Review

Small-molecule based musculoskeletal regenerative engineering

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Clinicians and scientists working in the field of regenerative engineering are actively investigating a wide range of methods to promote musculoskeletal tissue regeneration. Small-molecule-mediated tissue regeneration is emerging as a promising strategy for regenerating various musculoskeletal tissues and a large number of small-molecule compounds have been recently discovered as potential bioactive molecules for musculoskeletal tissue repair and regeneration. In this review, we summarize the recent literature encompassing the past 4 years in the area of small bioactive molecules for promoting repair and regeneration of various musculoskeletal tissues including bone, muscle, cartilage, tendon, and nerve.

Small molecules in regenerative medicine

Regenerative engineering is an emerging interdisciplinary field in which classical tissue engineering converges with advanced materials science, stem cell technology, and developmental biology toward the regeneration of complex tissues and organs [1]. In regenerative engineering, growth factors, synthetic scaffolds, and cells combine to form a construct that will be structurally, functionally, and mechanically similar to the native tissue that requires repair and regeneration (Figure 1) [2]. Growth factors are signaling polypeptides that guide cells during tissue development and have received considerable clinical interest [3]. Although promising, a significant limitation associated with therapeutic growth factors is immunogenicity, which can induce pleiotropic effects including the development of a high-affinity B cell-mediated humoral response directed against the therapeutic agents [4]. Therapeutic small molecules (<1000 Da) have unique advantages over and are an important alternative to growth factors [5–7]. Small molecules are unlikely to induce an immune response in the host because they are too small to do that [8]. Unlike polypeptides, higher-order structure is not a factor for small-molecule function [9]. Additionally, the

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Glossary

Aggrecan: a protein that is encoded by the ACAN gene in humans. The encoded protein is an integral part of the extracellular matrix of cartilage.
Bisphosphonates: a group of carbon-substituted analogs of pyrophosphate that are potent inhibitors of osteoclast-mediated bone resorption.
Bone morphogenetic proteins (BMPs): BMPs belong to the larger family of proteins known as the transforming growth factor (TGF)-β) superfamily. BMPs are low-molecular-weight secretory proteins that are active signaling molecules that influence proliferation, differentiation, and extracellular matrix synthesis of many different cell types.
Chondrocytes: the only type of cells found in healthy cartilage. They are contained in cavities called cartilage lacunae in the cartilage matrix and produce and maintain the cartilaginous matrix.
Embryonic myosin heavy chain: a protein found in muscle fibers that is used as a marker of developing muscle fibers.
Epiphyseal bone: The rounded end of a long bone.
Glycan: a carbohydrate polymer consisting of sugar subunits.
Hyaline cartilage: a type of cartilage found on many joint surfaces. It appears glassy, provides a low-friction surface, participates in lubrication in synovial joints, and distributes applied forces to the underlying bone. Articular cartilage refers to hyaline cartilage on the articular surfaces of bones.
Mesenchyme homeobox 1 (Meox1): a transcription factor that plays a key role in muscle development.
***Myf5*:** myogenic factor 5; a transcription factor that plays a key role in myogenesis.
Myoblasts: cells responsible for muscle growth.
Myogenesis: formation of muscular tissue.
Neuritogenesis: formation of neuritis.
Osteoarthritis: a painful disease of joints characterized by degeneration of healthy joint tissue including articular cartilage.
Osteoblasts: cells responsible for bone growth.
Osteoclasts: cells responsible for bone resorption.
Osteoporosis: a disease of unbalanced, natural bone turnover where too much bone is broken down and not enough new bone is created.
Paired box 3 (Pax3): a transcription factor that plays a key role in early muscle development.
Rat calvarial osteoblasts: osteoblasts isolated from the calvaria bone of rats.
Smad: Mothers against decapentaplegic homolog; transcription factors that function as downstream effectors for TGF-beta signaling.
Smurf1: SMAD specific E3 ubiquitin protein ligase 1; it functions as a negative regulator of BMP signaling pathway.
Sphingosine-1-phosphate: a lipid that has been implicated in different cellular signaling pathways.

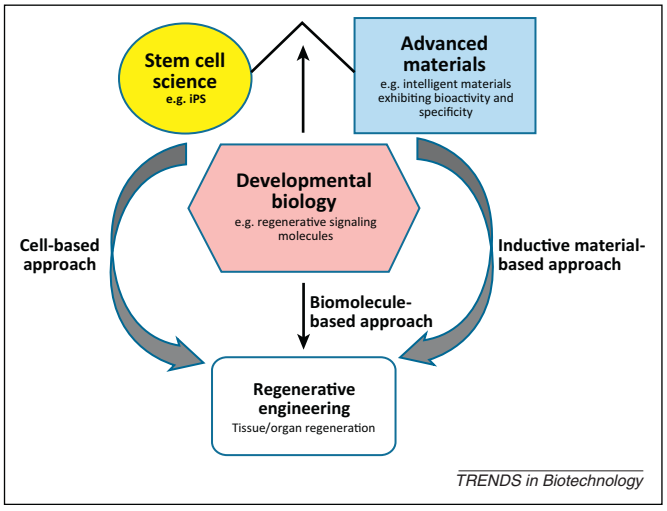

Figure 1. Schematics representation of the field of regenerative engineering. The field of regenerative engineering has been defined as 'the integration of tissue engineering with advanced material science, stem cell science, and areas of developmental biology' [1]. Regenerated tissues/organs via the regenerative engineering approach comprised on novel advanced biomaterials integrated with stem cells or biofactors, alone or in combination. Adapted from [80].

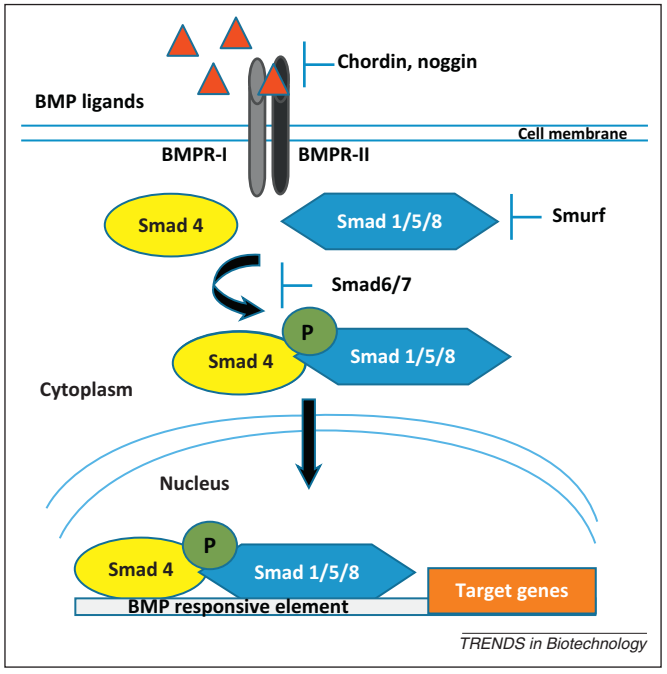

Figure 2. Schematic of canonical BMP signaling pathway. Binding of BMP ligands promotes dimerization of type I and type II BMP receptors (BMPR). The BMPR-II phosphorylates BMPR-I, which in turn phosphorylate phosphorylates the downstream effector proteins (Smad1/5/8). The phosphorylated Smad1/5/8 forms a complex with Smad4 and translocates from cytoplasm to nucleus and induces the expression of the osteoblast-related genes. Several extracellular protein including chordin and noggin, Samd6/7, and Smurf antagonize the BMP signaling pathway. Adapted from [18]. Abbreviations: BMP, bone morphogenetic protein; BMPR, bone morphogenetic protein receptor; Smad, Mothers against decapentaplegic homolog.

manufacturing cost and the risk of cross-species contamination can be significantly minimized with the use of small molecules versus recombinant protein-based growth factors [6,10]. Therefore, small molecules with therapeutic potential may represent the next generation of regenerative engineering for regenerative medicine and other applications. This review article focuses on recent developments of small molecules associated with musculoskeletal regeneration.

Small-molecule-mediated bone formation

Skeletal injuries are major bone disorders in human clinics. An estimated 1.5 million skeletal injuries require tissue graft reconstruction in the USA each year [11]. Thus, the development of effective strategies to regenerate bone tissue defects is a significant medical need. Stimulation of bone repair and regeneration using growth factors is a promising approach to these complications [12,13]. For instance, the FDA has approved several recombinant human bone morphogenetic protein (see Glossary) (rhBMP)-based products for use in various bone disorders including long bone fractures, spinal fusion, and oral surgery [12,13]. Although the clinical results are usually promising, there is an increasing demand for osteogenic small molecules because the rhBMPs raise concerns about safety and effectiveness in clinical settings [5,6,13,14]. In order to improve the effectiveness of BMPs, doses on the order of 1 million times the concentrations found in nature are used, potentially causing serious side effects. For instance, Taghavi *et al*. noted inflammatory reactions and ectopic bone formation associated with high doses of BMP [15]. Another study by Lad *et al*. concluded that the usage of BMP in lumbar spinal fusions is associated with increased rates of benign neoplasia [16]. In addition, the high cost of manufacturing BMP creates barriers for routine clinical use. A small molecule that could activate BMP signaling in the cell would be beneficial because it could reduce the patient's dose of BMP (thus minimizing the side effects) as well as the treatment cost [10,17].

BMP signaling is a tightly regulated process. Binding of BMP to the BMP receptor (BMPR) leads to the activation of intracellular signal transduction (Figure 2). BMPs bind to type I and type II serine/threonine kinase receptors and activate their kinase activities. The activated complex recruits and phosphorylates the transcription factors Smad1, Smad5, and Smad8. In turn, the phophorylated proteins then form a complex with Smad4, translocate from the cytoplasm to the nucleus, and induce the expression of the osteoblast-related genes [18]. Interestingly, several extracellular and intracellular proteins have been identified as specific negative regulators of the BMP signaling. For example, the E3 ubiquitin ligase for BMP-specific Smad proteins (Smurf1), secreted BMP antagonists (noggin, gremlin, cerberus, and chordin), and antagonistic Smads (Smad6 and Smad7) have been implicated as negative regulators of this signaling pathway (Figure 2) [19,20]. In contrast to negative regulators of BMP, activators of BMP signaling have not been widely reported. Identification of a small molecule activator targeting these negative regulators is a promising strategy to potentiate the BMP signaling.

Several small-molecule-based BMP signaling activators have been recently discovered using a variety of methods. In one study, using a customized cell-based reporter assay system, the small synthetic molecule SVAK-12 was identified as a novel BMP signaling activator for potentiating BMP-2-induced transdifferentiation of muscle cells into osteogenic lineages by interrupting the function of Smurf1 [21]. A single-dose of SVAK-12 (100, 250, or 500 μg)