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Gene therapy for repair and regeneration of bone and cartilage

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Gene therapy refers to the use of viral and non-viral vectors to deliver nucleic acids to tissues of interest using direct (*in vivo*) or transduced cell-mediated (*ex vivo*) approaches. Over the past few decades, strategies have been adopted to express therapeutic transgenes at sites of injury to promote or facilitate repair of bone and cartilage. Targets of interest have typically included secreted proteins such as growth factors and anti-inflammatory mediators; however, work has also begun to focus intracellularly on signaling components, transcription factors and small, regulatory nucleic acids such as microRNAs (miRNAs). In recent years, a number of single therapeutic gene approaches (termed 'monotherapies') have proven effective in preclinical models of disease, and several are being evaluated in clinical trials. In particular, an *ex vivo* TGF-β1 gene therapy was approved in Korea in 2017 for treatment of moderate-to-severe osteoarthritis (OA). The ability to utilize viral vectors for context-specific and combinatorial gene therapy is also being investigated, and these strategies are likely to be important in more robustly addressing the complexities of tissue repair and regeneration in skeletal disease. In this review, we provide an overview of viral gene therapies being developed for treatment of bone and cartilage pathologies, with an emphasis on emerging combinatorial strategies as well as those targeting intracellular mediators such as miRNAs.

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Introduction

Gene therapy refers to the strategy by which nucleic acids are delivered to target tissues using viral or non-viral vectors to prevent, treat or cure disease. Unlike recombinant protein therapy, gene therapy has the potential to achieve sustained and regulated delivery of therapeutic genes with cell-type specificity. This is particularly true for viral vectors given that their capsids have been evolutionarily optimized to target and deliver genetic payloads to virtually every mammalian cell-type and tissue.

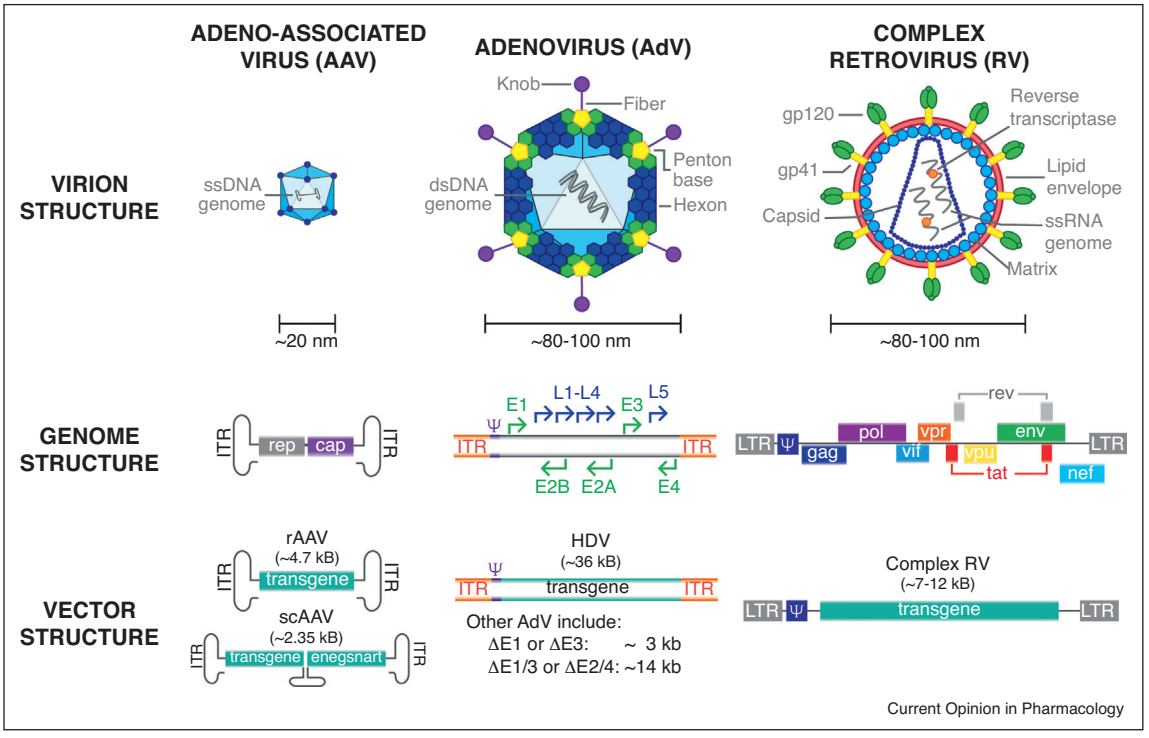
Many viral vectors have been examined for bone and cartilage gene therapy, and key features of the most commonly used, including adenovirus (AdV), helper-dependent AdV (HDV), adeno-associated virus (AAV) and complex retroviruses (RVs) such as lentivirus (LV), are illustrated in Figure 1. Non-viral gene therapies for skeletal disease have been reviewed elsewhere [1] and will not be discussed here. Optimal delivery is also key to any successful gene therapy strategy, and is achieved using an *in vivo* or *ex vivo* approach (Figure 2). In brief, the former involves direct administration of viral vectors, whereas the latter requires extraction of cells, transduction with a vector and reintroduction of those manipulated cells back to the site of interest.

Over the past decade, gene therapy strategies utilizing viral vectors for bone and cartilage repair have focused on pathologies such as non-union fracture, osteoarthritis (OA) and rheumatoid arthritis (RA). In this context, a single therapeutic gene (termed 'monotherapy') is delivered and expressed constitutively in an effort to reduce inflammation and/or modulate anabolic and catabolic pathways important for tissue repair. The targets of interest have often included secreted proteins such as growth factors and anti-inflammatory proteins; however, recent work has focused on signaling components, transcription factors and small, regulatory nucleic acids such as microRNAs (miRNAs). And while monotherapies have shown efficacy in animal models and late-stage human disease, the ability to utilize viral vectors for context-specific and combinatorial gene therapy is now being investigated.

Gene therapy strategies for bone repair

Primary bone healing resembles normal bone remodeling and occurs when the fracture gap is minimized with sufficient stabilization; in contrast, fractures that do not stabilize may instead repair via secondary healing — a multi-stage process involving formation of a hematoma and inflammatory cell infiltration followed by fibrocartilage callus formation and its subsequent replacement by bone [2]. Within the preclinical milieu, investigators evaluate therapeutic targets for their potential to form ectopic bone, heal critical-sized calvarial and femoral defects, and repair non-union fractures [3•,4]. And while

Figure 1



Common viral vectors used for skeletal gene therapy. Adeno-associated virus (AAV; *left*) is non-enveloped with an icosahedral capsid measuring ~20-nm in diameter that surrounds a linear, single-stranded DNA (ssDNA) genome consisting of inverted terminal repeats (ITRs) flanking 5′-rep and 3′-cap open reading frames. Recombinant AAV (rAAV) gene therapy vectors have a cloning capacity of ~4.7 kB and are generated by removal of all viral coding sequences excluding the ITRs. Self-complementary AAVs (scAAVs) have a reduced transgene capacity of ~2.5 kB but bypass the need for second strand synthesis post-infection making expression more immediate and robust. Adenovirus (AdV; *middle*) is a non-enveloped virus with a linear double-stranded DNA (dsDNA) genome surrounded by an ~80–100-nm icosahedral capsid formed of hexon and penton subunits with protruding fiber proteins. The AdV genome consists of early (E1–E4) and late genes (L1–L5) transcribed before and after viral DNA replication, respectively. Early AdV vector generations were constructed by deletion of E1, E2, E3 or E4 in various combinations and had cloning capacities ranging from ~3 to 14 kB. Helper-dependent AdVs (HDVs) lack all viral coding sequences except for the ITRs and packaging signal (Ψ) giving them a cloning capacity of up to 36 kB. Complex retroviruses (RVs; *right*) such as lentivirus (LV) are enveloped viruses (~80 to 100 nm in size) whose diploid positive-sense, single-stranded RNA (ssRNA) genome is surrounded first by a capsid followed by a host cell membrane-derived lipid bilayer containing virally-encoded glycoproteins. The LV genome consists of three essential genes (gag, pol and env), two regulatory genes (tat and rev) and four accessory genes (vif, vpr, vpu and nef) flanked by long-terminal repeats (LTRs). LV gene therapy vectors have a capacity of ~7 to 14 kB and are generated by removal of all viral coding sequences except the Ψ and LTRs.

in vivo approaches have been examined, studies that utilize *ex vivo* strategies in which transduced cells are combined with scaffolds are more common place.

Monotherapies

The early stages of bone repair, including inflammation and cellular invasion, are often not directly targeted. At the same time, studies utilizing bone morphogenetic protein (BMP) family members such as BMP-2 for their ability to drive bone formation have reported increased migration of endogenous progenitors to defect sites [5]. Similar findings have also been noted for the chemokine stromal cell-derived factor 1 (SDF-1) and for cyclooxygenase-2 (COX-2) [6,7]. In addition to promoting progenitor cell recruitment, *ex vivo* gene therapy utilizing COX-2 — an enzyme critical for inflammation, prostaglandin production and cartilage formation during fracture repair — has proven effective at enhancing angiogenesis and remodeling of the cartilage fracture callus in long bone defects [6].

Vascularization and subsequent remodeling of the fibrocartilage callus is also critical for fracture healing. The potent angiogenic and vasculogenic inducer vascular endothelial growth factor (VEGF) has been evaluated for its ability to enhance bone repair; however, use of VEGF alone has been met with mixed results [8,9] indicating a temporal spatial requirement of specific stimulatory factors. Investigators have also sought to indirectly enhance the amount of bone deposited within defects by suppressing bone resorption. In this regard, Liu and colleagues found that AdV-mediated expression of osteoprotegerin (OPG) in bone marrow stromal cells (BMSCs) seeded onto hydroxyapatite (HA) scaffolds