Local signaling specifies tissue-resident fibroblasts from multipotent sclerotome progenitors in zebrafish

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ABSTRACT

Fibroblasts play an important role in maintaining tissue integrity by secreting components of the extracellular matrix and initiating response to injury. Although the function of fibroblasts has been extensively studied in adults, the embryonic origin and diversification of different fibroblast subtypes during development remain largely unexplored. Using zebrafish as a model, we show that the sclerotome, a sub-compartment of the somite, is the embryonic source of multiple fibroblast populations, including tenocytes (tendon fibroblasts), blood vessel associated fibroblasts, and fin mesenchymal cells. High resolution imaging shows that different fibroblast subtypes occupy unique anatomical locations with distinct morphologies. Photoconversion-based cell lineage analysis reveals that sclerotome progenitors at different dorsal-ventral and anterior-posterior positions display distinct differentiation potentials. Single cell clonal analysis suggests that sclerotome progenitors are multipotent, and the fate of their daughter cells is biased by their migration paths and relative positions. Using a small molecule inhibitor, we show that BMP signaling is required for the development of fin mesenchymal cells in the peripheral fin fold. Together, our work demonstrates that the sclerotome contains multipotent progenitors that respond to local signals to generate a diverse population of tissue-resident fibroblasts.

AUTHOR SUMMARY

Fibroblasts are present in most organs in our body. They not only provide structural support to corresponding tissues, but also play important roles in wound healing and tissue fibrosis. Although the function of fibroblasts has been well appreciated, how different tissue-resident fibroblasts emerge during embryonic development is still poorly understood. Using the zebrafish model, we identify the sclerotome, a sub-compartment of the embryonic somite, as the main source of multiple fibroblast populations. Different fibroblast subtypes display distinct morphologies and locate at unique positions to support different tissues, including the muscles, blood vessels and the fin fold. Using cell tracing in live animals, we find that single sclerotome progenitors are able to generate more than one type of fibroblasts. The differentiation potential of sclerotome progenitors is biased by their initial locations in the trunk as well as their migration directions and relative positions. Local BMP signaling in the fin fold is essential for the proper development of sclerotome derived fin mesenchymal cells. Together, our results show that local microenvironment contributes to the diversification of multipotent sclerotome progenitors into distinct fibroblast subtypes.

INTRODUCTION

Fibroblasts are connective tissue cells that are present in most organs in animals. They are traditionally viewed as tissue support cells by synthesizing and remodeling extracellular matrix (ECM) components. Recent work has shown that tissue-resident fibroblasts also play important regulatory roles in wound healing, inflammation, tumor microenvironment, and tissue fibrosis (Kalluri, 2016; Kendall and Feghali-Bostwick, 2014; Thulabandu et al., 2018). Although fibroblasts are historically viewed as a homogenous population of cells, recent work using single-cell RNA sequencing (scRNA-seq) has revealed a high level of heterogeneity in the fibroblast population from different tissues as well as within the same tissue (Muhl et al., 2020; Tabib et al., 2018; Xie et al., 2018). It thus raises the question how different fibroblast subtypes are generated during embryonic development.

One mechanism to diversify fibroblast populations is through contributions from multiple embryonic sources. Previous studies suggest that fibroblasts of the same tissue can have heterogeneous embryonic origins. For example, in avian and mouse embryos, dermal fibroblasts in the head are of neural crest origin, while dermal fibroblasts in the dorsal and ventral trunk originate from the somite and the lateral plate mesoderm, respectively (Thulabandu et al., 2018). Similarly, during heart development in chick and mouse embryos, most cardiac fibroblasts originate from lateral plate mesoderm derived epicardium, while a small population arises from an endothelial/endocardial lineage (Tallquist, 2020).

The somite is the embryonic source of many tissue support cells, including fibroblasts. During development, the somite forms three separate domains: the dermatome, the myotome, and the sclerotome. The sclerotome contributes to the axial skeleton and cartilage of the animal. Work in mouse and chick has shown that the sclerotome is the developmental origin of tendon fibroblasts (tenocytes) (Brent et al., 2003; Schweitzer et al., 2001) and vascular smooth muscle cells (Pouget et al., 2008; Wiegrefe et al., 2007). In zebrafish, the sclerotome has a unique bipartite organization and contributes to tenocytes, perivascular fibroblasts, and pericytes in a stereotypical manner (Ma et al., 2018; Rajan et al., 2020). It raises the question about the cellular mechanisms that govern the diversification of fibroblast subtypes from sclerotome progenitors.

In fish and amphibians, fin mesenchymal cells are a population of fibroblasts present in the larval fin fold. They express many ECM components to provide structural support to the developing fin fold (Durán et al., 2011; Feitosa et al., 2012). However, the developmental origin of fin mesenchymal cells has been controversial. Dye labeling experiments in *Xenopus* and zebrafish suggest that the neural crest contributes to the fin mesenchyme (Smith and Hall, 1990; Smith et al., 1994). However, later experiments in *Xenopus* suggest that the mesoderm also contributes to fin mesenchymal cells in both

the dorsal and ventral fin (Garriock and Krieg, 2007; Tucker and Slack, 2004). Similarly, cell transplantation experiments in axolotls reveal dual origin of the fin mesenchyme from both the neural crest and the somites (Sobkow et al., 2006). More recent work using genetic lineage tracing in fish (zebrafish) and amphibians (*Xenopus* and axolotl) supports a model where fin mesenchymal cells originate exclusively from the mesoderm with no contribution from the neural crest lineage (Lee et al., 2013; Taniguchi et al., 2015). In particular, the dermomyotome compartment of the somite has been suggested to be the source of fin mesenchymal cells in zebrafish (Lee et al., 2013).

Here, we show that the sclerotome is a major source of multiple distinct fibroblast populations in zebrafish embryos. Different sclerotome derived fibroblast subtypes display unique morphologies and occupy distinct anatomical locations. In vivo time-lapse imaging and single cell clonal analysis reveal that sclerotome progenitors are multipotent, and their differentiation potential is determined by their positions as well as their migratory patterns. Specifically, fin mesenchymal cells are specified by local BMP signals at the peripheral fin fold. Together, our results suggest that distinct fibroblast subtypes are specified from multipotent sclerotome progenitors by local cellular environments.

RESULTS

The zebrafish sclerotome generates distinct fibroblast subtypes

To explore the lineage potential of the sclerotome, we developed a sclerotome-specific reporter line, *nkx3.1:Gal4; UAS:NTR-mCherry* (*nkx3.1^{NTR-mCherry}*, similar designations are used for all Gal4/UAS transgenic lines in this paper) (Ma et al., 2018). Due to the perdurance of the mCherry protein, we were able to label the initial sclerotome domains as well as all their descendants. We crossed *nkx3.1^{NTR-mCherry}* to the endothelial reporter *kdrl:EGFP* to examine the different populations of sclerotome derived cells at 2 dpf (days post fertilization). Based on their anatomical locations, we broadly defined four main groups of mCherry⁺ cells from the sclerotome lineage (Fig 1A and 1B): 1) cells located in both dorsal and ventral fin folds; 2) cells closely associated with blood vessels in the trunk, including the dorsal longitudinal anastomotic vessel (DLAV), intersegmental vessels (ISV), the dorsal aorta (DA), and the caudal vein plexus (CVP); 3) tenocytes between neighboring somites as we have previously described (Ma et al., 2018); and 4) interstitial cells located in the space between the notochord, the spinal cord and muscles. It is worth noting that the *nkx3.1^{NTR-mCherry}* line also drove expression in some muscle cells (Ma et al., 2018) and in some cuboidal cells at the edge of the fin fold (Fig 1B), neither of which were derived from the sclerotome lineage. We have previously shown that both tenocytes and ISV-associated perivascular fibroblasts express the pan-fibroblast markers such as *col1a2* and *col5a1* (Ma et al., 2018; Rajan et al., 2020). We asked whether other sclerotome derived cells are also fibroblasts. Indeed, all four groups of *nkx3.1^{NTR-mCherry}*-expressing cells were positive for *col1a2:GFP*, a pan-fibroblast reporter, at 2 dpf (Fig S1C). This result suggests that the sclerotome is the embryonic source of multiple types of fibroblasts in zebrafish.

At 2 dpf, the fin fold is subdivided into two lobes (Parichy et al., 2009) (Fig S1A). The major lobe arises from the dorsal edge of somite 7, wraps around the trunk and the tail, and ends at the ventral edge at somite 18 where it meets the end of the yolk extension. The minor lobe extends ventrally underneath the yolk extension. In *nkx3.1^{NTR-mCherry}* embryos, mCherry⁺ cells can be seen populating both the dorsal and ventral regions in the major lobe of the fin fold (Fig 1B and S1B). Using a mosaic *col1a2:Gal4; UAS:Kaede* line (referred to as *col1a2^{Kaede}*) (Sharma et al., 2019), we visualized the cell morphology at high resolution (Fig 1C). These Kaede⁺ fin cells were characterized by extensive “tree-like” cellular processes projecting towards the periphery of the fin fold (Fig 1B and 1C). The location, morphology, as well as the marker expression (shown later) suggest that these sclerotome derived fin fold residing cells correspond to fin mesenchymal cells as previously described (Feitosa et al., 2012; Lee et al., 2013). Interestingly, mCherry⁺ fin mesenchymal cells were absent in the fin fold at the tip of

the tail and the minor lobe (Fig S1B), suggesting that the sclerotome is not the only lineage that gives rise to fin mesenchymal cells.

Within the zebrafish trunk, many sclerotome derived fibroblasts were found associated with different vascular beds (Fig 1A and 1B). We have previously described ISV-associated perivascular fibroblasts, characterized by a globular cell body with a few short processes extended around the ISV (Rajan et al., 2020). By contrast, fibroblasts associated with DLAV or DA (referred to as DLAV fibroblasts and DA fibroblasts, respectively) were more elongated along the long axis of the blood vessel (Fig 1B and 1C). The CVP is a transient venous network with a stereotypical “honeycomb-like” structure. Fibroblasts marked by *nkx3.1^{NTR-mCherry}* were found occupying the space in the honeycomb pockets of the CVP (Fig 1B). Mosaic labeling with *col1a2^{Kaede}* revealed that individual Kaede⁺ cells had a small and elongated cell body with long cellular processes along the underlying endothelium and sometimes across the honeycomb pocket (Fig 1C). Their location and morphology suggest that these CVP-associated fibroblasts are previously described stromal reticular cells (SRCs) (Murayama et al., 2015).

Finally, tenocytes and interstitial fibroblasts were two fibroblast subtypes that populated in the trunk region but were not closely associated with blood vessels. We have previously shown that tenocytes, marked by *scxa* and *tnmd* expression, are located along the myotendinous junction (MTJ), extending long cellular processes into the intersomitic space (Fig 1B and 1C) (Ma et al., 2018). By contrast, interstitial fibroblasts were scattered throughout the interstitial space, displaying varied morphology (Fig 1B and 1C). Together, our results show that the sclerotome generates multiple types of fibroblasts in the zebrafish trunk, supporting different tissues, such as muscles, blood vessels and the fin fold. These fibroblast subtypes display unique morphologies locating in distinct anatomical locations, some of which can be defined by specific marker expression.

Both dorsal and ventral sclerotome domains give rise to multiple types of fibroblasts

We have previously shown that the sclerotome is comprised of a dorsal and ventral domain, both of which contribute to tenocytes and perivascular fibroblasts (Ma et al., 2018; Rajan et al., 2020). We asked how each sclerotome domain contributes to different fibroblast subtypes. Taking advantage of the photoconvertible Kaede protein, we generated the *nkx3.1:Gal4; UAS:Kaede* line (referred to as *nkx3.1^{Kaede}*) to perform lineage tracing on sclerotome domains (Fig 2A). Either the dorsal or ventral sclerotome domain of a single somite was photoconverted from Kaede^{green} to Kaede^{red} at 24 hpf (hours post fertilization). We then determined the fate of Kaede^{red} cells after 24 hours based on their anatomical locations and cell morphologies. For the ventral sclerotome, we also compared the lineage between anterior somites before the end of yolk extension (somites 16-18) and posterior somites

(somites 23-25) as stromal reticular cells and ventral fin mesenchymal cells were only present in the more posterior region of the fish.

First, we labeled the dorsal sclerotome domain by Kaede photoconversion at 24 hpf. The presence of some small projections from these Kaede^{red} cells suggests that cell migration may be initiating at this stage (Fig 2B). By 48 hpf, multiple Kaede^{red} cells can be seen populating the dorsal region of the fish (Fig 2B). Based on their morphology and location relative to the vasculature, we determined that the cell types generated by the dorsal sclerotome domain included dorsal fin mesenchymal cells, DLAV fibroblasts, tenocytes, ISV-associated perivascular fibroblasts, and interstitial fibroblasts. All dorsal sclerotome derived cells were restricted within the upper half of the zebrafish trunk dorsal to the notochord.

We next carried out photoconversion experiments to mark the ventral sclerotome domain with Kaede^{red} at 24 hpf. Similar to the dorsal domain, small cellular projections were visible from Kaede^{red} cells, suggesting that both dorsal and ventral sclerotome progenitors initiate their migration at the same stage (Fig 2C). Interestingly, in anteriorly converted somites (Fig 2C, top panels), cellular projections pointed exclusively to the dorsal direction, while in posteriorly converted somites (Fig 2C, bottom panels), cellular projections can be seen pointing both dorsally and ventrally. This result suggests that the migration pattern is potentially different in ventral sclerotome progenitors between anterior and posterior somites, which likely accounts for cells generated in the CVP and ventral fin fold region. In anterior somites, the ventral sclerotome generated cells in the trunk, including tenocytes, perivascular fibroblasts, and interstitial fibroblasts by 48 hpf (Fig 2C, top panels). Importantly, Kaede^{red} descendants were only found dorsal to their starting position, and therefore, no SRCs or ventral fin mesenchymal cells were labeled. In contrast, in posterior somites, the ventral sclerotome domain gave rise to both cells in the trunk, including tenocytes, perivascular fibroblasts, and interstitial fibroblasts, as well as cells in the periphery ventral to the photoconverted region, such as SRCs associated with the CVP and fin mesenchymal cells in the ventral fin fold. All cells generated by the ventral sclerotome domain were restricted within approximately the ventral 2/3 of the zebrafish trunk.

Together, our result suggests that both dorsal and ventral sclerotome domains are able to generate multiple fibroblast subtypes, but the progenitors in each domain generate an anatomically restricted population of cells according to their dorsal-ventral and anterior-posterior positions.

Fate of dorsal sclerotome progenitors is determined by the migration direction

Both dorsal and ventral sclerotome domains contribute to multiple types of fibroblasts. It is possible that sclerotome progenitors are multipotent, or alternatively, individual progenitors are “fate-restricted” to differentiate into a specific subtype of fibroblast. To distinguish these two possibilities, we

performed single cell clonal analysis on sclerotome progenitors as previously described (Sharma et al., 2019). We focused on the smaller dorsal domain to ensure precise single cell photoconversion and accurate cell tracing. We selected *nkx3.1^{Kaede}* fish with more mosaic Kaede expression to minimize the photoconversion of multiple sclerotome progenitors. At 24 hpf, a single Kaede^{green} cell in the dorsal domain between somites 16 and 18 was photoconverted to Kaede^{red} (Fig 3A). We then imaged the same fish after 24 hours to determine the identity of Kaede^{red} descendants. Most clones (35/42, 83%) contained at least 2 daughter cells, suggesting at least one cell division during the 24-hour period (Fig 3B). From a total of 42 dorsal sclerotome progenitors, 38% of them (16/42) produced clones with at least 2 fibroblast subtypes, and 2% (1/42) generated 3 different types of fibroblasts (Fig 3C). This result suggests that most dorsal sclerotome progenitors are at least bi-potent and possibly multipotent at 24 hpf. Consistent with the domain tracing, we found that dorsal sclerotome progenitors gave rise to all 4 types of fibroblasts, including dorsal fin mesenchymal cells, tenocytes, blood vessel associated fibroblasts, and interstitial fibroblasts (Fig 3D). As described earlier, the individually labeled Kaede^{red} cells at 24 hpf often had small projections that pointed either dorsally or ventrally, likely corresponding to their migration direction. To correlate the progenitor morphology with their lineage potential, we categorized different fibroblasts into two types based on their final positions (Fig 3A). In the type I group, fibroblasts are located dorsal to the somite, and these include dorsal fin mesenchymal cells and DLAV fibroblasts. Type II cells, such as tenocytes, ISV associated perivascular fibroblasts, and interstitial fibroblasts, are located medial to the somite within the trunk region. Interestingly, progenitors with initial dorsal projections predominantly generated type I cells (13/14, 93%), whereas cells projecting ventrally more likely gave rise to type II cells (11/19, 58%) than type I cells (3/19, 16%), and sometimes generated cells of both types (5/19, 26%) (Fig 3E and 3F). It is important to note that ventral projecting progenitors at 24 hpf never generated dorsal fin mesenchymal cells and all type I cells generated by this group were DLAV fibroblasts found along the ventral side of the DLAV (Fig 3F). Together, our results suggest that individual dorsal sclerotome progenitors at 24 hpf are still multipotent; however, their migration direction likely restricts the types of cells they differentiate into.

Fate of ventral sclerotome progenitors is determined by the position along the migratory path

In posterior somites, some ventral sclerotome progenitors migrate ventrally towards the CVP region to generate SRCs and ventral fin mesenchymal cells. To visualize this dynamic process, we performed time-lapse imaging in *kdrl:EGFP; nkx3.1^{NTR-mCherry}* embryos from 25 to 50 hpf (Fig 4 and Video S1). At 25 hpf, some migrating mCherry⁺ progenitors can already be found ventral to the somite. These cells migrated ventrally towards the fin fold as more mCherry⁺ cells emerged from the

ventral somite and joined the migration. To better describe the position of migrating cells, we referred to cells at the very front edge of the migration as “leading cells”, while all cells behind were called “lagging cells”. We observed a stereotypical migration pattern whereby mCherry⁺ cells “marched” ventrally in an orderly fashion and individual cells did not pass other cells that were ahead. This orderly migration was largely maintained even in the event of cell divisions. As a result, descendants of leading cells remained at the front of the migration throughout the movie and formed ventral fin mesenchymal cells, whereas descendants of lagging cells mostly generated SRCs associated with the CVP. This result suggests that the fate of ventral sclerotome progenitors is determined by their position along the migratory path.

To further compare the lineage potential between the leading and lagging cells, we performed single cell clonal analysis using *nkx3.1^{Kaede}* fish (Fig 5A). Single ventrally migrating Kaede^{green} progenitors between somites 21-25 were photoconverted to Kaede^{red} at 24 hpf, and their descendants were imaged after 24 hours. Similar to our time-lapse movies, the majority of photoconverted leading cells generated only ventral fin mesenchymal cells (17/20, 85%), but a small number of them gave rise to both SRCs and ventral fin mesenchymal cells (3/20, 15%) (Fig 5B and 5C). Interestingly, leading cells never generated SRCs only. By contrast, 41% of lagging cells generated only SRCs (9/22), while 36% generated both ventral fin mesenchymal cells and SRCs (8/22), and 23% produced only ventral fin mesenchymal cells (5/22) (Fig 5B and 5C). It should be noted that the closer a lagging cell was to a leading cell, the more likely it generated a ventral fin mesenchymal cell, suggesting that lagging cells have the potential to differentiate into fin mesenchymal cells in the appropriate environment. Combining time-lapse imaging and single cell tracing, our results suggest that the relative position along the migratory path biases the fate of these sclerotome derived cells.

Fin mesenchymal cells respond to local BMP ligands

Our results have shown that the sclerotome contributes to fin mesenchymal cells in both dorsal and ventral fin folds. Previous studies have shown that fin mesenchymal cells are marked by *hemicentin2* (*hmcn2*) and *fibulin1* (*fbln1*), while the neighboring cell layer of apical epidermal cells is labelled by *hmcn2* and *fras1* (Carney et al., 2010; Feitosa et al., 2012; Lee et al., 2013) (Fig 6A). Indeed, double staining revealed two juxtaposing cell layers in the fin fold: *hmcn2⁺fbln1⁺* fin mesenchymal cells in the proximal layer and *hmcn2⁺fras1⁺* apical epidermal cells in the distal layer (Fig 6B). As expected, double staining in *nkx3.1^{NTR-mCherry}* embryos showed that mCherry⁺ cells in the fin fold co-expressed *fbln1* but not *fras1* (Fig 6C), confirming that the sclerotome contributes to fin mesenchymal cells, a specific cell population in the fin fold.

We hypothesized that the development of fin mesenchymal cells is mediated by local signals in the fin fold. To test this model, we performed expression analysis to identify signaling ligands enriched in the fin fold. Interestingly, we found that a number of *bmp* genes, including *bmp2b*, *bmp4*, and *bmp6*, were expressed specifically in *fras1*⁺ apical epidermal cells, adjacent to *fbln1*⁺ fin mesenchymal cells (Fig 6D and S2). Accordingly, *nkx3.1*^{NTR-mCherry}-positive fin mesenchymal cells were positioned along a layer of *bmp4*-expressing cells (Fig 6C). This result suggests that BMP ligands secreted from apical epidermal cells might activate BMP signaling in fin mesenchymal cells. To test this possibility, we crossed the *nkx3.1*^{NTR-mCherry} line to the *BRE:GFP* reporter to label BMP responsive cells (Collery and Link, 2011). At 2 dpf, mCherry⁺ interstitial fibroblasts in the trunk were largely GFP⁻, suggesting the absence of active BMP signaling (Fig 6E). By contrast, cells at the periphery showed different levels of BMP signaling: stromal reticular cells were weakly positive for *BRE:GFP*, while ventral fin mesenchymal cells displayed strong BMP response (Fig 6E). Consistent with this result, *nkx3.1*^{NTR-mCherry}-positive fin mesenchymal cells showed elevated level of phospho-Smad (pSmad), a sensitive readout of BMP signaling (Fig 6F). Together, our results suggest that local BMP ligands from apical epidermal cells activate robust BMP response in fin mesenchymal cells.

Development of fin mesenchymal cells is regulated by BMP signaling

Since fin mesenchymal cells show strong BMP signaling compared to other sclerotome derived cells, we hypothesized that active BMP signaling is required for the proper development of fin mesenchymal cells. To test this possibility, we utilized DMH1, a specific BMP receptor inhibitor (Hao et al., 2010), to block BMP signaling. Treatment of *BRE:GFP* fish with DMH1 from 10 to 24 hpf substantially reduced the GFP expression (Fig S3), confirming DMH1 as an effective inhibitor of BMP signaling. To examine whether BMP signaling regulates fin mesenchymal cell development, we treated *kdr1:EGFP; nkx3.1*^{NTR-mCherry} embryos with DMH1 from 18 hpf (prior to the migration of sclerotome derived cells) to 48 hpf. Similar to what has been described (Wiley et al., 2011), DMH1-treated fish showed poorly formed CVP, a structure dependent on active BMP signaling (Fig 7A). Strikingly, DMH1 treatment resulted in an obvious expansion of the fin fold, accompanied by a substantial reduction of mCherry⁺ fin mesenchymal cells in both the dorsal and ventral fin folds (Fig 7A). Quantification of the height of the fin fold showed 51% (dorsal fin) and 39% (ventral fin) expansion in DMH1-treated fish (Fig 7B). By contrast, quantification of a 10-somite region showed that inhibition of BMP signaling lead to 49% reduction of dorsal fin mesenchymal cells (from 54.2 to 27.7), and 63% decrease in ventral fin mesenchymal cells (from 39.8 to 14.6) (Fig 7C). Interestingly, the few remaining fin mesenchymal cells in DMH1-treated fish showed an aberrant cell morphology with more cellular projections compared to controls (Fig 7A). To determine whether BMP signaling

304 regulates the branching morphogenesis of fin mesenchymal cells, we performed late DMH1
305 treatments on *kdrl:EGFP; nkx3.1^{NTR-mCherry}* embryos at 25-49 hpf (Fig S4). Similar to early DMH1
306 treatment, fin folds in DMH1-treated embryos were significantly expanded (Fig S4A and S4B).
307 Although the number of fin mesenchymal cells was largely unaffected in DMH1-treated fish (Fig S4C),
308 they displayed the similar hyperbranching morphology (Fig S4A). Our results suggest that BMP
309 signaling is required in not only the early migration but also the late morphogenesis of fin
310 mesenchymal cells. To further characterize the regulation of fin development by BMP signaling, we
311 analyzed different fin markers in embryos treated with DMH1 at 18-45.5 hpf. As expected, DMH1
312 treatment resulted in reduced *fbln1* expression in fin mesenchymal cells, but lead to a substantial
313 expansion of apical epidermal cells marked by *bmp4* and *fras1* expression (Fig 7D). This result
314 suggests that the inhibition of BMP signaling compromises the development of fin mesenchymal cells,
315 and the expansion of apical epidermal cells leads to the overgrowth phenotype of the fin fold.
316 Together, our results suggest that local BMP signaling in the fin fold is required for the migration and
317 morphogenesis of fin mesenchymal cells.

DISCUSSION

Our work establishes the sclerotome as a new system to explore cell fate diversification in embryonic development. Three major conclusions can be drawn from our studies. First, the sclerotome is the embryonic source of distinct tissue-resident fibroblasts. Second, sclerotome progenitors are multipotent, and their differentiation is determined by their axial positions and migratory paths. Third, development of fin mesenchymal cells from sclerotome progenitors requires local BMP signals in the fin fold. Together, our work suggests that the local microenvironment contributes to the diversification of fibroblast subtypes from multipotent sclerotome progenitors during embryonic development.

The sclerotome is the embryonic source of tissue-resident fibroblasts

The embryonic sclerotome is known to give rise to the axial skeleton, including the bones and cartilage of the body. Our previous studies (Ma et al., 2018; Rajan et al., 2020) and our current work provide strong evidence that the sclerotome is the embryonic source of multiple types of fibroblast populations in the trunk. Sclerotome derived fibroblasts share the expression of the pan-fibroblast marker *col1a2*, and likely provide structural support to a variety of tissues. Based on their marker expression, cell location and morphology, we identify at least four fibroblast subtypes: tenocytes, fin mesenchymal cells, blood vessel associated fibroblasts, and interstitial fibroblasts. Tenocytes express classic tendon markers *scxa* and *tnmd* (Chen and Galloway, 2014; Ma et al., 2018). Their cell bodies are positioned medially along the myotendinous junction while extending long cellular processes into the intersomitic space (Ma et al., 2018). Tenocytes function to stabilize muscle attachment, likely by secreting a number of ECM proteins (Ma et al., 2018; Subramanian and Schilling, 2014). Fin mesenchymal cells, on the other hand, are marked by the expression of ECM genes *hmcn2* and *fbn1*. They are located adjacent to apical epidermal cells in the fin fold, extending tree-like processes distally. Recent work has shown that fin mesenchymal cells guide fin formation by aligning collagen fibers of actinotrichia (Kuroda et al., 2020). Genetic ablation of fin mesenchymal cells in the pectoral and median fin folds results in a collapse of the respective fins (Lalonde and Akimenko, 2018). By contrast, although blood vessel associated fibroblasts are closely associated with the vasculature, their morphologies differ depending on the vascular bed. For example, ISV-associated perivascular fibroblasts are more globular, whereas DLAV fibroblasts are more elongated. We have previously shown that perivascular fibroblasts play dual roles in stabilizing nascent ISVs as well as functioning as pericyte precursors (Rajan et al., 2020). It is likely that other blood vessel associated fibroblasts play similar roles in stabilizing the corresponding blood vessels. Interestingly, stromal reticular cells closely

interact with the CVP, which functions as the early hematopoietic niche during development (Wattrus and Zon, 2018). Indeed, recent work has shown that defects in SRC maturation result in a compromised CVP niche that fails to support hematopoietic stem cell (HSC) maintenance and expansion (Murayama et al., 2015). Therefore, SRCs might not only provide the structural support to the CVP but also modulate the microenvironment required for HSC differentiation. Compared to other fibroblast subtypes, interstitial fibroblasts are the least well characterized. Based on their distribution around the notochord and the spinal cord, it is conceivable that interstitial fibroblasts give rise to osteoblasts required for the vertebral column development. Together, our results suggest that the sclerotome generates distinct fibroblast subtypes to support a variety of tissues, including muscles, fins and blood vessels.

The developmental origin of fin mesenchymal cells

There has been considerable debate as to where fin mesenchymal cells originate from during fish and amphibian development. Lineage tracing studies in zebrafish suggest that the paraxial mesoderm, specifically the dermomyotome, is the embryonic source of fin mesenchymal cells (Lee et al., 2013), which contrasts with our findings. To rule out sclerotome contribution, Lee et al. (2013) use the *ola-twist1:Gal4* transgenic line, in which Gal4 expression is driven by the medaka (*Oryzias latipes*) *twist1* promoter. Interestingly, we have previously shown that the expression of a similar *ola-twist1:EGFP* reporter is restricted to sclerotome derived cells around the notochord, but absent in both dorsal and ventral sclerotome domains (Ma et al., 2018). This observation explains why the *ola-twist1:Gal4* line fails to label any fin mesenchymal cells (Lee et al., 2013). Interestingly, our results show that the sclerotome only contributes to fin mesenchymal cells in the dorsal and ventral portion of the major lobe of the fin fold. No sclerotome contribution to the minor lobe or the caudal tail fin in the major lobe could be observed. Combining with previous work (Lee et al., 2013), it is likely that fin mesenchymal cells are generated by multiple different lineages depending on their locations. Fin mesenchymal cells in the dorsal and ventral fin fold are of sclerotome origin, those in the tail fin originate from the dermomyotome, while fin mesenchymal cells in the minor lobe of the fin fold have an unidentified embryonic source.

Multipotent sclerotome progenitors are fated by local signals

One fundamental question in development biology is how distinct cell types are differentiated from a single progenitor cell. To explore this question, we used *nkx3.1*-based transgenic reporters to analyze the lineage diversification from the sclerotome. Photoconversion-based clonal analysis reveals that single sclerotome progenitors are capable of generating 2-3 distinct fibroblast subtypes.

This result suggests that individual sclerotome progenitors are likely multipotent, rather than “lineage-restricted” to differentiate into one specific fibroblast subtype. Our studies reveal three key features in cell fate diversification of sclerotome progenitors (Fig 7E). First, sclerotome progenitors display regional differences in their differentiation potential along the dorsal-ventral and anterior-posterior positions. Dorsally located fibroblasts, such as dorsal fin mesenchymal cells and DLAV fibroblasts, are derived from the dorsal sclerotome, while ventrally located fibroblasts, such as stromal reticular cells and ventral fin mesenchymal cells, are generated by the ventral sclerotome. Similarly, the ventral sclerotome in anterior somites (before the end of the yolk extension) generates only fibroblasts in the trunk, whereas the ventral sclerotome in posterior somites contributes to the SRC and fin mesenchymal cell populations in addition to fibroblasts in the trunk. Second, fates of sclerotome progenitors are biased by their migratory path. For dorsal sclerotome progenitors, dorsal migration limits the cell to become either dorsal fin mesenchymal cells or DLAV fibroblasts, while ventral migration leads to the differentiation of predominately tenocytes or perivascular fibroblasts. Third, the relative order of migrating sclerotome progenitors largely determines their differentiation potential. Leading cells are more likely to become ventral fin mesenchymal cells, while lagging cells tend to differentiate into SRCs. The combination of these three mechanisms results in the generation of different fibroblast subtypes in a stereotypic manner (Ma et al., 2018; Rajan et al., 2020).

How does a sclerotome progenitor decide which cell type to differentiate into? Analysis of the fin fold strongly suggests that local microenvironment plays an instructive role in cell fate diversification. In the fin fold, a number of BMP ligands, including *bmp2b*, *bmp4*, and *bmp6*, are expressed distally in apical epidermal cells. Due to the proximity to the BMP source, leading cells of migrating sclerotome progenitors are exposed to likely the highest level of BMP ligands. This is consistent with stronger BMP response in fin mesenchymal cells compared to stromal reticular cells, mostly generated by lagging cells. Therefore, different levels of BMP signaling might guide the migration and maturation of different sclerotome derived cells. Indeed, inhibition of BMP signaling by DMH1 greatly reduces the number of fin mesenchymal cells, suggesting that BMP signaling is required for the development of fin mesenchymal cells. The role of BMP signaling in fin mesenchymal cell development at the periphery mirrors the dependence of Hh signaling in tenocyte specification in the trunk (Ma et al., 2018), suggesting that local cell signaling pathways contribute to the differentiation of sclerotome progenitors. Taken together, our work on the sclerotome lineage suggests a model where local microenvironment determines the fate of sclerotome progenitors. The cell type diversification of the sclerotome lineage is remarkably similar to the neural crest lineage. Previous studies have shown that migrating neural crest cells are multipotent (Baggiolini et al., 2015), and the ultimate cell fates are determined by their axial positions, as well as the migration timing and direction (Rocha et al., 2020).

BMP signaling plays dual roles in fin fold development

The zebrafish fin fold is comprised of two juxtaposing cell layers: the proximal layer of fin mesenchymal cells and the distal layer of apical epidermal cells. Our studies reveal that BMP signaling regulates the development of both cell populations. First, BMP signaling is essential for the migration and specification of fin mesenchymal cells. This is likely mediated by paracrine BMP signaling from BMP-expressing apical epidermal cells to BMP-responding fin mesenchymal cells. Inhibition of BMP signaling results in a substantial reduction in the number of fin mesenchymal cells, and the few remaining cells often display an aberrant hyperbranching morphology. This result suggests that BMP signaling is required not only for the migration of fin mesenchymal cell precursors into the fin fold, but also their morphogenesis once they reach the destination. BMP signaling has been previously implicated in the regulation of cell migration in other *in vivo* contexts, including the migration of neural crest cells (Fu et al., 2006; Goldstein et al., 2005; Sela-Donenfeld and Kalcheim, 1999) and cortical neurons (Saxena et al., 2018). Interestingly, despite the loss of fin mesenchymal cells, blockage of BMP signaling results in a significant outgrowth of the fin fold. This phenotype is likely caused by the expansion of apical epidermal cells. Therefore, BMP signaling plays a second role in restricting the outgrowth of apical epidermal cells. A recent study suggests that the fin fold expansion upon BMP inhibition is likely caused by prolonged cell proliferation, altered cell division orientation as well as excessive distal cell migration in the fin fold (Ka et al., 2020). Although our results strongly argue that BMP signaling is required autonomously in fin mesenchymal cells for its normal development, we cannot rule out the possibility that the altered ECM environment due to the expansion of apical epidermal cells contributes to certain aspects of the phenotype as previously suggested (Feitosa et al., 2012). Together, our results suggest a model where BMP signaling regulates the recruitment and morphogenesis of fin mesenchymal cells while controlling the size of the fin fold. Interestingly, fin mesenchyme migration defects have been previously proposed as a potential mechanism underlying the fin-to-limb transition during evolution (Nakamura et al., 2016; Zhang et al., 2010). Our findings raise the possibility that the re-wiring of BMP signaling might contribute to the evolution of tetrapod digits.

In summary, we demonstrate that the sclerotome is the embryonic source of multiple fibroblast populations in zebrafish. Our work shows that the sclerotome is an excellent model to study cell type diversification during embryonic development. The combination of positional and migratory information as well as local microenvironment likely determines the differentiation trajectory of

454 multipotent sclerotome progenitors. Although our work highlights the importance of extrinsic cues, cell
455 intrinsic programs likely also contribute to the cell fate diversification. Future work using single cell
456 RNA sequencing will likely identify key gene regulatory networks underlying the lineage progression of
457 sclerotome progenitors.

MATERIALS AND METHODS

Ethics statement

All animal research was conducted in accordance with the principles outlined in the current Guidelines of the Canadian Council on Animal Care. All protocols were approved by the Animal Care Committee at the University of Calgary (#AC17-0128).

Zebrafish strains

Zebrafish strains used in this study were maintained and raised under standard conditions. The following transgenic strains were used in this study: *Tg(BRE:GFP)mw29* (Collery and Link, 2011), *TgBAC(col1a2:Gal4)ca102* (Ma et al., 2018; Sharma et al., 2019), *TgBAC(col1a2:GFP)ca103* (Ma et al., 2018), *Tg(kdrl:EGFP)la163* (Choi et al., 2007), *TgBAC(nkx3.1:Gal4)ca101* (Ma et al., 2018), *Tg(UAS:Kaede)s1999t* (Davison et al., 2007), and *Tg(UAS:NTR-mCherry)c264* (Davison et al., 2007). The mosaic *col1a2:Gal4; UAS:Kaede* line was maintained by screening for embryos with more mosaic Kaede expression.

In situ hybridization and immunohistochemistry

Whole-mount in situ hybridization and immunohistochemistry were performed according to previously established protocols. We used the following RNA probes in this study: *bmp2b*, *bmp4*, *bmp6*, *hmcn2*, *fbln1*, and *fras1*. Double fluorescent in situ hybridizations were performed using different combinations of digoxigenin (DIG) or dinitrophenyl (DNP) labeled probes. For immunohistochemistry, rabbit polyclonal antibody to RFP (1:1000, MBL, PM005) and rabbit polyclonal antibody to phosphoSmad 1/5/9 (1:100, Cell Signaling Technology, 13820) were used. For fluorescent detection of antibody labeling, the appropriate Alexa Fluor-568 or Alexa Fluor-647 secondary antibodies (1:500, Thermo Fisher) was used.

Kaede photoconversion

Photoconversion was carried out using the 405nm laser and the 20x objective on the Olympus FV1200 confocal microscope. At the appropriate stage, Kaede-expressing embryos were anesthetized using tricaine and mounted in 0.8% low melting point agarose. The duration and intensity of laser required for complete green-to-red conversion depended on the expression level of Kaede and the size of the target region. For cell tracing of the sclerotome domains, typically a circular region of 20 by 20 pixels was converted using 1.5% 405nm laser for 4 seconds. For single cell clonal analysis of sclerotome progenitors, typically a circular region of 10 by 10 pixels was converted using

1.5% 405nm laser for 4 seconds. After photoconversion, embryos were confirmed for expression of Kaede^{red} and recovered in E3 fish water until necessary stages.

Time-lapse imaging and cell tracking

Time-lapse imaging of zebrafish embryos was carried out using the Olympus FV1200 confocal microscope. At the appropriate stage, fish were anesthetized using tricaine and mounted in 0.8% low melting point agarose. To keep the agarose hydrated and minimize the development of pigments as well as minimize movement of the fish, a small volume of E3 water with phenylthiourea and tricaine was carefully flooded around the agarose. Z-stack images of the region of interest were then collected at regular intervals (6-9 mins) for up to 25 hours. Images were then compiled into movies using the Olympus Fluoview software. Cells from each movie were then manually tracked using Fiji (Schindelin et al., 2012).

Drug treatments

Embryos at the appropriate stage were treated in DMH1 (Sigma Millipore, D8696) at a final concentration of 10 μ M in E3 fish water. Control embryos were treated similarly in an equal concentration of DMSO. Treated embryos were grown to the desired stage for analysis.

Statistical analysis

All the graphs were generated in the GraphPad Prism software. Data were plotted as mean $\pm$ SEM. Significance between two samples was calculated using Mann-Whitney *U* test. *p* values: *p* > 0.05 (not significant); *p* < 0.05 (*); *p* < 0.01 (**); *p* < 0.001 (***); *p* < 0.0001 (****).

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COMPETING INTERESTS

The authors declare that no competing interests exist.

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FIGURE LEGENDS

Fig 1. Characterization of sclerotome derived fibroblasts. (A) Schematic representation of sclerotome derived fibroblasts. Four major cell types are shown: blood vessel associated fibroblasts, fin mesenchymal cells, tenocytes, and interstitial fibroblasts. Each cell type is defined by its location in the trunk and its cell morphology. Lateral view of 2 somites is shown with the major blood vessels (green) indicated (DLAV: dorsal longitudinal anastomotic vessel; ISV: intersegmental vessel; DA: dorsal aorta; CVP: caudal vein plexus). n: notochord. (B) The *nkx3.1^{NTR-mCherry}* line (red) was crossed to the *kdr1:EGFP* line (green) to label the endothelial cells. At 2 dpf, mCherry⁺ cells were seen populated throughout the zebrafish trunk (top panels). Zoomed in views (bottom panels) show different mCherry⁺ fibroblast subtypes (arrows) in 4 boxed regions in the trunk. mCherry expression in some muscles and skin cells at the edge of the fin fold are denoted by asterisks and arrowheads, respectively. *n* = 13 embryos. (C) The mosaic *col1a2^{Kaede}* line (green) was crossed to the *kdr1:mCherry* line (red). High resolution images showed that individual blood vessel associated fibroblasts (cyan arrows), fin mesenchymal cells (white arrows), tenocytes (magenta arrow), and interstitial fibroblasts (yellow arrow) displayed distinct morphologies. The corresponding vascular bed is indicated for blood vessel associated fibroblasts. The corresponding fin fold is indicated for fin mesenchymal cells. Cell processes are indicated by arrowheads. *n* = 20 embryos. Scale bars: (B) 50 μ m; (C) 20 μ m.

Fig 2. Lineage analysis of sclerotome domains. (A) Schematic representation of lineage analysis. The entire dorsal or ventral sclerotome domain in *nkx3.1^{Kaede}* embryos were labeled by photoconversion at 24 hpf. The lineage of the converted cells was then imaged after 24 hours and analyzed for the cell types that were generated. (B) Photoconversion of the dorsal sclerotome domain in *nkx3.1^{Kaede}* embryos. Images of two examples are shown right after the conversion at 24 hpf and 24 hours later at 48 hpf. Cell projections at 24 hpf are denoted by white arrowheads. The dorsal sclerotome domain gave rise to dorsal fin mesenchymal cells (white arrows), DLAV fibroblasts (long cyan arrows), perivascular fibroblasts (cyan arrows), tenocyte (magenta arrows), and interstitial fibroblasts (yellow arrows). The outline of the vasculature is denoted by dotted lines. *n* = 20 embryos. (C) Photoconversion of the ventral sclerotome domain in *nkx3.1^{Kaede}* embryos. Images of two examples are shown at 24 hpf and 48 hpf. In anterior somites above the yolk extension (upper panel), the ventral sclerotome domain gave rise to perivascular fibroblasts (cyan arrows), tenocytes (magenta arrows), and interstitial fibroblasts (yellow arrows), but not SRCs or ventral fin mesenchymal cells. In posterior somites above the CVP region (bottom panel), the ventral sclerotome domain gave rise to SRCs (cyan arrowheads) and ventral fin mesenchymal cells (white arrows) in addition to tenocytes

(magenta arrows) and interstitial fibroblasts (yellow arrows) in the trunk. The ventral boundary of the CVP is indicated by dotted lines. $n = 20$ embryos. Asterisks in (B, C) denote pigment cells that show strong autofluorescence in the red channel. Scale bars: 50 μm .

Fig 3. Single cell lineage analysis of the dorsal sclerotome domain. (A) Schematic representation of lineage tracing experiment. A single dorsal sclerotome progenitor in *nkx3.1^{Kaede}* embryos was photoconverted at 24 hpf and imaged after 24 hours to determine the cell types generated. Dorsal sclerotome derived cells at 48 hpf were subdivided into two groups based on their dorsal-ventral positions: the more dorsal type I cells include dorsal fin mesenchymal cells and DLAV fibroblasts (above the dotted line), while the more ventral type II cells include tenocytes, perivascular fibroblasts, and interstitial fibroblasts (below the dotted line). (B) Quantification of the cell number in each clone. (C) Quantification of the number of fibroblast subtypes in each clone. (D) Distribution of different fibroblast subtypes from all decedents of traced cells. F: dorsal fin mesenchymal cells; T: tenocytes; V: blood vessel associated fibroblasts; I: interstitial fibroblasts. (E) Quantification of the types of cells generated by individually labeled dorsal sclerotome progenitors. Only photoconverted cells showing polarized cell projections at 24 hpf were graphed. $n = 14$ (with dorsal projection) and 19 (with ventral projection) photoconverted cells. (F) Photoconversion of a single dorsal sclerotome cell in *nkx3.1^{Kaede}* embryos. Two examples are shown at 24 hpf and 48 hpf. In the top panel (type I cell lineage), the photoconverted cell showing dorsal projections (arrowhead) gave rise to two dorsal fin mesenchymal cells (white arrows) and a DLAV fibroblasts (long cyan arrows). In the bottom panel (type II cell lineage), the photoconverted cell with ventral cell projections (arrowhead) gave rise to one DLAV fibroblasts (long cyan arrows), one perivascular fibroblast (cyan arrows) and one tenocyte (magenta arrows). The outline of the vasculature is denoted by dotted lines. $n = 42$ photoconverted cells traced from 40 embryos. Scale bars: 50 μm .

Fig 4. Time-lapse imaging of the migration of ventral sclerotome progenitors. Snapshots of a *kdrl:EGFP; nkx3.1^{NTR-mCherry}* embryo between 25 hpf and 50 hpf are shown with time stamps (hh:mm) indicated. Cell migration and divisions of representative lagging (cyan arrows) and leading (white arrows) cells were traced throughout their migration. The lagging cell never migrated ahead of the leading cell and generated 2 SRCs. The leading cell remained at the migration front ahead of the lagging cell and generated 2 ventral fin mesenchymal cells. $n = 7$ embryos. Scale bar: 50 μm .

Fig 5. Single cell lineage analysis of the ventral sclerotome domain. (A) Schematic representation of lineage tracing experiment. A single ventral sclerotome progenitor in *nkx3.1^{Kaede}*

embryos was photoconverted at 24 hpf and imaged after 24 hours to determine the cell types generated. Cells were classified into lagging or leading cells based on its initial position at 24 hpf. Leading cells were defined at the front of the migration path, while lagging cells were defined as any cell behind the leading cell. (B) Quantification of the types of cells generated by lagging and leading cells. (C) Examples of a photoconverted lagging cell (top panel) and a photoconverted leading cell (bottom panel) are shown at 24 hpf and 48 hpf. The lagging cell generated 4 SRCs (cyan arrowheads) and all photoconverted cells remained dorsal to the ventral edge of the CVP (dotted lines). The photoconverted leading cell migrated ahead of the CVP and generated 2 fin mesenchymal cells (white arrows). $n = 42$ photoconverted cells traced from 38 embryos. Scale bars: 50 μm .

Fig 6. Sclerotome derived fin mesenchymal cells are active in BMP signaling. (A) Schematic representation of the organization of the zebrafish fin fold. Both dorsal and ventral fin folds are populated by two adjacent cell layers: a distal layer of apical epidermal cells, and a proximal layer of fin mesenchymal cells. (B) Double fluorescent in situ staining of *fbn1* (red) with *fras1* or *hmcn2* (green) at 48 hpf. *fras1* labeled the distal apical epidermal cell layer (orange brackets), *fbn1* marked the proximal layer of fin mesenchymal cells (white brackets), while *hmcn2* was expressed in both layers. $n = 15$ embryos per staining. (C) *nkx3.1^{NTR-mCherry}* embryos were co-stained with *fbn1*, *fras1*, or *bmp4* probes (green) and anti-mCherry antibody (red) at 52 hpf. Apical epidermal cells and fin mesenchymal cells are indicated by arrowheads and arrows, respectively. *bmp4* was expressed in *fras1*⁺ apical epidermal cells adjacent to mCherry⁺ *fbn1*⁺ fin mesenchymal cells. $n = 10$ embryos per staining. (D) Double fluorescent in situ staining of *bmp4* (green) with *fbn1* or *fras1* (red) at 48 hpf. *bmp4* was expressed in *fras1*⁺ apical epidermal cells (orange brackets) adjacent to *fbn1*⁺ fin mesenchymal cells (white brackets). Asterisks denote *bmp4* expression in the dorsal spinal cord. $n = 15$ embryos per staining. (E) *nkx3.1^{NTR-mCherry}* fish were crossed to *BRE:GFP* to label BMP responsive cells. At 2 dpf, mCherry⁺ fin mesenchymal cells (white arrows) and SRCs (cyan arrows) were marked by strong and weak GFP expression, respectively. In contrast, no GFP expression was found in mCherry⁺ interstitial fibroblasts (yellow arrows) around the notochord (nc). $n = 9$ embryos. (F) *kdr1:EGFP; nkx3.1^{NTR-mCherry}* embryos were stained with pSmad antibody (cyan) at 2 dpf. mCherry⁺ fin mesenchymal cells showed elevated pSmad staining (arrows). $n = 14$ embryos. Magnified views of boxed regions in (B, D, F) are shown in corresponding panels. Scale bars: (B, D, E, F) 50 μm ; (C) 20 μm .

Fig 7. Regulation of fin mesenchymal cells by BMP signaling. (A) *kdr1:EGFP; nkx3.1^{NTR-mCherry}* embryos were treated with either DMSO or DMH1 between 18 hpf and 48 hpf. DMH1 treatment

636 resulted in the expansion of both dorsal and ventral fin folds (brackets), poorly developed CVP
637 (asterisk), and the reduction of mCherry⁺ fin mesenchymal cells in both the dorsal and ventral fin folds
638 (arrows). Remaining fin mesenchymal cells in DMH1-treated embryos appeared to have more
639 branches (arrowheads) compared to DMSO-treated controls in magnified views. $n = 19$ embryos per
640 condition. (B) Quantification of the fin fold expansion in DMH1 treated embryos. The heights of the
641 dorsal fin fold, ventral fin fold, and somite were measured at somite 26. Fin fold height was normalized
642 to the height of the somite of the same embryo. $n = 11$ (DMSO) and 9 (DMH1) embryos. (C)
643 Quantification of the number of fin mesenchymal cells in DMH1 treated fish. Fin mesenchymal cells
644 were counted in either the dorsal or ventral fin fold between somites 18 and 28. Each point represents
645 the total number of fin mesenchymal cells in 10 somite region of one fish. $n = 11$ (DMSO) and 10
646 (DMH1) embryos. Data in (B, C) are plotted as mean $\pm$ SEM. Statistics: Mann-Whitney U test; $p <$
647 0.01 (**); $p < 0.001$ (***). (D) Wild-type embryos were treated with DMSO or DMH1 from 18 hpf to 45.5
648 hpf and stained with *fbln1*, *bmp4*, and *fras1* probes. *fbln1* expression was markedly reduced in both
649 the dorsal and ventral fin folds (arrowheads and arrows, respectively), while *bmp4* and *fras1*
650 expression was expanded along the fin fold compared to DMSO controls. The anterior extent of *fbln1*
651 expression in the dorsal fin fold is denoted by asterisks. $n = 15$ embryos per condition. (E) Model of
652 diversification of fibroblast subtypes from sclerotome progenitors in zebrafish. Three mechanisms
653 contribute to the diversification of fibroblast subtypes from multipotent sclerotome progenitors. a)
654 Spatial regulation. Sclerotome progenitors at the different dorsal-ventral or anterior-posterior positions
655 have distinct differentiation potentials. b) Migration direction. The fates of sclerotome progenitors are
656 biased by the direction of their migration. c) Migration order. The fates of sclerotome progenitors are
657 determined by their position along the migratory path. Local microenvironment (for example, BMP
658 expression (indicated by the blue band) at the periphery of the fin fold) regulates the migration,
659 specification and morphogenesis of fin mesenchymal cells. n: notochord; CVP: caudal vein plexus.
660 Scale bars: (A) 50 μ m (20 μ m for magnified views); (D) 200 μ m.

SUPPLEMENTAL FIGURES

Fig S1. The sclerotome generates a population of fibroblasts. (A) Wild-type embryo at 2 dpf. The major lobe and minor lobe of the fin fold are outlined by dash lines. (B) A tiled confocal image of *nkx3.1^{NTR-mCherry}* embryo at 2 dpf showing mCherry⁺ sclerotome derived cells (red) throughout the trunk of the embryo. mCherry⁺ fin mesenchymal cells were present in both dorsal and ventral fin fold (arrows), but absent from the caudal fin and the minor lobe (asterisks). Arrowhead indicates mCherry expression in skin cells at the edge of the fin fold. (C) The *nkx3.1^{NTR-mCherry}* line (red) was crossed to the *col1a2:GFP* line (green). At 2 dpf, most mCherry⁺ sclerotome derived cells also expressed *col1a2:GFP*. The upper panel shows dorsal fin mesenchymal cells (white arrows), DLAV fibroblasts (long cyan arrows), ISV-associated perivascular fibroblasts (cyan arrows), tenocytes (magenta arrows), and interstitial fibroblasts (yellow arrows) in the dorsal region of the trunk. The middle panel shows DA fibroblasts (cyan arrows) associated with the dorsal aorta. The bottom panel shows stromal reticular cells (cyan arrows) and ventral fin mesenchymal cells (white arrows). *n* = 12 embryos. Scale bars: (A-B) 200 μ m; (C) 50 μ m.

Fig S2. Apical epidermal cells express BMP ligands. (A) Double fluorescent in situ staining of *bmp4* (green) with *hmcn2* (red) at 48 hpf. (B) Double fluorescent in situ staining of *bmp2b* (green) with *fbln1* or *fras1* (red) at 48 hpf. (C) Double fluorescent in situ staining of *bmp6* (green) with *fbln1* or *fras1* (red) at 48 hpf. *bmp4*, *bmp2b*, and *bmp6* were expressed in *hmcn2⁺fras1⁺* apical epidermal cells (orange brackets) adjacent to *hmcn2⁺fbln1⁺* fin mesenchymal cells (white brackets). Magnified views of boxed regions are shown in each staining. *n* = 15 embryos per staining. Scale bars: 50 μ m.

Fig S3. Effective inhibition of BMP signaling by DMH1. *BRE:GFP* embryos were treated with either DMSO or DMH1 between 10 hpf and 24 hpf and imaged for GFP expression. GFP expression was substantially reduced in the trunk of DMH1 treated embryos (arrows). *n* = 10 embryos per condition. Scale bar: 50 μ m.

Fig S4. BMP signaling regulates the morphogenesis of fin mesenchymal cells. (A) *kdrl:EGFP*; *nkx3.1^{NTR-mCherry}* embryos were treated with either DMSO or DMH1 between 25 hpf and 49 hpf. DMH1 treatment resulted in the expansion of both dorsal and ventral fin folds (brackets). Fin mesenchymal cells (arrows) in DMH1-treated embryos appeared to have more branches (arrowheads) compared to DMSO-treated controls in magnified views of boxed regions. *n* = 13 (DMSO) and 14 (DMH1) embryos. (B) Quantification of the fin fold expansion in DMH1 treated embryos. The heights of the dorsal fin

fold, ventral fin fold, and somite were measured at somite 23. Fin fold height was normalized to the height of the somite of the same embryo. (C) Quantification of the number of fin mesenchymal cells in DMH1 treated fish. Fin mesenchymal cells were counted in either the dorsal or ventral fin fold between somites 18 and 28. Each point represents the total number of fin mesenchymal cells in 10 somite region of one fish. Data are plotted as mean $\pm$ SEM. Statistics: Mann-Whitney *U* test; $p > 0.05$ (ns, not significant); $p < 0.001$ (**); $p < 0.0001$ (****). Scale bars: 50 μ m.

Video S1. Time-lapse imaging of the migration of ventral sclerotome progenitors. *kdr1:EGFP; nkx3.1^{NTR-mCherry}* embryos were imaged from 25 hpf to 50 hpf with time stamps (hh:mm) indicated. Cell migration and divisions of representative lagging (cyan arrows) and leading (white arrows) cells were traced throughout their migration. Daughter cells are indicated by arrows of the same color. The lagging cell never migrated ahead of the leading cell and generated 2 SRCs. The leading cell remained at the migration front ahead of the lagging cell and generated 2 ventral fin mesenchymal cells. Snapshots of this video are shown in Fig 4. $n = 7$ embryos. Scale bar: 50 μ m.

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