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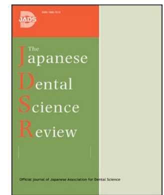

Multi-omics analysis in developmental bone biology

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A R T I C L E I N F O

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A B S T R A C T

Single-cell omics and multi-omics have revolutionized our understanding of molecular and cellular biological processes at a single-cell level. In bone biology, the combination of single-cell RNA-sequencing analyses and in vivo lineage-tracing approaches has successfully identified multi-cellular diversity and dynamics of skeletal cells. This established a new concept that bone growth and regeneration are regulated by concerted actions of multiple types of skeletal stem cells, which reside in spatiotemporally distinct niches. One important subtype is endosteal stem cells that are particularly abundant in young bone marrow. The discovery of this new skeletal stem cell type has been facilitated by single-cell multi-omics, which simultaneously measures gene expression and chromatin accessibility. Using single-cell omics, it is now possible to computationally predict the immediate future state of individual cells and their differentiation potential. In vivo validation using histological approaches is the key to interpret the computational prediction. The emerging spatial omics, such as spatial transcriptomics and epigenomics, have major advantage in retaining the location of individual cells within highly complex tissue architecture. Spatial omics can be integrated with other omics to further obtain in-depth insights. Single-cell multi-omics are now becoming an essential tool to unravel intricate multicellular dynamics and intercellular interactions of skeletal cells.

1. Introduction

Bone is a vital organ that plays an essential role in supporting fundamental biological functions. Over 200 pieces of bones in human body form the skeleton and help achieve locomotion. Moreover, bone is the primary site of hematopoiesis in the adult body [1]. At the cellular level, bone marrow is composed of bone-making skeletal cells, blood-making hematopoietic cells, and vascular cells that support these two cell types. Cells of multiple lineages interact with one another to create a highly functional bone marrow niche [2–9]. The current concept is that a special group of skeletal cells in the bone marrow, skeletal stem cells (SSCs), play a major role in bone growth, maintenance, and regeneration. SSCs represent a subclass of mesenchymal stem cells (MSCs) that are specific to bone tissues. SSCs have been identified as cells with the capacity to self-renew and differentiate into osteoblasts, chondrocytes, and adipocytes. These fundamental characteristics of SSCs have been mostly attributed through ex vivo cell culture experiments, which serve as the gold standard for SSCs [10,11]. Colony forming-unit fibroblasts (CFU-F) are first discovered in the 1960 s [12], and constitute the parental body of SSCs. Since that time, studies have investigated SSCs and their differentiation potential into 'trilineage cells' such as chondrocytes, osteoblasts, and marrow adipocytes [1, 13–15] in an ex vivo and transplantation setting. Over the last decade, SSCs have been extensively characterized using cell isolation techniques based on fluorescence-activated cell sorting (FACS), which utilize the combination of cell surface markers. These methods have been proven useful in isolating SSCs in both mice [16–18] and humans [19–21]. The major limitation of FACS-based approaches is that the information regarding in situ location of individual cells is permanently lost upon cell isolation. To circumvent this limitation, in vivo lineage-tracing approaches have been applied to define the cell fate of putative SSCs. These approaches utilize cell type-specific genetic tools in mice. Specifically, cell type-specific promoter-driven constitutively active *cre* or tamoxifen-inducible *creER* lines are combined with fluorescent reporter strains to permanently mark cells of interest, allowing the tracing of a specific cell type based on *cre-loxP* recombination [1, 13, 14, 22–24]. However, the bone marrow is composed of numerous numbers of cells that are tightly packed and intricately intertwined in vivo. Therefore, it is technically challenging to precisely define the fates of individual skeletal cells in the bone marrow at a single-cell level. In recent years,

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single-cell RNA-sequencing (scRNA-seq) and other omics approaches, including assay for transposase-accessible chromatin with sequencing (ATAC-seq) and combined multi-omics approaches, have further delineated the fundamental diversity of skeletal cell populations in the bone marrow and elsewhere. These approaches have led to the discovery of previously unidentified cell populations, therefore significantly updating our knowledge of the skeletal cell lineage. The findings from recent omics analyses revealed molecularly defined "micro-heterogeneity" within skeletal cell populations, revising the prevailing notion that skeletal cells represent monolithic groups of cells without defined cellular subsets. In this review, we discuss how single-cell omics and multi-omics have advanced our understanding of skeletal cells and skeletal biology in recent years.

2. Multi-omics analysis at a single-cell level

Omics analysis aims to comprehensively characterize the various molecules that support the fundamental function of a living organism. However, individual omics data from only one type of assay is largely correlative and cannot capture the intricacy of molecular events and their interactions [25]. Multi-omics refers to the analysis of multiple data of different omics groups such as genome, transcriptome, proteome, epigenome, metabolome, lipidomics, and microbiome, and the integrated analysis of the resulting data [26,27]. Multi-omics analysis allows us to investigate the relationship between each level of the hierarchy in addition to the single omics analysis, thus providing a more comprehensive understanding of the molecular changes that contribute to development, cellular responses, and potentially, diseases. The benefit of multi-omics is that one omics technology can make up for the deficiencies of another and provide a comprehensive overview of molecular complexity [25].

Multi-omics analysis is applied at an accelerating rate due to the rapid development of technologies and computational analyses as well as successful commercialization of the platforms. Further, with the advent of single-cell omics technologies, multi-omics analyses have further developed into the single-cell level. When scRNA-seq is combined with single-nuclei ATAC-sequencing (snATAC-seq), gene expression can be linked to open chromatin regions in the genome. As a result, we can not only identify cell types by gene expression but also predict cell status and dynamics [28–30]. Although transcription factor binding is transient, transcriptional responses unfold across minutes to hours [27]. By learning from differences, the current and future state of the cell becomes predictable, thereby strengthening the ability to reveal the gene regulatory program. The combined method to simultaneously assess chromatin accessibility and DNA methylation status in a single cell has successfully generated insight into the functional interplay between these two distinct regulatory layers and their contribution to the onset of gene expression [31,32]. Combined analysis of gene expression, chromatin accessibility, and DNA methylation has also been performed in some studies [33,34]. Furthermore, multi-omics analyses of protein-RNA, protein-chromatin, and protein-RNA-chromatin combinations are currently being developed, as demonstrated in recent studies [35–38]. Thus, various modalities of multi-omics analyses are becoming technically feasible, adding new insights into various areas of biology, including bone biology.

3. Multi-omics in developmental bone biology

Multi-omics approaches have been widely applied to developmental bone biology in recent years, providing substantial new insights regarding bone development. Bone, especially bone marrow, contains a variety of cells, including those of the skeletal, hematopoietic, and endothelial lineages. SSCs, or otherwise known as MSCs, have been highlighted as useful cells for regenerative medicine. Subsequently, ex vivo cell culture [39] and cell sorting approaches using cell surface markers [16,19] have been used to isolate SSCs. These approaches have also been used as evidence that a variety of SSCs reside in the bone marrow. However, with numerous subclasses of cells present in the bone marrow, these methods – colony formation and stem cell identification by cell surface markers – do not provide information regarding the anatomical location of putative SSCs in the bone marrow. Histologically, most of bone marrow stromal cells (BMSCs) – the nomenclature inclusive of both primitive and differentiated skeletal lineage cells – simultaneously express C-X-C motif chemokine ligand 12 (CXCL12) and leptin receptor (LepR), particularly in adult stages. However, fundamental cellular heterogeneity of CXCL12^{+}LepR^{+} BMSCs has remained unresolved, without definitive information regarding in vivo localization of putative SSCs. This is due at least in part to technical difficulties in calibrating cell surface marker expression in histology and flow cytometry.

Omics approaches, particularly scRNA-seq analyses, have enabled the examination of thousands of gene expressions at a single-cell level. The genome-wide interrogation of gene expression of individual cells allows to uncover the cellular heterogeneity in the bone marrow in an unbiased manner. In 2019, a series of scRNA-seq studies documented the fundamental heterogeneity of cells of the skeletal lineage at a single cell level. First, the transcriptomic landscape of vascular, perivascular, and osteoblast cell populations was mapped in the mouse bone marrow at a single-cell resolution both in homeostasis and under conditions of fluorouracil-administrated stress-induced hematopoiesis [40]. Another study performed a single-cell analysis of non-hematopoietic bone marrow cells and generated the atlas of BMSCs under steady-state as well as leukemic states [41]. Similarly, profiling of mouse BMSCs by scRNA-seq revealed distinct subtypes of BMSCs, including pre-adipocyte-like, pre-osteoblast-like, and chondrocyte-like cells [42]. Subsequently, detailed cellular heterogeneity of bone marrow stromal cells was clarified, establishing the concept that CXCL12^{+}LepR^{+} BMSCs are composed of two major types of osteogenic stromal cells with pre-osteoblast-like (Osteo-CAR cells) and pre-adipocyte-like (Adipo-CAR cells) characteristics [43,44]. This Adipo-CAR subset is almost equivalent to marrow adipogenic lineage precursors (MALPs), which was identified by scRNA-seq analysis of cells marked by *Col2a1-cre* [45]. These studies have collectively defined the fundamental diversity of skeletal cell lineages in the bone marrow, wherein an adipogenic stromal cell subset highly expresses *Lepr*, *Cxcl12*, and *Adipoq*, while an osteogenic stromal cell subset strongly expresses *Periostin (Postn)* and *C-type lectin domain containing 11a* (*Clec11a*). Computational trajectory analysis using Monocle predicts that the osteogenic stromal cell populations are destined toward other stromal populations, including adipogenic stromal cells [44,46]. In another study, however, RNA velocity revealed that osteogenic stroma and adipogenic stroma have distinct cells-of-origins [47], indicating that the computational analysis alone is insufficient to provide a definitive answer to the cell lineage.

In recent years, it has become clear that BMSCs undergo rapid changes in cell populations according to distinct stages of development – for examples, young, adult, and aging stages [48–51]. ScRNA-seq analyses of BMSCs reveal that the fraction of adipogenic stromal cell subsets highly expressing CXCL12 increases in aging [50]. *Paired related homeobox 1* (*Prrx1*)*-cre* marks all cells of the skeletal cell lineage in the appendicular skeleton i.e., limb long bones. Single-cell multi-omics analyses of cells marked by *Prrx1-cre* at young (3 weeks) and old (1.5 year) stages in mice revealed the landscape of the skeletal cell lineage in long bones. Multi-omics analyses led to several important discoveries (Fig. 1). First, these analyses identified a previously unidentified stromal cell population carrying characteristics of both osteoblasts and chondrocytes, namely osteoblast-chondrocyte transitional (OCT) stem cells. Importantly, these OCT stem cells are particularly abundant in young bone marrow but become depleted in old bone marrow. Interestingly, these OCT stem cells simultaneously express an intermediate level of markers for chondrocytes, osteoblasts, and adipocytes. *Fibroblast growth factor receptor 3 (Fgfr3)* is highly expressed in the OCT population. RNA velocity analysis [52,53] demonstrates that OCT stem cells are likely to

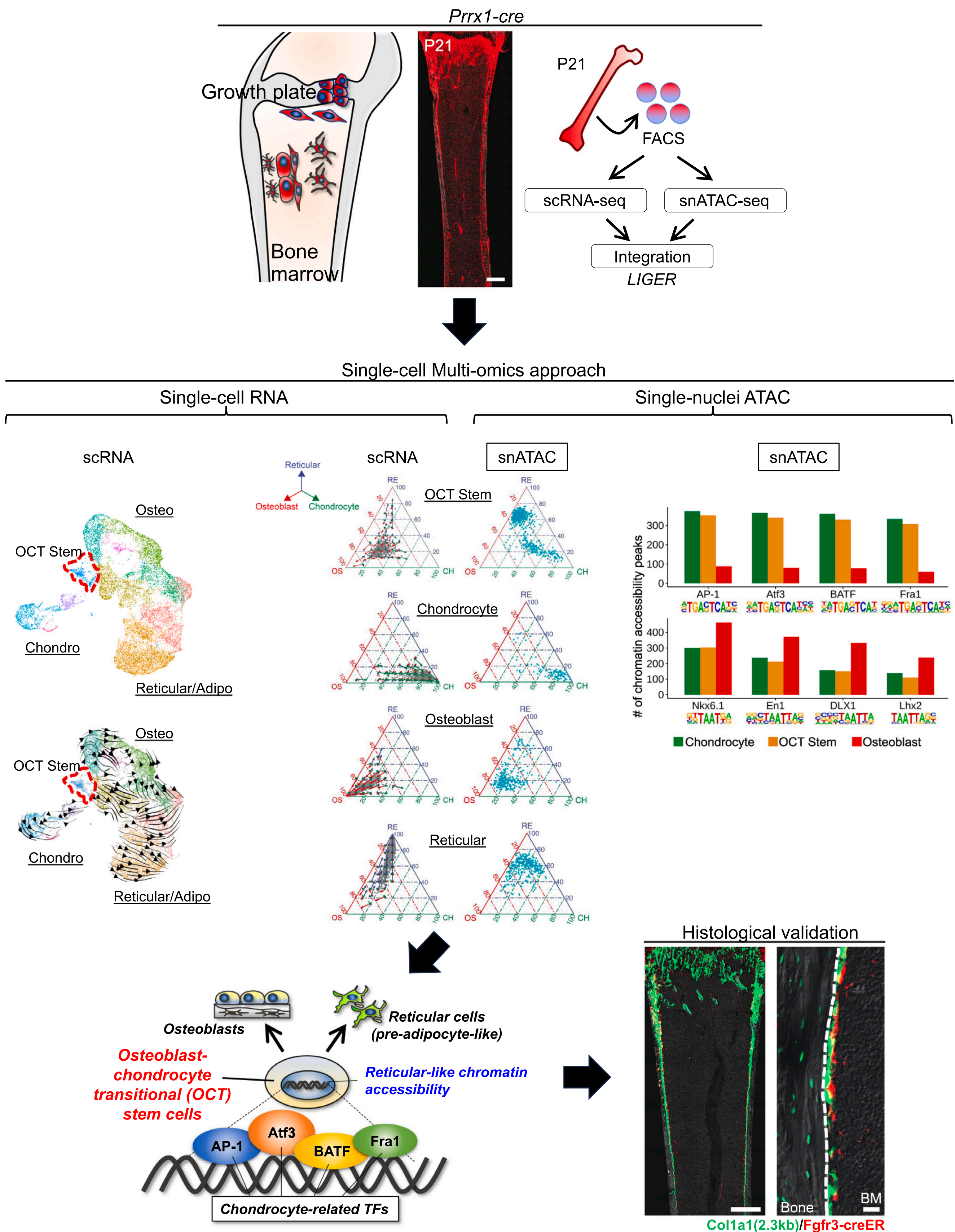

Fig. 1. Representative strategy of single-cell multi-omics approach (scRNA-seq + snATAC-seq) in bone. FACS-isolated *cre* driver-marked tdTomato+ cells are analyzed by scRNA-seq and snATAC-seq, and then, the data are integrated by appropriate algorithms such as LIGER. Combined multi-omics analyses reveal not only cellular diversity but also the state and dynamics. Validation analyses using in vivo genetic lineage-tracing unveil cell fates of omics-identified cell types. Matsushita et al. [50] Nat Commun 14:2383 2023.

provide a cells-of-origin of both osteoblasts and pre-adipocyte-like reticular stromal cells. In contrast, the potential cell-of-origin shifts to the CXCL12^{+} adipogenic stromal cell subset in old bone marrow, with pre-adipocyte-like reticular cells being a much higher probability as a cells-of-origin. This indicates that the cells-of-origins may change between young and old stages. Therefore, these studies demonstrate the utility of scRNA-seq analyses in uncovering putative spatiotemporal-specific skeletal stem cell populations.

Moreover, single-cell multi-omics (snATAC-seq + scRNA-seq) analyses reveal detailed states of OCT stem cells, in terms of their transcriptomic or epigenomic similarity to those of chondrocytes, osteoblasts, and pre-adipocyte-like reticular cells. Particularly, OCT stem cells demonstrate an equal similarity to three differentiated cell states, indicating a relatively unprimed and multipotent cell state. In contrast, chondrocytes, osteoblasts, and reticular cells have a strong similarity to only one fate, suggesting that these cells are more committed to a specific terminally differentiated state with less malleability. In addition, the combined RNA velocity analysis demonstrates that OCT stem cells possess "trilineage" potential for all three fates, predominantly inclined toward osteoblast and reticular fates. Intriguingly, the chromatin accessibility profiles of OCT stem cells indicate a stronger inclination toward the reticular state than predicted from the gene expression profile. These results suggest that the stromal cells' epigenomic regulatory landscape is primed for the pre-adipocyte-like reticular state, and the cells require stimuli from the environment to activate their osteoblast fates. The analysis of transcription factor (TF) binding motif enrichment in OCT stem cells showed enrichment of chondrocyte-related motifs in chromatin accessibility peaks, suggesting the OCT stem cells are still being regulated primarily by chondrocyte-related TFs. Thus, single-cell multi-omics successfully unveiled the fundamental molecular identities and regulatory mechanisms of putative skeletal stem cell populations that are particularly abundant in young bone marrow [50].

4. Validation of omics analysis in developmental bone biology

The application of various omics and multi-omics approaches has advanced the field of developmental bone biology, as noted above. However, the caveat is that these omics analyses can only provide predictions regarding the gene regulatory network based on highly complex computational analyses. Therefore, it is essential to validate the omics-based computational prediction using independent biological approaches. Most importantly, current omics approaches lack spatial information regarding the location, which is permanently lost during the process of cell isolation. This necessitates a careful validation of the outcomes of omics analyses in most instances. In developmental bone biology, in vivo lineage-tracing approach using mouse genetic models is the gold standard to define cell fates of identified cell types. This approach is widely used to label a particular group of cells at a given time point. Single-cell omics analyses can define cellular diversity at various stages of development. However, scRNA-seq alone only provides a snapshot of diverse skeletal cell groups at a given time. We argue that, if we combine scRNA-seq analyses are combined with in vivo lineage-tracing approaches, a static cell population map based on transcriptomic profiles without spatial information can transform into a dynamic cell population map encompassing the cell location and its differentiation trajectory. This will become a highly valuable tool that comprehensively interrogates the fate of each cell population. Therefore, these high-dimensional combinatory approaches can bring innovations in developmental bone biology, if multiple *cre* or *creER* lines with sufficient spatiotemporal specificity are utilized to assess cellular and molecular dynamics.

Prrx1-cre represents a useful mouse genetic tool to investigate the entire spectrum of the skeletal cell lineage. *Prrx1-cre* marks essentially all skeletal cells in limb bud, but it also marks other cells in the interlimb flank mesoderm of the latissimus dorsi muscle as well as a subset of cells derived from periocular mesenchyme [54]. At the cellular level, *Prrx1-cre* marks chondrocytes, cells of the perichondrium and periosteum, osteoblasts, and connective tissue fibroblasts including tendon and synovial cells [55]. The future skeletal element is defined by mesenchymal condensations occupied by SOX9-expressing cells. SOX9 is a key chondrogenic transcription factor that instructs undifferentiated mesenchymal cells to condense and differentiate into chondrocytes and perichondrial cells [55–58]. SOX9 expression is absolutely necessary for undifferentiated mesenchymal cells to remain organized within the condensation [55,59]. In vivo lineage-tracing experiments using *Sox9-cre/creER* reveal that Sox9^{+} cells in the condensation act as osteo-chondro-progenitor cells, as these cells differentiate into chondrocytes and perichondrial cells in the following stage [55,56,60]. Recently, scRNA-seq analysis at the condensation stage using *Prrx1-cre* (E11.5 in mice) identified the presence of peri-condensation cells expressing Notch effector Hes1. These Hes1^{+} peri-condensation cells contribute to perichondrial cells and invade into the condensation to differentiate into chondrocytes within the cartilage template during the early embryonic stage, which has been demonstrated by in vivo lineage-tracing experiments using *Hes1-creER* (Fig. 2a) [61].

The cartilage template and its surrounding perichondrium are formed in the following stage of mesenchymal condensations. Chondrogenic cells in the cartilage template are marked by *Sox9-cre/creER*, *Col2a1-creER*, or *Acan-creER* lines [56]. However, these *cre/creER* lines also target some populations of perichondrial cells in addition to chondrocytes within the cartilage template. Therefore, these lines cannot precisely define the fate of chondrocytes within the cartilage template. The perichondrium is located immediately outside the cartilage template. Cells in the perichondrium express a variety of markers such as *Ptch1*, *Gli1*, *Osx*, and *Col1a1* [62,63]. During the formation of the primary ossification center, perichondrial cells marked by *Osx-creER* translocate into the cartilage template along with blood vessels, and subsequently contribute to osteoblasts and marrow stromal cells [63]. However, descendants of Osx-expressing perichondrial cells are transient and eventually disappear from their destination during the postnatal stage [64]. Therefore, the identity of early perichondrial cells preceding Osx^{+} perichondrial cells remained undefined.

Again, scRNA-seq has been proven useful in identifying novel potential cells-of-origins at this stage. Computational cell fate mapping of cells marked by *Col2a1-cre* at E13.5 using RNA velocity and CellRank predicts two distinct cells-of-origins of the chondrocyte-perichondrial cell lineage, one being chondrocytes highly expressing *fibroblast growth factor receptor 3 (Fgfr3)* and another being perichondrial cells highly expressing *Dlx5* encoding a homeobox protein [65]. Notably, Fgfr3-expressing cells are histologically more restricted than Sox9- or Col2a1-expressing cells, and highly specific to chondrocytes within the cartilage template. Similarly, Dlx5-expressing cells are histologically distinct from Osx-expressing cells in the perichondrium, primarily located in the outer perichondrium. In other words, Fgfr3 is expressed in the central region of Sox9^{+}Col2a1^{+} cartilage template, while Dlx5 is expressed in the outer perichondrium immediately adjacent to Col1a1^{+} and Osx^{+} osteogenic perichondrium (Fig. 2b). In vivo lineage-tracing analyses using *Fgfr3-creER* and *Dlx5-creER* reveal that Fgfr3^{+} fetal chondrocytes in the center of the cartilage template contribute to metaphyseal skeletal cells, including growth plate chondrocytes, osteoblasts, and BMSCs. In contrast, Dlx5^{+} early perichondrial cells differentiate into skeletal cells in the diaphysis of long bone. Therefore, the combination approach of single-cell omics analysis followed by in vivo lineage-tracing approaches reveals in situ cellular dynamics in developing bones, particularly pertaining to fetal chondrocytes in the center of the cartilage template and early perichondrial cells in the outer layer of the perichondrium that become distinctively defined subsets of skeletal cells in the adult bone marrow.

The utility of cell type-specific *cre/creER* lines is further demonstrated below. As described above, Gli1 is expressed in the perichondrium, and *Gli1-creER* predominantly labels perichondrial cells and a

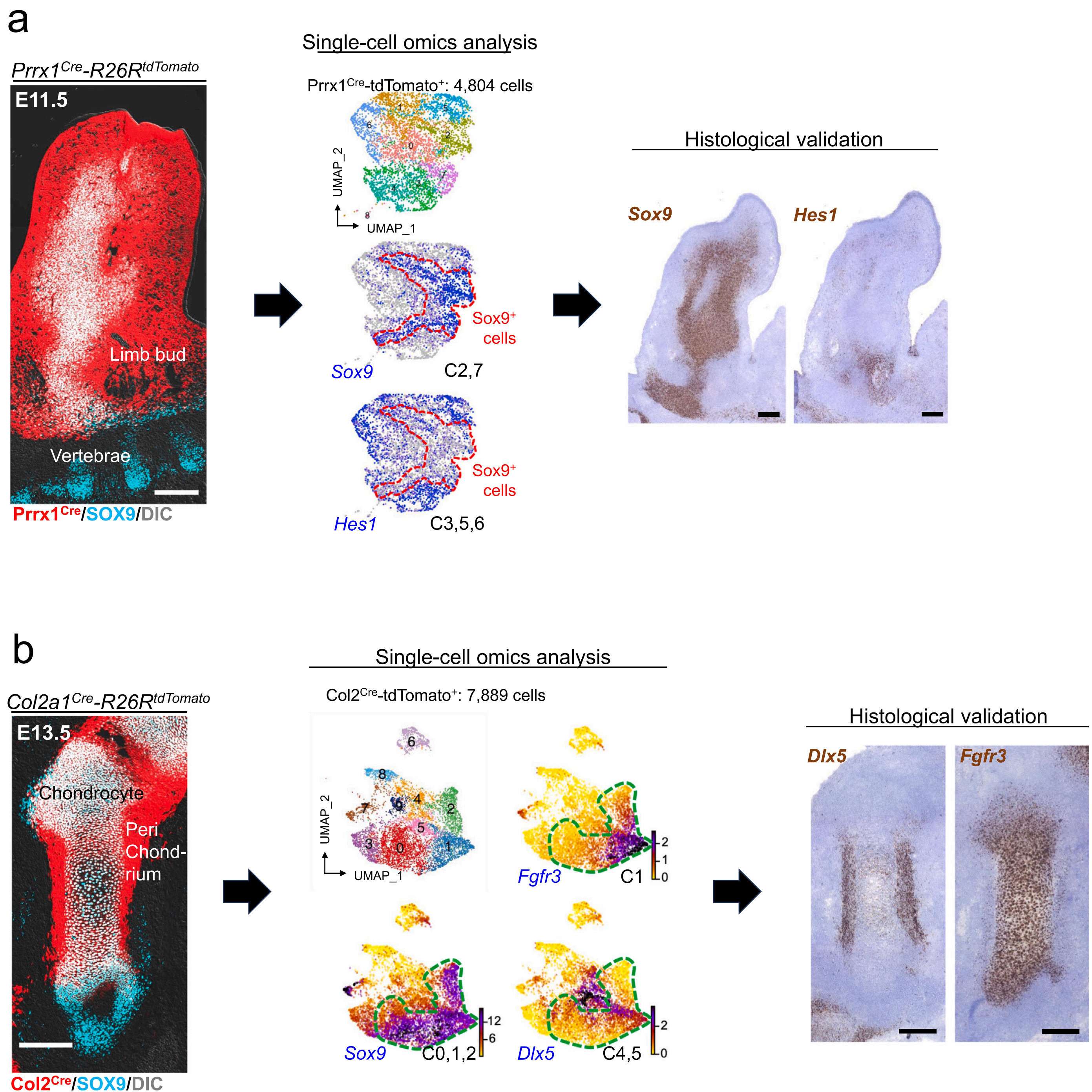

Fig. 2. Single-cell omics approach and subsequent validation analysis discover the new cell population in developing bone. **a.** scRNA-seq analysis using *Prrx1-cre*, which marks all mesenchymal cells in limb bud, at the condensation stage (E11.5 in mice) revealed the presence of peri-condensation cells expressing *Hes1*. **b.** scRNA-seq analysis using *Col2a1-cre*, which marks most skeletal cells, at the cartilage template stage (E13.5 in mice) elucidated the outer layers of perichondrial cells expressing *Dlx5*.
a. Matsushita et al. [61] J Biol Chem 299:104805 2023. b. Matsushita et al. [65] Nat Commun 13:7319 2022.

small number of chondrocytes at this stage. These cells generate multiple cell types within the skeleton [66]. *Col10a1-cre* or *Col10a1-creER* marks hypertrophic chondrocytes that directly contribute to postnatal osteoblasts and marrow stromal cells without mediating through perichondrial or periosteal cells. [67,68]. *Cathepsin K (Ctsk)-cre* marks cells in the perichondrial area during the embryonic stage, and these cells contribute to periosteal cells [69]. The landscape and dynamics of the skeletal cell lineage in developing bones have been elucidated by the combination of single-cell omics and subsequent in vivo lineage-tracing analyses.

The utility of combined scRNA-seq and in vivo lineage-tracing studies extends beyond early bone development. Postnatally, in young stage at P21, the multi-omics analysis of cells marked by *Prrx1-cre* in long bones demonstrates the heterogeneity of BMSCs, which are composed of distinct subpopulations. Fgfr3 is strongly expressed in OCT stem cells, whereas Gas1 is expressed in its immediate downstream population. In vivo lineage-tracing studies using *Fgfr3-creER* and *Gas1-creER* reveal that a subpopulation of $Fgfr3^{+}$ cells reside in the endosteal space and behave as SSCs that physiologically contribute to osteoblasts and marrow stromal cells, whereas $Gas1^{+}$ cells contribute as transit-

amplifying cells [50]. In the adult stage, MALPs marked by *Adipoq-cre/creER* have a substantial role in maintaining marrow vasculature and suppressing bone formation in the bone marrow. MALPs possess pre-adipocyte-like properties based on scRNA-seq [45] with overlapping properties with Adipo-CAR cells [43,44]. Thus, molecular and cellular dynamics of a given skeletal cell population can be clarified at a finer scale through the combination of multi-omics and in vivo lineage-tracing approaches. Further progress in multi-omics is expected to reveal additional in-depth intercellular interactions that support fundamental mechanisms of bone development and regeneration.

5. Spatial omics approach in bone biology

As mentioned above, the most important limitation of the current omics approaches is the loss of spatial information. To solve this problem, spatial omics approach has been rapidly developed in recent years [70]. The term “spatial transcriptomics” was first used in 2016. This approach enables visualization and quantification of the transcriptome in a manner linked to the cell’s location in tissue sections using unique positional barcodes [71]. Moreover, the combination of microarray-based spatial transcriptomics and scRNA-seq data from the same sample allows to uncover complex intercellular interactions within tissues [72]. Although the concept is groundbreaking, the size of the barcodes of current commercially available platforms often exceeds far beyond the size of each individual cell, with a diameter of 100 μm and a center-to-center distance of 200 μm, which limits their applicability to single-cell experiments [71].

Spatial transcriptomics with an improved resolution has been developed in recent years. Slide-seq achieves a nearly single-cell level by uniquely DNA-barcoded 10 μm microparticle beads [73]. This approach successfully revealed the spatial gene expression patterns in the Purkinje layer of the mouse cerebellum, and tracked the temporal evolution of cell type-specific responses in a mouse model of traumatic brain injury [73]. A computational framework has been developed by combining Slide-seq with targeted in situ RNA sequencing, which accurately localizes testicular cell types in individual seminiferous tubules. The framework demonstrates significant differences in the cellular composition of spermatogonial microenvironments between mouse and human testes [74]. The efficiency of the transcriptome detection of Slide-seq is now rapidly improving, almost approaching that of droplet-based single-cell RNA-seq techniques. For example, in the new version Slide-seqV2, bead synthesis and array indexing are improved to achieve an RNA capture efficiency of ~50% that of single-cell RNA-seq (~10-fold greater than Slide-seq) [75].

Deterministic barcoding in tissue for spatial omics sequencing (DBiT-seq) co-map mRNA and proteins in a formaldehyde-fixed tissue slide. The study uncovered the spatial arrangement of major tissue types in mouse embryos, including the retinal pigmented epithelium and microvascular endothelium at a cellular level [76]. Sci-Space can maintain single-cell resolution while simultaneously analyzing spatial heterogeneity at larger scales. It elucidates approximate spatial coordinates and whole transcriptomes of about 120,000 nuclei of developing mouse embryos [77]. Seq-Scope, a technology utilizing a spatial barcoding method with a resolution comparable to an optical microscope, can achieve submicrometer resolution and efficient transcriptome capture using a special barcode known as the high-definition map coordinate identifier. The center-to-center resolution of the image was found to be approximately 0.6 μm on average, which allowed for the spatial analysis of individual cells and subcellular structures in both the liver and colon [78]. Stereo-seq with a center-to-center distance of 500 or 715 nm uses approximately 220 nm DNA nanoball-patterned arrays and in situ RNA capture enables large field-of-view spatial transcriptomics at cellular resolution and reveals the spatial cell-type heterogeneity and the cell fate specification in mouse embryonic tissues [79]. The resolution of spatial transcriptome analyses has significantly improved in recent years.

Multi-omics approach with spatial technologies has made substantial progress. To investigate the epigenetic mechanisms underlying gene expression regulation, several new technologies have been developed. Spatial CUT&Tag revealed the spatial histone modification profiling. It combines the deterministic barcoding with the cleavage under targets and tagmentation (CUT&Tag) chemistry [80]. Spatially resolved, genome-wide, joint profiling of the epigenome and transcriptome by co-sequencing chromatin accessibility and gene expression (spatial ATAC–RNA-seq), or histone modifications (H3K27me3, H3K27ac or H3K4me3) and gene expression on the same tissue section at near-single-cell resolution (spatial CUT&Tag–RNA-seq) are of great interest in biomedical research [81]. These newer generations of omics technologies are expected to generate substantial new insights into developmental bone biology [82–87].

6. Conclusion

Developmental bone biologists now have two valuable tools to interrogate molecular and cellular profiles of individual cells: single-cell omics and multi-omics, which have the power to uncover skeletal cell diversity and predict their developmental paths, and mouse in vivo lineage-tracing approaches, which enable spatiotemporal validation of the computational predictions in vivo. Unraveling the dynamics of skeletal lineage cells has contributed to enhanced views on the fundamental process of bone development, providing potentially important insights into bone diseases. Platforms and algorithms for single-cell omics analyses are rapidly advancing. Single-cell omics and multi-omics allow us to learn more deeply about the state and dynamics of individual cells. The most recent single-cell spatial transcriptomic method is expected to significantly advance our understanding on fundamental molecular and cellular mechanisms of bone development and regeneration.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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