Guest Editorial

What's New in Musculoskeletal Basic Science

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As practicing orthopaedic surgeons, we strive to improve our patients' outcomes. However, surgical technique, perioperative measures, and modern implant designs can only accomplish small to moderate improvements. Knowledge of the biological foundation that results in orthopaedic maladies and an understanding of the cellular and molecular mechanisms that govern regeneration will eventually result in orthobiological treatments that may affect thousands of patients and accelerate and ease their return to function after an orthopaedic injury. Here we will highlight some of the most groundbreaking and relevant basic science articles that were published in the past year. This summary aimed to be inclusive, yet, due to the sheer number of publications, had to be limited to certain topics. Basic science breakthroughs in musculoskeletal oncology are not covered herein, as they are part of a separate update in *The Journal of Bone & Joint Surgery*.

Fracture Healing

The skeleton has a remarkable ability to regenerate in response to injury without forming scars. Although this process is successful in the large majority of fractures, a certain percentage of fractures still fail to unite, and it is this fraction that would benefit either from early recognition using radiographic or biological readouts or from therapeutic adjuvants that could jumpstart or guide successful regeneration. Skeletal stem and progenitor cells are the cornerstone of fracture repair. Although extensive research in the past has focused on the marrow-derived skeletal stem and progenitor cells, more recent efforts have focused on the periosteum as a source for stem cells. In fact, one could argue that the periosteum is the prime source of skeletal stem and progenitor cells during fracture repair. In a recent paper, Kegelman et al. nicely highlighted the role of YAP (Yes-associated protein) and TAZ (transcriptional co-activator with PDZ-binding motif) in promoting periosteal progenitor cell expansion and differentiation during fracture healing[1]. Utilizing a transgenic mouse strategy, they showed that YAP/TAZ deletion in Osterix-expressing cells (osteoprogenitor cells) during adult fracture healing did not affect the development of the chondrogenic soft callus; however, the deletion yielded impaired osteoprogenitor cell expansion and osteogenic differentiation resulting in delayed callus mineralization and thus impaired fracture healing. Julien et al. further elevated the importance of periosteal stem and progenitor cells as the key for successful bone-healing[2]. Using a transgenic mouse line (*Prx1*^{Cre};*Fgfr3*$^{Y367/+}$) in which fibroblast growth factor receptor 3 (FGFR3) signaling is overactivated in periosteal progenitor cells, the authors demonstrated that the cartilage-to-bone transformation by periosteal cells during bone regeneration is regulated by FGFR3 signaling. In addition, using elegant transplantation models, they showed that periosteal cells transplanted into a nonunion site can overcome the detrimental microenvironment that led to the failure of union and can therefore be considered as a potentially powerful, cell-based therapy for the treatment of delayed union or nonunion. Novak et al. examined the role of Notch signaling in periosteal cells during fracture healing[3]. Increased Notch signaling in osteochondroprogenitor cells in the periosteum resulted in increased proliferation, decreased soft callus size, increased mineralized tissue, and an overall stronger and stiffer callus, promoting Notch signaling as a potential therapeutic target to augment fracture healing. Aging is a known risk factor for poor fracture healing, and there is ample evidence now that the aging innate and adaptive immune system exerts a detrimental effect on all aspects of fracture healing. Clark et al. recently identified aging macrophages as culprits for delayed fracture healing[4]. Using RNA sequencing analysis, they demonstrated a clear shift toward an M1/pro-inflammatory phenotype in the aging fracture callus in addition to a general dysregulation of immune-related genes. When they prevented migration of macrophages into the aging fracture callus, they observed improved fracture healing, suggesting a negative impact of circulating monocytes and infiltrating macrophages during fracture healing in the aging animal.

Precision medicine has entered the clinical arena in cancer treatment, but orthopaedic care is still relying on the same methods and techniques that we used decades ago. We still use radiographs and clinical examination to define union of a long-bone fracture, and we have not made any substantial strides toward predicting the development of a nonunion rather than diagnosing it when it has already happened. For the affected patients, this decades-old diagnostic approach means waiting for up to 9 months before a decision is made to proceed with a revision surgical procedure. Working et al. recently

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What's New in Musculoskeletal Basic Science

demonstrated that the collagen X degradation fragments (collagen X marker [CXM]) can be measured in serum and correlated with the progression of endochondral fracture healing in mice[5]. Together with a human study showing that CXM can serve as a real-time marker for bone growth[6], one can imagine a precision medicine approach utilizing blood tests in addition to serial radiographs during the early stages of fracture repair to predict fracture union, and, in the untoward event of predicted aberrant union, early intervention could be initiated.

Cartilage and Osteoarthritis

Surgical management of isolated cartilage defects still represents a challenging task, and procedures such as microfracture have only resulted in temporary restoration of the articular surface by forming fibrocartilage. In a groundbreaking study, Murphy et al. demonstrated that the skeletal stem cells that are recruited to the microfracture site can be triggered to regenerate articular cartilage by delivering bone morphogenetic protein-2 (BMP-2) and a soluble vascular endothelial growth factor receptor 1 (VEGFR1)[7]. Atomic force microscopy revealed that the cartilage regenerate had a similar elastic modulus compared with uninjured cartilage, and animals showed improved mobility parameters as measured by gait analysis. This study showed, for the first time, that biochemical manipulation of the local stem cell niche environment can activate a regenerative response of resident skeletal stem cells that results in a durable articular cartilage regenerate.

Heterotopic Ossification

Wound-healing relies on a well-orchestrated sequence of molecular events that result in the correct programming of adult progenitor cells within the wound environment. In some instances (for example, after burns, brain injury, or massive trauma), this programming can be misguided, resulting in heterotopic bone formation. Recent discoveries have shed light on the cell type and the molecular pathways that are responsible for this aberrant regenerative response. Using transgenic reporter mice and single-cell RNA (scRNA) sequencing, Pagani et al. demonstrated that regionally specific multipotent progenitor cells respond to trauma and form heterotopic bone[8]. Mechanistically, Lee et al. identified the role of nerve growth factor-tropomyosin receptor kinase A (NGF-TrkA) signaling as an essential regulator of neural ingrowth and subsequent formation of heterotopic ossification following soft-tissue trauma[9]. After NGF expression in the zone of injury, NGF-responsive axons invade the site and induce osteocartilaginous differentiation. Surgical denervation and biochemical manipulation of NGF-TrkA signaling resulted in delays in axonal ingrowth, which then caused delayed osteochondral differentiation. Clinical observation has demonstrated an effect of joint mobilization on heterotopic ossification formation. Using an in vivo mouse model of severe trauma and joint immobilization, Huber et al. showed that the altered physical environment after immobilization results in extracellular matrix changes that trigger mesenchymal progenitor cell fate changes[10]. This study provided promising evidence that aberrant wound-healing and heterotopic ossification formation can be prevented by modulating mechanotransductive pathways using immobilization or pharmacologic inhibitors.

Tendon

Tendon injuries represent one of the most common orthopaedic injuries, often seen in young, active patients. Despite being so common in young patients with presumably favorable regenerative capacity, tendon injuries are often plagued by improper regeneration with resultant loss of strength, decreased range of motion, and joint instability. Therefore, research focusing on improved tendon regeneration has gained meaningful momentum in the past decade. In addition to this, in a recent publication, Wang et al. demonstrated the influence of the extracellular matrix protein periostin on the tendon stem and progenitor cell maintenance and proliferation[11]. The incorporation of recombinant periostin (rPOSTN) into a biomimetic parallel-aligned collagen scaffold resulted in improved tendon regeneration in a rat Achilles tendon defect model. The authors showed that the rPOSTN-loaded scaffold resulted in enhanced tendon stem and progenitor cell (TSPC) recruitment, tendon regeneration, and repair with native-like hierarchically organized collagen fibers. Also, the rPOSTN-treated tendon demonstrated recovery of mechanical properties and locomotion functions. Using rPOSTN treatment to initiate and promote endogenous stem cell recruitment to a tendon injury site has potential advantages with regard to clinical translation, as it avoids the regulatory hurdles associated with cell therapy.

Scleraxis-lineage cells play an essential role during tendon development, and, because regeneration often utilizes developmental processes, one could assume that this cell lineage contributes to adult tendon regeneration. In a surprising finding, Best et al.[12] revealed that genetic ablation of the scleraxis lineage prior to tendon injury and repair resulted in enhanced tendon regeneration with improved mechanical properties. However, the depletion during postnatal tendon growth and adult tendon homeostasis resulted in changes in matrix alignment and organization with an as-yet-unknown effect on the mechanical properties of the adult tendon. These findings add to our fundamental knowledge of tendon homeostasis and repair and further highlight the complexities encountered during adult tissue regeneration.

Understanding the Cellular Bone Marrow Landscape Using Single-Cell Transcriptomics

In the past few years, our understanding of skeletal stem cell identity and hierarchy has increased to a great degree. With the advent of scRNA sequencing, we are now in the position to unravel the skeletal stem cell field with detailed transcriptomic resolution, offering new insight into the workings of these now well-defined cell types within the bone and bone marrow and their computationally predicted differentiation trajectories.

Zhong et al. provided detailed insight into the bone marrow mesenchymal lineage using scRNA sequencing[13]. Using trajectory

What's New in Musculoskeletal Basic Science

analysis, they identified a population of cells as the most primitive of the mesenchymal lineage, which they called early mesenchymal progenitors, expressing common stem cell markers such as Sca1, CD34, and Thy1. The authors also identified a previously uncharacterized type of fat cell without lipid droplets that controls bone marrow osteoblast and vasculature homeostasis called marrow adipogenic lineage precursors, and these cells are marked by Lepr, which was previously identified as a skeletal stem cell marker (Zhou et al.[14]). The scRNA sequencing analysis of older mice, 16 months of age, revealed an expansion of the adipocyte clusters as well as a reduction in early mesenchymal progenitor number and a drift toward adipogenic status. This observation confirms a fate shift of marrow cells toward an adipogenic lineage with age that was previously observed. Similarly, Baccin et al. performed a detailed single-cell-level survey of bone marrow niches by integrating both scRNA sequencing and spatially resolved transcriptomics[15]. They developed a method of laser-capture microdissection on fixed bone marrow sections coupled with bulk RNA sequencing to provide detailed information on bone marrow cell types, their subtypes, and their location within the bone marrow. They found that Cxc12-abundant reticular (CAR) cells could be split into 2 subsets, Adipo-CAR cells, which differentially localize to sinusoidal endothelia, and Osteo-CAR cells, which differentially localize to arteriolar endothelia or nonvascular regions, providing evidence that CAR cells with different transcriptional potential can occupy distinct spatial niches. One shortcoming of scRNA sequencing technology is the loss of spatial information. However, Baccin et al. offered an approach to combine transcriptional and spatial information, providing a platform for a more detailed and complete picture of the bone microenvironment that includes both transcriptional data and spatial data. Finally, Matsushita et al. used scRNA sequencing, lineaging tracing, and genetic manipulation to define the role of Cxcl12-creER+ labeled reticular cells during regeneration and found that these cells represent quiescent perisinusoidal Cxcl12+ cells[16]. They found that these labeled cells express cytokines and adipocyte-related genes, have little colony-forming activity, and give rise to adipocytes but not osteoblasts during homeostasis. By using a drill-hole cortical injury, they demonstrated through lineage tracing, in combination with scRNA sequencing, that these cells adapt a stem cell identity and give rise to new osteoblasts during injury through activation of canonical Wnt signaling in their trajectory to osteoblasts. Using genetic ablation, they found that β-catenin deficiency in these cells caused deficits in healing, confirming the role of Wnt signaling in the activation of these cells during regeneration. This finding also demonstrates that, although perisinusoidal cells have an adipogenic signature, as also noted by Baccin et al., these cells can change their transcriptional profile and fate in response to injury. This highlights the need for a combination of scRNA sequencing with techniques such as lineage tracing and genetic manipulation to fully understand the function of heterogenous cell types within bone both during homeostasis and during regeneration.

Muscle Regeneration and Aging

Although considerable efforts have been mobilized to better understand the regenerative ability of skeletal muscle, we are still lacking a clinically applicable therapeutic that would allow our patients to recover faster and more reliably after muscle injury. Recent advances have identified an altered immune response to muscle injury during aging as a potential culprit for the poor healing response. Blanc et al. identified inflammatory-related CC-chemokine-receptor 2 (Ccr2) expression in myogenic progenitors during regeneration, which resulted in impaired myogenic progenitor fusion, a hallmark of successful muscle regeneration[17]. Inhibition of Ccr2 during muscle injury in older individuals revealed enhanced muscle regeneration and functional recovery in those older individuals.

Edwards et al. examined the role of toll-like receptor (TLR) signaling in muscle fibrosis after ischemia-reperfusion injury[18]. Using hydroxychloroquine, a small molecule inhibitor of TLR7/8/9, resulted in decreased muscle fibrosis and increased myofiber regeneration after ischemia-reperfusion injury.

The role of satellite cells in muscle regeneration has been extensively studied. Leung et al. identified a subset of satellite cells that express Lgr5 (leucine-rich repeat-containing G protein-coupled receptor 5), a receptor for Rspo1-Rpo4, which are potent Wnt signaling enhancers[19]. These Lgr5-positive cells are contributing only to muscle regeneration but not to muscle homeostasis. In response to injury, Lgr5 is upregulated in this subset of *Pax7*-positive cells and results in regeneration of muscle fibers and replenishment of the quiescent stem cell pool.

Upcoming Meetings and Events Related to Orthopaedic Basic Science

- The Gordon Research Conference, "Bones and Teeth," will be held on January 16 to 21, 2022, in Galveston, Texas.
- The 2022 Annual Meeting of the Orthopaedic Research Society (ORS) will be held on February 4 to 8, 2022, in Tampa, Florida.
- The annual meeting of the International Society for Stem Cell Research (ISSCR) will be held on June 15 to 18, 2022, in San Francisco, California.
- The American Society for Bone and Mineral Research (ASBMR) 2022 Annual Meeting will be held on September 9 to 12, 2022, in Austin, Texas.
- The World Orthopaedic Research Congress will be held on September 7 to 9, 2022, in Edinburgh, United Kingdom.

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WHAT'S NEW IN MUSCULOSKELETAL BASIC SCIENCE

References

1. Kegelman CD, Nijsure MP, Moharrer Y, Pearson HB, Dawahare JH, Jordan KM, Qin L, Boerckel JD. YAP and TAZ promote periosteal osteoblast precursor expansion and differentiation for fracture repair. J Bone Miner Res. 2021 Jan;36(1):143-57.

2. Julien A, Perrin S, Duchamp de Lageneste O, Carvalho C, Bensidhoum M, Legeai-Mallet L, Colnot C. FGFR3 in periosteal cells drives cartilage-to-bone transformation in bone repair. Stem Cell Reports. 2020 Oct 13;15(4):955-67.

3. Novak S, Roeder E, Sinder BP, Adams DJ, Siebel CW, Grcevic D, Hankenson KD, Matthews BG, Kalajzic I. Modulation of Notch1 signaling regulates bone fracture healing. J Orthop Res. 2020 Nov;38(11):2350-61.

4. Clark D, Brazina S, Yang F, Hu D, Hsieh CL, Niemi EC, Miclau T, Nakamura MC, Marcucio R. Age-related changes to macrophages are detrimental to fracture healing in mice. Aging Cell. 2020 Mar;19(3):e13112.

5. Working ZM, Morris ER, Chang JC, Coghlan RF, Johnstone B, Miclau T 3rd, Horton WA, Bahney CS. A quantitative serum biomarker of circulating collagen X effectively correlates with endochondral fracture healing. J Orthop Res. 2021 Jan;39(1):53-62.

6. Coghlan RF, Oberdorf JA, Sienko S, Aiona MD, Boston BA, Connelly KJ, Bahney C, LaRouche J, Almubarak SM, Coleman DT, Girkontaite I, von der Mark K, Lunstrum GP, Horton WA. A degradation fragment of type X collagen is a real-time marker for bone growth velocity. Sci Transl Med. 2017 Dec 6;9(419):eaan4669.

7. Murphy MP, Koepke LS, Lopez MT, Tong X, Ambrosi TH, Gulati GS, Marecic O, Wang Y, Ransom RC, Hoover MY, Steininger H, Zhao L, Walkiewicz MP, Quarto N, Levi B, Wan DC, Weissman IL, Goodman SB, Yang F, Longaker MT, Chan CKF. Articular cartilage regeneration by activated skeletal stem cells. Nat Med. 2020 Oct;26(10):1583-92.

8. Pagani CA, Huber AK, Hwang C, Marini S, Padmanabhan K, Livingston N, Nunez J, Sun Y, Edwards N, Cheng YH, Visser N, Yu P, Patel N, Greenstein JA, Rasheed H, Nelson R, Kessel K, Vasquez K, Strong AL, Hespe GE, Song JY, Wellik DM, Levi B. Novel lineage-tracing system to identify site-specific ectopic bone precursor cells. Stem Cell Reports. 2021 Mar 9;16(3):626-40.

9. Lee S, Hwang C, Marini S, Tower RJ, Qin Q, Negri S, Pagani CA, Sun Y, Stepien DM, Sorkin M, Kubiak CA, Visser ND, Meyers CA, Wang Y, Rasheed HA, Xu J, Miller S, Huber AK, Minichiello L, Cederna PS, Kemp SWP, Clemens TL, James AW, Levi B. NGF-TrkA signaling dictates neural ingrowth and aberrant osteochondral differentiation after soft tissue trauma. Nat Commun. 2021 Aug 16;12(1):4939.

10. Huber AK, Patel N, Pagani CA, Marini S, Padmanabhan KR, Matera DL, Said M, Hwang C, Hsu GC, Poli AA, Strong AL, Visser ND, Greenstein JA, Nelson R, Li S, Longaker MT, Tang Y, Weiss SJ, Baker BM, James AW, Levi B. Immobilization after injury alters extracellular matrix and stem cell fate. J Clin Invest. 2020 Oct 1;130(10):5444-60.

11. Wang Y, Jin S, Luo D, He D, Shi C, Zhu L, Guan B, Li Z, Zhang T, Zhou Y, Wang CY, Liu Y. Functional regeneration and repair of tendons using biomimetic scaffolds loaded with recombinant periostin. Nat Commun. 2021 Feb 26;12(1):1293.

12. Best KT, Korcari A, Mora KE, Nichols AE, Muscat SN, Knapp E, Buckley MR, Loiselle AE. Scleraxis-lineage cell depletion improves tendon healing and disrupts adult tendon homeostasis. Elife. 2021 Jan 22;10:e62203.

13. Zhong L, Yao L, Tower RJ, Wei Y, Miao Z, Park J, Shrestha R, Wang L, Yu W, Holdreith N, Huang X, Zhang Y, Tong W, Gong Y, Ahn J, Susztak K, Dyment N, Li M, Long F, Chen C, Seale P, Qin L. Single cell transcriptomics identifies a unique adipose lineage cell population that regulates bone marrow environment. Elife. 2020 Apr 14;9:e54695.

14. Zhou BO, Yue R, Murphy MM, Peyer JG, Morrison SJ. Leptin-receptor-expressing mesenchymal stromal cells represent the main source of bone formed by adult bone marrow. Cell Stem Cell. 2014 Aug 7;15(2):154-68. Epub 2014 Jun 19.

15. Baccin C, Al-Sabah J, Velten L, Helbling PM, Grünschläger F, Hernández-Malmierca P, Nombela-Arrieta C, Steinmetz LM, Trumpp A, Haas S. Combined single-cell and spatial transcriptomics reveal the molecular, cellular and spatial bone marrow niche organization. Nat Cell Biol. 2020 Jan;22(1):38-48.

16. Matsushita Y, Nagata M, Kozloff KM, Welch JD, Mizuhashi K, Tokavanich N, Hallett SA, Link DC, Nagasawa T, Ono W, Ono N. A Wnt-mediated transformation of the bone marrow stromal cell identity orchestrates skeletal regeneration. Nat Commun. 2020 Jan 16;11(1):332.

17. Blanc RS, Kallenbach JG, Bachman JF, Mitchell A, Paris ND, Chakkalakal JV. Inhibition of inflammatory CCR2 signaling promotes aged muscle regeneration and strength recovery after injury. Nat Commun. 2020 Aug 20;11(1):4167.

18. Edwards NJ, Hwang C, Marini S, Pagani CA, Spreadborough PJ, Rowe CJ, Yu P, Mei A, Visser N, Li S, Hespe GE, Huber AK, Strong AL, Shelef MA, Knight JS, Davis TA, Levi B. The role of neutrophil extracellular traps and TLR signaling in skeletal muscle ischemia reperfusion injury. FASEB J. 2020 Dec;34(12):15753-70.

19. Leung C, Murad KBA, Tan ALT, Yada S, Sagiraju S, Bode PK, Barker N. Lgr5 marks adult progenitor cells contributing to skeletal muscle regeneration and sarcoma formation. Cell Rep. 2020 Dec 22;33(12):108535.