The genetic profile of bone repair

Rozalia Dimitriou[1]
Peter V. Giannoudis[2]

[1] Academic Department of Trauma and Orthopaedics, Leeds General Infirmary, Leeds, UK
[2] Academic Department of Trauma and Orthopaedics, School of Medicine, University of Leeds, Leeds General Infirmary, Leeds, UK

Address for correspondence:
Peter V. Giannoudis BSc, MD, FRCS
Academic Department of Trauma and Orthopaedics
School of Medicine, University of Leeds
Leeds General Infirmary, Clarendon wing Level A
Great George Street
LS1 3EX, Leeds, United Kingdom
Phone: +44 (0) 113-392-2750; Fax: +44 (0) 113-392-3290
E-mail: pgiannoudi@aol.com

Summary

Bone repair following a fracture is a complex, well orchestrated, physiological process in response to injury. Even though the exact number of the genes and expressed proteins involved in fracture healing remains unknown, the molecular complexity of the repair process has been demonstrated, and it involves numerous genes and molecules, such as extracellular matrix genes, growth and differentiation factors, matrix metalloproteinases, angiogenic factors and others. Discrepancies in fracture healing responses and final outcome seen in the clinical practice may be attributed among other factors to biological variations between patients and different genetic "profiles", resulting in "altered" signalling pathways that regulate the bone repair process. Preliminary human studies support a "genetic" component in the pathophysiology of impaired bone repair seen in atrophic non-unions by correlating genetic variations of specific molecules regulating fracture healing with non-union. However, the role of the genetic "profile" of each individual in fracture healing and final outcome, and its possible interaction with other exogenous factors remains a topic of extensive research.

KEY WORDS: genetic profile; genetic variation; bone repair; fracture healing.

Introduction

Bone repair is a complex, well orchestrated, physiological process aiming to restore or maintain skeletal function. Particularly in fracture healing and unlike other tissues that heal by the formation of scar tissue, bone is regenerated in response to the injury with its pre-existing properties being largely restored and the newly formed bone being, sometimes, eventually even indistinguishable from the adjacent uninjured bone (1). During the repair process, the pathway of normal embryonic development is recapitulated with the coordinated participation of several cell types (2), involving the interaction of various complex signalling pathways for bone induction and conduction, endochondral and intramembranous ossification, and angiogenesis (3).
Although, bone regeneration remains to a great extent an unknown cascade of biological events, the ongoing research in skeletal development, bone biology and fracture healing improved our understanding of the cellular and molecular pathways that govern the complex process of bone formation. A number of local and systemic regulatory factors that interact with several cell types, such as mesenchymal stem cells (MSCs) and osteoprogenitor cells recruited at the fracture-injury site or from the circulation have been found to initiate and regulate the cascade of events. Such molecules include pro-inflammatory cytokines [Interleukins 1 (IL-1) and 6 (IL-6), and tumour necrosis factor-alpha (TNF-α)], bone morphogenetic proteins (BMPs) and other members of the transforming growth factor-beta (TGF-β) superfamily, as well as other growth factors, including platelet-derived growth factors (PDGFs), fibroblast growth factors (FGFs), insulin-like growth factors (IGFs), angiogenic factors and metalloproteinases, as well as their receptors and inhibitors, forming complex and interacting signalling pathways (3,4).

Gene expression during bone repair

The synthesis, activation and overall function of these molecules/proteins during bone regeneration are being controlled by different genes and regulated by complex mechanisms, which still need to be elucidated. Even the exact number of the genes and expressed proteins involved in fracture healing remains unknown. In an animal study, evaluating the transcriptional profiling of bone repair in a rat femoral fracture model, using suppressive subtractive hybridization (SSH) to identify up-regulated genes within the fracture callus, the molecular complexity of the repair process has been demonstrated, with almost 600 known genes and over 100 presumably novel genes being expressed (5). Such genes included known extracellular matrix (ECM) genes, like bone collagen types I, V, VI, and XII, cartilage collagen types II, VI, and XI, osteopontin, osteonectin, etc., various cytokines (e.g., IL-1, IL-6), growth and differentiation factors and their receptors (e.g., BMPs, IGF-1, TGF, FGF, PDGF), transcription factors, adhesion molecules, proteolytic enzymes, matrix metalloproteinases, and other molecules (5). More recently, the expression of the angiogenic vascular endothelial growth factor (VEGF) and its receptors, during endochondral bone repair, has also been shown in a mouse rib fracture model (6).

Bone repair and clinical outcomes

Despite the complexity of bone healing at the molecular and cellular level, and our lack of understanding regarding the exact number of genes and molecules that regulate this biological process, in the clinical practice, the clinicians are very "familiar" with the clinical and radiological progression of the bone

repair process, as fractures of the skeleton are common, with for example over 6,200,000 fractures occurring annually in the United States (7). Although there are several factors that are known to be associated with different "healing responses" seen during bone repair, such as the type of fixation used and the provided mechanical stability, the presence of gap at the fracture site, the degree of soft tissue damage, open fractures, administration of NSAIDs, smoking, etc. (8,9), and the bone repair process is usually uneventful with healing rates up to 90-95% (7), different "healing responses" are being observed among fracture patients in the clinical setting. These include for example differences in time to fracture union, and variations in the amount of callus formation.

Moreover, "enhanced" or "impaired" healing responses following a fracture can also be occasionally observed. For example, spontaneous repair of large femoral bone defects (up to 15 cm) without associated head injury that healed unexpectedly in patients waiting for a definitive skeletal reconstructive procedure has been reported (10). On the other hand, impaired healing response in the form of delayed union or non-union in an overall 5-10% incidence of impaired healing responses (7) can occur within "fracture patients" with similar risk factors and fracture patterns, and even after low-energy closed fractures.

Bone repair and genetic variations

Given the still unknown complex "genetic" component of fracture healing (as of any biological process) and the observed "diversity" of bone healing responses; the different final clinical outcomes could be attributed, among other factors, to biological variations between individuals. Furthermore, the importance of genes as causes of diseases or as predisposing factors is indisputable and genetic variations among individuals do exist with an unknown possible clinical significance and role to phenotypic diversity (11). Therefore, it could be that genetic variants within the genes expressed during fracture healing and the unique genetic "profile" of each patient may result in a possible increased or impaired inherent potential for bone regeneration, by "altering" signalling pathways of bone formation at the molecular level.

Even though little is known about the possible significance of the genetic variations among patients within the genes expressed during the "normal" uneventful fracture healing process, there are a few studies that advocate a "genetic component" in the pathophysiology of the impaired healing response seen in fracture non-unions, and a possible genetic predisposition/susceptibility in addition to their multifactorial nature (8). The definite role of genetic predisposition as an etiological factor for impaired bone healing has not been currently confirmed, but some evidence from preliminary human studies correlate genetic alterations of the molecules regulating fracture healing and their expression with impaired bone repair and the development of atrophic non-union. These studies used specific genetic markers, the so-called single nucleotide polymorphisms (SNPs), to evaluate the different genetic "profile" of fracture patients and specifically to assess for genetic variants within known genes involved in fracture healing in patients with or without the development of atrophic non-unions. In the field of genetic research, SNPs are new generation genetic markers (12), occurring when a single nucleotide is changed (substituted), deleted or inserted into the DNA sequence, and are identified using polymerase chain reaction techniques (PCR) and genome sequencing (13). SNPs are used to show genetic variability and the unique genetic "profile" of each individual and relate genetic variations to phenotypic diversity and disease propensity (14), also used to assess the likelihood that someone will develop a disease or a complex trait (susceptibility). However, they do not cause a disease and therefore they are associated (predisposing or protective) and not causing factors (11).

In summary these studies, showed that specific genetic variants within various mediators of the fracture healing cascade may be associated with the impaired bone repair process seen in atrophic non-unions. Two SNPs within the genes encoding for two known BMP inhibitory molecules (Noggin and Smad6) were found to be associated with a statistically significant greater risk of fracture non-union (15). The BMP pathway is essential during bone repair, as BMPs are known powerful osteoinductive factors (3); and therefore a different genetic "profile" within these genes may potentially reflect diversity in bone formation during bone repair. In addition, another study has shown a significant association of a specific PDGF haplotype and non-unions, indicating that polymorphisms within the PDGF gene may represent a genetic risk factor for the development of non-unions (16). Finally, a recent study investigating genetic variations within genes regulating local antimicrobial response and bone healing demonstrated that specific genetic variants of the Toll-like pathogen recognizing receptors TLR 4 and the TGF-β genes were observed in patients with impaired bone repair, which may indicate a possible susceptibility to impaired pathogen recognition and elimination, leading to prolonged pathogen existence in the fracture site and impairment of the healing response (17).

Although there are only a few preliminary human studies that correlate the genetic "profile" with the bone repair process, there are also animal studies that suggest the possible role of a "genetic element" for the different bone formation responses seen in healing fractures. *In vivo* studies demonstrated that genetic variability among different inbred mouse strains significantly contributes to the process of bone regeneration (18), and genetic differences between mice strains seem to affect the length of each stage of fracture healing and the overall healing rate (19). Also, it has been suggested that genetic alterations in negative regulators of fracture healing may also alter fracture healing response and even accelerate bone formation (20).

Nevertheless, and to complicate things even further, the genetic profile and specific genetic variants may interact with environmental variants, without always resulting in loss of function. For example, in an animal model on postnatal skeletal development, it was shown that genetic variants affecting skeletal growth and strength may be buffered by environmental variants, and only when they are not fully compensated, they are expected to alter skeletal growth forming complex genotype-phenotype relationships (21). Furthermore, the genetic profile of each individual may be also influenced further by other biological processes that can occur during gene expression and protein production, increasing further the biological complexity of phenotypic and genetic heterogeneity. For instance, alternative splicing, which can occur up to 90% of all human genes, allows the production of multiple proteins (isoforms) from one gene, and they can contribute to the differences seen in normal and pathological physiological processes (22).

Genetic profile and other orthopaedic conditions and bone diseases

Research is also ongoing to assess the genetic profile of various bone diseases and other orthopaedic conditions, in an effort to elucidate the role of genetics in their pathophysiology. Such bone diseases and conditions include osteoporosis, osteoarthritis, heterotopic ossification (HO), Paget's disease, osteonecrosis of the femoral head (ONFH), and others. Extensive research on genetic variations and osteoporosis, has verified, to date, at least 15 genes including vita-

min D receptor (VDR), lipoprotein receptor-related protein 5 (LRP5), sclerostin (SOST), osteoprotegerin (OPG), RANK/RANKL, and collagen type I alpha1 (COLIA1), as osteoporosis susceptibility genes, whereas, another >30 genes are promising candidate genes (23). Large genetic studies have also identified genetic variants within genes in signalling pathways involved in cartilage and bone biology, like the BMP pathway (e.g. GDF5), genes in inflammatory pathways and variants in the 7q22 region to be associated with increased risk of osteoarthritis (24). Certain polymorphisms close to four specific genes were also found to attribute the majority of the genetic risk for Paget's disease (25); and even for the pathophysiology of stress fractures, specific genetic "profiles" have also been suggested as potential predisposing factors for increased fracture risk within military recruits (26). Moreover, certain genetic variants in angiogenesis- and hypoxia-related genes were found to be associated with an increased risk for development of non-traumatic ONFH (27) and three other variants with the development of post-traumatic HO (28). Finally, specific genetic "profiles" with several genetic variations within different genes (like VDR, IL-6, BMP-2) have also been associated with various spinal conditions, such as disc degeneration, adolescent idiopathic scoliosis, and ossification of the posterior longitudinal ligament (29-31).

Clinical significance and future directions

From the clinical perspective, the significance to elucidate the role of the genetic "profile" of bone repair may be beneficial, as simple genetic testing and analysis of genetic variants linked to "normal" or "impaired" bone healing could be used to early identify patients at risk of developing complications associated with impaired bone healing like atrophic non-union. This could expedite the on-time intervention at the biologic aspects of bone healing with biological response modifiers to enhance bone formation and it could expand their clinical applications, if the hypothesized role of genetic variations in the bone repair is established. Finally, such knowledge will enable the design of novel, more effective treatment strategies that promote bone regeneration and even modified ones to suit the genetic profile of the patient.

However, the role of the genetic "profile" in fracture healing and final outcome, and its possible interaction with other exogenous factors remains a topic of extensive research. Further studies are necessary to validate the genetic profile of bone repair.

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