

Bone and Cartilage Repair

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Every cell in our body is subjected to stress! This may take various forms such as chemical stress from neurotransmitters and exogenous agents to physical such as elevated shear stress and bone loading through to traumatic injury. Many of the biochemical and molecular stimuli that regulate response to stress are common in different cell types and I will emphasise how an understanding of stress response systems in the cardiovascular system may provide clues to new targets and therapeutic approaches in tissue repair. In this presentation I will discuss how the shear stress responsiveness of the transcription factor Egr-1 in vascular endothelial cells may provide us with a unique way to target multiple growth factor cascades and promote tissue repair in both skin and bone.

Despite intensive research effort and many clinical trials, the potential of growth factors, when delivered as single proteins, to stimulate soft and hard tissue repair has not been fully realised. This may be on account of a number of factors, which include efficacy of the growth factor protein in its formulation, its penetration to the target tissue and the heterologous nature of growth factor expression and cellular pathology in patients with complex disease. At present, recombinant human platelet derived growth factor-B (PDGF-B) is the only biological indicated for the treatment of diabetic foot ulcers and has shown some, but limited efficacy in clinical trials. To counter the limited success attainable with a single growth factor, autologous cocktails of growth factors derived and purified from the patient's plasma has received some attention but met with restricted use owing to cost implications, reimbursement and lack of efficacy data from any clinical trial. The use of transcription factors, as either activators or repressors of transcription, may circumvent the use of single or multiple proteins as these factors themselves may serve to stimulate or repress the natural signaling cascades that serve to direct tissue repair. In this presentation, I will describe the use of transcriptional modulators for use in tissue repair and regeneration and illustrate this utility with a transcriptional activator proteins; Egr-1, and a transcriptional corepressor protein, NAB2.

Egr-1 for promotion of soft and hard tissue repair and regeneration

Early growth response factor-1 (Egr-1; also termed NGFI-A, Krox24, Zif268 and TIS8) is an immediate early gene coding for an 80 kDa phosphoprotein transcription factor. Egr-1 belongs to the C₂H₂ class of zinc finger proteins which include Egr-2 (also called krox-20), Egr-3 and Egr-4 (also called NGFI-C;). Like HIF-1, Egr-1 is naturally produced in response to acute stimuli, but unlike hypoxia inducible factor-1 (HIF-1), the range of stimuli to which Egr-1 is responsive is considerably more pleiotropic. The Egr-1 promoter comprises transcription factor binding sites which allow stimulation of Egr-1 transcription by a number of physiological stimuli. These stimuli include hypoxia (through a non-HRE dependent mechanism), laminar fluid shear stress generated *in vitro* or *in vivo*, mechanical stretch, bone loading and acute tissue injury. Egr-1 transcription may also be induced by non-physiological stimuli such as electric shock, phorbol ester radiation and urea. The induction of Egr-1 protein stimulates the production of many

genes whose products play a role in cellular growth, development and differentiation. These include genes encoding cytokines (tumour necrosis factor- α), adhesion molecules (intercellular adhesion molecule-1), members of the coagulation cascade (tissue factor, urokinase-type plasminogen activator), and growth factors such as acidic FGF, basic FGF, transforming growth factor- β_1 , (TGF- β_1), platelet derived growth factor-A (PDGF-A) and PDGF-B, hepatocyte growth factor (HGF), VEGF and insulin like growth factor-II. We have studied the effects of Egr-1 expression at the site of dermal wounding in healthy rodents whose response to wounding is not impaired by, for example, diabetes. We found that Egr-1 promoted angiogenesis *in vitro* and *in vivo*, enhanced re-epithelialisation, increased collagen production and accelerated wound closure. In addition to our work in wound healing, we have shown that Egr-1 is capable of inducing bone formation in a rodent model of ectopic osteogenesis. These studies demonstrate that Egr-1 gene therapy can accelerate the normal healing process and raises the potential use of this therapeutic transcription factor for any aspect of tissue repair. This includes repair of tendon, ligament, muscle and spinal cord and regeneration of lung tissue in patients with chronic obstructive pulmonary disease.

NAB2 for applications in limiting the healing process

NGFI-A-Binding proteins (NAB1 and NAB2) bind to the Egr family of transcription factors. Binding of the NAB proteins to Egr-1 and Egr-2 results in repression of Egr-1 and Egr-2 *trans*-activation of target genes and both NAB1 and NAB2 have been termed transcriptional corepressors. Recent studies have shown that after balloon injury of the rat aorta, expression of Egr-1 and NAB2, but not NAB1 is rapidly stimulated. The paralleled increase in expression of both NAB2 and Egr-1 suggest that NAB2 may function in a feedback manner to limit the *trans*-activating effect of Egr-1. NAB2 may repress the NGF-induced differentiation of PC12 cells and inhibit Egr-2 mediated activation of the bFGF promoter in fibroblasts. We have shown that NAB2 may inhibit Egr-1 activation of PDGF-AB, TGF- β_1 , HGF and VEGF and the endogenous expression of PDGF-AB and TGF- β_1 in vascular smooth muscle cells. In addition, expression of a wild type but not dominant negative NAB2 mutant abrogated Egr-1 mediated angiogenesis. It has been suggested that the NAB proteins act as a transcriptional 'brake' for Egr-1 activated gene expression in the vessel wall. Thus, NAB2 appears to repress a number of key growth factors which have been linked to VSMC proliferation and remodelling in restenosis and to dermal fibroblast hyperproliferation manifested in the production of hypertrophic and keloid scarring. Given the ubiquitous expression of both NAB and Egr proteins it is likely that the NABs act to restrict Egr activity in all tissue and may have therapeutic utility in any condition where the over-expression of, for example growth factors, is disadvantageous or undesirable to the tissue or organ. In addition to therapeutic utility in restenosis and dermal scarring, NAB2 may have use in inhibiting Egr-1 driven osteogenesis such as may be found at sites of surgery in patients with the bone forming disease fibrodysplasia ossificans progressiva.

In summary, we believe that as the science of tissue repair with applications to regenerative medicine develops, there will be an increased role for the use of this class of 'master regulators' of transcription. The topical application and therefore restricted

systemic exposure of transcription factors in simple delivery vehicles at sites of tissue injury or loss should be both efficacious and economically viable. The use of protein matrices and stem cells in stimulating both cartilage and bone repair is in its infancy, but given the rapid advances in scientific understanding and the burden of unmet need for many degenerative diseases, successful clinical trials and new medical products are on the horizon. The recent FDA fast-track status award for Prochymal (Osiris Therapeutics) for acute graft versus host disease heralds the start of stem cell therapies entering phase II clinical trials. Chondrogen (Osiris Therapeutics) a further stem cell product, is currently under FDA review for the treatment of joint damage and the outcomes of these clinical trials will be eagerly awaited.

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