Developmental Cell
Previews

Embryonic Immune Cells Remodel the Heart

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https://doi.org/10.1016/j.devcel.2019.02.017

How the products of transient hematopoiesis in the yolk sac, dorsal aorta, and developing heart tube function at their sites of production is poorly understood. In this issue of *Developmental Cell*, Shigeta et al. (2019) elegantly demonstrate that macrophages derived from the heart tube contribute to local tissue remodeling during valve development.

During development, hematopoietic cells are generated at several sites, including the yolk sac, aorta, and the heart tube. Although we are beginning to understand their relative contributions to the adult and embryonic blood supplies, the reasons for the emergence of these cells at such varied anatomical sites has remained a mystery. Many cell types are generated at these sites, but of these diverse cell types, macrophages (or professional phagocytes) have been shown to be critical to the development and homeostasis of the tissues in which they reside. Additionally, it has become recently evident that most of the tissue-resident macrophages in the adult arise during development, and that these long-lived cells are maintained in specific niches throughout life (Mass, 2018). However, precisely defining the origin of hematopoietic cells is inherently difficult because of their mobile nature. As a result, there has been controversy surrounding the origins of tissue-resident macrophages because it is still unclear whether these cells arise *in situ*, or whether they are dependent upon an upstream hematopoietic stem cell precursor.

Tissue resident macrophages also play critical roles in tissue remodeling during development; the macrophages are involved in killing and removing interdigital cells in the mouse paw (Hopkinson-Woolley et al., 1994), developing the mouse eye hyaloid vessels and pupillary membrane (Bishop et al., 2016), bridging blood vessel anastomoses in the zebrafish embryo (Fantin et al., 2010), and branching morphogenesis in the murine kidney (Munro and Hughes, 2017). On the basis of the observation that the hemogenic activity of the endocardium in the heart tube coincides with the developmental remodeling of cardiac tissue, the authors hypothesized that these events might be intrinsically linked. More specifically, that tissue-resident macrophages might arise in the heart tissue and contribute to remodeling at this time.

To define the origin of macrophages that are present in the heart tube during development, the authors used a heart-beat-deficient mouse model to derive heart explants that are free from circulating cells, allowing the authors to examine the hematopoietic cells arising only from the heart tube itself. Using explant cultures and then colony-forming assays, the authors showed that macrophages arise *in situ* from the heart tube. The authors then tested the ability of the cardiac endothelium to produce macrophages using the Nfatc1-cre line, which specifically labels cells of the endocardium. Using this strategy, they identified that 58% of mature macrophages in the endocardial cushion arose from Nfatc1^{+} precursor cells. Moreover, these cells seem to arise independently of hematopoietic stem cells or other multipotent progenitors, suggesting that these macrophages originate directly from the endocardium.

Once the authors established that these macrophages arise directly from the developing heart, they then showed that these cells are alternatively-activated M2 macrophages, which most classically function in wound healing. These have high phagocytic activity during embryonic development, although this drops off in the adult, consistent with a putative role in the clearing of excess tissue. During cardiac development, the endocardial cushion cells undergo an epithelial-to-mesenchymal transition (EMT) to build thick, cellularized valves that require remodeling to eventually form the thin, delicate, mature cardiac valves. This remodeling is partially accomplished through apoptosis, though the method of clearing apoptotic debris was unclear. The authors speculated that these cardiac-tissue-resident macrophages might be involved in this process. In support of this notion, Shigeta et al. (2019) found that these cardiac-tissue macrophages were more phagocytic than other tissue-resident macrophages not derived from the endocardium, and the authors also observed these cells engulfing apoptotic debris in the cushion mesenchyme.

Genetic ablation of these endocardium-derived macrophages led to lower overall survival at weaning and a phenotype consistent with a lack of phagocytosis-driven pruning of the cardiac valves. Interestingly, there were no other gross abnormalities detected, indicating that these endocardially derived cardiac-tissue macrophages are only required in the tissue from which they are derived. Another major question in the field has been whether circulating monocytes can generate macrophages that are able to functionally compensate for tissue-resident macrophages. In the study by Shigeta et al. (2019), the authors found an increased number of circulating monocytes in the absence of the endocardially derived tissue macrophages, but the circulating cells were unable to compensate functionally for the loss of tissue-resident macrophages.

Altogether in this work, Shigeta et al. (2019) demonstrate that tissue-resident macrophages in the heart tube arise directly from the endocardium, and that this new population is specifically required for valvular remodeling during development. These cells arise independently from hematopoietic stem cell precursors, and their loss cannot be

compensated for by circulating monocytes. This work sets the stage for determining both how other tissue-resident macrophages arise and their potential roles in local tissue remodeling during development, homeostasis, and disease.

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A New Player in Tissue Mechanics: MicroRNA Control of Mechanical Homeostasis

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https://doi.org/10.1016/j.devcel.2019.02.019

How the homeostasis of tissue mechanics is controlled remains an open question. In a recent issue of *Nature Cell Biology*, Moro et al. (2019) reveal a novel role for miRNAs in regulating mechanotransduction in cells, tissues, and wound healing.

In living organisms, animal cells can be exposed to a wide range of varying mechanical stimuli as a result of their environment and thus need to constantly adapt to these changes (Humphrey et al., 2014; Petridou et al., 2017). A reduced capacity for mechanical adaptation can lead to severe diseases such as cancer and the formation of metastases (Butcher et al., 2009). In recent years, a special emphasis has been put on assessing how cells could adapt to environments with different mechanical properties and how such environments could affect their shape, mechanics, or even differentiation (Discher et al., 2005).

It was recently shown that when cells adhere to a rigid matrix, the expression of cytoskeletal proteins and the reinforcement of the actin cytoskeleton are enhanced via mechanotransduction and transcriptional activity (Elosegui-Artola et al., 2016). In particular, Hippo signaling, via the translocation of the transcription regulators YAP and TAZ, has been shown to be the main effector of this transcriptional modulation (Dupont et al., 2011). This further implies the differential regulation of multiple molecular players such as actin, and adhesion or matrix associated proteins, in response to the mechanics of the substrate. How this regulation of cell mechanics could be achieved still remains an open question.

In a recent issue of *Nature Cell Biology*, Moro et al. identify miRNAs as a novel player in the control of the mechanical properties of cells and tissues (Moro et al., 2019). Using an unbiased screen to identify interaction between miRNAs and mRNAs in human endothelial cell lines, the authors found 122 miRNAs that specifically target 127 mRNAs, out of which 73 mRNAs specifically encode cytoskeleton, ECM, and adhesion-related proteins. The authors termed this group of proteins CAM (contractility adhesion matrix proteins). Interestingly, this post-transcriptional regulation of CAM proteins by miRNAs is modulated by substrate stiffness and therefore points toward a mechanically regulated mechanism to control cell mechanics.

To get more insight into the control of cytoskeleton architecture and overall cell mechanics by miRNAs, the authors altered miRNA levels by using endothelial cells lacking Argonaute2 (AGO2) or DROSHA, two key proteins for miRNA biogenesis (Pasquinelli, 2012). While in control cells, higher contractility is only observed for higher substrate stiffness, for cells lacking AGO2 or DROSHA, an increased contractility also occurs on softer substrates. Importantly, mutant cells apply higher traction forces on both substrate stiffnesses and show higher YAP nuclear localization. Similar observations are also present in 3D cultures of fibroblasts and confirm a role of miRNAs in the regulation of cell contractility.