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Authors

Marcucio, R

Bahney, C

Miclau, Theodore

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A Shifting Paradigm: Transformation of Cartilage to Bone during Bone Repair

R.S. Marcucio[1], T. Miclau III[1], and C.S. Bahney[1,2]

Abstract

While formation and regeneration of the skeleton have been studied for a long period of time, significant scientific advances in this field continue to emerge based on an unmet clinical need to improve options to promote bone repair. In this review, we discuss the relationship between mechanisms of bone formation and bone regeneration. Data clearly show that regeneration is not simply a reinduction of the molecular and cellular programs that were used for development. Instead, the mechanical environment exerts a strong influence on the mode of repair, while during development, cell-intrinsic processes drive the mode of skeletal formation. A major advance in the field has shown that cell fate is flexible, rather than terminal, and that chondrocytes are able to differentiate into osteoblasts and other cell types during development and regeneration. This is discussed in a larger context of regeneration in vertebrates as well as the clinical implication that this shift in understanding presents.

Keywords: fracture repair, paligenosis, metaplasia, chondrocyte-to-osteoblast transformation, endochondral ossification, intramembranous ossification

Introduction

Bone healing throughout appendicular, axial, and cranial skeleton occurs via a similar set of highly stereotyped events (extensively reviewed in Bahney et al. 2019). Immediately after fracture, inflammatory cells are recruited to the fracture site via cytokine signaling. These cells debride the injured site and then help stimulate the repair process. Simultaneously, cells in the periosteum and endosteum become activated and begin proliferating to increase the number of osteo- and osteochondral progenitor cells that will form the skeletal tissues that comprise the fracture callus (Colnot 2009). Clinically, most fractures heal through a combination of endochondral and intramembranous ossification, which is directed by the mechanical environment. At the fracture site, a cartilage callus forms that is eventually replaced by bone through endochondral ossification. Proximal and distal to the fracture site and in the bone marrow cavity, periosteal and endosteal cells, respectively, differentiate directly into osteoblasts to form bone by intramembranous ossification. The newly formed bone is constantly remodeled until the injured bone has regained its preinjury strength. Most of the mechanistic studies described in this review are performed in small animal models, primarily because these models allow investigators to examine cellular and molecular details that can be used to frame directed hypotheses that can be tested clinically. Of course, one must be wary that not all mechanisms operating in animal models may equally affect human bone healing.

Relationship between Skeletal Development and Regeneration

The skeleton is divided into axial, appendicular, and cranial to reflect distinct developmental origins of each region. The axial skeleton forms the vertebral column and is derived from somites, and the appendicular skeleton forms the appendages and is derived from lateral plate mesoderm comprising the limb bud. The cranial skeleton forms the braincase, jaw, and pharyngeal skeleton and is derived from neural crest cells and paraxial mesoderm originating in the anterior region of the embryo. Many studies on these different skeletal elements have provided major insight into mechanisms of patterning and evolution of the skeleton. However, in terms of mechanisms of skeletogenesis, little information is gleaned from these anatomical distinctions, and there is even less concordance with site of development and mechanisms of bone regeneration.

Bone forms by 2 processes. Intramembranous ossification occurs when skeletal progenitor cells differentiate into

[1]University of California, San Francisco (UCSF), Orthopaedic Trauma Institute, San Francisco, CA, USA
[2]Steadman Philippon Research Institute, Vail, CO, USA

Corresponding Author:
R.S. Marcucio, University of California, San Francisco (UCSF), Orthopaedic Trauma Institute, 2550 23rd St. San Francisco, CA, 94110, USA.
Email: Ralph.Marcucio@UCSF.edu

osteoblasts and bone formation occurs directly. In contrast, endochondral ossification occurs when progenitor cells differentiate into chondrocytes and a cartilage template is replaced by bone. These modes of bone formation correspond roughly to the cranial and axial/appendicular skeleton, respectively, but there are many examples of endochondral ossification in the developing skull, such as the retroarticular process of the dentary bone (mandible), while intramembranous ossification forms some of the flat bones of the axial skeleton such as the sternum. Interestingly, the mode of bone formation is not retained during regeneration; bones that develop via either intramembranous or endochondral ossification can heal by either of these 2 processes, depending on the mechanical environment. In this review, we discuss the clinical importance of understanding bone regeneration, examine the different modalities of bone repair across the cranial and appendicular skeleton, and review new evidence supporting the plasticity of repair wherein cartilage transforms into bone through metaplasia. Finally, we suggest the utility of using "endochondral ossification" as a therapeutic modality to care for patients after injury or reconstructive surgery.

Clinical Considerations

Understanding the molecular mechanisms of bone formation is important in determining clinical fixation protocols and will be the key in driving new therapeutic approaches. Impaired bone healing remains a significant clinical challenge in individuals with high-velocity injuries or comorbidities, including diabetes, obesity, aging, estrogen deficiency, malnutrition, and smoking (Hellwinkel et al. 2020). Problematic healing results in a substantial reduction in quality of life for the patient and remains difficult and costly for the physician to treat. In addition to fracture repair, there is also a need to regenerate bone following removal of tumors, for fusion of spinal segments, around orthopedic and dental implants, and after surgical procedures that correct growth discrepancies and other congenital disorders such as cleft lip and palate. For these to be successful, new bone must form and integrate with native bone to ensure mechanical integrity. Therefore, to develop therapies for treating patients, understanding mechanisms of bone regeneration is necessary.

Role of Mechanical Microenvironment in Regulating Bone-Healing Pathways

There is substantial evidence that motion across the healing fracture site plays a critical role in the rate, quality, and mechanism of bone formation (as recently reviewed in Bahney et al. 2019). Clinically, fractures heal primarily via endochondral ossification with a small amount of direct bone formation. While the precise amount and direction of motion is not defined, recent biomechanical studies support clinical evidence that limited motion across a fracture site promotes endochondral callus formation to improve healing (Dailey et al. 2021), but excessive motion is a key contributor to nonunion. The effects of motion on healing are also dependent on the direction and magnitude of motion: while axial compression can increase callus formation, shear motion is detrimental to fracture healing and is considered the underlying cause of hypertrophic nonunion (Steiner et al. 2014). There is also evidence to suggest that the frequency of loading/displacement events is related to healing rates. A small study of tibial repair in sheep found the frequency of early loading events was highly correlated ($R = 0.98$) with fracture callus area at 8 wk (Windolf et al. 2020). The strength of this relationship in humans and the optimal timing/rates for resuming weightbearing and gait after fracture fixation have not been well studied. Novel biomarkers may help realize the clinical implication of the mechanical microenvironment in a translational manner, with clinical cohort studies showing differential rates of healing with different fixation techniques and dependent upon time to weightbearing (Working et al. 2022). Importantly, mechanical stability influences healing independently of how the bones formed.

The Embryonic Basis for Mode of Bone Regeneration

Given that many bones in the cranial skeleton form through intramembranous ossification, there is interest in determining if these embryonic programs are recapitulated during regeneration. There are molecular differences between the periosteum in bones that develop in different anatomical locations. For example, the periosteum of bones that are derived from neural crest cells appears to retain gene expression profiles that reflect their neural crest origin (Ichikawa et al. 2015). Whether these changes reflect fundamental differences in the response to injury is unknown. However, other work has shown that differences in expression of HOX genes may also distinguish periostea from each other. HOX-free periosteum was found covering calvarial bones that were derived from both neural crest and mesoderm, and these cells had exclusively osteogenic potential. In contrast, HOX-expressing periosteal cells were found in bones of the jaw skeleton and limbs, and these cells had multilineage potential (Bradaschia-Correa et al. 2019). While expression of HOX genes was associated with the fate potential of these cells, research confirming this and showing underlying mechanisms has not been completed. Nonetheless, these results suggest that the developmental origin of bones does not dictate periosteal responses of either long bones or bones of the cranial skeleton; rather, these responses are intrinsic to the bones themselves. Furthermore, cells located within the endosteum also contribute to bone healing (Colnot 2009), but these cells have an exclusive osteogenic potential in long bones even when transplanted into periosteal regions, suggesting that multiple cell populations line bones that have unique fate trajectories. Whether this is true for the cranial skeleton is not known.

Despite differences in lineage potential, embryonic origin does not appear to affect the mode of bone fracture healing. The mode of healing of long bones that have formed by endochondral ossification is directed by the mechanical environment at the fracture site. Similarly, healing of the dentary bone

depends on the mechanical environment. The dentary bone is derived from neural crest cells and forms the lower jaw skeleton. When a trephine defect is created, the injury heals through direct bone formation, while an osteotomy through the diastema heals through endochondral ossification, although there may be a predilection for osteoblast differentiation (Yu et al. 2012; Wong et al. 2021). In contrast, the calvarium forms from neural crest and mesoderm through intramembranous ossification, and these bones appear to heal exclusively through intramembranous ossification. While these tissue-level processes are shared among different parts of the skeleton, whether there are more subtle local differences is largely unknown.

The mechanisms that direct endochondral versus intramembranous ossification during bone repair has been an area of research over the past decades. Recent research in mandibular distraction osteogenesis reveals a unique role for focal adhesion kinase in regulating chromatin accessibility, gene expression, and bone formation. Interestingly, during distraction osteogenesis of the mandible, the periosteal progenitor cells reactivate a neural crest program of gene expression as these cells formed the regenerating tissue (Ransom et al. 2018).

Zebrafish have also been used to assess healing of mandible injuries. Fractures in the mandible of zebrafish heal via endochondral ossification like in rodents. This work has shown that signaling by the Notch pathway is active in mesenchymal cells and chondrocytes that differentiated after injury and that pharmacologic inhibition of Notch signaling significantly affects fracture healing leading to a nonunion. In contrast, activation of the Notch pathway accelerates mandibular repair (Kraus et al. 2022). Furthermore, work in zebrafish indicates that initial differentiation of the chondrocytes requires signaling by Indian Hedgehog (Paul et al. 2016). How these pathways are induced in response to mechanical information is not known.

Other factors that are thought to contribute to the differences in healing include the blood supply and hypoxia at the fracture site. The region around the fracture site of a tibial fracture is hypoxic but becomes normoxic quickly, and no differences in local oxygen tension and subsequent differentiation of osteoblasts and chondrocytes were observed. However, other research concluded oxygen levels regulate differentiation of chondrocytes and osteoblasts during healing. For example, increasing the rate of angiogenesis is associated with increased intramembranous ossification (reviewed in Miclau et al. 2017).

Models of Endochondral Ossification

Within the past decade, the process of endochondral ossification has come under intense scrutiny that has challenged concepts held in the field for more than a century. This has been enabled in part by modern genetic labeling of cells for fate mapping. Our work and others has now clearly demonstrated that chondrocytes give rise to the new bone during repair of fractures in appendicular and facial bones (Bahney et al. 2019; Kaji et al. 2020; Wong et al. 2021). Chondrocytes also contribute to the osteoblast lineage during long bone development. While technically challenging to accurately quantify, studies have shown that chondrocytes give rise to between 35% and 68% of the osteoblasts depending on the region and timing of quantification (Zhou et al. 2014; Park et al. 2015). Taken together, there is a large amount of evidence that cartilage transforms into bone and that chondrocytes are a primary source of osteoblasts (Javaheri et al. 2018; Bahney et al. 2019). We propose that this transformation is part of the conserved mechanism of paligenosis as discussed later.

Molecular Regulation of Cartilage to Bone Transformation

Molecular mechanisms underlying cartilage to bone transformation during healing remain unclear. Part of the challenge is significant molecular overlap between hypertrophic chondrocytes and osteoblasts. While this was originally viewed as a confounding factor, perhaps it supports that these cell types are part of a lineage continuum (Javaheri et al. 2018). Using in situ hybridization and immunohistochemistry, we observe a sharp transition from cells expressing the chondrocyte marker genes *Col2*/*Col10* to cells expressing the osteoblast marker *Col1a1* (Hu et al. 2017). The cells expressing *Col1a1* remained large and morphologically similar to hypertrophic chondrocytes, but changes in gene expression are abrupt and definitive. Moreover, in this work, expression of transcription factors associated with cellular reprogramming was observed in hypertrophic chondrocytes. Together, these data suggest that wholesale alteration of the transcriptome from chondrocyte to osteoblast occurs in a very short period of time.

In contrast, work in zebrafish suggests that the cartilage cells that differentiate in response to resection of the jawbone are a hybrid skeletal cell. In this work, a large cartilage "callus" formed that spanned the injured mandible. The cells coexpressed markers of chondrocytes and osteoblasts, suggesting they are a composite cell type that expressed both the chondrogenic and osteogenic molecular programs (Paul et al. 2016). Whether this reflects species or site-specific differences in bone regeneration, differential effects of the injury type (fracture vs. large scale resection), or another unknown difference in experimental design is not clear.

During metaplasia, fully differentiated cells appear to acquire stem cell–like properties and give rise to a host of other differentiated cell types. One model is that during endochondral ossification, hypertrophic chondrocytes transiently express transcription factors associated with multipotency that allows them to transform into other cell types (Fig.) (Bahney et al. 2014; Hu et al. 2017; Wong et al. 2021). This is supported by lineage-tracing studies showing chondrocytes can contribute to multiple mesenchymal lineages (Ono et al. 2014; Wong et al. 2020; Long et al. 2022). Collectively, these studies indicated that hypertrophic chondrocytes exhibit cellular plasticity and are progenitors for other cell types.

While multiple molecular signals have been suggested to be involved in activating the osteogenic fate, there are relatively few mechanistic studies. The canonical Wnt signaling pathway may act as a key molecular switch between chondrogenic and

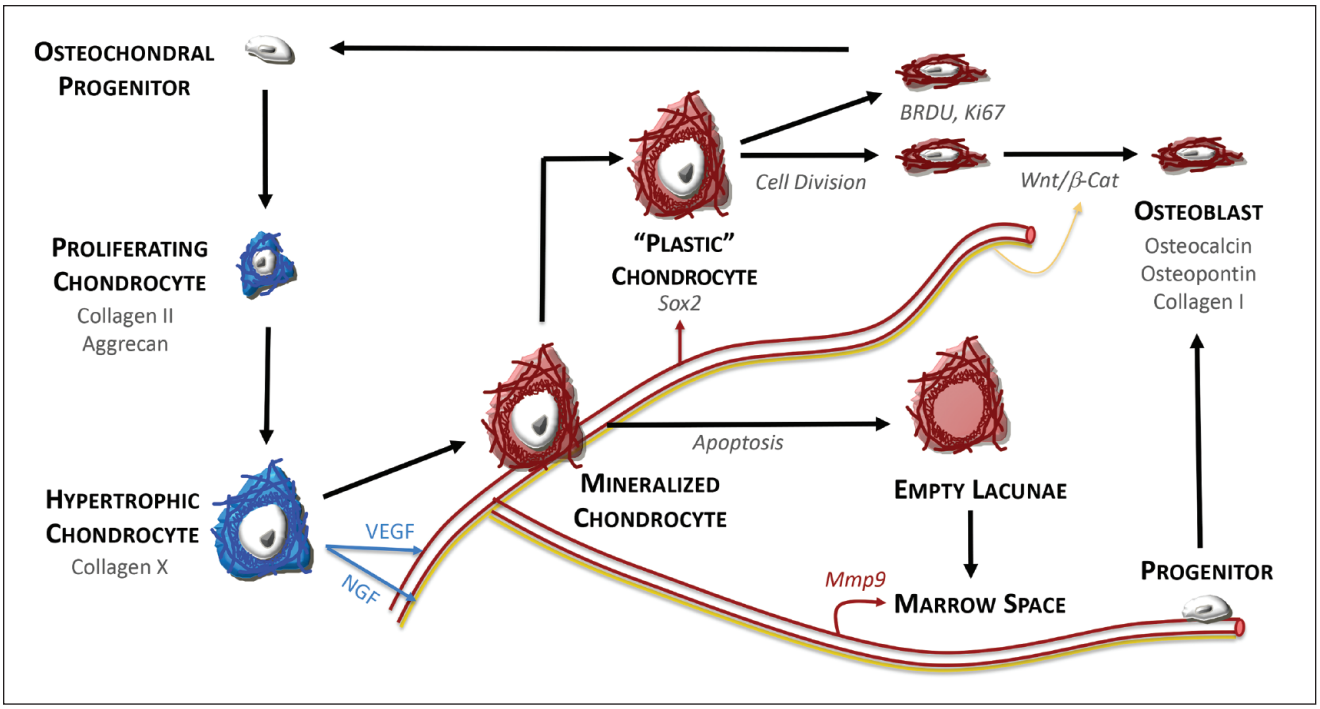

Figure. An updated model of endochondral ossification. Osteochondral progenitors from the periosteum and endosteum of long bones and the mandible differentiate into osteoblasts and chondrocytes to form bone and cartilage in the fracture site. The cartilage callus is formed by proliferating immature chondrocytes (blue). Eventually, the chondrocytes within the callus mature and become hypertrophic. They express angiogenic factors that induce vascular invasion. The hypertrophic cartilage (red) comprising the transition zone mineralizes adjacent to the invading neurovascular bundle (blood vessels = red, nerves = yellow). Some hypertrophic chondrocytes undergo apoptosis to create marrow space within the new bone. Other hypertrophic chondrocytes express transcription factors associated with "stemness" (e.g., SOX2). Hypertrophic cells in the transition zone reenter the cell cycle and begin dividing. These cells also express osteogenic genes and transform into osteoblasts. This model does not exclude previously proposed systems in which osteoblasts in the newly formed bone are derived from osteoprogenitors that are brought in by the invading vasculature. Whether regional differences in these processes exist is not widely known and open to investigation. This has been modified from Hu et al. (2017).

osteogenic fate. In the growth plate, a ubiquitously expressed cytoplasmic protein tyrosine phosphatase, SHP2, has been shown to regulate osteogenic differentiation of hypertrophic chondrocytes by balancing SOX9 and β-catenin signaling (Wang et al. 2017). Activating β-catenin in chondrocytes is sufficient to drive osteogenesis and endochondral bone repair (Wong et al. 2020). Moreover, chondrocyte-specific deletion of Wnt/β-catenin inhibits formation of bone during development (Houben et al. 2016) and healing (Wong et al. 2020). While numerous Wnt ligands and receptors are expressed during fracture repair, it remains unclear how endogenous Wnt/β-catenin is activated in chondrocytes. Interestingly, recent evidence suggests that nerve growth factor (NGF) signaling through high-affinity binding to the tropomyosin receptor kinase A (TrkA) receptor may contribute to the activation of the Wnt/β-catenin pathway (Tomlinson et al. 2017; Rivera et al. 2020).

In addition to the Wnt/β-catenin pathway, studies show that Hedgehog signaling may also serve as a switch between chondrogenic and osteogenic fates during development and repair (Kuwahara et al. 2022). At the chondro-osseous junction of the growth plate, hypertrophic chondrocytes have active Hedgehog signaling, and deletion of Indian Hedgehog (*Ihh*) results in a loss of trabecular bone during elongation (Maeda et al. 2007; Shi et al. 2017; Haraguchi et al. 2018). Similarly, reporter mice show that $Gli1^{+}$ cells contribute to both chondrocytes and osteoblasts during axial and mandibular fracture repair (Shi et al. 2017; Chen et al. 2022). In femur fractures, inhibition of Hedgehog signaling delayed bone healing but did not affect chondrogenesis or angiogenesis, suggesting dysregulation may be associated with conversion of cartilage to bone (Liu et al. 2017). Conversely, administration of a Hedgehog agonist accelerated repair through increased chondrocyte proliferation, an enlarged cartilage callus, and an increased number of cells expressing osteoblast markers within the callus (Kashiwagi et al. 2016). Similarly, we observed that Ihh signaling was highly upregulated after applying NGF to the fracture site (Rivera et al. 2020).

Significantly more research is necessary to understand the molecular and epigenetic mechanisms that regulate chondrocyte to osteoblast transformation. This includes understanding when and if these mechanisms are conserved across development, repair, and disease and whether they are conserved between the axial and cranial skeleton. Furthermore, the crosstalk between pathways and the order of operations needs to be disentangled through rigorous studies.

Paligenosis: A Process of Metaplasia in Multicellular Organisms

Metaplasia describes transformation of a fully differentiated cell type into other cell types as tissues change into entirely different tissue types. The concept gained popularity in the 1800s, was based on histological and anatomical observations, and is often discussed in terms of pathological changes in tissue in response to environmental insult such as with Barrett's esophagus (Zhang et al. 2021) or in the context of cancer as reviewed (Giroux and Rustgi 2017). However, recent evidence suggests that metaplasia and cellular plasticity contribute to development and regeneration within the musculoskeletal system and beyond (Kaji et al. 2020; Brown et al. 2022; Goldenring and Mills 2022). Metaplasia appears to be common in normal and pathologic processes in multicellular organisms. Heterotopic bone formation is termed *osseus metaplasia* because nonskeletal tissues are transformed into bone after environmental or genetic insult (Xu et al. 2018). However, this process is not restricted to skeletal lineages. For example, injury of the stomach lining stimulates gastric chief cells to assume a progenitor-like state that give rise to multiple cell types comprising the stomach lining (Goldenring and Mills 2022). In the gastrointestinal tract, this behavior provides protection from insults such as gastroesophageal reflux. While most of the changes are benign, metaplasia can lead to carcinogenesis of the stomach (Goldenring and Mills 2022), and acinar to ductal metaplasia may be a precursor to adenocarcinoma of the pancreas (Murtaugh and Keefe 2015; Giroux and Rustgi 2017).

The process of metaplasia occurring in these tissues has been named "paligenosis" (Messal et al. 2018). One of the defining features of paligenosis is reentry of mitotically inactive cells into the cell cycle (Miao et al. 2021). Interestingly, this is what we observe in hypertrophic chondrocytes adjacent to the blood vessels. Specifically, in the fracture callus, we observe that large hypertrophic chondrocytes located in the Transition Zone incorporate BrdU and undergo cytokinesis (Hu et al. 2017). In the stomach, this process is mediated by changes in mTOR (mammalian/mechanistic target of rapamycin) activity, but whether this is also the case during skeletal regeneration is not known.

Another aspect of paligenosis is that the cells undergoing transformation assume a more stem cell–like state and may have multilineage potential. This also appears to be the case for chondrocytes. In response to injury of the temporomandibular joint, chondrocytes acquire the ability to differentiate into adipocytes (Xu, Chu, et al. 2022). Impressively, during development of long bones, chondrocytes appear to have multiple cell fates that include osteoblasts, adipocytes, and skeletal progenitor cells that are located in the bone marrow cavity; during this transformation, the chondrocytes dedifferentiate and then form progenitor cells that serve as a source of osteoblasts (Long et al. 2022).

Clinical Implications of Mechanisms of Bone Regeneration

The clinical implication of this research is undeniable. As understanding of the cellular processes of bone healing become refined, understanding of mechanisms that delay healing and novel approaches to stimulate healing may become apparent. For example, healing is delayed in diabetic patients. Research in animal models suggests that the conversion of cartilage to bone is altered as large numbers of chondrocytes appear to undergo apoptosis in diabetic models due to increased production of Tnfα (Kayal et al. 2010). Similarly, persistent cartilage in hypertrophic nonunions (Panteli et al. 2022) suggests a failure of the conversion of chondrocytes to osteoblasts, indicating a mechanism that could be targeted to stimulate repair. In fact, a large area of research focuses on therapies designed to enhance bone formation. However, most therapeutics under investigation for fracture healing aim to promote intramembranous repair. This disconnect between current therapies and the endogenous mechanism of fracture repair represents a potential explanation for poor or inconsistent outcomes with existing osteoinductive therapeutics.

Bone morphogenetic proteins (BMPs) are the most widely recognized osteoinductive proteins. INFUSE (Medtronic) uses BMP2 delivered via surgically implanted collagen sponge to promote bone formation. INFUSE has approval from the US Food and Drug Administration (FDA) within a narrow indication of tibial fractures, but widespread off-label use was once reported. Clinical use of BMP has fallen out of favor due to the high cost, limited evidence of clinical efficacy, and severe off-target effects. Results of recent mechanistic work showing that Wnt signaling is required for transformation of chondrocytes to osteoblasts (Houben et al. 2016) may help explain why treatment with BMPs has not proven clinically effective.

Studies showing that Wnt/β-catenin signaling mediates transformation of chondrocytes to osteoblasts suggest that a Wnt-activating therapy may be a better choice to stimulate endochondral bone repair. Unfortunately, the Wnt pathway is challenging to manipulate, because Wnt ligands are lipidated. Lipid modification is required to enable ligand trafficking and pathway activation. As such, simple manufacturing and delivery of Wnt ligands is not economical, and commercial strategies have used antibodies to pathway inhibitors to indirectly activate Wnt signaling (Schupbach et al. 2020). One example is the sclerostin antibody EVENITY (Amgen) which has FDA approval to *prevent* osteoporotic fractures in postmenopausal women by increasing bone mineral density. While EVENITY has proven anabolic in treating osteoporosis, clinical studies testing efficacy in fracture repair have shown no benefit (Bhandari et al. 2020; Schemitsch et al. 2020), suggesting systemic delivery of antibodies is insufficient to stimulate repair perhaps due to limited activation of the pathway within the fracture callus.

Developing tissue engineering strategies to promote vascularized bone regeneration is another area of research. Bone autograft remains the gold standard for augmenting bone healing. While autograft is associated with good healing outcomes, bone harvest increases surgical time and risk of complications by ~60% and is associated with donor site morbidity, and there are insufficient bone fill large defects. Bone allograft is readily available in several forms, but product failure rates remain unacceptably high. Hence, developing cell-based therapies

may provide significant advantage. Many cell-based therapies are based on intramembranous ossification that develop a bone-based construct for transplantation. However, bone is a highly vascularized tissue, so developing a composite graft that comprises vascularized bone may be necessary for success.

Cartilage may have a more effective translational path. Endochondral cartilage matrix built by hypertrophic chondrocytes is highly bioactive, promoting vascular and neural invasion, along with calcification and mineralization of the matrix (Erickson 2021). Hence, using cartilage as a bone graft substitute may offer considerable advantage. In previous work, we showed that transplantation of cartilage capable of endochondral ossification is able to heal critical-sized defects in mouse tibia and dentary bones (Bahney et al. 2014; Wong et al. 2021).

Research emphasizes the critical importance that the periosteum and neurovascular bundle play in normal repair. Since it is now well established that the periosteum is the major source of osteochondral progenitors during repair, preservation of the periosteum during clinical procedures can improve outcomes. Murine models have demonstrated that surgical-, radiation-, or genetic-induced dysfunction of the periosteum results in delayed healing or nonunion (Garcia et al. 2013; Wang et al. 2019; Julien et al. 2020). Therefore, in addition to bone grafts, significant engineering efforts have recently been placed into developing biomimetic periosteum for bone repair and reconstruction (Yu et al. 2020; Zhai et al. 2021; Dai et al. 2022; Zhang et al. 2022). Replacement of the periosteum is not only critical for supplying the initial progenitor cells themselves but may be subsequently repopulated by the chondrocytes they give rise to (Julien et al. 2020; Wong et al. 2020; Long et al. 2022).

Similarly, both the vascular and neural plexus appear to have roles in bone repair, and surgical preservation, therapeutic stimulation, and/or tissue engineering strategies may be clinically targeted to promote repair. During endochondral healing, the role of the neurovascular bundle is intriguing, because cartilage is avascular and aneural prior to transforming into vascularized and innervated bone. Moreover, the periosteum is highly vascularized and innervated; hence, disruption of these structures within the periosteum by bone fracture may contribute to molecular activation of chondro- or osteogenic differentiation. The vasculature has an established role in the repair process. Recent evidence suggests that vascular invasion supports chondrocyte plasticity through secretion of factors that promote cellular proliferation and activation of pluripotent transcription factors such as *Sox2*, possibly through a tissue-specific angiocrine (Zhai et al. 2021).

A more emerging area of research is investigation into the role of neurotrophins during repair of the axial and craniofacial skeleton. Bone is highly innervated, yet, aside from a role in pain sensation, little is known about a functional role in healing. While loss-of-function studies are complicated by the essential and systemic role of neuronal signaling, the laterality of the bone-healing response with concomitant brain injury suggests there is underlying neuronal influence to endochondral repair (Morioka et al. 2019). Furthermore, mice lacking *Ngf* in myeloid cells demonstrate reduced migration of osteogenic precursors, which, together with the differential immune response found in the previous study, suggests a high level of connection between the neural and inflammatory responses (Morioka et al. 2019; Xu, Li, et al. 2022). Gain-of-function studies show that treatment of bone during development and repair with NGF promotes endochondral ossification and osteogenic progression (Tomlinson et al. 2017; Rivera et al. 2020). NGF is expressed after axial and cranial bone injury along with the high-affinity TrkA and low-affinity p75 receptors in a variety of cells, including chondrocytes, osteoblasts, and mesenchymal cells (Rivera et al. 2020; Xu, Li, et al. 2022). NGF has also been shown to be involved in intramembranous repair of long bone stress fractures and cranial bones (Li et al. 2019; Xu, Li, et al. 2022). During intramembranous repair, NGF has pleiotropic effects with TrkA signaling, triggering reinnervation, vascularization, and osteoblastic activity and the p75 pathway coordinating cell migration during early bone repair (Li et al. 2019). Together, these studies suggest that NGF contributes to intramembranous and endochondral repair and would be applicable in both axial and craniofacial injuries.

One clinical implication of understanding the molecular and cellular mechanisms of endochondral fracture healing is evident now. In a clinical setting, fracture healing is typically defined based on serial radiographs, physical examinations, and experience of the physician. As such, there has been a quest for objective detection modalities that could provide quantitative assessments of fracture repair with sensitivity to both the endochondral and intramembranous healing. Biomarkers to date have primarily measured bone turnover markers associated with intramembranous repair and remolding. These markers, while established for homeostatic bone maintenance in osteoporosis, are likely later indicators of bone healing that coincide with radiographic evidence of mineralization (Stewart et al. 2022). In contrast, we recently have validated a novel biomarker associated with the transient cartilaginous callus tissue present in fracture healing by detecting collagen X (ColX), which is the basis of matrix produced by hypertrophic chondrocytes (Erickson 2021; Working et al. 2021, 2022). Taken together with other novel approaches to detect the early phases of fracture repair through the use of impedance spectroscopy (Lin et al. 2019), the ability to measure progression of endochondral ossification may improve patient care and development of novel therapies.

Summary

There remains an unmet clinical need to improve bone repair and regeneration in the appendicular, axial, and craniofacial skeleton. Taking into account the molecular and cellular mechanisms of development and repair may allow tailored and functional therapies for specific clinical applications that consider both the region of repair and the relative stability of fixation. The development of therapies that promote, or enable, cellular plasticity that is endogenous during repair may ultimately improve the process of endochondral bone repair and represent a new class of therapeutics designed to promote metaplasia.

Most of the mechanistic studies in this review are based on animal studies and work performed in cell culture, and whether some of these processes occur in patients remains to be determined. Rodents heal with incredible robustness. They are able to heal bone fractures in the absence of any stabilization, and nonunions do not readily occur. In fact, the lack of impaired fracture healing in young, wild-type rodents is a problem for understanding healing failure in humans, and creation of nonphysiologic models of nonunions is used to address this problem. Whether these are adequate models to understand delayed healing or nonunions in humans is questionable. However, the work in animal models provides a substantial framework for developing hypothesis-based research studies that can be done in patients. Finally, historical studies on human tissues have shown that metaplasia of cartilage to bone is very likely to be occurring during fracture healing (Urist and Johnson 1943). Examining histological sections through human fracture calluses led Urist and Johnson (1943) to state,

> The cartilage appears to lose its basophilic staining and gradually disappears in the osseous tissue as though through transformation of the chondrocytes to the osteocytes. This transformation, regarded by some authors as metaplasia, is a prominent feature of the ossification of the callus.

Author Contributions

R.S. Marcucio, contributed to conception, data acquisition, analysis, or interpretation, drafted and critically revised the manuscript; T. Miclau III, contributed to data conception, critically revised the manuscript; C.S. Bahney, contributed to data conception, drafted and critically revised the manuscript. All authors gave their final approval and agree to be accountable for all aspects of the work.

Declaration of Conflicting Interests

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