Exploration of new platforms and potentials in regenerative medicine

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Abstract

Regenerative medicine is focused on the regeneration of various tissues and organs through the development of innovative therapeutic strategies and devices. Center of Excellence for Reproductive and Regenerative Medicine (CERRM) was established to provide necessary resources for conducting the state-of-the-art research in the field and to foster translation of developed therapeutic solutions to clinical practice. CERRM subunit for regenerative medicine has developed Osteogrow-C, a novel osteoinductive device for bone regeneration, which was successfully tested in various preclinical models including rabbit and sheep posterolateral spinal fusion (PLF) models as well as rabbit ulnar segmental defect model. Furthermore, CERRM has developed anti-BMP1.3 antibody-based therapy for various diseases that share fibrosis as a key pathological mechanism. This review paper provides the most important contemporary findings on the development of novel BMP-based devices for bone regeneration as well as the development of antifibrotic therapies.

Keywords: Osteogrow, Osteogrow-C, rhBMP6, bone regeneration, posterolateral spinal fusion, regenerative medicine

Sažetak

Istraživanje novih platformi i potencijala u regenerativnoj medicini

Regenerativna medicina je usmjerena na regeneraciju različitih tkiva i organa kroz razvoj inovativnih terapijskih strategija i novih lijekova. Centar izvrsnosti za reproduktivnu i regenerativnu medicinu (CERRM) osnovan je kako bi pružio potrebne resurse za provođenje suvremenih pretkliničkih istraživanja i potaknuo translaciju razvijenih terapijskih rješenja u kliničku praksu. Podjedinica CERRM-a za regenerativnu medicinu je razvila Osteogrow-C, novi osteoinduktivni lijek za regeneraciju kostiju, koji je uspješno testiran u različitim prekliničkim modelima uključujući modele posterolateralne spinalne fuzije kod kunića i ovaca te model segmentalnog defekta lakatne kosti kod kunića. Nadalje,

CERRM je razvio terapiju temeljenu na anti-BMP1.3 protutijelima za liječenje različitih bolesti koje dijele fibrozu kao ključni patološki mehanizam. Ovaj rad daje pregled razvoja i kliničkih studija novih terapija za koštanu regeneraciju temeljenu na koštanim morfogenetskim proteinima te pregled razvoja inovativnih antifibroznih terapija.

Keywords: Osteogrow, Osteogrow-C, rhBMP6, regeneracija kostiju, posterolateralna spinalna fuzija, regenerativna medicina

1. Introduction

Regenerative medicine is among the most propulsive scientific fields focused on the regeneration of various tissues and organs employing different strategies and approaches. In order to position Croatia as one of the leading EU countries in the field of regenerative medicine, Center of Excellence for Reproductive and Regenerative Medicine (CERRM) was established as a collaboration between leading academic institutions in Croatia including the University of Zagreb School of Medicine, University of Zagreb Faculty of Veterinary Medicine, University Hospital Center Zagreb, University Hospital Dubrava, University of Rijeka School of Medicine, Ruđer Bošković Institute as well as private companies Genera Research, Fidelta, and Smart Medico. In the past period (2017-2023) CERRM has conducted a major project „Reproductive and Regenerative Medicine – Exploration of new platforms and potentials (KK.01.1.1.01.0008)" that has resulted in unprecedented success in the development of novel diagnostic and therapeutic solutions in the field. Specifically, the CERRM subunit for Regenerative Medicine has developed Osteogrow-C, a novel osteoinductive device for bone regeneration, which was tested in various preclinical models with current efforts for initiation of clinical trials (1-8). Furthermore, CERRM has developed BMP1.3 antibody-based therapy for various diseases that share fibrosis as a key pathological mechanism (9-12). This review aims to provide an overview of the recent advancement in the development of novel devices for bone regeneration and BMP1.3-based anti-fibrotic therapy.

2. Development of novel BMP-based therapeutic strategies for bone regeneration

Bone morphogenetic proteins (BMPs) are growth factors and members of the TGFβ superfamily which are possessing potent osteoinductive properties (13-19). Due to their ability to induce bone formation, osteoinductive BMPs have been widely investigated as a key component of novel therapeutic solutions for bone regeneration and as a substitution for autologous bone graft (ABG) which is currently the gold standard for promoting spinal fusion as well as treatment of segmental defects and bone fracture nonunions (20-28). Effective BMP application requires carrier which is biocompatible, easily manufactured with desired biomechanical properties, enabling vascular and cellular invasion, and in the end, provide good retention of an osteoinductive molecule of interest (29-34). BMP carriers are divided into synthetic polymers, natural polymers, and inorganic materials (35, 36). Synthetic polymers (e.g., polylactic acid-PLA, polyglycolic acid-PGA, polyethylene glycol-PEG, poly-E-caprolactone-PCL, polypropylene fumarate-PPF) and inorganic materials (e.g., calcium phosphate ceramics, bioglass, calcium sulfate cement) have been evaluated as a BMP carrier in numerous preclinical trials, however they have not been introduced to clinical practice (35, 37-39). On the other hand, natural polymers (e.g., collagen, hyaluronic acid, fibrin, chitosan, gelatin) have been the most commonly used BMP carries in preclinical studies which ultimately led to the approval of currently only commercially available BMP-based osteoinductive device, Infuse, which uses collagen as a carrier in a form of absorbable collagen sponge in combination with recombinant human Bone morphogenetic protein (rhBMP2) (13, 31, 40-45). Infuse is currently approved for anterior lumbar interbody fusion (ALIF), acute tibial fracture treatment, and maxillofacial reconstructions (13). However, its *off-label* applications in various spinal fusion procedures have led to severe side effects, necessitating the development of safe, effective, and affordable autologous bone graft substitutes (ABGS) (46-49). Addressing this need involves ongoing research of novel BMP carriers, focusing on exploring combinations of natural or synthetic polymers with inorganic materials to optimize BMP delivery and consequently bone regeneration (13, 19, 29). Currently, one of the most promising BMP-based therapeutic solutions for bone regeneration is Osteogrow, an autologous bone graft substitute (ABGS) developed at the Laboratory for Mineralized Tissues at the University of Zagreb School of Medicine (1, 2). Osteogrow consists of recombinant human Bone Morphogenetic Protein 6 (rhBMP6) delivered within autologous blood coagulum (ABC) as a carrier (1, 2). In comparison to other osteoinductive BMPs, rhBMP6 showed superior ability

to promote osteoblast differentiation *in vitro* and induce bone regeneration *in vivo* when compared to BMP2 and BMP7 (50-52). Additionally, ABC serves as an ideal physiological carrier, suppressing foreign body responses, facilitating tight rhBMP6 binding with plasma proteins within the fibrin meshwork, and allowing sustained *in vitro* release of rhBMP6 (53-55).
Following successful preclinical studies, Osteogrow underwent Phase I/II trials in patients with distal radial fractures (DRF; EudraCT 2014-005101-21) and patients undergoing high tibial osteotomy (HTO; EudraCT 2015–001691-21) (56, 57). For applications where compressive forces are present, Osteogrow requires supplementation with a compression-resistant matrix (CRM). Initially, Osteogrow-A was developed, consisting of rhBMP6/ABC with the addition of allograft particles as CRM (58). Osteogrow-A underwent successful evaluation in rabbit and sheep posterolateral spinal fusion (PLF) models (2) and a Phase II clinical trial (EudraCT number 2017-000860-14) as part of the Horizon2020 project OSTEOproSPINE (2018-2023; GA 779340). However, the use of allograft is associated with several disadvantages, including immunogenicity and regulatory issues in different markets (24, 59, 60). Hence, our focus shifted towards identifying an alternative to allograft, leading us to explore calcium phosphate (CaP) synthetic ceramics as a viable substitute (60, 61). CaP ceramics offer versatility in shape, size, and chemical composition, with tricalcium phosphate (TCP) and hydroxyapatite (HA) being the most utilized variants (38, 62-75). Their key distinction lies in resorbability post-implantation, with TCP being highly resorbable while HA remains stable (76). Additionally, biphasic calcium phosphate (BCP) combines TCP and HA in varying ratios to achieve optimal resorbability (66, 76). Our efforts culminated in the development of novel Osteogrow formulation with addition of synthetic ceramics as a CRM - Osteogrow-C. Extensive animal studies were conducted to optimize implant properties and determine safety and efficacy in relevant preclinical models (3-7, 77, 78). Osteogrow-C was evaluated in several animal models ranging from initial to advanced models (3-7, 30, 53, 77-81). Rodent models (rats, mice) serve as the initial evaluation stage, facilitating rapid assessment of novel therapies potency and elucidating osteogenesis mechanisms (30, 80, 82-84). Promising therapies identified in rodent models progress to clinically relevant intermediate (rabbit) and larger animal models (sheep, dogs, non-human primates - advanced stage). In rat subcutaneous model, Osteogrow-C induced bone formation, with studies indicating that smaller particle sizes of rhBMP6 resulted in a higher bone volume (3). Moreover, the chemical composition of particles showed no significant impact on bone induction at the rat ectopic site (3).
In the subsequent phase of preclinical trials (Figure 1), optimal formulations of Osteogrow-C were selected for evaluation in pertinent models of posterolateral spinal fusion (PLF) and segmental defects (4, 5, 7). Specifically, chosen Osteogrow-C formulations underwent assessment in a rabbit PLF model with follow-up periods of 7, 14, and 27 weeks (5). Early observations indicated that Osteogrow-C facilitated rapid spinal fusion between adjacent transverse processes (5). Moreover, extended follow-up revealed significant differences in resorption between TCP ceramics and biphasic ceramics containing high HA proportion. Nonetheless, these differences in residual ceramic amounts did not affect the biomechanical properties of the newly formed bone for spinal fusion. Building upon the success of rabbit PLF studies, the safety and efficacy of Osteogrow-C were further validated in sheep PLF models (7). In these studies, Osteogrow-C with 74-420 μm and 1000-1700 μm ceramic particles induced radiographic solid fusion 9 weeks following implantation. Moreover, spinal fusion and osseointegration with native bone were confirmed at the end of the follow-up period (14, 27 and 40 weeks following surgery) by microCT and histological analyses as well as biomechanical testing (7). Additionally, Osteogrow-C underwent evaluation in a rabbit ulnar segmental defect model, showcasing superiority over both Osteogrow and Infuse. Finally, the safety and efficacy of Osteogrow-C in treating large segmental defects were demonstrated in a case involving a dog with a significant gunshot defect of the humerus, successfully restored through the application of Osteogrow-C implants containing TCP ceramics (8).

3. Development of novel antifibrotic therapy based on anti-BMP1.3 antibodies

Fibrosis is a key pathogenetic mechanism in many crucial diseases including chronic kidney disease, liver cirrhosis, and ischemic heart disease (85, 86). It is defined as the excessive accumulation of fibrous connective tissue in diverse organs and tissues. (86). Unfortunately, therapeutic interventions for fibrotic ailments remain scarce, often accompanied by grim prognoses (86, 87). Bone morphogenetic protein 1 (BMP1) was originally isolated from bone alongside other BMPs due to its heparin affinity but it diverges from the conventional BMP protein family and belongs to the astacin/BMP1/tolloid (TLD)-like family of zinc metalloproteinases (88-90). Recent advancements have unveiled multiple splice variants of BMP1 stemming from the same gene, denoted by sequential suffixes ranging from BMP1.1 to BMP1.7 (91-93). Both BMP1.1 and its isoform BMP1.3 play pivotal roles in converting various extracellular matrix (ECM) precursors into functional proteins. These include pro-collagens C I–III, small leucine-rich proteoglycans (such as decorin and biglycan), laminin, collagen VII, and perlecan within the basal membrane (94). Additionally, BMP1 isoforms facilitate the maturation of BMP antagonist chordin and pro-lysyl oxidases, crucial mediators of collagen crosslinking (95, 96). This process is indispensable for the proper assembly of insoluble collagen within the ECM and subsequent scar formation. Previously, employing liquid chromatography-mass spectrometry we have identified

the presence of BMP6, GDF15, and the BMP1.3 isoform of the Bmp1 gene in plasma samples collected from both healthy individuals and patients afflicted with chronic kidney disease (12). Subsequently, we isolated the endogenous BMP1-3 protein and provided evidence of its circulation as an active enzyme (12). Due to its role in ECM assembly, BMP1.3 emerged as a potential target for the prevention of fibrosis. To test the role of BMP1.3 in fibrosis we have conducted studies employing a rat model of chronic kidney disease (12). Administration of a BMP1.3 neutralizing antibody reduced renal fibrosis (CKD), preserved organ function and subsequently increased survival of rats with CKD. On the other hand, the administration of recombinant BMP1.3 increased renal fibrosis and reduced survival. Antifibrotic effects of BMP1.3 neutralizing antibody have been subsequently evaluated in rats with carbon tetrachloride (CCl4)-induced liver fibrosis (10). Results of this study revealed that administration of monoclonal BMP1.3 antibodies significantly decreased fibrosis and therefore might be a therapeutic solution for decreasing the progress of liver cirrhosis (10). Recently, we investigated BMP1.3 antibodies as the first antibody-based treatment for ischemic heart disease (9). Acute myocardial infarction is an extremely common disease with a high mortality rate for which there is currently no sufficiently effective therapeutic solution (97, 98). Our studies on rodents revealed that BMP1.3 inhibition reduces collagen deposition, decreases scar formation, and supports cardiomyocyte survival after myocardial infarction (9). Mechanistically, we have demonstrated that BMP1.3 monoclonal antibody inhibits the Transforming Growth Factor β (TGFβ) pathway. Furthermore, CERRM has explored anti-BMP1.3 antibodies as a therapy for merosin-deficient congenital muscular dystrophy type 1A (MDC1A) mice which is the second most prevalent congenital muscular dystrophy (CMD). Dystrophy is caused by a mutation in the lama2 gene encoding the laminin-a2 protein that contributes to merosin, an important protein present in the skeletal muscle basement membrane (99-102). To evaluate the potential therapeutic effects of antifibrotic therapy we evaluated anti-BMP1.3 antibodies in a mouse model of congenital muscular dystrophy with mutation in the laminin gene (103). The application of BMP1.3 antibodies had a stunning effect on mice with CMD and significantly increased their survival, mobility, body mass, and decreased fibrotic muscle area (Figure 2) (103). Promising results on the mouse CMD model indicate that BMP1.3 inhibition might be an effective therapy for patients suffering from this rare but extremely severe disease. In conclusion, antifibrotic therapy based on anti-BMP1.3 antibodies represents a potential therapeutic solution for a wide range of diseases in which fibrosis is a key pathogenetic mechanism, including but not limited to chronic kidney disease, liver cirrhosis, acute myocardial infarction, and congenital muscular dystrophy.

4. Conclusions

The development of regenerative medicine and novel therapeutic devices provides hope to improve the life quality of millions of patients worldwide. The establishment of the Croatian Center for Reproductive and Regenerative Medicine (CERRM) provided necessary resources for conducting the state-of-the-art research in the field of regenerative medicine and translation of developed therapeutic solutions to clinical practice. CERRM members have conducted extensive preclinical studies to develop Osteogrow-C, a novel osteoinductive device for bone regeneration as well as novel anti-BMP1.3 based antifibrotic therapy (3-7, 9, 10, 12, 77, 78, 81). Moreover, several Osteogrow formulations (Osteogrow, Osteogrow-A) have also been evaluated in Phase I/II clinical trials (56, 57). All phases of Osteogrow development have been conducted or coordinated by the University of Zagreb School of Medicine which is a unique example worldwide that an academic institution has carried out the development of a drug from discovery and preclinical research to advanced stages of clinical trials. The aforementioned studies have resulted in more than 30 papers published in prestigious scientific papers including Nature Communications, Nature Microbiology, and Nature Scientific Reports (Figure 3).

Furthermore, CERRM scientists have presented their work at numerous international conferences (Figure 4) including the Tissue Engineering and Regenerative Medicine Society (TERMIS) Conference and the European Calcified Tissue Society (ECTS) Congress. Moreover, CERRM has significantly contributed to the internationalization of research through numerous established co-operations with leading scientists in the field and by the organization of the 13th International Conference on Bone Morphogenetic Proteins in Dubrovnik (October 2022) (18). The 13th International BMP Congress gathered world-leading experts in the BMP field who delivered keynote lectures and young scientists who had the opportunity to present and discuss their work. Finally, an integral part of CERRM's mission and vision is the education of young scientists in the field of regenerative medicine. Therefore, CERRM has provided optimal conditions for the development and education of young scientists and as part of the project, eight students have completed or will soon complete their doctoral dissertations (104, 105).

To summarize, the establishment and implementation of CERRM's activities have had a transformative impact on the development of regenerative medicine in Croatia, resulting in significant scientific discoveries, the development of new therapeutic devices, the education of young scientists, and the establishing of new collaboration with different institutions and experts in the field. The established network of CERRM scientists and institutions will continue their endeavor to develop and bring to clinical practice cutting-edge therapies for bone regeneration and treatment of fibrosis.

Declaration of competing interests

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