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Review article

Engineering growth factors for regenerative medicine applications

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ABSTRACT

Growth factors are important morphogenetic proteins that instruct cell behavior and guide tissue repair and renewal. Although their therapeutic potential holds great promise in regenerative medicine applications, translation of growth factors into clinical treatments has been hindered by limitations including poor protein stability, low recombinant expression yield, and suboptimal efficacy. This review highlights current tools, technologies, and approaches to design integrated and effective growth factor-based therapies for regenerative medicine applications. The first section describes rational and combinatorial protein engineering approaches that have been utilized to improve growth factor stability, expression yield, biodistribution, and serum half-life, or alter their cell trafficking behavior or receptor binding affinity. The second section highlights elegant biomaterial-based systems, inspired by the natural extracellular matrix milieu, that have been developed for effective spatial and temporal delivery of growth factors to cell surface receptors. Although appearing distinct, these two approaches are highly complementary and involve principles of molecular design and engineering to be considered in parallel when developing optimal materials for clinical applications.

Statement of significance

Growth factors are promising therapeutic proteins that have the ability to modulate morphogenetic behaviors, including cell survival, proliferation, migration and differentiation. However, the translation of growth factors into clinical therapies has been hindered by properties such as poor protein stability, low recombinant expression yield, and non-physiological delivery, which lead to suboptimal efficacy and adverse side effects. To address these needs, researchers are employing clever molecular and material engineering and design strategies to both improve the intrinsic properties of growth factors and effectively control their delivery into tissue. This review highlights examples of interdisciplinary tools and technologies used to augment the therapeutic potential of growth factors for clinical applications in regenerative medicine.

Abbreviations: BMP, bone morphogenetic protein; BP, binding protein; CBD, collagen binding domain; CS, chondroitin sulfate; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; ECM, extracellular matrix; EGF, epidermal growth factor; EGFR, EGF receptor; FGF, fibroblast growth factor; FN, fibronectin; GAG, glycosaminoglycans; G-CSF, granulocytes-colony stimulating factor; HB, heparin binding; HGF, hepatocyte growth factor; hGH, human growth hormone; hGHR, human growth hormone receptor; HS, heparan sulfate; IFN, interferon; IGF, insulin growth factor; NGF, nerve growth factor; PCR, polymerase chain reaction; PDGF, platelet-derived growth factor; PEG, polyethylene glycol; Pla, plasmin sensitive sequence; PlGF, placental growth factor; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

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Contents

1. Introduction

Regenerative medicine is an interdisciplinary field where researchers aim to replace or repair damaged cells, tissues, and organs to effectively restore normal function and circumvent the need for donation [1]. Major strategies being pursued to achieve these goals include introducing materials and modulating agents, such as extracellular matrix (ECM)-inspired biomaterial scaffolds, cells, and growth factors [2], to the damaged site to stimulate regeneration [3].

Growth factor proteins are naturally secreted from cells and directly interact with or are sequestered by the surrounding ECM for presentation to cell surface receptors. Growth factors are essential to the regenerative process. Specific growth factor receptor binding stimulates cellular signal transduction pathways that trigger events such as cell migration, survival, adhesion, proliferation, growth, and differentiation [4–6] (Fig. 1). On a larger scale, these growth factor-stimulated cellular responses are involved in organism development, angiogenesis, and wound healing [5]. Clinically-approved growth factors include human growth hormone (hGH; Humatrope® [7]), which is used to treat children of short stature, platelet-derived growth factor-BB (PDGF-BB; Regranex® [8]) which is approved to treat lower extremity diabetic neuropathic ulcers, and bone morphogenetic factor-2 (BMP-2) and BMP-7 for lumbar spine fusion (InFUSE™ Bone Graft/LT-Cage™ [9]; OP-1 Putty [10]) and open tibial fracture (INFUSE® Bone Graft [11]; OP-1 Implant [12]).

While growth factors have had clinical success, their potential as therapeutic agents has generally been hindered by inherent limitations imposed by their native protein forms. In particular, nature has designed growth factors with properties such as low protein stability, short circulating half-life, rapid rate of cellular internalization, and localized tissue activity as mechanisms for controlling their function through restricted spatial and temporal effects. As an example, fibroblast growth factor (FGF-1) possesses intrinsically low stability, exhibiting a functional half-life of only 1 h in serum at 37 °C [13]. Additional challenges arise for utilizing exogenous growth factors as therapeutics, including poor recombinant expression yield, difficulty of purification, high cost of production, and lack of appropriate delivery methods [14]. Collectively, these limitations create a significant need for new tools and technologies that will render growth factors more amenable for therapeutic use.

In this review, we discuss progress to address these needs, including the design, engineering, and development of novel proteins and protein delivery systems. Although we focus on applications related to regenerative medicine, the concepts and strategies discussed here are broadly relevant to other therapeutic applications. We first focus on different protein engineering strategies used to create growth factors with improved biochemical and biophysical properties. We then present examples of engineered microenvironments, and discuss how these strategies have been used to develop enhanced growth factor delivery systems. The concluding section provides future outlook on the critical role that design and engineering will continue to play in these efforts.

2. Protein engineering technologies applied to growth factors

As described above, challenges exist for using natural growth factors in regenerative medicine applications that stem from their inherent limitations as proteinaceous materials; quite simply, nature designed growth factors for specific local and temporal tissue effects that do not translate well into their development or use as therapeutics. In the next sections, we highlight examples of combinatorial protein engineering methods, often referred to as 'directed evolution', that have been applied to engineer growth factors with improved properties, including increased protein stability, extended serum half-life, enhanced biodistribution, improved recombinant expression yield, and altered receptor binding affinity or internalization/recycling rate (Fig. 2).

3. Overview of combinatorial protein engineering strategies

3.1. Protein library creation

Directed evolution involves high-throughput screening of large libraries of protein variants (e.g. thousands to millions) as an efficient means to interrogate and identify beneficial growth factor