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REVIEW ARTICLE

Constructing the toolbox: Patient-specific genetic factors of altered fracture healing

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Abstract The multifaceted sequence of events that follow fracture repair can be further complicated when considering risk factors for impaired union, present in a large and growing percentage of the population. Risk factors such as diabetes, substance abuse, and poor nutrition affect both the young and old, and have been shown to dramatically impair the body's natural healing processes. To this end, biotherapeutic interventions such as ultrasound, electrical simulation, growth factor treatment (BMP-2, BMP-7, PDGF-BB, FGF-2) have been evaluated in preclinical models and in some cases are used widely for patients with established non-union or risk/indication or impaired healing (i.e. ultrasound, BMP-2, etc.). Despite the promise of these interventions, they have been shown to be reliant on patient compliance and can produce adverse side effects such as heterotopic ossification. Gene and cell therapy approaches have attempted to apply controlled regimens of these factors and have produced promising results. However, there are safety and efficacy concerns that may limit the translation of these approaches. In addition, none of the above mentioned approaches consider genetic variation between individual patients. Several clinical and preclinical studies have demonstrated a genetic component to fracture repair and that SNPs and genetic background variation play major roles in the determination of healing outcomes. Despite this, there is a need for preclinical data to dissect the mechanism underlying the influence of specific gene loci on the processes of fracture healing, which will be paramount in the future of patient-centered interventions for fracture repair.
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Introduction

Fracture treatment relies on the timely principles of restoration of anatomy and appropriate osseous stabilization, which will lead to restoration of bone structure and function.[1,2] Despite the intrinsic ability of the body to heal fractures, patient risk factors can significantly impair skeletal repair.[3] The rate of delayed fracture healing or non-union is highest amongst subpopulations with specific risk factors such as smoking, advanced age, steroid use, use of certain pharmaceuticals (i.e. anti-cancer drugs) and metabolic diseases such as diabetes mellitus (DM).[3] An increased mechanistic understanding for impaired osseous healing associated with specific high-risk populations will provide fundamental information necessary to design a regenerative approach for fracture patients with specific risk factors for non-union. This complexity is further increased when "the patient factor" is introduced. Namely, each individual has a unique genetic makeup, which influences the processes of fracture repair. In addition, genetic mutations caused by external patient factors (comorbidities, environmental influences) may further distinguish healing processes amongst our world's population as truly heterogeneous.

Of the 6.2 million fractures sustained in the United States each year, these patient factors have resulted in a 10% incidence of delayed union or non-union.[4] To address these clinical concerns, there are a number of treatments available including autologous or allogeneic bone grafts and a variety of bone substitutes such as demineralized bone matrix (DBM).[5,6] Adjunctive measures such as low intensity pulsed ultrasound (LIPUS) to provide biomechanical stimulation[7] have also been used. More recently, biological factors including the bone morphogenic proteins (BMPs) have been successfully used to promote bone repair.[8] BMP2 (Infuse) in particular has been administered to patients with established non-union or risk of non-union due to the fracture location. While these and other currently available agents hold promise in accelerating fracture healing, they have limited usefulness or efficacy and do not account for the genetic component or "the patient factor".[9,10]

The development of a predictive "toolbox" to assess how individual patients will respond to particular treatment regimens should be the next leap forward in treating a growing global population, many of whom have comorbidities that increase the likelihood of compromised bone repair. The collection of preliminary data to construct this "toolbox" may be garnered through large-scale preclinical studies which examine the genetic influences of isolated point mutations on bone repair using models of closed fracture and established non-union. This information can be used to personalize therapeutic regimens for fracture repair, similar to existing personalized medicine for genetic screening for certain cancers (i.e. BRCA gene for breast cancer) and screening for risk of cystic fibrosis in expected parents.

In this review, we will begin with a brief discussion of fracture repair, followed by a description of patient factors, which have been shown to inhibit regenerative processes. Several clinically implemented biotherapeutics and promising gene therapy approaches for patients with these risk factors will be described and their use/effectiveness will be discussed. Finally, the potential of patient centered medicine will be presented, considering potential pitfalls and alternative paths forward.

Bone fracture healing

Following injury, bone has the unique ability to repair itself through mechanisms similar to its post-natal development process. Fracture healing involves two distinct but important mechanisms leading to bone formation, primary and secondary fracture healing. Primary fracture healing occurs when bones unite across the fracture site via direct bone formation to bridge the gap. This type of healing occurs in the presence of rigid internal fixation and a near absence of interfragmentary strain.[11] Secondary fracture healing (endochondral ossification) occurs when there is significant micro-motion at the fracture site. It is characterized by responses from the periosteum, marrow, and external soft tissue that lead to formation of a callus to bridge the gap, and occurs in three stages: inflammation, repair, and remodeling.[1,2] Despite the sequenced description of these processes, these phases actually occur in an overlapping spacial and temporal pattern.

Blunt trauma associated with a fracture results in an interruption of skeletal integrity, coupled with a disruption of the normal vascular structures and nutrient flow at the fracture site. This leads to reduced oxygen tension and disruption of bone marrow architecture (Fig. 1A). The inflammatory phase of fracture healing proceeds with inflammatory cell, macrophage, and degranulating platelets infiltration of the fracture site during hematoma formation.[2,12] Platelets and inflammatory cells within the hematoma release several factors that are important for chemotaxis, proliferation, angiogenesis and mesenchymal stem cell differentiation into osteoblasts or chondroblasts.[13,14] The early events that take place during the inflammatory phase set the stage for the cartilaginous phase and the progression of endochondral ossification.

During the cartilaginous phase of healing in a long bone fracture, two discrete crescent shaped cartilage tissue masses develop, symmetric to the fracture line (Fig. 1B). Cartilage, which provides initial stability to the healing fracture, is produced through a process beginning with signaling molecule directed differentiation of mesenchymal cells into chondroblasts. Once chondroblasts become embedded in the extracellular matrix, they mature to become chondrocytes. Non-hypertrophic chondrocytes are capable of proliferation and continue to synthesize cartilage matrix. Maturing chondrocytes which previously expressed aggrecan and type II collagen undergo hypotrophy, terminal differentiation, and characteristically express type X collagen.[15] The matrix is subsequently calcified and remaining cartilage is resorbed, setting the stage for the bony phase of fracture repair.

Following calcification, the callus is invaded by newly formed blood vessels. The vasculature provides a conduit for the recruitment of osteoblastic progenitors, as well as chondroclasts and osteoclasts needed to resorb the calcified tissue and early mineralized tissue (Fig. 1C). The

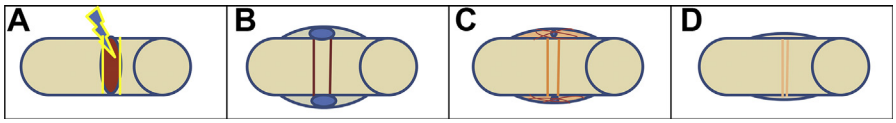

Figure 1 The progression of fracture healing (A) injury and hematoma formation (B) cartilaginous callus and intramembranous bone formation (C) bony/vascularized callus formation (D) callus remodeling and fracture resolution.

osteoblastic progenitors differentiate into osteoblasts and produce woven bone within the gap region of the fracture callus.[16] The woven bone produced in this stage acts as a peripheral buttress, which is subsequently remodeled in the final phase of fracture healing.

The final stage of fracture healing is characterized by remodeling of woven bone to form a structure with similar tissue structure and mechanical integrity of non-fractured bone (Fig. 1D).[16] Despite the efficient design of these bone healing processes, they may be impaired through substance abuse, pathologic conditions, estrogen loss, and malnutrition.

Factors that impair bone fracture healing

The clinical importance of impaired fracture healing cannot be overemphasized. Several past orthopedic challenges have been overcome with the advent of collaboration between basic science and clinical research such as accelerated repair of fracture healing, particularly in the presence of risk factors associated with increased rate of infection and non-union. Impaired fracture healing outcomes, particularly those associated with co-morbidities represents an ongoing hurdle for initial fracture management.[3] Although the fracture repair process is well understood, relatively little is known about the coordinated regulation of events necessary to achieve successful repair. Even less is understood about how this process can fail, leading to cases of delayed union or non-union. Bone healing generally proceeds in a highly reproducible manner, producing a tissue virtually indistinguishable from the original bone. However, certain systemic conditions significantly impair the body's ability to repair fractured bone (Fig. 2).

Vitamin D and some of its metabolites have demonstrated roles in bone metabolism and fracture healing.[17] Vitamin D deficiency is known to induce secondary hyperparathyroidism, as well as increased bone turnover and bone mineral loss.[18,19] Vitamin D deficiency has been shown to block formation of mature bone during fracture healing. Osteoid deposition however, remains unchanged with Vitamin D deficiency.[20] Similar to Vitamin D deficiency, the deficiency of many other nutrients that influence bone health and/or signaling can significantly inhibit fracture healing. Local growth factor deficiencies which include platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-I (IGF-I), and transforming growth factor-beta (TGF-β) amongst others have been identified as playing critical roles in the early stages of the musculoskeletal healing process.[21,22] Thus, malnutrition such as Vitamin D deficiency can significantly impair the processes of fracture repair, particularly bone turnover. Similar to how nutritional regulation can influence callus remodeling, hormonal regulation has also been shown to be critical to this stage of fracture healing.

Estrogen is an important protective hormone for bone homeostasis and loss of this protection after menopause contributes to the pathogenesis of postmenopausal osteoporosis. Although this estrogen's protective effect is thought to be largely mediated by inhibition of bone resorption, recent evidence suggests that estrogen may also stimulate osteoblast activity, critical to appropriate fracture healing.[23–25] This provides evidence that in addition to nutritional regulation, hormonal regulation is important to the mechanisms of fracture repair, yet sometimes environmental factors (i.e. substance abuse) may significantly impair the healing processes.

Clinical and experimental settings have shown a relationship between smoking and a variety of orthopaedic conditions, including delayed union and nonunion amongst human and animal fracture models.[26–28] Fracture repair is slower in smokers compared to non-smokers. The rate of nonunion also is higher amongst smokers. This observation has been shown in clinical trials of open and closed tibia shaft fractures and spinal fusions.[26,29,30–32] The negative impact of smoking on fracture healing could be explained by nicotine's inhibition of osteoblast activity[33,34] as well as its constrictive effect on the micro-vasculature.[35–37] This vasoconstrictive effect has been observed during revascularization of cancellous bone graft during spinal fusion.[38] It is also possible that nicotine attenuates cytokine expression during bone formation.[39] In addition to the established inhibition of fracture healing with nicotine use, other substance abuse can also increase the incidence of non-union.

Few studies exist which examine the effects of alcohol on fracture repair. Available data suggests that alcohol abuse does not increase the incidence of non-unions, osteonecrosis, or other complications,[40,41] but is associated with an increase in healing time for fractures with

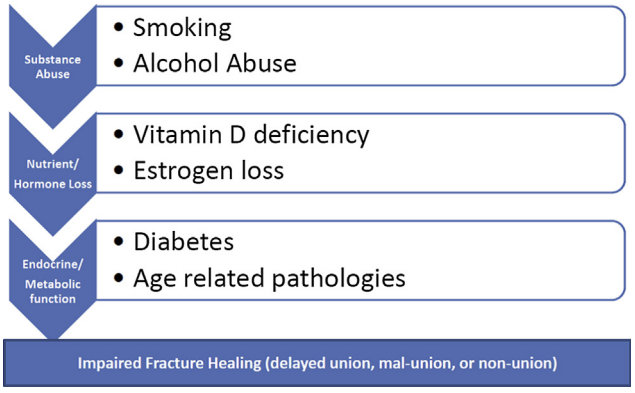

Figure 2 Pathologic factors associated with impaired fracture repair.

transverse patterns.[40] The mechanism underlying this observed impairment of fracture callus tissue formation is deregulation of the Wnt signaling protein β-catenin signaling, and associated disruption of Wnt-mediated transcription.[42] Although maintenance of nutritional health and avoidance of environmental risk factors can reduce the risk of non-union, certain metabolic conditions can significantly impair several facets of the fracture healing processes.

Diabetes mellitus is one such condition associated with impaired fracture healing, presenting a major therapeutic challenge as over 21.0 million Americans are diagnosed with Type I/Type II diabetes.[43] Impairments associated with the diabetic osteopathology include local growth factor deficiencies within the DM fracture callus which delay/compromise healing, advanced glycation end products (AGEs) which accumulate within the bone and have been shown to affect osteoblast function and the structural and mechanical properties of bone, and impaired osteoclastogenic regulation at the end of the cartilaginous phase, leading to accelerated resorption of calcified cartilage. AGEs are produced when reducing sugars, such as glucose, react with amino groups in proteins, lipids, and nucleic acids through a series of reactions forming Schiff bases and Amadori products.[44] The accumulation of AGEs leads to tissue damage through structural modification of proteins,[45] stimulation of cellular responses via receptors specific for AGE proteins,[46] and generation of reactive oxygen intermediates.[47,48] In patients with DM, AGE formation is markedly accelerated because of the increased availability of glucose and increased oxidative stress. AGEs have been found to accumulate in bone tissue and affect osteoblast function and its structural and mechanical properties.[49,50] In addition to the damaging effects of AGE accumulation, bone remodeling is significantly impaired in DM fractures.

Insight into the influence of DM on the extent of osteoclastogenesis can be determined from several studies analyzing the effect of DM upon release and expression of various inflammatory cytokines critical for osteoclastogenesis.[51,52] Suzuki et al measured the bone mineral content and fasting levels of serum intact parathyroid hormone (i-PTH), intact osteocalcin (i-OC), tartrate-resistant acid phosphatase (TRAP), and osteoclastogenesis inhibitory factor/osteoprotegerin (OCIF/OPG) among male type 2 diabetic subjects and their age matched non-diabetic controls.[53] These proteins are essential in both bone homeostasis and fracture healing, and are all potentially compromised by diabetes. Suzuki et al determined that serum levels of i-PTH and i-OC were significantly lower in DM patients than in non-diabetic controls indicative of reduced bone formation. Serum levels of TRAP were significantly higher in diabetic patients indicative of increased osteoclastogenesis. Suzuki et al found no definitive correlation when comparing i-OC and OPG serum levels within the same group. This was unexpected as expression of i-OC is known to regulate bone turnover, and OPG may inhibit osteoclastogenesis. TRAP serum values, associated with osteoclastogenesis and OCIF/OPG, associated with vascular physiology and pathology, were both shown to have a negative correlation with bone mineral density. Therefore, diabetic individuals with higher TRAP and OCIF/OPG serum levels had lower bone mineral densities than those with lower serum values. In addition to AGE induced bone damage and disruption of bone remodeling, metabolic conditions such as diabetes also increase the risk of infection following trauma.

Infection still represents a major surgical challenge, despite improvements in operative techniques and antibiotic therapy. Deep infections can result in significant patient morbidity following open fractures or surgery for closed fractures. Osteomyelitis, an infection of the bone, may occur in as many as 5%–18% of operatively managed closed calcaneal fractures.[54,55] Depending on the size of an open wound, osteomyelitis may arise in up to 27% of cases with open calcaneal fractures.[56] Despite the plethora of risk factors for mal-union/non-union and with infections as a secondary complication, the fracture healing processes can alternatively be enhanced through use of bone healing adjuncts and potentially by promising gene therapeutic approaches.

Biotherapeutics for fracture repair

The use of locally delivered adjuncts to accelerate bone fracture healing has truly progressed over the years. With the remarkable success of BMPs, the orthopedics market leapt forward. Despite recent advances in technology (Fig. 3), current Food and Drug Administration (FDA) approved bone healing adjuncts/therapies either suffer from risks[9,10] including ectopic bone formation (BMPs), or are reliant on patient compliance (LIPUS).[7,57,58] Further, the costs of each intervention are quite expensive for both the manufacturer and patient. Unpredictable responses to these therapies may further be complicated when considering a diverse population with differing genetic backgrounds.

As seen with many orthobiologics, growth factors/biologics such as BMP-2 and BMP-7 (OP-1), platelet derived growth factor BB (PDGF-BB), and TGF-β demonstrate a biphasic effect at higher doses, either failing to accelerate[59] or possibly inhibiting osseous healing.[60,61] A study by Boerckel et al found that bone volume and connectivity were significantly reduced with increasing doses of recombinant human bone morphogenic protein 2 (rhBMP-2), above 1 μg rhBMP-2 in a critical size segmental defect rat model at 12 weeks post-osteotomy.[60] Boerckel et al found that effective and biphasic dosages of rhBMP-2 were dependent on the delivery mechanism.[60] In contrast, clinical results for OP-1 reported no advantage in treating tibial non-unions with rhBMP-7 compared to over autograft controls.[61] As such, despite promising results for BMPs in fracture repair, there is still a need to optimize treatment regimens and explore alternative approaches to combat non-union.

With this under consideration, alternative orthobiologics, administered at low doses or specific to bone have investigated in clinical trials. Based on the effectiveness of parathyroid hormone (PTH) to increase osteoblastic activity and bone mineral density in animal studies of osteoporosis,[62] a clinical trial was initiated to evaluate the efficacy of oral PTH (1–34) (teriparatide) to treat fracture in postmenopausal women following fracture of the distal

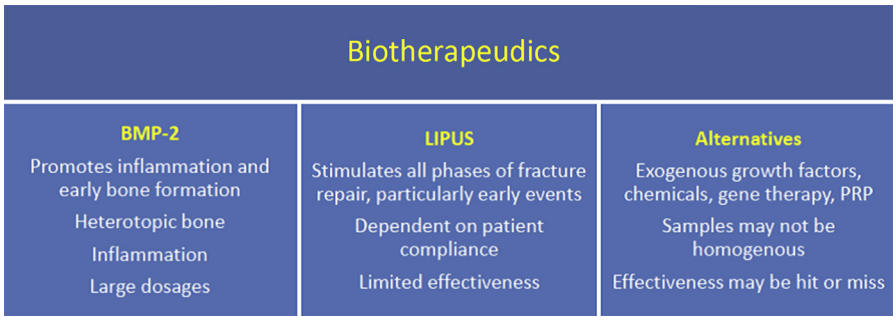

Figure 3 Biotherapeutics for fracture repair.

radius. This trial reported that the 20 μg dose yielded a significantly shorter time to healing, but the 40 μg was not significantly different from the control.[62] As a result of the clinical trial success of PTH for fracture healing in post-menopausal women, other osteoporotic medications were investigated for the same indication. Despite promising early data in preclinical models, an anti-sclerostin antibody romosozumab phase II clinical trial for another pharmaceutical was recently abandoned, in favor of focusing efforts on more favorable data on osteoporosis.[63] A large multicenter study investigating the bisphosphonate risedronate, to repair fractures found that administration of risedronate during surgery did not significantly alter the progression of intertrochanteric fracture repair or incidence of complications.[64] In contrast to the results of the risedronate trial, following early promising evidence, a phase III clinical trial involving injection of the bisphosphonate denosumab[65] in a tibial fracture model, is currently underway in China. In addition to the PTH, sclerostin antibody and bisphosphonate clinical trials for fracture repair in post-menopausal women, clinical trials using growth factors have also been recently initiated.

Clinical trials in Japan evaluating the fracture healing efficacy of local administration of fibroblast growth factor 2 (FGF-2) into the fracture gap of tibial shaft fractures following intramedullary nailing, revealed that significantly more patients achieved bony union compared to the placebo group at 24 weeks post-administration, with no adverse events noted with the higher dose of FGF-2.[66] Similarly, large scale human clinical trial in periodontal osseous defects found that recombinant human PDGF-BB had a biphasic effect at 1 mg/ml on bone mineralization within the defect region, compared to the 0.3 mg/ml group at 6 months post-treatment.[67] Besides these promising treatment modalities, there are many other alternatives that are currently being investigated in preclinical models.

Various preclinical animal models have been established and used to investigate bone fracture healing and to facilitate the preclinical evaluation of new treatment options such as osteoinductive drugs. Animal models are indispensable for demonstrating the benefit of any new modality sufficiently different from the standard treatment. Additionally, preclinical testing of new treatments or devices is required to prove efficacy and safety. The US Food and Drug Administration (FDA) recommend that agents be evaluated in at least two different animal species. Experimental animal models that mimic blunt trauma have been developed. Many of these models are described in the fracture repair literature, and have been implemented to investigate the local and systemic delivery of pharmaceutical agents to enhance fracture repair.

The numerous animal studies on this subject differ in terms of species, fracture site, and fixation method. Rabbit models have been used to study the effect of transforming growth factor-β (TGF-β),[22,68] fibroblast growth factor-1 (FGF-1), and fibroblast growth factor-2 (FGF-2)[69,70] on tibia fracture repair. Dogs, baboons, monkeys, pigs, sheep, rabbits, and goats all have been used to study the effects of exogenous growth factors and cytokines on fracture healing.[71–75] However, the rodent closed femur fracture model described by Bonnarens and Einhorn[76] is the most frequently used model due to fracture reproducibility, availability or rats in large numbers, and affordability.[77–86,21] The success and limitations of these and other biotherapeutics in translational animal models and in some patients lead to the investigation of gene therapy/cell therapy approaches to potentially improve the efficiency of augmented fracture repair.

Gene therapy/cell therapy approaches to bone healing

Recent advances in tissue engineering and cell biology have opened avenues to improve our methods for enhancement of bone repair especially in large bone defects. Various gene therapy techniques using HSV, Adenovirus, Lentivirus or AAVs have been successfully used to deliver osteoinductive agents to the site of fracture healing. Delivery of the cDNA of a number of BMPs using in vivo and ex vivo transfer techniques have demonstrated successful enhancement of the healing of critical sized defects in preclinical models.[87] Other transgenes including VEGF PDGF, FGF, Osterix, Nell-1 and Runx-2 have also been used with success in the animal models of fracture and long bone defect healing.[88] Virk et al introduced a "same day" gene therapy protocol using a Lentiviral vector with a 2-h transduction step to overexpress BMP-2 in fresh rat bone marrow mononuclear cells and showed the efficacy of this method in healing 6-mm long critical-sized defects in the rat femur.[89] They also compared the use of the Lentiviral and Adenoviral vector-based ex vivo gene therapy methods. They showed superior bone healing using the Lentiviral delivery of BMP-2 and postulated that prolonged release of BMP-2 in a more physiologic dose was responsible for improved defect healing.[90] Betz et al demonstrated that

delayed delivery of Adenoviral BMP-2 cDNA (5–10 days) after induced fracture, significantly enhanced radiological union and biomechanical strength, following critical-size segmental defects.[91] Despite the promise of gene therapy to overcome the difficulties of challenging bone repair scenarios, the use of these techniques in humans is limited due to the safety concerns such as mutagenic or carcinogenic nature of these genetic modifications.

To address the safety concerns associated with gene therapy, Alaee et al developed a dual gene expression vector overexpressing BMP-2 and HSV-Tk in an effort to enhance the safety of ex vivo gene therapy by killing the transduced cells with Ganciclovir after the bone healing process was completed. They showed reduced numbers of viable transduced cells in a mouse critical sized defect model, but complete elimination of these cells was not achieved.[92] In summary, there is a need for development of effective gene therapy strategies with established safety profiles in well controlled trials before they can be used in the humans. A promising alternative to gene therapy is the application of novel cell-based therapeutic strategies.

Cell-based therapies including embryonic stem cells for bone regeneration purposes have also been evaluated by many investigators. Tianai et al used an osteoporosis related impaired fracture model in mice and showed enhanced fracture healing after 4 weeks of implanting murine ES-derived osteoblasts.[93] Enhanced healing of calvarial defects in immune-compromised mice after implantation of human ES cells was reported by Kuhn et al.[94] They showed that the hESCs were able to recapitulate the mesenchymal developmental pathway and were able to repair the bone defect semi-autonomously without preimplantation differentiation to osteo- or chondroprogenitors. There are still concerns regarding the efficacy of the cell based therapies as well as ethical issues surrounding the use of embryonic stem cells. More refinements in these cell-based strategies are also warranted before they can be used as a viable option for enhanced bone formation in humans. These gene/cell therapy approaches continue the propagation of grouping patients based on case indications and pathologic diagnosis. Despite this, there is growing evidence of a genetic component to fracture repair.

Genetic components of fracture repair

Although orthobiologic treatments present options and will advance in the future, the genetic components of fracture healing should be considered to optimize the development of patient centered othobiologics. Several studies have shown that there exists a genetic predisposition to fracture non-union which is complicated by patient risk factors.[95–98] A study examining single nucleotide polymorphisms (SNPs) in the BMP signaling cascade found that advanced age was an important covariate in development of atrophic non-union and that SNPs located on NOGGIN and SMAD6 (inhibitors of the BMP pathway) are associated with a greater risk of fracture non-union.[95] Similarly, others have shown a significant association between SNPs of the PDGF gene (particularly PDGF-A and MMP-13) and incidence of non-union for lower extremity fractures.[98] SNPs have also been shown to be important influencers of fracture risk, based on genetic background in patients.[99] This emphasizes the need for large-scale preclinical studies, which evaluate the mechanisms underlying genetic predisposition to impaired fracture healing and identifying novel targets associated with SNP-impaired healing amongst preclinical models.

The early clinical evidence supporting the importance of genetic variations on fracture repair, prompted preliminary preclinical studies, comparing the ability of various strains of mice to heal surgically induced fractures.[100,101] Manigrasso and O'Connor compared C57BL/6NTac (B6/N) with DBA/2NTac (D2/Tac) and C3H/HeNTac (C3H/Tac) mouse stains and found that the B6/N strain exhibited an accelerated rate of healing compared to the other two strains, with C3H/Tac mice healing the slowest.[100] In another study, Jepsen and colleagues compared fracture healing in the C57BL/6J (B6) with C3H/HeJ (C3H) and A/J mice. They found that B6 and A/J strains exhibited faster healing compared to C3H based on mechanical properties of healed bones[101] and that B6 mice expressed the highest percentage of cartilage gene products. This was associated increases in chondrocyte maturation and hypertrophy. In contrast, C3H mice had a premature osteogenic response, and an associated slower healing rate than the other strains. Although these preclinical studies were the first to demonstrate a genetic component to fracture healing, they did not evaluate the influence of specific SNPs of impaired fracture healing.

There are limitations to clinical studies investigating SNP variations in the general population that create a need for sophisticated preclinical studies which dissect and evaluate the influence of individual SNPs, independent of patient risk factors (which may not be previously diagnosed). One such limitation is that clinical studies are designed to be non-invasive, and thus are limited in their assessment of biomechanical tissue integrity, high-resolution imaging capacity, and the availability of histological/immunohistochmical tissue samples. For these reasons, the mouse model, which can be targeting specific gene loci, presents a unique translational model to study the influences of SNPs on fracture healing in the absence or presence of fracture repair. Future studies using mouse models to evaluate the influence of specific gene loci in fracture repair may serve as a stepping stone to transition to higher order translational animal models, and further the transition from bench to bedside.

Conclusions

With the plethora of genetic, pathologic, and environmental factors that pose a risk for proper fracture healing, there has been substantial characterization regarding the effects of these risk factors on fracture healing. However, there is still an urgent need to enhance the healing process and eradicate the incidence of non-unions. As such, the development of adjuncts that are designed to be effective for individual patients is of paramount importance. The concept of a single gene regulating a complex phenomenon such as bone repair must transition into a more comprehensive assessment of the causes underlying impaired fracture healing. This is underscored by various patient

factors and co-morbidities that contribute to delaying the repair process. Thus, future studies ought to link fracture repair with genetic variability. Studies using genetically diverse populations of mice can indeed bring unique insight into the contribution of genomics to impaired or enhanced fracture healing. Such studies are now possible using the collaborative crosses or the diversity outcross mice generated by a consortium of mouse geneticists. These studies will also permit sub-analyses regarding the effects of risk factors for impaired fracture healing and their association with specific single nucleotide polymorphisms. Ideally, strategies that can be tuned to the patients' genetics and potential risk factors, would create optimized orthobiologics for what we could envision as "personalized orthopaedics".

Conflicts of interest

All authors state that they have no conflict of interest.

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