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Stimulation of transforming growth factor-beta-1 and contact with type I collagen cooperatively facilitate irreversible transdifferentiation in proximal tubular cells

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ABSTRACT

Background: By transdifferentiation, proximal tubular cells (PTC) have been considered as a source of interstitial myofibroblasts. We examined the combined effect of transforming growth factor-beta-1 (TGF-β1) stimulation and contact with type I collagen on PTC transdifferentiation.

Methods: Human kidney-2 cells were grown on type I substratum with the concurrent stimulation of TGF-β1.

Results: Following addition of TGF-β1, cells acquired an elongated fibroblastic appearance and an increase in α-smooth muscle actin (α-SMA) expression, a myofibroblastic marker. Upon addition of TGF-β1, E-cadherin expression, an epithelial marker, was reduced, while cytokeratin expression, another epithelial marker, remained unaltered. Following removal of TGF-β1, PTC regained an epithelial appearance and E-cadherin expression reverted to the unstimulated level, suggesting incomplete and reversible transdifferentiation. Addition of TGF-β1 to cells grown on type I collagen demonstrated a cooperatively increased α-SMA expression and decreased E-cadherin and cytokeratin expressions, suggesting more complete transdifferentiation. Co-stimulation of TGF-β1 and contact with type I collagen led to a stable cell phenotype and persistently decreased E-cadherin, which was not reversed upon removal of TGF-β1, indicating irreversible transdifferentiation. Addition of TGF-β1 or type I collagen caused a 4-fold increase in migratory cell number as compared to the control, whereas addition of both TGF-β1 and type I collagen led to an 11-fold increase.

Conclusions: TGF-β1 alone results in a reversible and incomplete transdifferentiation. The combination of TGF-β1 and exposure to type I collagen leads to an irreversible and complete PTC transdifferentiation.

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At a glance commentary

Scientific background of the subject

Proximal tubular cells (PTC) have been considered as a source of interstitial myofibroblasts and transforming growth factor-beta-1 (TGF-β1) is crucial to induce phenotypic alterations of PTC to form fibroblastoid morphology. But previous studies imply that there are other factors required to cooperate with TGF-β1 to complete terminal transdifferentiation.

What this study adds to the field

This study demonstrates that TGF-β1 alone causes incomplete and reversible transdifferentiation. In contrast, combination of TGF-β1 stimulation and contact with type I collagen substratum cooperatively causes complete and irreversible PTC transdifferentiation.

It is now clear that progression of renal insufficiency is closely correlated to the degree of renal interstitial fibrosis [1,2]. Despite conflicting evidence arguing the origin of myofibroblasts [3,4], it has been demonstrated that renal proximal tubular cells (PTC) can contribute to progression of renal fibrosis through a process called epithelial-mesenchymal transition (EMT) or transdifferentiation, in which PTC exert a phenotypic conversion to acquire characteristics of mesenchymal cells [5–7]. Early work of Strutz *et al.* suggested that PTC may express fibroblast-specific markers *in vitro* and *in vivo* in a murine model of anti-tubular basement membrane and anti-glomerular basement membrane model of nephritis [8]. Ng *et al.* have demonstrated *de novo* expression of α-smooth muscle actin (α-SMA), a marker of myofibroblast phenotype, by PTC, associated with disruption of the tubular basement membrane in 5/6 nephrectomized rats [9]. Li *et al.* have also demonstrated that advanced glycation end products and induced epithelial-myofibroblast transdifferentiation in PTC [10].

Transforming growth factor-beta-1 (TGF-β1), a prototypic member of the TGF-β superfamily, exerts a broad range of biological activities. It plays pivotal roles during embryonic development where it is involved in induction of cell differentiation and organogenesis. Furthermore, TGF-β1 has been implicated in the pathogenesis of renal fibrosis in both experimental and human disease [11–14]. In addition, TGF-β1 has been shown to be involved in oncogenesis as it can induce EMT of mammary epithelial cells, thought to be important during transformation of squamous carcinoma to invasive spindle cell carcinoma [15,16]. *In vitro* studies also suggest that TGF-β1-induced phenotypic alterations of PTC to form fibroblastoid morphology and α-SMA expression and loss of epithelial markers, suggesting induction of EMT [17–19].

Early studies have demonstrated that tubular basement membrane is an essential structure for maintenance of epithelial phenotype as EMT of PTC is frequently associated with damage of tubular basement membrane [9,20–22]. Zeisberg *et al.* have clearly shown that disruption of the structure of type IV collagen, a major component of basement membrane, is essential for PTC transdifferentiation. These studies would therefore suggest that although TGF-β1 is a critical cytokine in the process of transdifferentiation, other factors are likely to act in a cooperative way to complete the process.

Since type I collagen is the most abundant extracellular matrix protein in the renal interstitium and the most possible candidate that transdifferentiated PTC may frequently contact with following migration through disrupted basement membrane into the interstitium, the aim of this study was to examine the combined effect of TGF-β1 stimulation and contact with type I collagen on PTC phenotype and function. The data demonstrate that contact with type I collagen have synergistic effects with TGF-β1, promoting irreversible PTC transdifferentiation.

Materials and methods

Cell culture

Human kidney-2 (HK2) cells (human renal PTC immortalized by transduction with human papilloma virus 16 E6/E7 genes [22]) were purchased from American Type Culture Collection (Manassas, VA, USA). Cells were cultured in DMEM/Ham's F12 medium (Invitrogen Ltd.) supplemented with 10% fetal calf serum (Biological Industries Ltd.), 2 μM glutamine (Life Technologies Ltd.), 20 mM HEPES buffer (Gibco BRL), 5 μg/mL insulin, 5 μg/mL transferrin and 5 ng/mL sodium selenite (Sigma). Cells were grown at 37 °C in 5% CO_2 and 95% air. Cells were grown to confluence and then serum deprived for 48 h prior to experimental manipulation. All experiments were performed under serum free conditions. For cell culture plate coated with type I collagen, 2 mg/mL of sterilized type I collagen (Sigma) was added to tissue culture plate overnight to form a monolayer of gel and cells were then seeded upon the gel on the next day.

Immunocytochemistry

Immunocytochemistry was performed on cells grown in 8-well chamber slides (Nunc, Gibco/BRL Life Technologies Ltd.) [23]. Cells were grown to confluence and stimulated under serum free conditions. Culture medium was subsequently removed and the cell monolayer washed with sterile phosphate buffered saline (PBS). For immunostaining of cytokeratin, cells were fixed in acetone-methanol (1:1 vol/vol) for 20 min at −20 °C. For the staining of α-SMA and E-cadherin, cells were fixed in 3.5% paraformaldehyde for 15 min at room temperature, and permeabilized with 0.1% triton in PBS for 5 min at room temperature. Following fixation, slides were blocked with 5% Bovine serum albumin (BSA) for 1 h at room temperature prior to a further washing step with PBS. Subsequently slides were incubated with the primary antibody diluted in 1% BSA/PBS for 1 h at room temperature. Primary antibodies included: Murine monoclonal anti-α-SMA clone 1A4 (Sigma), murine monoclonal anti-cytokeratin (DAKO), and murine monoclonal anti-E-cadherin (Transduction Laboratories). Following a further washing step slides were incubated with the appropriate fluorescein isothiocyanate-