Matrix Biology 28 (2009) 324–335

Contents lists available at ScienceDirect

Matrix Biology

journal homepage: www.elsevier.com/locate/matbio

Effects of transforming growth factor-β1 and vascular endothelial growth factor 165 gene transfer on Achilles tendon healing

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ARTICLE INFO

Article history:
Received 20 December 2008
Received in revised form 26 March 2009
Accepted 10 April 2009

Keywords:
TGF-β1
$VEGF_{165}$
Gene therapy
Adenovirus
BMSC
Achilles tendon

ABSTRACT

Repaired Achilles tendons typically take weeks before they are strong enough to handle physiological loads. Gene therapy is a promising treatment for Achilles tendon defects. The aim of the present study was to evaluate the histological/biomechanical effects of Transforming growth factor-β1 (TGF-β1) and vascular endothelial growth factor 165 ($VEGF_{165}$) gene transfer on Achilles tendon healing in rabbits. Bone Marrow-Derived Mesenchymal Stem Cells (BMSCs) were transduced with adenovirus carrying human TGF-β1 cDNA (Ad-TGF-β1), human $VEGF_{165}$ cDNA (Ad-$VEGF_{165}$), or both (PIRES-TGF-β1/$VEGF_{165}$) Viruses, no cDNA (Ad-GFP), and the BMSCs without gene transfer and the intact tendon were used as control. BMSCs were surgically implanted into the experimentally injured Achilles tendons. TGF-β1 distribution, cellularity, nuclear aspect ratio, nuclear orientation angle, vascular number, collagen synthesis, and biomechanical features were measured at 1, 2, 4, and 8 weeks after surgery. The TGF-β1 and TGFβ1/$VEGF_{165}$ co-expression groups exhibited improved parameters compared with other groups, while the $VEGF_{165}$ expression group had a negative impact. In the co-expression group, the angiogenesis effects of $VEGF_{165}$ were diminished by TGF-β1, while the collagen synthesis effects of TGF-β1 were unaltered by $VEGF_{165}$. Thus treatment with TGF-β1 cDNA-transduced BMSCs grafts is a promising therapy for acceleration and improvement of tendon healing, leading to quicker recovery and improved biomechanical properties of Achilles tendons.

1. Introduction

Transforming growth factor-β1 (TGF-β1) and vascular endothelial growth factor (VEGF) have been previously shown to induce tendon or ligament formation (Klein et al., 2002; Anaguchi et al., 2005; Wang et al., 2005; Chan et al., 2008). TGF-β1 is a prototypic multifunctional cytokine which regulates a wide range of biological processes including cell proliferation, migration, differentiation, apoptosis, and extracellular matrix deposition, and plays a key role in the tendon healing process. TGF-β1 exerts its neovascularization effects at several levels: control of vascular cell proliferation, maintenance of differentiation, and increased immunomodulatory and anti-inflammatory actions. Some traditional drugs such as aspirin (Redondo et al., 2003; Nakamura et al., 2006) and pioglitazone (Redondo et al., 2005; Ruiz et al., 2007) act on the TGF-β1 pathway. TGF-β1 is also known to enhance tendon repair during the fibrosis period in vitro and vivo by increasing cell proliferation, migration, and the synthesis of both collagen and proteoglycan. TGF-β1 was shown to be released from cultured tendon fibroblasts in response to mechanical loading, and has been confirmed to play a role in mechanical regulation of local collagen type I and type III synthesis in tendon-related connective tissue in vivo (Anaguchi et al., 2005; Sporn 2006; Xia et al., 2007). However, although TGF-β1 can accelerate tendon repair, the spatio-temporal distribution of TGF-β and its receptor restrict its function, and even cause postoperative adhesions in the normal healing progress (Chan et al., 2008). Potentially, exogenous TGF-β1 may change this distribution and accelerate tendon healing.

VEGF plays an essential role in vascular development during granulation where VEGF is required for the formation of the initial vascular plexus early in granulation tissue development (Ferrari et al., 2006; Gavard et al., 2008). VEGF can promote endothelial survival, proliferation, and migration by acting on its cognate cell-surface tyrosine-kinase receptors, VEGFR2 (Flk1, KDR) and Tie2 (Tek) (Jones et al., 2001), and contributes to vascular bud formation and endotheliocyte migration during neovascularization, which occurs at the primary stage of tendon healing. Importantly, VEGF and TGF-β1 are often co-expressed in tendon healing, and several studies have demonstrated that together they can enhance each other's synthesis (Anitua et al., 2005, 2007; Wang et al., 2005, 2008). Furthermore, TGF-β1 is involved in endothelial cell apoptosis, which is required for pruning the forming vascular network, acting as a downstream factor following VEGF in the granulation progress.

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doi:10.1016/j.matbio.2009.04.007

Without persistent exposure, the biological functions caused by growth factors would be lost with sequential passage. For the purpose of persistent gene expression, bone marrow-derived mesenchymal stem cells (BMSCs) have been used as a platform for gene transfer. Furthermore, the BMSCs themselves act as an individual healing factor. The therapeutic action of transplantation of BMSCs is mainly attributed to their paracrine activities, including production of large amounts of cytokines and growth factors. Furthermore, this unique stem cell population is able to differentiate into tenocytes in vivo and vitro (Awad et al., 1999; Hoffmann et al., 2006; Chong et al., 2007).

In the present study, we hypothesized that (1) the healing of experimentally injured Achilles tendons in rabbits would be enhanced by cell-based gene transfer of VEGF and TGF-β1, and (2) there would be further improvement when these two genes were co-expressed. To perform these studies, we transfected BMSCs with VEGF and/or TGF-β1, surgically implanted the BMSCs into the Achilles tendon lesion area in a rabbit model, then examined the rate and extent of tendon-healing.

2. Results

2.1. Gross findings

There were no macroscopic differences between the five tendon treatment groups and no repair ruptures. At two weeks, the fibrin glue in the repair site had almost completely degraded. The repair site was hypertrophic and appeared semi-translucent. At four weeks, there was no trace of fibrin around the repair site and the repair site was less thick. By eight weeks, the repair site appeared continuous with the proximal and distal ends of the tendon in terms of thickness and opacity. Compared with the normal tendon, the cross-sectional area of the repair site was increased significantly at two and four weeks in all the treatment groups except the $VEGF_{165}$ treatment group ($p<0.01$). There were no differences between the four groups which showed improvement. The cross-sectional areas in all groups except the VEGF treatment group decreased to values similar to the normal group at four weeks, which remained constant at eight weeks.

2.2. Western blot analyses

Western blot of the proteins collected from the transfected BMSCs demonstrated the gene modified BMSCs secreted the desired proteins (Fig. 1A). Western blot of the proteins collected from the paratendon cells cultured with different supernatants showed that the Smads pathway (activated by TGF-β) and the Erk1/2 pathway (activated by VEGF) were activated by the supernatants collected from the TGF-β1 treated BMSCs (Fig. 1B), the TGF-β1/VEGF co-expression treated BMSCs (Fig. 1C,D), and the $VEGF_{165}$ treated BMSCs (Fig. 1E).

2.3. Enzyme-linked immunospecific assay (ELISA) analyses

One-way ANOVA analysis revealed that the concentration of TGF-β1 or $VEGF_{165}$ exhibited significant differences between all the treatment groups ($p<0.01$), post hoc comparisons demonstrated that both TGF-β1 and $VEGF_{165}$ concentration increased significantly in the supernatant from BMSC with $VEGF_{165}$, TGF-β1 or co-expression gene

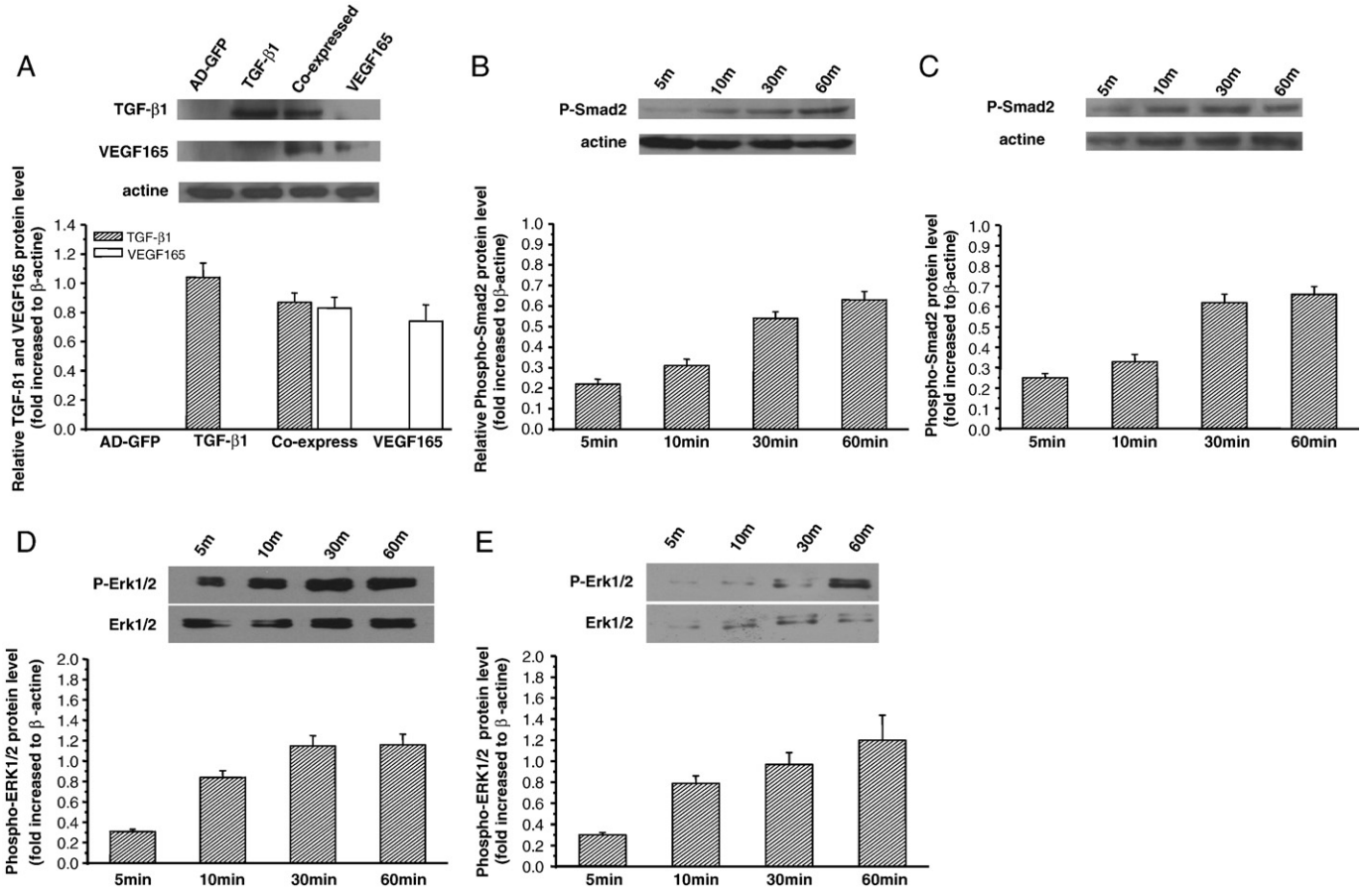

Fig. 1. Western blot analysis. (1A) Protein expression of BMSCs which were transfected the recombinant virus of AD-GFP, TGF-β1, $VEGF_{165}$ or co-expression of TGF-β1/$VEGF_{165}$. (1B) Smad pathway in paratendon cells was activated by TGF-β1 from supernatant collected from BMSCs transfected with the recombinant virus of TGF-β1. (1C) Smad pathway in paratendon cells was activated by TGF-β1 from supernatant collected from BMSCs transfected with the recombinant virus of TGF-β1/VEGF co-expression. (1D) Erk1/2 pathway in paratendon cells was activated by $VEGF_{165}$ from supernatant collected from BMSCs transfected with the recombinant virus of $VEGF_{165}$. (1E) Erk1/2 pathway in paratendon cells was activated by $VEGF_{165}$ from supernatant collected from BMSCs transfected with the recombinant virus of TGF-β1/VEGF co-expression.