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Review

Cellular and molecular bases of skeletal regeneration: What can we learn from genetic mouse models?

Rana Abou-Khalil, Céline Colnot *

INSERM UMR1163, Université Paris Descartes-Sorbonne Paris Cité, Institut Imagine, Paris, France

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ABSTRACT

Although bone repairs through a very efficient regenerative process in 90% of the patients, many factors can cause delayed or impaired healing. To date, there are no reliable biological parameters to predict or diagnose bone repair defects. Orthopedic surgeons mostly base their diagnoses on radiographic analyses. With the recent progress in our understanding of the bone repair process, new methods may be envisioned. Animal models have allowed us to define the key steps of bone regeneration and the biological and mechanical factors that may influence bone healing in positive or negative ways. Most importantly, small animal models such as mice have provided powerful tools to apprehend the genetic bases of normal and impaired bone healing. The current review presents a state of the art of the genetically modified mouse models that have advanced our understanding of the cellular and molecular components of bone regeneration and repair. The review illustrates the use of these models to define the role of inflammation, skeletal cell lineages, signaling pathways, the extracellular matrix, osteoclasts and angiogenesis. These genetic mouse models promise to change the field of orthopedic surgery to help establish genetic predispositions for delayed repair, develop models of non-union that mimic the human conditions and elaborate new therapeutic approaches to enhance bone regeneration.

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Introduction

Bone repair is a dynamic regenerative process involving many cell types and molecular pathways to insure fracture consolidation [1–6]. Repair is initiated during the inflammatory phase, characterized by the formation of a hematoma, an immune response and progenitor cell activation and recruitment to the site of injury. The regenerative phase consists of revascularization and bone formation by intramembranous and/or endochondral ossification, followed by callus remodeling to restore the normal bone structure. Healing occurs in general through a combination of intramembranous and endochondral ossification. However, sites that are more mechanically stable and naturally in compression predominantly heal with primary bone formation in an intramembranous pathway, while mechanical instability leads to the formation of cartilage, which becomes calcified, vascularized and eventually replaced by bone [7–10]. Perturbations in any of these phases of repair may cause delayed or impaired consolidation. Although most bone injuries heal normally if they are properly reduced and fixed,

* Corresponding author at: INSERM UMR1163, Université Paris Descartes-Sorbonne Paris Cité, Institut Imagine, 24 Boulevard du Montparnasse-75015 Paris, France.
E-mail address: celine.colnot@inserm.fr (C. Colnot).

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tremendous risk factors subsist such as avascularity, large fracture gaps or concomitant infections that are associated with poor fracture healing [11]. Genetic risk factors are less well defined.

Over the past ten years, genetic manipulations of animals have changed our understanding of the cellular and molecular mechanisms of bone regeneration. These advances came first from the identification of genes involved in skeletal development through the analysis of skeletal anomalies in various genetic screens. Mouse models have been particularly relevant to apprehend the biology of human bone diseases, such as Osteogenesis Imperfecta or achondroplasia [12,13]. As molecules regulating bone development and postnatal bone growth are re-expressed during fracture healing and bone regeneration, many candidate genes involved in fracture repair have been characterized, including genes in the Wnt, Hedgehog (HH), bone morphogenetic protein (BMP), parathyroid hormone (PTH) and notch pathways [14–17]. Yet the functions of most of these genes remain to be fully defined in the context of fracture repair. For example, the Wnt pathway is key to bone development and repair. However, Wnt regulation of cartilage and bone formation during skeletal regeneration is influenced by variations in the mechanical environment, which does not affect skeletal development through the same mechanisms [18,19]. During development, Wnt signaling regulates osteogenesis and chondrogenesis based on the patterning of skeletal elements [20]. Other events are unique to bone healing such as the inflammatory response. Hence, mouse models of inflammatory defects have been essential to better understand the role of inflammatory mediators in bone repair [21–23]. In addition, the mutation of a given gene may not have consequences on bone development due to compensatory mechanisms, while the absence of the same gene during repair may lead to fracture non-union or delayed-union [24,25]. With a growing number of genetically modified mice available, rodent fracture healing studies are instrumental to further elucidate the cellular and molecular bases of bone regeneration [26–29].

Rodents are attractive animal models to uncover new gene functions, in part due to their lower cost and short gestation time compared to large animal models. Mouse models of fracture non-union and bone graft healing have been established to test therapeutic approaches to enhance bone regeneration [23,30–38]. Rodents complement other large animal models that are employed to validate surgical techniques and are required for preclinical studies. Taken together, these models provide a broad toolkit for regenerative bone research. In this review, we classified the genetic mouse models in six groups that illustrate the key components of bone regeneration, i.e. inflammation, skeletal cell lineages, signaling pathways, extracellular matrix, osteoclasts and angiogenesis. However, many mouse models exhibit alterations in more than one of these interconnected processes and may be used to study several aspects of bone healing.

Genetic tools for mouse models of skeletal regeneration

Genetically modified mouse models for bone repair can employ a wide range of genetic manipulations including (i) the targeted inactivation of specific genes or specific expression of mutated genes to understand gene functions, and (ii) the specific expression of reporter genes to assess gene expression or track cell lineages during bone repair.

Mouse knockout (KO) models are particularly informative to elucidate the function of genes during bone repair in the adult. However, the conventional KO approach is only possible for mutations that do not lead to embryonic/perinatal lethality or severe skeletal phenotypes [9,22,39,40]. To circumvent the limitations of conventional gene targeting strategies, considerable progress has been achieved with the Cre/loxP system [41,42]. The loxP sites are flanking a critical portion of a target gene or genomic region of interest to induce, after Cre recombination, gene deletion (KO), gene replacement/point mutations or insertion/overexpression (KI), and chromosomal translocation [43,44]. Expression of Cre recombinase under a cell- or tissue-specific promoter can induce gene modification in a conditional manner to assess gene function during bone repair without being hampered by its functions elsewhere in the body [44–48]. If conditional gene targeting is still embryonic lethal or leads to severe skeletal anomalies, inducible Cre recombination can be achieved with the tetracyclin- or the tamoxifen systems, offering both cell type/tissue-specific and temporal control of conditional gene modifications to study adult bone regeneration [44,49–54].

The Cre/loxP system and classical transgenic (Tg) approaches provide valuable tools for spatial and temporal localization of gene expression and cell lineage analyses to visualize the contribution of various cell types to fracture repair. The Cre/loxP system has been extensively used for lineage tracing in mouse development, organogenesis and tissue repair by crossing Cre with floxed reporter strains containing loxP sites in combination with fluorescent or β-galactosidase/lacZ markers [50,55–60]. In the case of Tamoxifen-inducible Cre lines, Tamoxifen can be injected during embryonic development, after birth or in the adult before bone injury to follow the fate of diverse cell types involved in bone regeneration. In addition, these lines are essential to monitor the recombination event when Cre lines are employed for conditional and/or inducible gene inactivation [60–62]. Below we illustrate how these various strategies have been employed to elucidate the endogenous bone healing process.

Inflammation

Bone fracture triggers an immediate inflammatory response. Upon injury, blood vessels, the periosteum and the surrounding soft tissues are ruptured and a hematoma is formed. The hematoma serves as an important source of hematopoeitic cells, mainly neutrophils/macrophages, and platelets that initiate the inflammatory response [63–65]. During the inflammatory phase of repair, several inflammatory cytokines and growth factors including interleukin-1a (IL-1a), IL-1b, IL-6, IL-18, and tumor necrosis factor alpha (TNF-α) are released at the fracture site [3]. These cytokines play an essential role in initiating the repair cascade by recruiting other inflammatory cells, enhancing extracellular matrix resorption and angiogenesis [21,66–69]. Most importantly, these growth factors and cytokines promote activation, recruitment and differentiation of stem cells that are essential for bone regeneration and proper fracture healing [6,70,71]. Although the inflammatory response is essential to initiate the repair cascade, an excessive or prolonged inflammatory response is detrimental to repair as illustrated by the complications observed in polytraumatic patients [72]. In addition, some components of the inflammatory response are favorable, while others are not. Due to the multiple inflammatory factors and cell types involved, it is a challenge to distinguish their specific effects on bone healing. Genetic mouse models have proven to be valuable tools to dissect the role of various inflammatory factors or cell types and to define new therapeutic targets.

Mouse mutants lacking T and B cells, such as Recombination Activating Gene (RAG) KO or T Cell Receptor (TCR) KO mice exhibit enhanced bone repair, revealing that the adaptive immune system has a negative influence on bone repair (Table 1) [73,74]. The exact roles of these cell types remain to be fully established. Other cell types involved in innate immunity have opposite effects on repair as the absence of macrophages has been shown to delay healing [40,75]. Mice lacking macrophages were obtained by inactivating the chemokine (C–C motif) receptor 2 (CCR2) gene or using the Macrophage fas-induced apoptosis (Mafia) transgenic mouse model, which express a ligand-inducible Fas-dependent suicide receptor under the control of the myeloid-specific csf1r promoter (Tables 1 and 4) [76]. CCR2 inactivation reduces the early macrophage infiltration to the site of bone fracture and impairs the function of osteoclasts leading to a delay in fracture healing (Table 1) [40]. Other components of the innate immune response may have negative effects, as Toll-Like Receptor 4 (*TLR4*) gene inactivation