Title:

Cell migration in the cardiovascular system. Force to reckon with?

Author:

Anke M. Smits

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Commentary on:
Tissue stiffening coordinates morphogenesis by triggering collective cell migration in vivo.
Barriga EH, Franze K, Charras G, Mayor R. Nature 2018;**554**:523–527

The correct formation of an embryo requires three essential aspects of developmental biology: cell differentiation, cell growth, and morphogenesis: the spatial distribution of cells. The literal translation of morphogenesis is “beginning of the shape”, and as the name suggests, it is responsible for the shape of an organ. Morphogenesis requires a coordinated migration of (groups of) cells, sometimes over long distances to reach their final position. The movement of groups of cells is known as collective cell migration. This process occurs mainly during embryonic development but is also observed in tissue regeneration, wound healing, and cancer progression. While single cell migration occurs mainly in response to chemical signals like chemotactic protein gradients, collective cell migration can additionally be driven by mechanical cues. The interplay or hierarchy between these different migration inducing forces is not yet completely understood.

Barriga and colleagues[1] now show that mechanical forces resulting from tissue stiffening may be the primary regulator for collective cell migration, by using a simple, yet elegant model. During embryonic development of the frog *Xenopus Laevis,* a group of cells known as neural crest follow a defined migratory path towards the head mesoderm. The authors defined two distinct developmental stages where neural crest cells were either non-migratory, or about to migrate (pre-migratory). By transplanting non-migratory neural crest into a pre-migratory host and vice versa,

they were able to conclude that collective cell migration is dictated by the environment in the host rather than the state of the transplanted neural crest. Using atomic force microscopy they showed that stiffening of the head mesoderm during the pre-migratory stage provided the mechanical cue for collective cell migration, and that this stiffening resulted from an increased cell density in the head region. Interestingly, in vitro both non- and pre-migratory neural crests responded equally to Stromal Cell-Derived factor-1 stimulation showing that a chemoattractant gradient is in this case inferior to environmental cues. Since migration is an essential component of cardiovascular development and contributes to cardiovascular disease, its regulation by stiffness might play a potentially underestimated role.

In cardiac morphogenesis, several waves of tightly regulated cell movement are required for the correct formation of a four-chambered heart. These include for instance the migration of precardiac mesodermal cells towards the embryonic head region to form the cardiac crescent, the addition of cells that form the second heart field which will eventually give rise to large parts of the in- outflow tract and right ventricle, and the influx of neural crest cells required for septation of the outflow tract and the generation of cardiac ganglia[2]. The importance of migration for correct heart development is illustrated by the cardiac defects that arise when components of the SLIT-ROBO signalling pathway (involved in regulation of cell migration), are dysfunctional[3].

In order to obtain a migratory capacity, neural crest (and other epithelial-like cells) must undergo epithelial-to mesenchymal transition (EMT) to loosen their cell-cell contact, and to gain mesenchymal features including the rearrangement of the cytoskeleton and an increased ability to deposit extracellular matrix. While many studies have shown that EMT is induced by growth factors like Transforming Growth Factor-β, Barriga now shows that EMT can also be triggered by stiffening of the tissue[1]. This finding may be important for cardiac development. During formation of the heart, EMT is an indispensable process because most of the non-cardiomyocyte cells in the myocardium are the result of EMT of either the endocardium or epicardium[4]. Endocardial cells lining the atrioventricular cushions undergo endothelial EMT and become valve precursors, while the epicardium undergoes EMT to form epicardial-derived cells that migrate into the developing myocardium; a process imperative for cardiac vessel formation and maturation, and compaction of the myocardium[5].

Obviously, during cardiac development, aberrant regulation of either EMT or migration will disrupt proper formation of the heart and result in embryonic lethality or severe cardiac abnormalities. While many of the molecular determinants in cardiac development are well studied, tissue stiffening as a regulator is not yet fully understood[6]. Recent studies however suggest that mechanical forces

may indeed be part of the equation[7]. Therefore it will be interesting to take stiffening and force into account when investigating the underlying causes of congenital heart disease in the future.

In pathological conditions of the heart, cell migration and EMT are essential processes. Upon acute myocardial infarction (MI), a massive influx of inflammatory cells is followed by invasion of fibroblasts and myofibroblasts into the injured area that deposit extracellular matrix. Local EMT of cardiac endothelial cells may also contribute to matrix deposition and thereby to the formation of a scar[8]. The epicardium undergoes EMT after MI, and arisen epicardial-derived cells potentially migrate into the injured myocardium. In light of the current finding that tissue stiffening in the frog was the result of an increase in cell density[1], a role for stiffness in the processes following acute MI can be foreseen. While the initial inflammatory response after MI is likely mediated by chemotactic factors, the resulting change in cell density itself could alter the mechanical properties of the tissue. In combination with the deposition of extracellular matrix by other incoming cells, a feed forward loop can be envisioned where an increase in cell number results in stiffening and a subsequent induction of EMT and migration, thereby exacerbating the fibrotic response. A similar situation may occur in the fibrotic hypertrophic heart[9] , and atherosclerosis[10], as both have a potential contribution of EMT. Moreover, in heart failure with preserved ejection fraction, stiffening of the left ventricle as a result of increased collagen content and passive cardiomyocyte stiffness underlies the LV-diastolic dysfunction. Mechanical forces could therefore play a prominent role in development of the disease[11]. Conversely, cell migration into the damaged heart may also have beneficial effects on repair of damaged tissue: for instance, angiogenesis is also considered a collective cell migration process, this too may be influenced by tissue forces.

Investigating how EMT and cell migration are influenced by tissue stiffening in cardiac disease, and if these processes are susceptible to therapy will be an exciting new direction to pursue.
Especially since an interesting feature of the presented frog model was that migration could be influenced. The stiffness of the head mesoderm was manipulated by locally altering the expression of contractile proteins, or by releasing tension via a simple cut into the tissue[1]. This ability to locally change stiffness could potentially be of use in several ex vivo models for cardiovascular disease. For instance, adapting the mechanical properties may be very relevant when developing tissue engineered constructs or organoids. A correct cellular organisation is paramount to mimicking diseased myocardium in a dish. Additionally, recent therapeutic approaches where patches of biomaterial containing (stem)cells or their derivatives are placed on the outside of the injured heart[12] may benefit from taking into account the mechanical properties of their material and/or the underlying tissue. An incorrect gradient between tissue and graft may prevent cell migration out of the patch or may attract local cells towards it. Mechanical cues could also influence (cardiac) stem

cells. For instance, early cardiogenic differentiation of cardiac progenitor cells increased the expression of vinculin[13], a protein Barriga proved to be involved in migration[1].
Sprouting angiogenesis is also considered a form of collective cell migration, since sprouting endothelial cells migrate as a collective strand, guided by a single tip cell which "pulls along" the connected stalk cells. While vessel formation is known to be partially regulated via extracellular gradients of angiogenic factors including vascular endothelial growth factor, a role for tissue stiffness has become increasingly clear[14]. Therefore changing local stiffness genetically or mechanically may offer new approaches in situations where guided vessel formation is required, or conversely needs to be halted e.g. in tumour angiogenesis.

The take home message of this paper is that cells respond to a complex environment of both molecular and mechanical cues in which the latter may have a greater influence than previously anticipated. The research in the manuscript by Barriga describes very basic biological processes, but it represents a great example of how these types of insights may offer new options for therapy in the future; changing the local stiffness to enhance the endogenous cellular response to injury may provide an additional approach for patients suffering from heart disease. In the coming years, when research aims at trying to understand the cellular processes underlying cardiac development and repair, mechanical force should definitely be reckoned with.

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