The effects of hemodynamic force on embryonic development

JAMES C. CULVER and MARY E. DICKINSON, Ph.D.

1 Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas

Abstract

Blood vessels have long been known to respond to hemodynamic force, and several mechanotransduction pathways have been identified. However, only recently have we begun to understand the effects of hemodynamic force on embryonic development. In this review, we will discuss specific examples illustrating the role of hemodynamic force during the development of the embryo, with particular focus on the development of the vascular system and the morphogenesis of the heart. We will also discuss the important functions served by mechanotransduction and hemodynamic force during placentation, as well as in regulating the maintenance and division of embryonic, hematopoietic, neural, and mesenchymal stem cells. Pathological misregulation of mechanosensitive pathways during pregnancy and embryonic development may contribute to the occurrence of cardiovascular birth defects, as well as to a variety of other diseases, including preeclampsia. Thus, there is a need for future studies focusing on better understanding the physiological effects of hemodynamic force during embryonic development and their role in the pathogenesis of disease.

Keywords

Hemodynamic force; vascular development; cardiac morphogenesis; hematopoiesis; stem cells

Mechanisms of mechanotransduction

The idea that the physical forces imparted by flowing blood play important physiological roles was first postulated over a century ago by Thoma [136]. His experiments demonstrated that blood vessels morphologically remodel over time and either widen or regress in order to adapt to the amount of flow that they carry [59,122,136]. Early explanations of this phenomenon by Murray and others held that vessels do this by sensing shear stress in order to remodel and change size as heart rates and blood volumes change [85,105]. The basic premise of Murray’s law is that the cost of moving blood can be minimized by expanding the diameter of the vessels and is balanced by the metabolic cost of enlarging the vessels and of blood production itself [85,105,116]. Murray’s law has now been validated in many systems, from mammals to sponges, but how these principles influence morphogenesis in developing vascular systems is just beginning to be understood [57,58,105,116].
The molecular basis of mechanosensitivity

Flowing blood exerts several forces on vessels (Figure 1A). These include: the normal force exerted on the vessel wall by blood pressure; the associated circumferential stress that occurs as the vessel stretches in response to that pressure; and finally, the frictional force exerted by flowing blood as it drags along the vessel wall, also known as shear stress [15]. By virtue of their direct contact with flowing blood, vascular endothelial cells, which comprise the inner layer of blood vessels, are able to sense these forces, and are thought to do so by making use of a variety of membrane-localized molecules [15, 61, 96]. These endothelial cells then respond to such force by initiating cytoskeletal rearrangements that help them align in a direction parallel with flow [15]. When exposed to laminar flow, in which fluid flows in parallel layers and in a steady and orderly fashion, endothelial cells also downregulate genes that promote proliferation and the inflammatory response [15]; however, disturbed flow, in which fluid flows in a disorderly fashion, is known to have the opposite effect and induces increased proliferation [15, 19]. Particular levels of cyclic pressure have also been shown to upregulate endothelial cell proliferation in a VEGF-C (vascular endothelial growth factor-C) dependent manner [118, 119].

In some cases, in vitro experiments have yielded evidence suggesting that some molecules are preferentially activated by one particular type of force over another. For example, it has recently been shown that a molecular complex of PECAM-1 (platelet endothelial cell adhesion molecule-1), VEGFR2 (vascular endothelial growth factor receptor 2), and VE-cadherin (vascular endothelial cell cadherin) is necessary for cultured endothelial cells to respond specifically to shear stress [139]. Normally, endothelial cells initiate a cascade of intracellular signaling events in response to laminar shear through VEGFR2-dependent activation of PI3K (phosphatidylinositol-3-OH kinase) [139]. However, endothelial cell lines deficient in either Pecam-1 or Ve-cadherin expression are unable to do so; furthermore, ectopic expression of these two genes along with Vegfr2 is sufficient to confer this mechanosensitivity to cell lines that are otherwise unresponsive to shear stress [139]. The ability of endothelial cells to respond specifically to circumferential stretch, on the other hand, is thought to preferentially involve integrin-mediated interactions with the extracellular matrix [3,13]. In particular, it has been shown that integrins can interact with FAK (focal adhesion kinase) to activate MAPK (mitogen-activated protein kinase) in response to stretch [3].

However, in practice, it is difficult to distinguish between these distinct forces in vivo, and so they are usually not considered separately; instead, they are often referred to collectively as hemodynamic force. The subtle roles played by each distinct force in the live animal have also been further obscured by the difficulty of extrapolating in vitro data to predict physiological circumstances in vivo. For instance, cell culture work suggests PECAM-1 may be necessary for the response of the endothelium to shear stress [139]; however, Pecam-1 knockout mice are able to survive through adulthood with only minor cardiovascular defects [14,22,112]. This may be explained by genetic compensation, or by the existence of overlapping and potentially redundant mechanosensory mechanisms. Another possibility is that survival of these knockout mice during embryonic development might be explained by a difference in the magnitude of hemodynamic force that is experienced by adult vessels as compared to embryonic vessels. Our relative lack of understanding of these differences is reflected in the fact that, despite notable successes in measuring hemodynamic forces during development [52], a detailed understanding of how these forces change over time remains elusive. Finally, although it is clear that endothelial cells respond to hemodynamic force during development [53,59,68], it may also be true that different mechanisms are used in developing cells than are used in adult cells. The unanswered questions related to this one example therefore illustrate the challenges inherent to the study of these complex molecular pathways in vivo.

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The large number of molecular mechanisms that have been implicated in conferring mechanosensitivity to endothelial cells serves as another indication of the complexity of the cellular response to hemodynamic force [61]. For example, the non-motile (9+0) microtubular primary cilia of endothelial cells have been shown in some cases to protrude into the lumen of the vessel and to mediate the opening of calcium channels in response to shear stress [20,88,140]; interestingly, mice lacking essential components of this mechanosensitive primary cilium are dysfunctional in cardiovascular development and suffer from focal hemorrhaging and defects in cardiac morphogenesis [9,121]. Other lines of evidence have shown that cell surface components in particular, including several G-protein coupled receptors [12,61,73], TRP (transient receptor potential) ion channels [10,97], and the endothelial glyocalyx [151] are also important constituents of mechanosensory pathways. However, at present, the importance of each of these components during development is unclear. The mechanisms by which endothelial cells respond to hemodynamic force may in fact be dependent on a combinatorial activation of any number of these molecules, and may change during development depending on context or on the availability of different intracellular signaling pathways. Future efforts in the field may therefore focus on elucidating the complex interactions between these different pathways. Exactly how some of these molecules are biomechanically activated also remains largely unknown.

In contrast, the downstream effects of hemodynamic force on intracellular pathways are better understood. A number of major signaling pathways are quickly activated after the onset of flow, including notably the PI3K/AKT pathway and the MAPK pathway [15,35,139]. A variety of transcription factors also show responsiveness to hemodynamic force, including Klf2 (Krüppel-like factor 2), which is necessary for heart valve formation [36,37,60,142], and SP1, which is important for arterial specification [93]. Furthermore, histone methylation and acetylation, which can directly affect transcription, have been shown to be induced by shear stress [50,51].

In addition to regulating these cell-intrinsic signaling events, hemodynamic force also regulates endothelial cell paracrine signaling to other cell types. For example, the secretion of a variety of growth factors, including VEGF, PDGF (platelet-derived growth factor), and TGFβ (transforming growth factor beta), is tightly regulated in endothelial cells by hemodynamic force [17,47,77,94]. Nitric oxide release, an important mediator of vascular tone that is regulated by eNOS (endothelial nitric oxide synthase), is also sensitively controlled by shear stress [31,36,37,68,135]. Finally, hemodynamic force has been observed to regulate the expression of cell adhesion molecules on endothelial cells [16]. In these ways, endothelial cells are able to respond to blood flow by transducing downstream signaling events in a paracrine manner. The exposure of endothelial cells to shear stress therefore initiates a series of cell-cell signaling events that can influence the development of adjacent tissues. This broad effect is reflected in the wide-range of roles played by mechanical force, not only in the cardiovascular system, but in other systems as well.

The physiological effects of mechanical force

What we have learned about the effects of hemodynamic force has been surprisingly applicable to the study of the mechanotransduction of other forces as well. The nervous system, for example, relies on mechanosensitive pathways very similar to those seen in endothelial cells [40,92,96]. Such mechanisms serve as the molecular basis of hearing and touch [25,92,96]. Surprisingly, kidney development is also dependent on mechanotransduction pathways [20,87]. The same primary cilia that have recently been implicated in the cardiovascular response to shear stress were originally shown to sense similar forces exerted by fluid flow in the kidney tubule and to thereby regulate the cell...
cycle; pathological misregulation of this mechanism often leads to cyst formation [9,87,88,121].

In the context of both the embryonic and adult cardiovascular systems, however, hemodynamic force is the predominating force. The large body of work that has focused on elucidating the physiological effects of this force has increased our understanding of a variety of pathologies in the adult vascular system [56,79,125,134,155]. However, some of the newest breakthroughs in our understanding of the physiological roles of hemodynamic force have been in the context of embryogenesis [1,68,91]. This review will focus on the current state of what we know about the effects of hemodynamic force during pregnancy and embryonic development (summarized in Figure 1), their roles in regulating the maintenance and division of stem cells, and their implications in a variety of diseases.

The effects of hemodynamic force on cardiovascular development

Vascular remodeling during early development

Shortly after gastrulation, extraembryonic mesodermal cells in the yolk sac are specified to form the blood islands [108]. These blood islands are the first sites of hematopoiesis during development, and also contain angioblasts, which coalesce to form the primitive capillary plexus by E8.5 (embryonic day 8.5) in a process termed vasculogenesis [108,109]. However, the polygonal arrangement of this initial plexus quickly begins remodeling shortly after the start of the heartbeat (~3 somite stage) and the onset of flow (~6 somite stage) [68,107,109]. Within a day, this plexus remodels into a hierarchical network of large vessels carrying high volumetric flow and small vessels carrying low flow (Figure 2) [68,107]. This finalized, low-resistance vascular network is highly efficient at perfusing the yolk sac and delivering blood to the embryo proper, and aberrant formation of this vessel network results in growth retardation, cardiac failure, and eventual embryonic death [35,68].

It is now clear that hemodynamic force is an important factor regulating this process of yolk sac vascular remodeling. Dramatic reductions in hemodynamic force during development, through the disruption of heart function for example, are able to prevent proper remodeling. Knocking out either Mlc2a (atrial myosin light chain 2) or Ncx1 (sodium calcium exchanger 1), two molecules that are required for proper heart function and that are expressed only in the heart, abolishes vascular remodeling (Figure 2) and causes mid-gestation embryonic death [48,145]. The cardiac-specific manner in which these genes are expressed supports a model by which altered heart function can cause secondary effects on the remodeling of the yolk sac vasculature. Genetic rescue experiments have also been reported that further bolster this argument. Vascular remodeling defects in N-cadherin (neuronal cadherin) knockout mice, for instance, can be rescued by cardiac-specific transgenic expression of N-cadherin or E-cadherin (epithelial cadherin) [71]. Similarly, vascular remodeling defects seen in mice lacking the cardiac homeobox transcription factor Nkx2.5 can be rescued in mouse chimeras in which wildtype cells contribute to the heart at rates of 85% or higher [132]. Cardiac specific cellular defects are therefore capable of causing non-cell autonomous failures in vascular remodeling in the yolk sac.

However, only recently has evidence been reported that attributes these secondary effects to the hemodynamic consequences of heart defects, as opposed to the changes in the circulation of oxygen, nutrients, and signaling molecules that also accompany heart failure [68]. By sequestering primitive erythroblasts in the blood islands using polymerized acrylamide, hemodynamic force in a developing embryo can be dramatically reduced by decreasing blood hematocrit, and therefore blood viscosity. When this is done, yolk sac vascular remodeling is abolished, and the embryo no longer undergoes proper turning; however, restoration of blood viscosity by injection of hetastarch, a viscous plant starch, is
capable of rescuing both normal embryonic turning and normal vascular remodeling [68]. Together, these data make a strong case for the fact that hemodynamic force is both necessary and sufficient for yolk sac vascular remodeling. Similar conclusions have been made from observing the failure of vascular remodeling in mice which are deficient in primitive and definitive hematopoiesis, and therefore have low blood hematocrit and viscosity [42]. Hemodynamic force may also prove to be important during vascular remodeling in the embryo proper [42]. Endothelial cell migration, which must be highly regulated for proper vascular remodeling to occur [8,35], is one cellular process that might be regulated by hemodynamic force; however, future studies will be needed to address this possibility.

**Vessel maturation and arterial/venous specification**

After the initial phase of vascular remodeling in the yolk sac and embryo nears completion, the vessels undergo a process of stabilization that is largely mediated by interactions between endothelial cells and mural cells [4,30,109]. During this stage of development, endothelial cells secrete PDGFB (platelet derived growth factor B). PDGF binds to PDGF receptors on pericytes and smooth muscle cells to recruit them to participate in the formation of the vessel wall [4,30,109]. At the same time, these recruited mural cells secrete Ang-1 (angiopoitin-1) [30,109,127,146]. Upon binding to Tie2 receptors on endothelial cells, this glycoprotein transduces a number of downstream signaling events that lead to vessel stabilization, a necessary step in the maturation of the vessels which brings them to homeostasis [109,127,128]. Without proper function of this paracrine signaling loop, cardiovascular development cannot proceed; this is underscored by the finding that the knockout of either \textit{Pdgfb} or \textit{Ang-1} in mice results in embryonic lethality due to hemorrhaging and defective vascular integrity [64,128].

\textit{In vitro} experiments that more closely examine this paracrine loop suggest that it too may be regulated to some extent by hemodynamic force. Shear stress, for example, can stimulate the expression and secretion of PDGFB by endothelial cells [47,77]. Hemodynamic force may thus strengthen high-flow vessels by leading to mural cell recruitment, thereby reinforcing vessel maturation in an ANG-1 dependent manner. Furthermore, once smooth muscle cells and pericytes are recruited to the vessel wall, hemodynamic force may continue to play a role in regulating the interactions of mural cells with neighboring endothelial cells. Using customized flow chambers, smooth muscle cells have been cocultured with endothelial cells on opposite sides of a porous membrane in a manner such that the endothelial cells can be subjected to flow while the smooth muscle cells are maintained in an environment without shear stress [16,28,86]. Interestingly, at different levels of flow, the endothelial cells are able to stimulate significantly different levels of smooth muscle cell proliferation by communicating through the porous membrane [86]. It also seems that endothelial cells are able to direct the cytoskeletal reorganization of cocultured smooth muscle cells so that they align in a direction perpendicular to that of flow; this is the configuration which smooth muscle cells are known to adopt \textit{in vivo} [16]. Signals are propagated in the opposite direction as well, and smooth muscle cells are able to induce the expression of various genes in endothelial cells; this effect too is modulated by the exertion of shear stress on the endothelium [16]. In these ways, hemodynamic force continues to play a role in the later stages of vascular development, even after vessels have reached homeostasis.

As vessels form, they also become specified to differentiate into either arteries or veins. Predetermined genetic mechanisms largely control this choice between arterial or venous fate through a variety of pathways, including the Notch and ephrin signaling pathways [109,149,156]. However, experimental evidence indicates that hemodynamic force is also involved in this differentiation process. In fact, shear stress has been shown to drive endothelial progenitor cells toward an arterial fate [93]. Prolonged exposure to shear stress...
both in vitro and in vivo causes endothelial cells to upregulate classical arterial markers, such as ephrin-B2, and to downregulate classical venous markers, such as Eph-B4 (the conjugate receptor of ephrin-B2) [59,93,149]. These downstream effects of flow are even powerful enough to respecify arteries and veins. For example, the recruitment of perivascular cells to the vessel wall in response to hemodynamic force, as discussed above, appears to play an important part in the process of arterialization [84,100,141], a fact which has important implications for the use of vein grafts to replace arteries [33,65,66].

Transplantation studies first demonstrated the plasticity of endothelial cells by showing that endothelial cells from the dorsal aorta of the quail are able to colonize both arteries and veins in the developing chick up through late development [83]. Subsequent studies in chick have also shown that changes in shear stress and pressure, brought on either by venous ligation or vascular incision, are capable of inducing changes in arterial/venous fate [36,37,45,59]. These data further emphasize the importance of hemodynamic force in the process of vessel maturation.

Cardiac development and morphogenesis of the aortic arch

Given the extensive role of hemodynamic force in directing vessel morphogenesis, it is not surprising that mechanical forces appear to exert a similar influence over cardiac development [43,46,102,129,130]. Proper heart morphogenesis is an intricate program by which the early embryonic heart tube remodels into a complex, multi-chambered pump [41]. Disruption of normal flow through the heart, however, disrupts this finely tuned process [36,43,45,48]. As mentioned above, genetic manipulation of heart function via ablation of various cardiac-specific genes can cause failures in vascular remodeling; in a similar fashion, those same manipulations cause defects in cardiac septation and valve formation [48,132]. Defects in cardiogenesis can also be caused by knocking out Pkd1 (polycystic kidney disease 1) and Pkd2, genes that are involved in ciliary mechanotransduction in the mouse [9,121]. Together, these data suggest that sufficient blood flow and the ability to sense it are crucial for the heart to form properly.

However, results gleaned from genetic loss-of-function experiments in the heart are hard to interpret. For example, it is difficult to rule out the possibility that cardiac-specific mutations disrupting heart function do not also disrupt intrinsic morphogenic programs. To partially address these problems, alternative methods have been used to examine the role that hemodynamics play in this system. For instance, both pharmacological and surgical techniques, such as the ligation of the atria or the vitelline arteries of the chick, have confirmed that changes in blood flow patterns can result in defects in heart septation and valve formation [36,37,45,69,113,142]. The use of these techniques, in combination with similar investigations which instead use aortic banding, suggest that pressure in particular may be important for regulating cardiac morphogenesis [53,130,131,138]. Occluding blood flow through the zebrafish heart by cardiac injection of glass beads has also demonstrated that altering hemodynamic force in the heart can dramatically impair cardiac morphogenesis [46]. Because hemodynamic force and heart function are tightly coupled, however, further investigation will be needed to confirm these results and ensure that shear stress and pressure do indeed directly affect cardiogenesis.

A closely related and likely more tractable question is whether or not hemodynamic forces play a role in the morphogenesis of the aortic arch. Due perhaps to the close proximity of the developing aortic arch to the heart, the asymmetric formation of the great arteries has proven to be particularly sensitive to shear stress [2,55,122,150,154]. Recent studies have shown that the asymmetric formation of the adult aortic arch is a direct consequence of shear stress-induced remodeling of the embryonic BAA (branchial arch artery) system [122,154]. Normal development of the heart includes a spiraling of the outflow tract, which then sets up an asymmetric distribution of blood flow through the right and left BAAs; however, deletion
of the gene for the paired-like homeodomain transcription factor Pitx2, or deletion of the
gene’s asymmetric enhancer, results in abolished spiraling of the outflow tract, and
subsequently, symmetric blood flow and randomization of the laterality of the aortic arch in
mice [2,122,154]. Similar pathologies can be achieved by microsurgical disruption of
embryonic blood flow, or by manipulation of the levels of VEGF or PDGFA in the BAA
system [154]. The morphogenesis of the aortic arch, therefore, is a process which is
dependent on a complex interplay between a multitude of genetic and epigenetic factors,
including hemodynamic force. Similarly, future advances in our understanding of cardiac
development will need to take into account all of these complex interactions between
intrinsic and extrinsic factors during embryogenesis.

Implantation and placental development

Evidence also exists to suggest that placental development is dependent on
mechanosensitivity and hemodynamic force. Soon after implantation of the developing
embryo into the uterine wall, the maternally-derived, largely avascular deciduum surrounds
the embryo [49]. The oxygen gradient created by this hypoxic environment then induces
cytotrophoblasts to extend from the embryo toward the more oxygen-rich tissues in the
uterus [49,103]. After spanning this gap, these cytotrophoblasts will invade the uterine wall,
and finally reach the spiral arteries of the endometrium; there, they undergo an epithelial to
endothelial transition, and change their expression of a wide array of integrins so that they
may intercalate into the walls of the maternal vessels [7,26,29,49,67,103]. In doing so, these
syncytiotrophoblast cells thereby invade the maternal vasculature and remodel it so that
maternal blood flow is rerouted into the intervillous space of the developing placenta
[26,67,103].

Several studies suggest that hemodynamic force regulates this process by which the
cytotrophoblasts remodel the spiral arteries. First, cytotrophoblasts have been shown to be
mechanosensitive in vitro [67,101,123]. Intriguingly, shear stress upregulates the expression
of β1 integrin in cytotrophoblasts, a molecule which is also known to be upregulated as the
cells invade maternal vessels [123]. Shear stress can also induce the motility of
cytotrophoblasts in culture [67,123,124]. Furthermore, endothelial cells that are cocultured
with cytotrophoblasts are able to further enhance the degree of cytotrophoblast migration,
particularly in the direction opposite to flow [124]. These data therefore raise the possibility
that the hemodynamic force exerted by maternal blood flow plays a role in directing
cytotrophoblasts to invade and remodel the maternal arteries in the endometrium. This
hypothesis is supported by the in vivo observation that over time, cytotrophoblasts migrate
progressively further upstream within the maternal arterioles that they invade; in contrast,
after cytotrophoblasts invade venules, they do not migrate any further [7,67].

Poor cytotrophoblast invasion of the spiral arteries is linked with a high incidence to
preeclampsia [29,49,103,147]. Failure of the developing placenta to obtain an adequate
blood supply after insufficient vessel invasion leads to the release of vasoactive substances
into the maternal bloodstream that are thought to underlie the sudden appearance of
hypertension, proteinuria, and edema that is characteristic of preeclampsia [29,137,147]. If
hemodynamic force is important in regulating cytotrophoblast invasion of the spiral arteries,
then defects in cytotrophoblast mechanotransduction may very well play a central role in the
pathogenesis of this serious condition [81,101], highlighting the clinical importance of more
fully understanding the mechanisms of mechanotransduction in a variety of systems. It has
been suggested [104] that in the future, tetraploid complementation techniques may help us
to investigate this question by allowing genetic manipulation of the placenta without
affecting the embryo proper.
Hemodynamic force as a regulator of stem cell proliferation and differentiation

One other field in which mechanotransduction is emerging as an important factor is stem cell biology. Various in vitro studies have shown, for instance, that mechanical forces regulate certain aspects of ES cell (embryonic stem cell) differentiation [110,111,117], and that laminar shear stress in particular can be used to drive embryonic stem cells toward an endothelial cell lineage [51,153]. These data, together with evidence showing that blood vessels play important roles in a variety of stem cell niches, suggest that hemodynamic force may play a role in the regulation of stem cell maintenance.

Hematopoiesis

Hematopoietic stem cells comprise one population of cells that are newly recognized as being sensitive to the effects of hemodynamic force. During early development, primitive hematopoiesis begins in the extraembryonic yolk sac, where HSCs (hematopoietic stem cells) arise in the blood islands [24,72,108]. These cells produce the primitive erythroblasts that are crucial for early embryonic development; however, their importance is quickly supplanted by the emergence of new hematopoietic sites within the embryo proper [23,24,72]. This second wave of what is known as definitive hematopoiesis occurs in a variety of places at different times during development, including the PSp/AGM (para-aortic splanchnopleura/aorta-gonads-mesonephros), fetal liver, spleen, and eventually the adult bone marrow [1,23,24]. Though it was originally thought that the HSCs in the yolk sac colonized all of these sites in order to initiate definitive hematopoiesis, quail-chick transplantation studies eventually disproved that idea, demonstrating instead that definitive HSCs in the adult arise from a lineage that is completely independent of the cells that first appear in the blood islands [21,23]. These sites of definitive hematopoiesis have been the focus of several recent studies [1,32,91,99], and the AGM region in particular has been of considerable interest, due largely to the fact that the AGM is capable of initiating autonomous hematopoiesis even in explant cultures [23,75,90,91,99].

Recent investigations have suggested that hemodynamic force controls hematopoiesis in the AGM [1,91], possibly by acting on a specialized population of endothelial cells called the hemogenic endothelium, which is capable of producing blood cells [32,91]. Changing the in vivo rates of blood flow in zebrafish, either genetically or pharmacologically, can cause changes in the levels of definitive hematopoiesis in the AGM [91]. Experiments performed on mouse AGM explant cultures have also yielded similar results; exposing such cultures to shear stress is sufficient to increase the hematopoietic colony forming potential of the tissue, and to induce its expression of hematopoietic markers [1]. Induction of the runt-related transcription factor Runx1 in these cultures may be of particular importance, due to the fact that Runx1 is required for the emergence and function of HSCs throughout development [1,89,90,95]. NO (nitric oxide) signaling is another important component to AGM hematopoiesis; in fact, overproduction of NO can rescue the defects in hematopoiesis that are observed in flow-compromised sih (silent heart) zebrafish, while reduction in blood flow-dependent NO signaling causes significant reductions in blood production [1,91].

These observations, in combination with in vitro data, and the fact that mutant mice with cardiac defects often have defects in hematopoiesis as well, have begun to reshape our understanding of both hematopoiesis and mechanotransduction [1,72,99,132]. It appears that hemodynamic force may have even farther reaching effects than previously realized.
Hemodynamic force and the stem cell niche

Each distinct stem cell population is often observed to exist in its own specialized microenvironment, also known as a stem cell niche. NSCs (neural stem cells), for example, which divide to populate the developing brain, reside during embryonic development in specialized microenvironments known as the VZ (ventricular zone) and the SVZ (subventricular zone) [38]. They also continue to function throughout adulthood in specialized environments in the SEZ (subependymal zone), which lines the lateral walls of the lateral ventricles (Figure 3), and in the SGZ (subgranular zone) of the hippocampus [114,115,133]. Collectively, these environments comprise what we know as the neural stem cell niche.

Interestingly, blood vessels seem to play an important role in this stem cell niche. In fact, there is both in vitro and in vivo evidence to suggest that these blood vessels exert some control over the differentiation and expansion of the neural progenitors in the niche [98,114,115,133]. It has been shown in coculture experiments, for example, that endothelial cells secrete trophic factors that induce both symmetric and asymmetric divisions of neural stem cells in vitro [114]. Observations in vivo have also demonstrated a striking physical association between blood vessels and proliferating NSCs in the SEZ and the SGZ [98,115,133].

Based on this evidence, some have speculated that NSC maintenance and division in the neurovascular niche may be another process which is regulated by hemodynamic force. Such a mechanism would be consistent with the fact that shear stress regulates the secretion of a number of growth factors by the endothelium, many of which are known to influence neurogenesis [80,126,144]. It would also fit with data showing that the changes in cerebral blood flow that are induced in mouse models of stroke result in a stimulation of neural proliferation and neuroblast migration [34,62,157]. Actually, another step toward verifying this hypothesis has recently been made with the demonstration that surgical induction of chronic cerebral hypoperfusion increases levels of neurogenesis in the SEZ [78]. If the changes in blood flow and shear stress that are so often experienced by cerebral vessels after brain trauma are indeed a trigger for neurogenesis, then it would also explain how neural stem cells in the SEZ are able to divide in response to non-local trauma [34]. However, future studies will need to carefully separate the effects of hemodynamic force from the effects of hypoxia and cell damage that also occur in these situations.

These promising observations therefore merit further investigation into the possible role of hemodynamic force in the regulation of neurogenesis. Confirmation of such a mechanism could potentially lead to therapies for the stimulation of endogenous brain repair after stroke [5], or for the correction of the histopathological effects of reduced cerebral blood flow during embryonic development [76]. A better understanding of this process could also lead to new treatments for the changes in neurogenesis and cerebral blood flow that are observed in those diagnosed with some mental illnesses such as schizophrenia and depression [63,70,74].

Hemodynamic force may also prove to be important in other stem cell niches as well. MSCs (mesenchymal stem cells), for example, reside within the bone marrow in a vascularized niche of their own [18]. These MSCs are known to mediate bone deposition and cartilage formation through a regulated production of osteoblasts and chondrocytes [120,152] and are also thought to regulate mechanosensitive bone remodeling, possibly through sensitivity to interstitial fluid flows created in response to bone compression [6,39,54,143,152]. MSCs may be sensitive to hemodynamic forces within the bone as well; in fact, accumulating evidence suggests that MSCs can differentiate into vascular cell types [6,44,106,148], and that shear stress and cyclic strain may regulate this process [6,44,106,148]. The role of
matrix stiffness in determining MSC fate further supports the idea that the mechanical environment of the bone marrow niche may be important [27]. Due to their proposed role in producing perivascular cells that contribute to vessel function [6], as well as their close association with HSCs, it would therefore not be surprising if MSCs are also eventually shown to be sensitive to hemodynamic force.

Conclusions

Hemodynamic force was first observed to play a role in flow-induced vascular remodeling over a century ago. Today, those classical observations have come to serve as the foundation of an entire field focused on the roles of hemodynamic force in the body. Even now, we are still continuing to uncover new ways in which hemodynamic force exerts important effects on various aspects of embryonic development. Considering the important roles played by mechanotransduction in the pathogenesis of such a wide variety of diseases, further elucidation of the effects of hemodynamic force on embryonic development should remain a research priority, now and in the future.

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References


Figure 1. Summary of the effects of hemodynamic force on embryonic development

(A) Three mutually perpendicular hemodynamic forces are exerted onto the vessel wall by flowing blood (a). First, blood pressure exerts a normal force against the vessel wall (b). Second, as a result of this normal force, the cells comprising the wall also experience circumferential stretch (c). Finally, because flowing blood exerts drag on the vessel wall, the endothelial cells that line the vessel experience a frictional force known as shear stress (d). These hemodynamic forces are important at a variety of developmental time points and in a diverse range of vessel architectures, including those seen in: the heart (e – adapted with permission from Oxford University Press: <Hogers B, et al. (1999). Extraembryonic venous obstructions lead to cardiovascular malformations and can be embryolethal. Cardiovasc Res 41:94. Figure 6a.>) [45]; the extraembryonic yolk sac (f); the bone (g – adapted with kind permission from Springer Science+Business Media: <Morini S, et al. (2006). Microvascular adaptation to growth in rat humeral head. Anat Embryol (Berl) 211:407. Figure 8.>) [82]; the placenta (h – © Society for Reproduction and Fertility (2009). Reproduced by permission. Adapted from: <Burton GJ, et al. (2009). Regulation of vascular growth and function in the human placenta. Reproduction 138:897. Figure 3b.>) [11]; and the brain (i).

(B) Hemodynamic force can have effects on a variety of cell types. The heart (a) creates hemodynamic force (b) by pumping blood; reciprocally, this force affects the morphogenesis of the heart as it develops. This hemodynamic force is also sensed by endothelial cells that line the walls of vessels (c), and thereby directs a variety of intrinsic cellular responses, including those that are important for vascular remodeling and arterial/venous specification. Some of these endothelial cells, particularly in the aorta-gonads-mesonephros, may be hemogenic endothelial cells (depicted in yellow) that divide to produce blood cells in a flow-dependent manner (d). Hemodynamic force may also further regulate the self-renewal and differentiation of hematopoietic stem cells that are not part of the vessel wall (e) by using paracrine signals released by the endothelium. Other paracrine signals released by the endothelium in response to hemodynamic force may also influence the self-renewal and differentiation of progenitor cells in other stem cell niches, including neural stem cells (h) and mesenchymal stem cells (m). Finally, flow-dependent release of paracrine signals by endothelial cells have also been implicated in the recruitment of mural cells (k) to the walls of developing vessels, and in the invasion of maternal vessels in the endometrium by cytotrophoblasts (l) during placentation.
Figure 2. Defective yolk sac vascular remodeling in embryos with deficient heart function
Shown are littermate mouse embryos at the 7 somite (A, B), 10 somite (C, D), and 23 somite (E, F) stages, which are either heterozygous (A, C, E) or homozygous (B, D, F) for the recessive null allele of \textit{Mlc2a}. Blood vessels are visualized through use of the fluorescent reporter Tg(\(\varepsilon\)-globin-KGFP), which is transgenically expressed in primitive erythroblasts. Shortly after gastrulation, extraembryonic mesodermal cells in the yolk sac coalesce to form the blood islands (A). Shortly thereafter, angioblasts in these blood islands form the primitive capillary plexus of the yolk sac (C), and by the 23 somite stage, this early capillary plexus has remodeled into a hierarchical structure of large and small vessels (E). Mice which are homozygous for the \textit{Mlc2a} null mutation, and therefore have deficient heart function, are still able to form the blood islands (B) and the primitive capillary plexus in the yolk sac (D). However, they are not able to remodel their yolk sac vasculature as the embryo grows (F), and therefore die in mid-gestation. This process of vascular remodeling is thought to depend on the hemodynamic force created by the robust flow of blood through the capillary plexus in wildtype mice (adapted with permission from the Company of Biologists: \textless Lucitti JL, et al. (2007). Vascular remodeling of the mouse yolk sac requires hemodynamic force. Development 134:3321. DOI:10.1242/dev.02883. Figure 4.\textgreater) [68].
Figure 3. The adult neural stem cell niche
This figure shows a flatmount preparation of the highly vascular subependymal zone of the adult mouse brain. Functional vessels are visualized with a fluorescently labeled dextran that fills the vessel lumen. Neural stem cells persist in this region of the adult brain, which is directly adjacent to the lateral ventricle.