TISSUE REGENERATION

Better growth factor binding aids tissue repair

Enhancing the binding of growth factors to heparan sulphate proteoglycans in the extracellular matrix and on cell surfaces improves wound healing and bone regeneration in mice.

Megan Lord, John Whitelock & Jeremy E Turnbull

By interacting with cells and the surrounding extracellular matrix (ECM), growth factors regulate cellular processes such as proliferation, differentiation and wound healing. Hence, these signalling proteins hold great promise in regenerative medicine applications, in particular for tissue repair. However, despite some clinical success, suboptimal efficacy and limitations in the delivery and slow clearance of growth factors have led to treatment regimens that administer them in supraphysiological doses and that often lead to unwanted adverse effects. Strategies for addressing some of these issues involve improving the binding affinity of growth factors to ECM molecules, for greater stability and control over growth factor release, bioavailability and spatiotemporal cellular signalling, and boosting the signalling strength of growth factors by modifying their affinity for cell-surface proteins rather than those in the ECM. Proteoglycans — a class of complex glycoproteins containing glycosaminoglycan (GAG) chains attached to core proteins — are ideally suited as binding targets for such engineered growth factors. This is because proteoglycans are ubiquitously expressed in the ECM and at the cell surface of almost all mammalian tissues, and because they exert diverse biological functions via the selective binding of the GAG chains to growth factors and other proteins[1].

Growth factors can be modified[2] to display higher affinity for the transmembrane-protein family of heparan sulphate proteoglycans (HSPGs). HSPGs control the activity of multiple HS-binding growth factors, including those involved in tissue-repair processes such as fibroblast growth factors, vascular endothelial growth factors (VEGFs) and platelet-derived growth factors[3] (PDGFs). Reporting in *Nature Biomedical Engineering*, Mikaël Martino and colleagues now show that growth factors modified for improved control of their release from ECM-based carriers and for improved binding to cell- surface proteoglycans enhance the efficacy of tissue repair in mice[4].

Martino and co-authors added additional HS - binding sites to VEGF-A and PDGF-BB by using a peptide derived from the heparin binding region of the alpha subunit of laminin. The peptide, which is commonly known as AG73, binds to cell- surface HSPGs of the syndecan family via their HS side- chains. For VEGF-A165, which lacks the natural HS-binding site found in the longer VEGF variants, the addition of the AG73 peptide yielded a construct (VEGF-A-SB; with SB meaning syndecan binding) with high affinity binding to HS (2–4-fold higher affinity for syndecans than VEGF-A165). By contrast, for PDGF-BB, which contains a natural HS -binding domain, the authors fused the AG73 peptide as an additional SB site, resulting in a construct (PDGF-BB-SB) with 4–6-fold higher affinity for syndecans. Although these effects were consistent with expectations from previous studies[3], signalling assays indicated that VEGF-A-SB and PDGF-BB-SB elicited altered kinetics in, respectively, endothelial cells and in mesenchymal stem cells. Whilst wild-type VEGF-A165 and PDGF-BB led to 'burst' signalling (that is, elevated but short-lived; Fig. 1a), VEGF-A-SB and PDGF-BB-SB displayed 'tonic' signalling (that is, sustained and of lower intensity; Fig 1b). In addition, VEGF-A-SB and PDGF-BB-SB were retained for longer periods on cell surfaces, owing to their expected binding to cell- surface syndecans (and possibly to other HSPGs). Also, they triggered significantly lower desensitization of their cognate receptors (measured as internalisation and degradation) than their wild-type counterparts. These altered kinetics suggest that the engineered growth factors might be advantageous for tissue repair. Moreover, the authors added an additional fibrin-binding site based on residues 1–8 from the alpha-2 plasmin-inhibitor (α_2PI_{1-8}), with an intervening matrix-metalloprotease cleavage site. These constructs, termed α_2PI_{1-8}-M-PDGF-BB-SB and α_2PI_{1-8}-VEGF-A-SB, could bind to the ECM and to HS. Cell-based assays showed that these constructs had further increased potency, with α_2PI_{1-8}-M-PDGF-BB-SB showing higher and more sustained activity in promoting the proliferation and colony formation of mesenchymal stem cells. Likewise, α_2PI_{1-8}-M-VEGF-A-SB displayed higher and sustained activity in endothelial cells, promoting their proliferation as well as angiogenesis.

To test the ability of α_2PI_{1-8}-M-PDGF-BB-SB and α_2PI_{1-8}-VEGF-A-SB to repair tissue *in vivo*, Martino and co-authors delivered the constructs in a fibrin matrix at doses lower than those required for the wild-type variants in the context of bone repair. α_2PI_{1-8}-M-PDGF-BB-SB was delivered within a fibrin matrix placed around critical-size calvarial defects in mice. Whilst wild-type PDGF-BB did not significantly increase bone regeneration, α_2PI_{1-8}-M-PDGF-BB-SB and α_2PI_{1-8}-VEGF-A-SB led to significant increases in bone coverage and volume. In the context of wound healing, the authors delivered VEGF proteins to full-thickness skin wounds in diabetic mice and quantified granulation tissue (new connective tissue and blood vessels) as well as the extent of wound closure evidenced by re-epithelialization. The wild-type variants VEGF-A121 and VEGF-A165, and also α_2PI_{1-8}-M-VEGF-A165, did not promote healing, yet α_2PI_{1-8}-M-VEGF-A-SB led to enhanced granulation and wound closure. Furthermore, angiogenesis (key in sustaining newly-formed granulation tissue) was most strongly promoted by α_2PI_{1-8}-M-VEGF-A-SB.

A key criterion for evaluating the potential clinical value of such engineered growth factors is the relative risk of them causing adverse effects (PDGF-BB increases cancer risk, and VEGF-A increases vascular permeability). Martino and colleagues show that α_2PI_{1-8}-M-PDGF-BB-SB did not lead to the accelerated tumour growth observed in mice treated with PDGF-BB or M-PDGF-BB, and that in classic in vivo vascular-permeability assays in mice α_2PI_{1-8}-M-VEGF-A-SB induced markedly lower

levels of vascular leakage when compared with α_2PI_{1-8}-M-VEGF-A121 and α_2PI_{1-8}-M-VEGF-A165. These results suggest that the engineered constructs are safer than the wild-type growth factors and than the constructs only incorporating SB. Hence, the 'multi-site' strategy of adding additional ECM and proteoglycan binding domains into growth factors is a promising strategy for enhancing tissue repair.

Additional work is however needed to fully elucidate the mechanisms of action of the engineered growth factor constructs. In particular, the AG73 peptide is likely to bind generically to HS on HSPGs present in the ECM and on the cell surface, rather than just to syndecans, and the extent and location of binding is likely to be variable in different tissues, depending on the HS binding site used. Further investigation of the tonic signalling is warranted to ensure that unwanted longer-term side-effects are not induced in multiple tissues. Increased understanding of such factors will underpin the development of engineered growth factors with multiple optimized properties, including ECM and cell-surface affinities and localization, and growth factor release, stability and signalling activity. This could result in reduced dosages, adverse effects and costs. Furthermore, this strategy may provide opportunities to tune many more tissue-repair signalling molecules. Similar strategies for enhancing the affinity of growth factors for their receptors include the addition of the heparin-binding domain of the epidermal growth factor, of binding domains for matrix molecules such as collagen, fibronectin, fibrin and matrix metalloproteinases, and of plasmin cleavage sites; these modifications altered the binding and residence time of the growth factors in tissues, and enabled their controlled release from biomaterial scaffolds[3,5].

Typically, tissue-repair approaches have focused on biomaterial scaffolds presenting GAGs as growth factor-binding partners[6]. The most widely explored GAG in these applications is heparin, because of its commercial availability, and because it often improves performance when presented alone or with exogenous growth factors[6–8]. Owing to heparin's frequent suboptimal growth factor signalling and adverse effects as an anticoagulant, the development of natural[9,10] and chemoenzymatic[11] GAG saccharide libraries may help discover potent HS structures that can replace heparin in these approaches. Also, non-mammalian polysaccharides such as chitosan and alginate are abundant, biocompatible and readily available, and have been used as biomaterials alone or in hybrid scaffolds after chemical modification to promote endogenous growth factor interactions[12]. The incorporation of proteoglycans into tissue-repair strategies hasn't been widely explored, most likely because of their low abundance and high cost, and because of challenges in their expression with functional GAGs[7].

Gene-delivery approaches using biomaterial scaffolds have led to wound repair by simultaneously inducing proteoglycan and growth factor expression[13], and gene-editing techniques for engineering libraries of proteoglycans with designer GAGs are starting to be tested[14]. The potential for synergy of genetic interventions and the administration of engineered proteoglycan-binding growth factors could further facilitate the control of tissue-repair processes.

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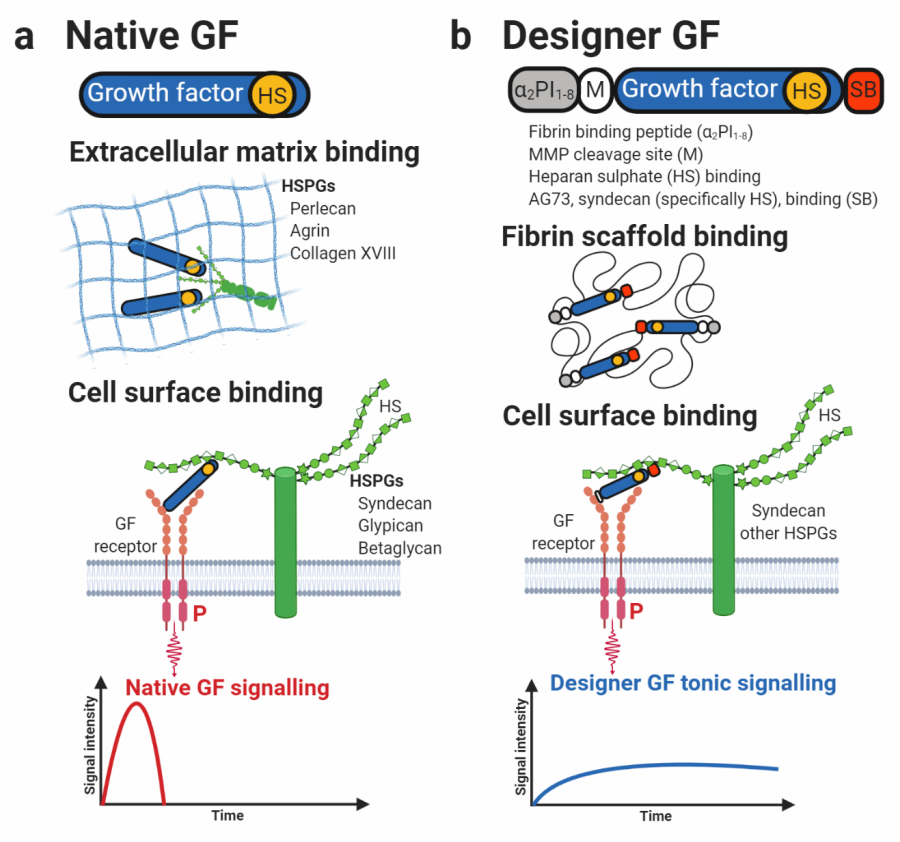

Fig. 1 | Engineering growth factors for tonic signalling. a, Many native growth factors contain heparan sulphate (HS) binding sites that enable them to bind to HS proteoglycans (such as perlecan, agrin and collagen type XVIII) in the extracellular matrix and on the cell surface (through syndecans, glypicans and betaglycans). Native growth factors are rapidly released from the extracellular matrix, and when presented to proteoglycan receptors on the cell surface elicit rapid 'burst' signalling. **b**, Growth factors engineered to contain an additional HS-binding peptide that also binds to syndecans (denoted as SB, for syndecan binding), a fibrin-scaffold-binding motif (α_2PI_{1-8}-M) and a matrix-metalloproteinase cleavage site (M) are slowly released from fibrin-based scaffolds, resulting in sustained low-intensity signalling (tonic signalling) and enhanced tissue repair.

[Note for the art editor: All panels are original. See attached file for a vector version of the figure. Please replace 'Native GF signalling' with 'Burst signalling', and 'Designer GF tonic signalling' with 'Tonic signalling'. 'Betaglycan' should read 'Betaglycans'. 'Syndecans' and 'Glypicans' should also be in their plural form. Please also add hyphens in 'Extracellular-matrix binding', 'Fibrin-scaffold binding', 'Cell-surface binding'. 'GF receptor' should read 'Growth-factor receptor'. 'Other' should be capitalized. Please remove 'HSPGs' and the four lines of text below the top structure in panel b.]