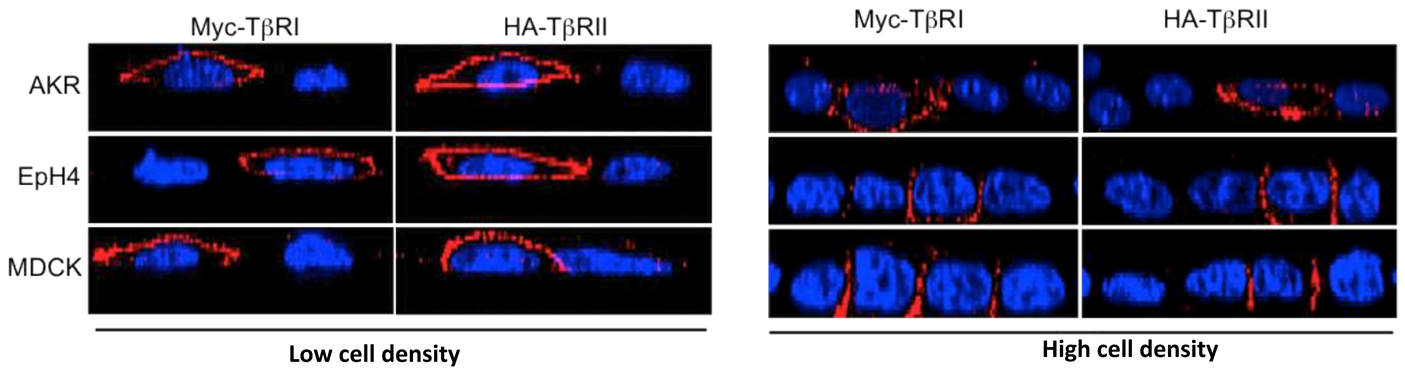


Figure 1 : Study of the paracellular permeability of EpH4 and HaCat cells at high cell density : Cells were seeded into inserts (12 mm diameter) to the density 2,5.105 cells / insert , and cultured for 3 days. Since the TGFβ ligand is 25KDa, FITC-Dextran (4, 20 and 40 kDa) was added to the apical compartment (1mg / ml) and 50 ml aliquots are taken from the basal compartment. The values of the fluorescence, expressed in unit arbitrary (ua) are the average of two independent measurements per condition. the condition "+ Ca^{2+}" is represented by the solid curves, and the 3-Ca^{2+}" provided by the discontinuous curves. Each color represents a size of FITC-Dextran: 4KDa red, 20kDa purple and green for 40 kDa. Measurement of paracellular permeability EpH4 cells (left panel), and HaCaT cells (right panel).

TGF-β receptors are located exclusively to the basolateral domain in polarized epithelial cells

Figure 2 : IF detection of transiently transfected Myc-TβRI and HA-TβRII in low density (LD, upper panel) and high density (HD, lower panel) monolayer cultures of AKR, MDCK, and EpH4 cells. Images are represented as XZ cross-sections of transfected cells. The AKR cells are fibroblasts that do not form a polarized epithelium, thus used as a control and show the same result as the HaCat cells (data not shown).



The establishment of polarity blocks TGF-β signaling

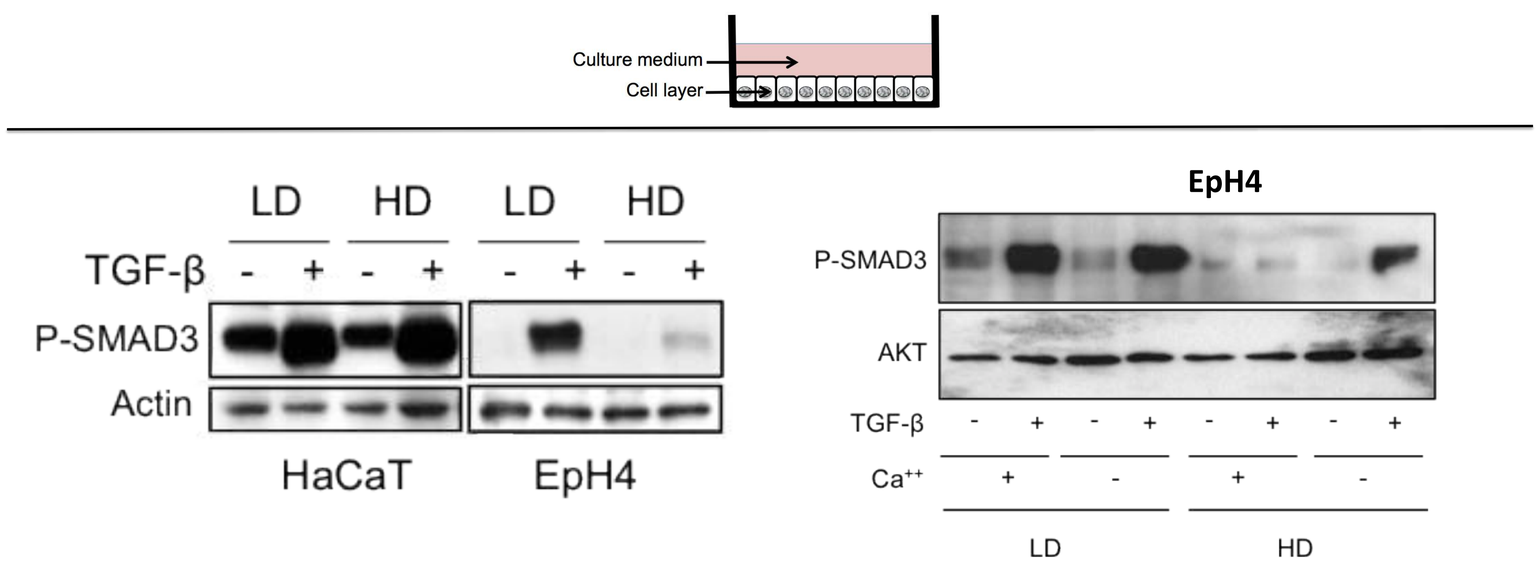


Figure 3 : Western analysis of P-SMAD3 levels without or with 30-min TGF-β stimulation in monolayer (schematic) . Actin levels were measured as a control for the specificity of P-SMAD3 changes under each experimental condition. Results from one representative of several independent experiments are shown.

Figure 4 : Western analysis of P-SMAD3 levels in EpH4 cells grown without (-) or with (+) TGF-β for 30 min. at low or high density in monolayer (schematic) cultures in the presence (+) or absence (-) of Ca2+.

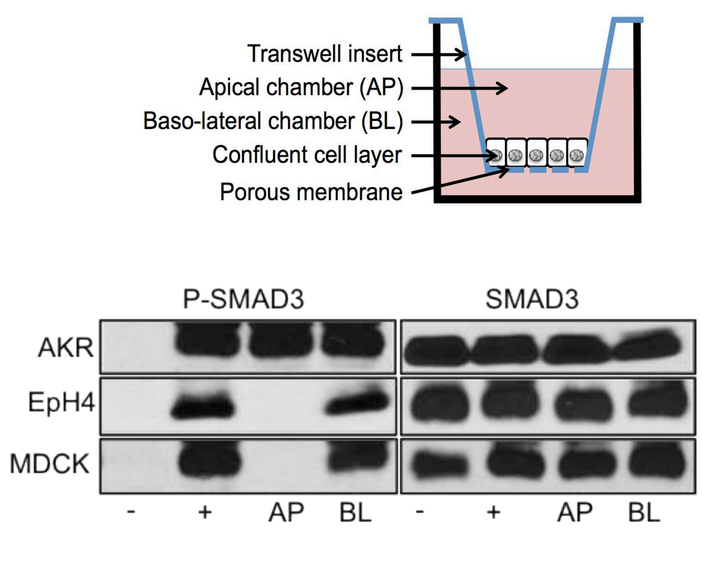
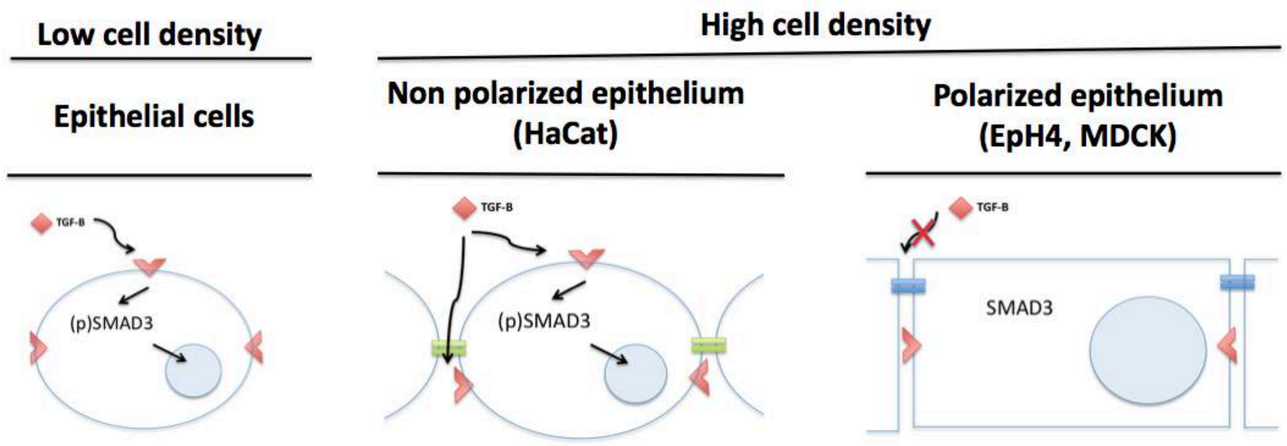


Figure 5 : Western analysis of P-SMAD3 (left panel) and SMAD3 levels (right panel) in AKR, MDCK, and EpH4 cells grown in Transwell cultures (schematic) following 60-min apical (AP) or baso-lateral (BL) stimulation with TGF-β.

CONCLUSION

The density-driven loss of TGF-β responsiveness, exclusively observed in polarized epithelial cells, is due to the establishment of Ca^{2+}-dependent cell-cell contacts that prevent the apical ligand from binding to, and activation of, basolaterally expressed TGF-β receptors. This, in turn, prevents SMAD phosphorylation, subsequent nuclear accumulation and gene responses.



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