Published in final edited form as:

Curr Osteoporos Rep. 2018 April ; 16(2): 146–154. doi:10.1007/s11914-018-0429-9.

Transcriptional mechanisms of secondary fracture healing

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Abstract

Purpose of review: Growing evidence supports the critical role of transcriptional mechanisms in promoting the spatial and temporal progression of bone healing. In this review we evaluate and discuss new transcriptional and post-transcriptional regulatory mechanisms of secondary bone repair, along with emerging evidence for epigenetic regulation of fracture healing.

Recent findings: Using the candidate gene approach has identified new roles for several transcription factors in mediating the reactive, reparative, and remodeling phases of fracture repair. Further characterization of the different epigenetic controls of fracture healing, and fracture-driven transcriptome changes between young and aged fracture has identified key biological pathways that may yield therapeutic targets. Furthermore, exogenously delivered microRNA to post-transcriptionally control gene expression is quickly becoming an area with great therapeutic potential.

Summary: Activation of specific transcriptional networks can promote the proper progression of secondary bone healing. Targeting these key factors using small molecules or through microRNA may yield effective therapies to enhance and possibly accelerate fracture healing.

Keywords

Transcription factor; gene expression; bone; microRNA; epigenetics; transcriptome

Introduction

In the United States the cost of healthcare services and lost productivity from musculoskeletal disorders, including impaired fracture healing, is estimated at \$215 billion annually [1]. Of the 6.2 million fractures that occur annually in the US, approximately 5 to 10% undergo non-union or delayed union [2, 3]. Thus, impaired fracture healing constitutes a significant public health dilemma. This is due to the high complexity of the sequential events as well as the cellular cues that govern the healing process. In addition to the fracture characteristics, patient factors including diabetes, prolonged steroid treatment, substance

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Conflict of Interest: The authors declare that they have no conflict of interest.

Recently published papers of interest have been highlighted as: (•): Important; (••): Very important

abuse, aging and other co-morbidities also impede the rate of healing [4–6]. Therefore, a large number of elegant studies focused on identifying the molecular mechanisms involved in fracture healing, with the common objective to identify new efficacious and cost-effective therapeutic modalities to enhance and/or accelerate fracture healing. These studies relied on preclinical models of primary, secondary and critical sized bone healing to reveal the function of specific growth factors, inflammatory mediators and inhibitors, and small molecules. Several key transcription factors have been validated as master regulators of specific stages of fracture healing. However, candidate gene approaches often raise new questions about potential hierarchies amongst them. In this review, we aim to provide an update on the state of knowledge particularly in the discovery of new transcriptional mechanisms of secondary fracture healing, new and exciting areas of post-transcriptional control of fracture healing as well as discuss the promise of a systems biology approach to fracture.

Transcriptional control of secondary bone healing

Fracture healing is a complex process that is orchestrated through interactions between cells, growth factors, and biological pathways. Fractures can heal through primary (intramembranous ossification) or secondary (endochondral ossification) bone healing depending on the degree of anatomic reduction. Clinically, the majority of fractures heal through secondary bone repair, which does not require accurate anatomical reduction [7]. In order for proper secondary fracture healing to occur specific genes are expressed in both a temporal and spatial pattern to promote progression through the reactive phase (inflammation and hematoma formation), reparative phase (cartilaginous callus and bony callus formation), and remodeling phases of bone repair (Figure 1) [8]. Below we discuss recent advances in the transcriptional mechanisms underlying each phase of secondary fracture healing.

Reactive Phase—After trauma, the proprioceptive system attempts to restore the gross shape of the fractured bone through a process called natural reduction. This process requires movement of the two fractured ends, which is mediated by ossification of the cartilaginous callus and muscle contraction. Expression of Runx3, a member of the runt-related factors x (Runt) family, in proprioceptive neurons was recently shown to be essential for natural reduction of fractured humeri to occur [9]. This intriguing finding provides further insight into the pleiotropic action of this transcription factor that has an established role in mediating not only neurogenesis, but also chondrogenesis through control of both late and early chondrocyte differentiation [10–13].

Immediately following fracture a hematoma forms as a result of broken blood vessels that then drive the inflammatory reaction [14]. This phase of fracture healing is also highly enriched in reactive oxygen species (ROS) that promote the activation and recruitment of inflammatory cells to initiate repair [15]. An overabundance of ROS interferes with the natural progression of fracture repair, and up-regulation of specific antioxidant genes and enzymes is required. Perhaps one of the most important regulators of oxidative stress is nuclear factor erythroid-2 related factor 2 (Nrf2), which binds to the antioxidant response element to induce expression of key genes involved in antioxidant defense [16]. Genetic

disruption of Nrf2 increased macromolecular damage within the cartilaginous callus, decreased chondroprogenitor cell proliferation and differentiation, and resulted in an overall delay in the healing process [17]. Forkhead box O (FoxO) transcription factors also play critical roles in regulating the expression of genes important in oxidative defense, proliferation, and apoptosis. ROS are strong activators of FoxO, which has been linked to impaired fracture healing in diabetes and chronic alcohol abuse [18]. For instance, in fractured diabetic mice there is a 5.8-fold increase in the percentage of all nucleated cells with FoxO1 localized to the nucleus. Furthermore, episodic alcohol exposure increased FoxO1 activation and protein expression within the callus at day 3 post-fracture [19]. These activating effects on FoxO1 were concluded to be driven by the heightened oxidative load that accompanies alcohol metabolism, as co-treatment with the antioxidant N-acetylcysteine blunted the changes in FoxO1 [19]. These studies not only highlight how dysregulation of the ROS-rich environment of the inflammatory phase can impair healing, but also identify critical transcription factors that may be therapeutically targeted to enhance fracture healing in conditions associated with overproduction of ROS (e.g., diabetes, smoking) [20, 21].

Reparative phase—The inflammatory environment at the fracture site recruits progenitor cells to the site of injury that facilitate the formation of the initial cartilaginous (soft) callus, which is then vascularized, resorbed, and ossified to form a bony (hard) callus. Of particular interest are mesenchymal stem cells (MSCs) that undergo rapid proliferation and differentiation to form cartilage-producing chondrocytes. Recent work has identified a novel role of the transcriptional co-activator Yes associated protein 1 (Yap1) in promoting this early chondrocyte proliferation after fracture [22]. Furthermore, that while Yap1 drives chondrocyte proliferation, it acts as an inhibitor of maturation and hypertrophy by negatively regulating Col10a1 expression [22]. To induce these transcriptional changes Yap1 must interact with other transcription factors like Runt-related factor X1 (Runx1) and Runx2. In this regard, Yap1 inhibits Runx2 a regulator of osteogenesis and chondrocyte hypertrophy, but activates the chondrogenic Runx1. Our group recently shed new light on the importance of Runx1 in this early stage of healing, in which global loss of Runx1 function resulted in delayed fracture healing, primarily through slower soft callus formation and remodeling [23]. Furthermore, conditional deletion of Runx1 from periosteal cells led to significantly reduced amounts of cartilaginous and bony tissues [23]. Impaired fracture healing in our model likely stems from inhibited chondrocyte differentiation, especially considering the role of Runx1 in promoting expression of key chondrogenic genes (e.g., Sox5, Sox6, and Sox9) [23]. Hox11 has also been recently implicated in controlling chondrocyte differentiation during fracture healing, as illustrated by reductions in cartilage formation in Hox11 null mice [24]. This effect appears to stem from defects in late chondrocyte differentiation as expression of Sox9 and Sox5 were not different, but expression of Col2a1 and Col10a1 was lower in the absence of Hox11.

Scleraxis (Scx) is a member of the basic helix-loop-helix family of transcription factors that has been classically studied for its role in tendon development. However, it was recently implicated in having an important role in cortical bone biomechanics and fracture healing [25]. McKenzie et al. [25] utilized a Scx GFP reporter and a Scleraxis knockout mouse model to evaluate whether Scx is a critical transcriptional regulator of fracture healing,

and where it was localized throughout fracture healing. They found that Scx gene expression was elevated at 24 hours after fracture, as BMP-4 was decreasing and that Scx GFP+ cells were distributed in numerous musculoskeletal tissues throughout the healing process. Their findings suggest that Scx is a key transcriptional regulator of fracture repair, as fracture repair was asymmetric and impaired in Scx knockout mice [25]. Specific target genes of Scx that are responsible for this phenotype remain an area of active investigation.

After proliferation, chondrocytes within the fracture callus will undergo hypertrophy followed by apoptosis that in turn triggers vascularization and ossification of the cartilaginous matrix. This calcified cartilage is then replaced with woven bone via the coordinated activities of chondroclasts and osteoblasts. Classically, endochondral ossification is thought to occur when hypertrophic chondrocytes undergo apoptosis, which is then followed by vascular and osteoprogenitor cell invasion of the cartilage callus. However, a paradigm shifting study has challenged this model of cartilaginous to bony callus transition [26]. Hypertrophic chondrocytes within the cartilage-to-bone transition zone appear to be capable of transdifferentiating into osteoblasts through induction of key pluripotency programs mediated by Nanog, Sox2, and Oct4 [26]. Furthermore, this effect was primarily coordinated by vascular invasion of the avascular soft callus [26]. This exciting finding that hypertrophic chondrocytes have a high degree of plasticity raises new questions regarding the functional mechanisms that promote the activation of pluripotency programs. Moreover, one wonders if these signals play a significant role in promoting trans-differentiation of somatic cells into the necessary cells for the repair tissue.

Our group has recently identified a new function of the pleiotropic transcription factor Runx3 in controlling the reparative phase of fracture healing. In particular, conditional deletion of Runx3 in mesenchymal cells resulted in both accelerated and enhanced fracture healing that was indicated by smaller cartilaginous calluses, earlier mineralized callus formation, enhanced blood vessel formation, and improved biomechanical properties of healed femurs [27]. Our subsequent studies using periosteal cell cultures found that Runx3 conditional knockout enhanced both the osteogenic and angiogenic potential of these cells, as measured by mineralized nodule, branch tube formation, and gene expression analyses [27]. Furthermore, our data suggest that this effect stems from enhanced expression of IL-17a and its receptor in the Runx3 conditional knockouts, corresponding to induction of VEGF and Hif1- α , which is transiently increased during the early inflammatory phase (day 7 post-fracture) of fracture repair [28, 27]. Its homologue IL-17b and its receptor were also enhanced in the Runx3 conditional knockouts which was sustained until day 14 post-fracture [28]. Collectively, these findings suggest that, within periosteal cells, Runx3 acts as an inhibitor of IL-17 thereby leading to diminished osteogenesis and angiogenesis [28, 27].

Recently we found that another essential transcriptional regulator of organogenesis, Runx1, not only acts as a key regulator of osteoclast differentiation and function during skeletal homeostasis [29, 30], but also controls the remodeling of both mineralized cartilage and bony callus in a model of secondary femoral fracture healing (unpublished data). Our observations that impaired transition from cartilaginous callus to bony callus results in mechanically unstable repaired tissue identifies a critical need to characterize the role of chondroclasts as well as osteoclasts. We also previously reported at the American Society

for Bone and Mineral Research annual meeting that transcriptomic evaluations of osteoclasts and chondroclasts laser captured from mouse femoral fracture calluses revealed differential regulation of more than 800 genes between the two cell types [31]. Of these differentially regulated genes, a number were transcriptional molecules that regulate migration, environmental sensing, and microenvironment interactions (unpublished data). Identifying the function of these factors is likely to shed some new light on differences between cartilage and bone matrix remodeling and their respective contribution to the healing process.

While much attention has been placed on identifying growth and other secreted factors (e.g., RANKL, M-CSF, TNF- α) that orchestrate these events, our understanding of the transcriptional cues that govern the transition of cartilaginous callus into bony callus are largely unknown. A recent study by Xu et al. [32] identified a novel role of the Sry-related high-mobility group box 11 (Sox11) transcription factor in promoting fracture healing. Sox11 has an established role in controlling organogenesis and fetal development and has been implicated in governing the stemness of MSCs. Interestingly, when Sox11 overexpressing MSCs were intravenously injected into fractured rats it initiated callus ossification and led to an overall improvement in bone healing [32]. These osteogenic effects likely stem from Sox11-mediated transcriptional activation of Runx2, the master-regulator of osteogenesis [32]. We also characterized the role of the osteogenic transcription factor osterix in promoting ossification of soft callus [33]. This study demonstrated that, contrary to chondrogenic makers (e.g., Col2a1 and Col10a1), osterix expression was maximally expression during the period of bone formation and callus remodeling (day 14 post-fracture) [33]. This highlights the important role of osterix in committing MSCs to the osteoblast lineage as opposed to chondrocytes to promote formation of the bony callus.

Because of its essential role in osteogenesis, Runx2 and along with other chondrogenic transcriptional regulators were postulated to regulate chondrocyte differentiation and the transition to a bony callus following fracture. As such, several studies have evaluated the roles of Runx2, Sox5, Sox6, and Sox9 in these processes, and identified their key regulatory roles. Both Sox9 and Runx2, in particular, regulate the differentiation of mesenchymal cells into chondrocytes, with Sox9 and Runx1 (Runx2 homologue) being induced earlier in the healing process than Runx2 [34]. Sry-related family members of the HMG box DNA binding proteins, Sox5/6/9 have also been identified to be coexpressed in precartilaginous condensations during embryogenesis through hypertrophy. Although Sox5 and 6 are homologous, they have different transcriptional roles outside the HMG box domain [35]. During cartilage matrix deposition following fracture, Sox5/6/9 have been shown to be colocalized via immunohistochemistry, while Sox5 and Sox9 were significantly downregulated during hypertrophy as Col10 was upregulated [36]. This family of transcription factors have also been implicated in the mechanism of action underlying induction of chondrogenesis and cartilage matrix production by BMP-2 following fracture [36].

An elegant study by Winger et al. found that acute phosphate restriction in murine diet 2–3 days prior to fracture to simulate rickets/osteomalacia, did not affect cell chemotaxis to the fracture site or lineage commitment (based on Sox5 and Sox9 levels), but hypophosphatemia

impaired the differentiation of these cells compared to controls, coinciding with BMP-2 resistance [37]. This group also characterized the distinct regulatory roles of Runx2 and its homologue Runx3 during endochondral bone remodeling and following bone marrow ablation [12]. They found that while only Runx2 was upregulated following bone marrow ablation, Runx3 expression in fracture callus chondrocytes coincided with chondrocyte proliferation and chondrocyte matrix production [12]. Similar to Sox5/6/9, Runx2 was primarily expressed in chondrocytes during hypertrophy and primary osteogenesis, suggesting it has a larger role in cell differentiation during fracture healing rather than chemotaxis or lineage commitment [12].

It is particularly noteworthy that genes that play key roles in organogenesis continue to demonstrate fundamental roles in tissue regeneration. While we continue to discover proof that fracture healing does not recapitulate embryonic development, one cannot ignore the ability of progenitor cells to trigger molecular events that govern tissue organogenesis in response to wound healing. We are certainly poised to determine hierarchical relationships between the transcription factors known to promote organogenesis as systems biology approaches are used in the context of fracture tissue repair and regeneration.

Remodeling phase—Woven bone deposited during the reparative phase of fracture healing must be remodeled into lamellar bone to fully restore the biomechanical properties of the fractured bone. In long bones, successful remodeling is illustrated by reestablishment of the central medullary canal and cortical bridging. This phase appears to be regulated, at least in part, by Hox11 as animals lacking this transcription factor exhibited a high number of non-unions and delays in remodeling [24]. Mechanistically, this defect in remodeling may stem from altered osteoclast function as many osteoclasts were detached from bony matrix in mice lacking Hox11 [24]. Ultimately, additional molecular work is warranted to determine how Hox11 transcriptional regulation mediates the remodeling phase of fracture healing.

Targeting transcription factors to enhance healing—Targeting transcription factors for therapeutic benefit has become increasingly feasible. One of the most intriguing strategies is through the use of small molecules. To date a number of small molecules have been investigated for their osteoinductive capacity. One of which is Kartogenin, which activates the transcription factor Runx1 and exhibited a robust ability to slow the progression of osteoarthritis in preclinical studies [38, 39]. Identifying other modalities and small molecules that can activate or inhibit specific transcription factors, including the ones discussed above, has immense therapeutic potential to enhance secondary bone healing. Considering the aging population and burgeoning epidemic of fragility fractures this strategy has immense clinical relevance.

Transcriptome of fracture healing

Initial transcriptome analyses of properly healing fractures provided invaluable insight into genes that likely play roles in facilitating the complex repair process [40, 41]. Two recent studies have described the transcriptional differences between young and aged animals that may contribute, at least in part, to the delayed healing phenomena observed in older patients.

The first focused on transcriptional changes that occur within the post-fracture hematoma, with a focus on the interaction between fixation (semi-rigid vs. rigid) and age (12 vs. 52 weeks). This analysis identified a number of genes differentially expressed that were associated with cell migration and blood vessel formation [42]. Of the 1103 genes that were differentially expressed, matrix metalloproteinase (MMP)-9 and MMP-13 were the most strongly influenced by the interaction of age and mechanical stability [42]. These enzymes play essential roles in degrading the cartilaginous matrix of the soft callus to facilitate vascular invasion [43]. A more recent analysis of differentially expressed genes in fractured (day 1, 3, 5 post-fracture) young (6 weeks) and aged (8 months) mice revealed 207 genes that were consistently up-regulated in both ages [44]. Interestingly, genes involved in chemokine activity, chemokine receptor binding, and inflammatory response were consistently downregulated in the aged mice [44]. These findings may provide some mechanistic insight into the impairments in healing observed in aged individuals, especially considering the importance of the inflammatory response in initiating the fracture healing sequelae. Collectively, these studies further our understanding of different gene clusters that may be targeted for enhanced bone repair.

Emerging evidence for fracture healing through epigenetic modifications

Epigenetic modifications play important roles in mediating skeletal development, homeostasis and disease. Though there are many types of epigenetic modifications, DNA methylation is the most widely studied. A genome-wide methylation analysis of MSCs from osteoporotic patients with hip fractures revealed significant differences in methylation patterns that were predominantly located in enhancer regions distant from the promoter and gene body [45]. Interestingly, genes with hypomethylated enhancers and upregulated expression belonged to critical fracture healing pathways, including MSC proliferation, osteoblast differentiation, and bone mineralization [45]. This study sets the stage for further investigation into the fracture-driven molecular mechanisms that promote shifts in the epigenome to facilitate healing. This will help identify whether there is therapeutic potential in targeting these factors for improved healing. Efforts dedicated to identifying the role of epigenetic modifications other than DNA methylation in fracture healing will hopefully be undertaken in the near future. Histone modifications will certainly bring some fundamental insight into other epigenetic aspects of fracture healing with the potential to discover new drug targets to accelerate fracture repair in the context of aging, fragility, and chronic conditions.

Post-transcriptional regulation

Over the last few years, elegant studies have clarified our understanding of important transcriptional regulators of fracture repair. The role of post-transcriptional regulatory mechanisms, primarily by microRNA (miRNA), has also gained much attention. miRNAs are short (18–25 nucleotides), non-coding RNAs that elicit their regulatory effects by binding to the 3' UTR of target mRNAs thereby leading to translational repression or mRNA degradation depending on sequence complementarity. Expression of miRNAs is transient and influenced by disease and injury, including fracture. Thus, characterizing temporal changes in miRNA expression during the fracture healing process, along with specific targets, has been the focus of several studies due to the promising prospect of using miRNAs

to enhance bone healing. Hadjiargyrou et al. [46] was the first to identify temporal changes in the miRNA transcriptome during the early stages (days 1–14) of fracture repair. Using the murine mid-diaphyseal fracture model they discovered that target genes of down-regulated miRNAs were involved in bone and skeletal development, and ossification; whereas, target genes of miRNAs that negatively regulate bone remodeling and resorption were upregulated. In a separate study, aberrant miRNA expression patterns in non-healing rat femoral fractures was also investigated using a miRNA microarray. When compared to non-healing fractures, five highly expressed miRNAs (miR-140–3p, miR-140–5p, miR-181a–5p, miR-181d–5p, and miR-451a) were identified in standard healing fractures at day 14 [47]. It is also well established that age is a significant risk factor for delayed fracture healing and changes in miRNA expression may contribute to this effect. A comparison of miRNA expression patterns in femoral fracture callus in young (9 months old) and aged (24 months old) mice identified 53 differentially expressed miRNAs [48]. These elegant studies provide the necessary foundation to begin investigating the specific functions of differentially expressed miRNAs at the fracture site.

To date, only a handful of studies have examined the role of specific miRNAs in promoting fracture healing. In a murine transverse osteotomy model a single injection of miR-29b expressing plasmid that was transfected into cells within the callus using a micro-bubble ultrasound system led to improved fracture healing by stimulating osteogenesis [49]. Conversely, a recent study provided compelling evidence that injection (once per week for 4 weeks) of miR-142–5p into the fracture site can promote bone healing through stimulation of osteoblast activity [50]. Two groups have also investigated the therapeutic utility of inhibiting specific miRNAs that act as negative regulators of angiogenesis, chondrogenesis, and osteogenesis. Murata et al. [51] demonstrated that both systemic and local inhibition of miR-92a enhanced fracture healing by promoting neovascularization of the fracture callus in a murine femoral fracture model. Whereas, Yoshizuka et al. [52] established that injection of a collagen matrix containing anti-miR-222 promoted bony union by enhancing osteogenesis and chondrogenesis in a rat femoral fracture model of non-union. Though scant, the existing studies highlight the promising therapeutic potential of exogenously delivered miRNAs or anti-miRNAs to enhance and/or accelerate fracture healing. The practicality of this strategy is evidenced by the emergence of several new miRNA-based drugs. One example is Miravirsin that targets miR-122 and has shown exciting effectiveness in treating Hepatitis C [53]. Ultimately, further in vivo functional studies are warranted to truly ascertain the precise role of the ever-increasing list of miRNAs implicated in controlling fracture repair.

In addition to studies evaluating microRNAs, Lin et al. [54] quantified the temporal expression pattern of Cyclooxygenase (COX)-1, COX-2, and 5-lipoxygenase (5-LO) derived lipid mediators, inflammatory cytokines, and mRNA levels using mass spectroscopy, and multiplex bead assays. They found that the early inflammatory phase of fracture healing days 0–4, coincided with elevated levels of COX-derived lipid mediators and inflammatory cytokines, and decreased levels of 5-LO lipid mediators. This increase in COX-derived lipid mediator was sustained for at least a week following fracture. Differences between corresponding cytokines and lipid mediator levels suggest that COX-derived lipid mediators have a post-transcriptional role in the early inflammatory phase of fracture healing, while 5-LO derived lipid mediators generally negatively affect the initial stages of bone repair.

The importance of exploring wound healing through forward genetics

Much of our understanding of the key genes and transcriptional mechanisms that facilitate and oppose fracture healing was established using reverse genetic techniques. While this strategy has significantly advanced our understanding of critical genes for bone healing, we have now entered the era where analyzing whole genomes to identify casual variants is both economical and feasible. The exploratory nature of forward genetics, coupled with its unbiased approach, make it an extremely powerful tool to detect genetic differences that contribute to differences in bone healing. Along these lines, studies examining the influence of genetic diversity in mice of different strains have been published. Manigrasso and O'Connor compared the fracture healing rate between C57BL/6NTac (B6/N), DBA/2NTac (D2/Tac) and C3H/HeNTac (C3H/Tac) strains of mice, finding that B6/N mice demonstrated an enhanced healing rate, while C3H/Tac mice healed the slowest [55]. Jepsen et al. [56] evaluated the fracture healing rate amongst similar mouse strains including C57BL/6J (B6), C3H/HeJ (C3H) and A/J mice. They found that B6 and A/J mouse fracture healed more rapidly than C3H, based on biomechanical testing of the fracture callus. Cartilage gene products were significantly higher in the B6 strain, compared to the other strains, leading to increased chondrocyte maturation and hypertrophy. The reduced healing rate of the C3H mice, relative to the other strains was associated with a premature osteogenic response [56].

A later study by Grimes et al. evaluated differences in BMP-2/TGF- β signaling, associated with coordination of skeletal and vascular development during endochondral ossification [40]. The authors found that strain-dependent differences in cardiovascular development were phenocopied during peripheral vascular regeneration in fracture repair. Additionally, angiogenic mRNA (VEGF, VEGFc, CD31, and Vegfr2) and mRNA of transcriptional regulators of chondrocyte differentiation and cardiac morphogenesis (Hand2 and FoxC2) were expressed earlier and induced to a greater extent in B6, compared to C3H mice, implicating their potential as transcriptional regulators of fracture healing [40].

More comprehensive studies using sophisticated models such as the Diversity Outcrossed (DO) and Collaborative Cross (CC) populations [57] are needed in the future to further dissect and delineate the contribution of genetic diversity in mouse models to distinct fracture phenotypes and associated signaling. Quantitative trait loci (QTL) studies can be designed beginning with these diverse populations to attempt to link genetic polymorphisms with distinct fracture phenotypes. These discoveries will, in turn, facilitate the development of therapies that can be tailored to a patient's genetics to enhance healing. This will be of critical importance particularly in the context of precision medicine.

Conclusions

Musculoskeletal disorders, including impaired fracture healing, remain a significant public health and economic burden and are underscored by a plethora of genetic, pathologic, and environmental risk factors [1]. While currently available studies provide a detailed picture of the complex biological mechanisms that coordinate stages of bone healing, much remains to be understood regarding the interaction of transcriptional networks to promote progression of the healing process. Novel post-transcriptional regulation studies and forward genetics models are providing data that will be instrumental in characterizing transcriptional and

post-transcriptional regulatory networks. Mapping these networks is rapidly becoming more feasible, particularly as we move into the systems biology era. Translation of all the impactful data researchers are gleaning from preclinical and clinical transcriptomic studies will allow the transition to more patient-centered medicine, which considers how an individual patient will respond to a particular treatment, and allows the possibility of therapeutic customization. With the common knowledge that impaired fracture healing is often associated with specific single nucleotide polymorphisms, there is a need for “personalized orthopaedics” to develop an optimal treatment for each patient. The DO and CC mouse populations are only the starting point, with the overarching goal being a greater understanding of how to help the population heal musculoskeletal injuries on an individual basis.

Acknowledgments

Funding: This study was supported by the National Institutes of Health (grant number R01AR063661).

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Figure 1.

The temporal progression of clinical fracture healing and important transcriptional regulators of bone repair. The reactive phase is induced immediately following injury and proceeds through natural reduction, hematoma formation, and inflammation. As healing progresses into the reparative phase a soft callus forms that is subsequently vascularized and replaced with a bony callus. This hard bony callus is finally remodeled to restore the pre-fracture morphology of the bone. These processes are mediated through the coordinated action of several cell types, including inflammatory cells, mesenchymal stem cells (MSCs), chondrocytes, hypertrophic chondrocytes, vascular cells, chondroclasts, osteoblasts, and osteoclasts. Many transcription factors (black) have been identified as driving these processes, and more recently the role of several novel transcription factors (red) in secondary bone repair has been characterized. *Abbreviations:* Nrf2, nuclear factor erythroid 2-related factor 2; FoxO1, forkhead box protein O1; Hox11, homeo box 11; Runx1, runt-related transcription factor 2; Runx2, runt-related transcription factor 2; Runx3, runt-related transcription factor 3; Sox9, SRY-Box 9; Sox5, SRY-Box 5; Sox6, SRY-Box 6; Yap1, Yes-associated protein-1; Hif-1α, hypoxia-induced factor 1-alpha; MSCs, mesenchymal stem cells; Sox11, SRY-box 11; Nfatc1, nuclear factor of activated T-cells 1; Ap1, activator protein 1; Mitf, microphthalmia-associated transcription factor.