Advanced Drug Delivery Reviews 64 (2012) 1320–1330

Contents lists available at SciVerse ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Gene therapy approaches to regenerating bone☆

Nadav Kimelman Bleich [a,*], Ilan Kallai [a], Jay R. Lieberman [b], Edward M. Schwarz [c], Gadi Pelled [a,d], Dan Gazit [a,d,*]

[a] Skeletal Biotech Laboratory, The Hebrew University, Hadassah Faculty of Dental Medicine, Ein Kerem, Jerusalem, Israel
[b] New England Musculoskeletal Institute, Department of Orthopaedic Surgery, University of Connecticut School of Medicine, Farmington, CT, USA
[c] Center for Musculoskeletal Research, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA
[d] Department of Surgery and Cedars-Sinai Regenerative Medicine Institute (CS-RMI), Cedars Sinai Medical Center, Los Angeles, CA, USA

ARTICLE INFO

Article history:
Received 25 October 2011
Accepted 5 March 2012
Available online 10 March 2012

Keywords:
Gene therapy
Bone regeneration
Tissue engineering
Viral vectors
Nonviral vectors

ABSTRACT

Bone formation and regeneration therapies continue to require optimization and improvement because many skeletal disorders remain undertreated. Clinical solutions to nonunion fractures and osteoporotic vertebral compression fractures, for example, remain suboptimal and better therapeutic approaches must be created. The widespread use of recombinant human bone morphogenetic proteins (rhBMPs) for spine fusion was recently questioned by a series of reports in a special issue of *The Spine Journal*, which elucidated the side effects and complications of direct rhBMP treatments. Gene therapy – both direct (in vivo) and cell-mediated (ex vivo) – has long been studied extensively to provide much needed improvements in bone regeneration. In this article, we review recent advances in gene therapy research whose aims are in vivo or ex vivo bone regeneration or formation. We examine appropriate vectors, safety issues, and rates of bone formation. The use of animal models and their relevance for translation of research results to the clinical setting are also discussed in order to provide the reader with a critical view. Finally, we elucidate the main challenges and hurdles faced by gene therapy aimed at bone regeneration as well as expected future trends in this field.

Contents

1. Introduction . . . 1320
2. In vivo gene therapy for bone regeneration . . . 1321
   2.1. Introduction . . . 1321
   2.2. Viral vectors . . . 1322
   2.3. Nonviral vectors . . . 1323
3. Ex vivo gene therapy for bone regeneration . . . 1324
   3.1. Introduction . . . 1324
   3.2. Cell-mediated gene therapy using viral vectors . . . 1324
   3.3. Cell-mediated gene therapy using nonviral vectors . . . 1326
4. Animal models and clinical translation . . . 1326
5. Challenges, hurdles, and future trends . . . 1327
6. Summary . . . 1328
Acknowledgment . . . 1328
References . . . 1328

1. Introduction

Bone formation and regeneration therapies continue to require optimization and improvement because many skeletal disorders remain undertreated. Nonunion fractures, especially those in anatomical locations suffering from a low blood supply such as the distal radius or scaphoid bone, do not have optimal therapies [1–3]. Five percent of all scaphoid factures are nonunion injuries that cannot

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Targeted delivery of therapeutics to bone and connective tissues".

* Corresponding authors at: Skeletal Biotechnology Laboratory, Hebrew University, Hadassah Faculty of Dental Medicine, P.O. Box 12272, Ein Kerem, Jerusalem 91120, Israel. Tel.: +972 2 6757625 (Lab.); fax: +972 2 6757628.
E-mail addresses: nadavk@ekmd.huji.ac.il (N. Kimelman Bleich), danga@ekmd.huji.ac.il (D. Gazit).
URL: http://gazitlab.huji.ac.il (D. Gazit).

0169-409X/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.addr.2012.03.007

heal and are accompanied by severe pain and morbidity [3]. Ten percent of all fractures are nonunion injuries that never heal [4]. Osteoporotic vertebral compression fractures are the most common fragility fractures in the United States. Accounting for approximately 700,000 injuries per year, these injuries lead to prolonged hospitalizations and result in high health care costs [5]. Current therapeutic strategies include implantation of autologous bone grafts for nonunion fractures [6–8], vascularized bone grafting in one- or two-stage operations for fractures in anatomical sites with a poor blood supply [3], and vertebroplasty or balloon tamp reduction for vertebral fractures [9]. Those methods are hampered by donor-site morbidity; a limited supply of autologous bone grafts [8]; complicated two-step surgeries [3]; and in some cases, such as vertebral fracture repair, by lack of clinical results [10,11].

Recombinant human bone morphogenetic protein-2 (rhBMP-2) and rhBMP-7 have been used clinically throughout the last decade to promote fracture repair and bone formation in cases of spinal fusion [7,8,12,13]. However, recent evidence has indicated serious flaws in the use of rhBMP-2 as well as in published reports regarding its application in spinal fusions. A recent review of 13 original industry-sponsored rhBMP-2 studies found that the authors reported 10 to 50 times fewer complications in cases treated with rhBMP-2 than were found in the manufacturer's original FDA summaries [14]. Another review highlighted side effects in the central and peripheral nervous systems associated with rhBMP-2 use, which could explain the high rates of nerve root irritation seen in clinical practice [15]. Moreover, osteolysis (bone resorption by the body) following spinal fusion procedures utilizing rhBMP-2 was found in 54% of cases [16]. All these critical reports were published in a special issue of *The Spine Journal* dedicated to revision of the growing use of rhBMPs in orthopedic medicine. However, it is important to note that therapies based on the use of rhBMP-2 and rhBMP-7 are the only biological solutions currently available to avoid bone harvesting [7,8,12,13]. A systematic review performed in the UK determined that addition of BMP treatment to conventional intervention is more effective than conventional intervention alone for establishing union of acute open tibial fractures [4]. Moreover, a 44% reduction in tibia failure to heal was noticed when rhBMP-2 was administrated on a collagen sponge [17]. rhBMP-7 was also used with beneficial results for long-bone nonunion fractures [8]. However, this treatment requires megadoses of the protein – as high as 1.5 mg protein/ml matrix [8,18] – and thus is not always cost-effective [4].

Gene therapy approaches to bone regeneration are being studied extensively to provide much needed improvements in bone regeneration. Unlike protein-based therapy, gene delivery induces the production of physiological, rather than pharmaceutical, amounts of growth factor over time. Delivery of the gene is much cheaper than delivery of the protein and can be better controlled; in addition, when compared with rhBMP delivery, ex vivo gene therapy was found to be more efficient [19]. Gene therapy, therefore, may provide a better clinical solution to pathological disorders currently treated with rhBMPs. However, among the more than 1300 clinical trials conducted between 1990 and 2007, only 8.2% involved the delivery of growth factors and most of these targeted the cardiovascular system [20]. The fact that rhBMP use is now being reevaluated might help to promote the massive research that has been performed in various animal models into the clinical arena.

Viral vectors for gene delivery are the most popular vectors used in clinical trials as well as in research due to their high efficiency. Use of nonviral vectors is increasing [20], however, in response to safety issues associated with the use of retroviral vectors [21,22] and adenoviral vectors [23]. Since brief expression of osteogenic genes is sufficient for bone formation, the use of nonviral vectors can be valuable for bone regeneration applications [24,25]. Following direct adenovirus-mediated gene delivery, *BMP-6* and *BMP-9* were found to be the most potent inducers of osteogenic differentiation among 14 different *BMP* genes, followed closely by *BMP-2* [26,27]. This finding was also apparent using nonviral techniques [28–30], demonstrating the potential of gene therapy in the orthopedic field.

Gene therapy is usually categorized as either in vivo, in which the gene is delivered directly into recipient cells in the site of interest, or ex vivo, in which the gene of interest is inserted in vitro into a targeted cell population (usually stem cells or fibroblasts) and the cells are delivered to the desired site in vivo [31]. Those two gene delivery strategies are usually termed “in vivo gene delivery” and “cell mediated gene delivery”, respectively. When gene is delivered into the desired tissue, either directly or by using a cell mediator, a complex cascade of events follows that results in expression of the inserted DNA and in an effect on the expressing cells or the cellular environment. Multitude of factors can affect this process, as recently reviewed [32,33]. Only few studies compared in-vivo and cell-mediated gene therapy for bone repair. One of those studies demonstrated that while bone formation capacity was similar using both strategies, the use of transfected cells allowed for better control of bone formation [34]. In addition, ex-vivo gene therapy enables better control over the identity of recipient cells in contrast to in-vivo gene delivery in which it is difficult to target the gene to a specific population of cells. The main advantage of in vivo gene delivery approach is that it does not require the complex process of cell isolation, characterization and expansion. Yet, recent studies have shown possible strategies to overcome these disadvantages. Kimelman-Bleich et al. showed that potential is possible to target gene delivery to a population of host progenitors using an implantation of a biodegradable scaffold [35]. Another study suggested a “same-day” approach in which stem cells were transduced on the same day of isolation and implanted in vivo without an expansion phase [36]. Most orthopedic-oriented approaches include ex vivo gene therapy because of the added benefits of a cellular component, which allows for fast and predictable bone formation. BMP-expressing mesenchymal stem cells (MSCs) are usually used because of their osteogenic potential [37] and ability to act in both an autocrine and paracrine fashion by differentiation of implanted cells and recruitment of host cells [38].

Here, we review recent advances in gene therapy research aimed at bone regeneration or formation ex vivo and in vivo. The use of animal models and their role in clinical translation are also discussed in order to provide the readers with a critical view. Finally, we discuss the main challenges and hurdles faced by gene therapy aimed at bone regeneration as well as expected future trends.

2. In vivo gene therapy for bone regeneration

2.1. Introduction

Table 1 summarizes the studies reviewed in this section.

The first attempts at direct gene delivery aimed at bone regeneration were reported as early as 1996 [39]. Nonviral vectors used at the time included naked DNA delivery and an array of methods designed to enhance the poor efficiency of gene delivery associated with naked DNA delivery, such as dividing DNA delivery into several constitutive injections [40], implementation of gene-activated matrices (GAM) [32], and use of sonoporation and electroporation [28–30,35]. Viral vectors, which were more efficient but, alas, raised some safety issues, were also used. Adenoviral vectors were used first and met with satisfying results [26,41], which were somewhat hampered by the immune system's response to bone formation [42]. Adeno-associated viral vectors (AAVs) were used successfully, mainly when combined with bone allografts [43]. Finally, retroviral and lentiviral vectors were used as well and had a positive influence on bone formation and regeneration [44]. In this section we will review studies performed using in vivo gene delivery for bone formation and regeneration.