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Review

Virus-based gene therapy strategies for bone regeneration

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Abstract

Gene therapy has emerged as a promising strategy for the repair and regeneration of damaged musculoskeletal tissues. Application of this paradigm to bone healing has shown enhanced efficacy in preclinical animal studies compared to conventional bone grafting approaches. This review discusses current and emerging virus-based genetic engineering strategies for the delivery of therapeutic molecules which promote skeletal regeneration. Viral gene delivery vectors are discussed in the context of bone repair in order to illustrate the challenges and applications of these methods with tissue-specific examples. Moreover the concepts discussed can be broadly applied to promote healing in a wide range of tissues. We also present important considerations involved in the application of these gene therapy techniques to a variety of osteogenic (e.g. bone marrow-derived cells) and non-osteogenic (e.g. fibroblasts and skeletal myoblasts) cell types. Criteria for the selection of regenerative molecules with soluble versus intracellular modes of action and emerging combinatorial approaches are also discussed. Overall, gene transfer technologies have the potential to overcome limitations associated with existing bone grafting approaches and may enable investigators to design therapies which more closely mimic the complex spatial and temporal cascade of proteins involved in endogenous bone development and repair.

Keywords: Gene therapy; Viral vectors; Bone regeneration; Bone tissue engineering

Contents

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1. Introduction

Six million bone fractures are reported each year in the United States and roughly 10% of these require some form of orthopedic graft [1,2]. As a result, approximately 500,000 grafting procedures are performed annually in victims of non-healing or delayed union defects caused by age-related bone deterioration, traumatic injury, tumor resection, or osteolytic disease [3]. Conventional skeletal grafting therapies typically involve the implantation of autogenic bone harvested from the patient's iliac crest or allogenic bone from cadaver tissue banks. Autograft implantation is considered the gold standard in bone repair, but the widespread clinical success of this procedure has been hindered by variable results associated with the quality of the bone graft, inadequate tissue supply, and donor site morbidity [4]. Allografts offer advantages for off-the-shelf tissue availability, but display reduced biological activity and mechanical properties due to tissue processing and carry the risk of disease transmission [5,6]. Synthetic materials such as metals, calcium phosphate ceramics, bioactive glasses, and polymers have also been explored for bone grafting applications, but generally display insufficient regenerative potential to warrant the inflammatory host reaction [7]. Although these traditional approaches to augment bone formation have had significant medical impact, it is clear that their limitations leave a pressing need for alternative bone regeneration strategies.

Various growth factors and cytokines have been investigated for their capacity to promote bone regeneration in vivo [8–11]. Among these, recombinant human bone morphogenetic proteins (rhBMPs) induce robust ectopic bone formation and healing of orthotopic bone defects in several different animal models [12–14]. Formulations based on bone morphogenetic protein-2 (BMP-2) and BMP-7 have been approved by the FDA for the treatment of severe orthopedic conditions such as spinal fusion and skeletal nonunion [15–17]. However, the doses of recombinant protein required to accelerate healing in humans are significantly higher than the levels expressed during normal bone repair, likely due to suboptimal delivery vehicles and rapid in vivo protein degradation. These supraphysiologic concentrations are cost-prohibitive to widespread clinical usage and may be problematic if the non-selective targeting of neighboring non-osseous tissues leads to ectopic bone formation [12,18]. Because of these complications, gene therapy has been pursued as an alternative strategy for the sustained delivery of therapeutic proteins to a compromised tissue site.

The general paradigm for gene therapy involves introduction of engineered nucleic acid material into a patient's cells for the local production of therapeutic factors at the site of injury or disease. In addition, delivery of genetic sequences, such as antisense or RNA interference (RNAi) oligonucleotides, that downregulate the expression and/or activity of endogenous molecules has recently emerged as a promising approach to elicit controlled healing responses. Genetic engineering strategies have shown significant promise for the treatment of a wide range of inherited congenital disorders (e.g. cystic fibrosis, phenylketonuria, adenosine deaminase deficiency, hemophilia B) and acquired diseases (e.g. AIDS, cancer, cardiopathy) [19]. More recently, gene transfer vectors have been developed for the overexpression of bioactive factors to induce repair of damaged neural, cardiovascular, and musculoskeletal tissues. Among these, orthopedic applications may provide gene therapy with its first widespread clinical success because long-term expression of the therapeutic gene is not required following tissue healing. This review discusses current and emerging virus-based gene therapy technologies employed for tissue regeneration. Although we focus on skeletal tissue as the model system, the methods/techniques discussed in this manuscript are applicable to many other tissue repair systems.

2. Bone anatomy and physiology

Bone is a specialized connective tissue which provides mechanical support and protection for the body's internal organs. Beyond this structural role, bone tissue also serves as a major reservoir for calcium and phosphate ions and contains a population of hematopoietic and stromal cell precursors within its marrow cavity. Thus, despite its inert appearance, bone is a metabolically active organ that undergoes continuous remodeling throughout life in order to maintain serum homeostasis and the structural integrity of the skeleton [20]. This remodeling process involves a complex series of highly regulated steps that primarily depend on the interplay of two cell types, the osteoblast and the osteoclast. Osteoblasts promote bone formation by regulating deposition of osteoid and mineral nucleation. Osteoclasts are primarily responsible for bone resorption through the secretion of hydrogen ions and acid proteases. Mature osteoblasts (termed osteocytes) are embedded within the dense extracellular matrix in lacunae and extend microfilament-rich canaliculi processes. These processes contact canaliculi originating from other cells through gap junctions, enabling the propagation of signals to the interior of the highly dense bone matrix.

Bone contains both cortical (compact) and trabecular (spongy) tissue types. Although these tissues are structurally and functionally different, they contain the same fundamental constituents, including a highly organized extracellular matrix, bone resorbing cells (osteoclasts), and