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Research review paper

Recent progresses in gene delivery-based bone tissue engineering

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ABSTRACT

Gene therapy has converged with bone engineering over the past decade, by which a variety of therapeutic genes have been delivered to stimulate bone repair. These genes can be administered via in vivo or ex vivo approach using either viral or nonviral vectors. This article reviews the fundamental aspects and recent progresses in the gene therapy-based bone engineering, with emphasis on the new genes, viral vectors and gene delivery approaches.

Contents

Abbreviations: MSCs, mesenchymal stem cells; BMSCs, bone marrow-derived mesenchymal stem cells; ASCs, adipose-derived stem cells; MDSCs, muscle-derived stem cells; EPCs, endothelial progenitor cells; EC, endothelial cells; iPSCs, induced pluripotent stem cells; ECM, extracellular matrix; VEGF, vascular endothelial growth factor; BMP, bone morphogenetic protein; BMP-2, bone morphogenetic protein 2; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor β; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α; IL-6, interleukin 6; FGF-2, fibroblast growth factor-2; HIF-1α, hypoxia-inducible factor 1α; Cox-2, cycloxygenase-2; SATB2, special AT-rich sequence-binding protein 2; RANKL, receptor activator of nuclear factor κB ligand; CXCR4, CXC chemokine receptor-4; TRAP, tartrate-resistant acid phosphatase; Sca-1, stem cell antigen-1; AAV, adeno-associated virus; AdBMP2, adenovirus expressing BMP-2; AcMNPV, *Autographa californica* multiple nucleopolyhedrovirus; miRNA, microRNA; siRNA, small interfering RNA; GAM, gene activated matrix; DBM, demineralized bone matrix; i.v., intravenous; LLP2A, peptidomimetic ligand; FLP, flippase; Frt, flippase recognition target; NZW, New Zealand White; TLR-3, toll-like receptor 3; PET, positron emission tomography; CT, computed tomography.

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1. Introduction

1.1. Medical need for bone repair and current options

Approximately 6–6.5 million fractures are reported per year in the United States (Praemer et al., 1992). Although bone tissue has regenerative capabilities that enable the self-repair of fractures (Pelled et al., 2010), ≈5–10% of these fractures result in nonunion or delayed union (Praemer et al., 1992). Additionally, large critical-size bone defect (one spanning >2 cm) resulting from serious trauma or tumor surgery cannot union without bone grafting (Hollinger and Kleinschmidt, 1990). Management of such massive defects still poses a tremendous challenge for orthopedic surgeons (Tseng et al., 2008), partly because the injury impairs blood supply and results in ischemia, osteonecrosis, bone loss and ultimately non-union (Buckwalter et al., 1996).

Currently available materials for clinical bone reconstruction include autologous bone grafts, allogeneic banked bone grafts and synthetic bone substitutes (Bueno and Glowacki, 2009). Autologous bone grafting is considered the gold standard to fill a bone defect (Kimelman et al., 2007; Kneser et al., 2006; Marino and Ziran, 2010), but autografting may be restricted by donor site morbidity, bone availability and the need for bone harvesting procedures (Kneser et al., 2006). Furthermore, the repair by autografting is not always satisfactory (Gamradt and Lieberman, 2004). Allografting can initiate the healing process and recruit cells from the surrounding soft tissue (Smith et al., 2011), but the donor shortage and potential risk of disease transmission remain to be the major drawbacks of allografting. Conversely, various synthetic bone substitutes have been developed (for review see Szpalski et al., 2010; Bose et al., 2012), but they are still unable to provide an osteogenic capacity as good as the autograft (Bueno and Glowacki, 2009).

1.2. Bone cell types, healing process and vascularization

Bone formation entails orchestrated activities by osteoblasts, osteocytes and osteoclasts (Shegarfi and Reikeras, 2009). Osteoblasts are responsible for synthesizing the organic extracellular matrix (ECM) and regulating matrix mineralization while osteocytes function in mineral homeostasis, mechanical sensing and signaling. Osteoclasts can resorb bones and play roles in skeletal growth and bone remodeling (Bueno and Glowacki, 2009). Bone formation proceeds through either endochondral or intramembranous ossification pathway. Endochondral ossification is the process by which mesenchymal stem cells (MSCs) differentiate toward chondrocytes and produce a cartilaginous template, which contributes to longitudinal growth of most bones (e.g. long bones). Conversely, intramembranous ossification occurs without a cartilage template and contributes to the formation of calvarial bone.

One critical factor to successful bone formation/healing is vascularization (Santos and Reis, 2010). New blood vessels enable the transport of oxygen and nutrients to the metabolically active callus, thus angiogenesis plays crucial roles in the onset of fracture healing and promotion of ossification (Santo et al., 2013). Angiogenesis is regulated by a variety of growth factors, notably vascular endothelial growth factor (VEGF). Exogenous VEGF enhances blood vessel formation and ossification and promotes bony bridging (Street et al., 2002).

1.3. Roles of host immunity on bone healing

Recently, it was shown that bone fracture healing may be retarded by endogenous adaptive/innate immune responses. For instance, γ/δ T cells, the innate lymphocytes involved in tissue repair, can repress bone healing by influencing the fate of other responder cells and the ultimate callus formation (Colburn et al., 2009). Pro-inflammatory T cells also inhibit the ability of exogenously added bone marrow-derived MSCs (BMSCs) to mediate bone repair, owing to interferon (IFN)-γ-induced down-regulation of RunX2 pathway and enhancement of tumor necrosis factor (TNF)-α signaling in the stem cells (Y. Liu et al., 2012). Conversely, reduction of IFN-γ and TNF-α concentrations, by systemic infusion of $Foxp3^+$ regulatory T cells or by local administration of aspirin, markedly improves BMSC-based calvarial defect repair in mice (Y. Liu et al., 2012). Furthermore, delayed fracture healing correlates with enhanced levels of terminally differentiated $CD8^+$ effector memory T (TEMRA) cells in peripheral blood (Reinke et al., 2013). These $CD8^+$ TEMRA cells are enriched in fracture hematoma and are the major producers of IFN-γ/TNF-α, which inhibit osteogenic differentiation (Reinke et al., 2013). These data collectively highlight the role of recipient T cells in BMSC-based bone engineering (Y. Liu et al., 2012).

1.4. Protein therapy and gene therapy

Successful bone healing/repair requires 4 critical factors that act in concert: (1) osteoconduction, (2) osteoinduction, (3) cells that can respond to osteogenic signals and (4) vascular supply. Bone tissue engineering aims to offer these components in order to stimulate bone regeneration, either in vitro or in vivo. Osteoconduction can be provided by sophisticated scaffold design that permits cell attachment, bone matrix deposition and bone bridging (for review, see Bose et al., 2012). Osteoinduction is often mediated by osteogenic growth factors such as bone morphogenetic proteins (BMPs), platelet-derived growth factor (PDGF) and transforming growth factor β (TGF-β) (Lieberman et al., 2002). Among these growth factors, BMP-2 and BMP-7 have been approved by the Food and Drug Administration (FDA) for clinical use (Boden, 2005; De Biase and Capanna, 2005). BMP-based therapy is superior to the use of autologous grafts alone (Villavicencio et al., 2005), but the graft may not suffice to completely heal massive segmental defects in long bones. Moreover, recombinant human BMP-2 is

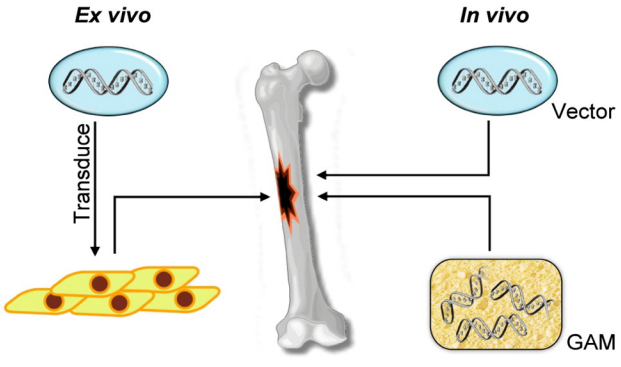

Fig. 1. Ex vivo and in vivo gene therapy for bone healing/regeneration.

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