Literature Analysis of Piezo1 Signaling Pathway for Arteriovenous Malformations

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Endothelial cells are critical regulators for blood supply as they form the lining of blood vessels (Alberts et. Al., 2002). They are the first perturbed in systemic changes and are therefore the first responders. This is not limited to changing cell supply, because they are also critical for cell migration as they extend and remodel blood vessel formation, networks, and connections. The endothelial cell in mature blood vessels has sensory abilities through its membrane mechanosensors which detect the flow of the blood across its surface, allowing it to sense the shear stress exerted on the vessel and therefore adapt to the dynamic flow by modifying the vessel diameter or wall thickness. Shear stress is therefore, the key initiating factor that triggers the cascade causing the resulting phenotypes observed.The endothelial cells are also critical for angiogenesis, which creates transient and pruned capillary connections between arteries and veins (Alberts et. Al., 2002). With genetic mutations, these normally transient and small capillary beds can become permanent direct connections between an artery and vein, which are also called Arteriovenous Malformations (AVMs). These direct connections are characteristic of Hereditary Hemorrhagic Telangiectasia (HHT), an autosomal dominant vascular disorder. The HHT disorder arises due to Alk1/ACRVL1, Endoglin, or SMAD4 haploinsufficiency, encoding for components in the TGFβ signaling pathway and for endothelial Bone Morphogenetic Protein (BMP) signaling which are all necessary to prevent the AVMs. Dr. Roman's lab, at the University of Pittsburgh Graduate School of Public Health, has discovered a two step mechanism in which AVM formation occurs by studying *alk1*-deficient zebrafish embryos. Endothelial cell movement within lumenized arterial vessels is against the flow of blood, as they migrate

in a distal-to-proximal direction towards the heart and areas of high shear stress. AVM development's first step is the loss of flow-dependent Alk1 signaling that enhances endothelial cell migration in the direction of flow within lumenized vessels, resulting in cell accumulation and arterial caliber of segments distal to the heart. Next, the normally transient capillaries get retained in a flow-dependent manner which forms high-flow AVMs (Rochon et. al., 2016).

Although the two step formation is laid out, the molecular basis--including mechanical forces and cellular pathways--behind why this takes place is not completely understood. There are many possible leads as to why this process takes place, including perturbations to circulating BMP molecules, changes in cell polarity, and changes in haemodynamics in flow, but the spatiotemporal location characterization of AVMs derived shunts in *alk1* mutants had not been attempted. When characterized, it was found that the AVMs in these cranial vessels formed within 36-48 hours post fertilization (hpf) in zebrafish embryos. These shunts were characterized to appear in one of three locations, but 51% of shunts developed in the Basal Communicating Artery (BCA) to the Primordial Midbrain Channel (PMBC). The rest of the observed shunts developed between the Posterior Communicating Segment (PCS) and Primordial Hindbrain Channel (PHBC), and between the Basilar Artery (BA) and the PHBC.

Through previous literature investigation, it has been deduced that the Piezo1 Signaling Pathway of endothelial cells could be the source of the variation in the shunt formation and its locations. We therefore hypothesize that agonists of Piezo1 will exacerbate AVM phenotype through its ability to transduce biological signals leading to shunt enlargement and that antagonists of Piezo1 will rescue phenotype by blocking the

cellular response to increased shear stress. Using zebrafish embryos as the method of analysis will be crucial as zebrafish embryos are transparent, and through the use of transgenic lines that track red blood cells, accurate quantification of the effects on haemodyanmic responses to the differential influencers on the Piezo1 channel activity can be deduced. Zebrafish embryos being transparent also allows for visualization of the heart under dissecting microscopes, allowing for heartbeat quantification to be possible as well. In this paper, I will explain the background of the Piezo1 signaling pathway, the implications the pathway has on the properties of the Piezo1 channel, and the way the agonists and antagonists have on the pathway and the resulting haemodynamic changes they cause.

As previously mentioned, endothelial cells play a critical role in vessel formation which has been shown to be affected by blood flow and its haemodynamic changes. To be able to sense these changes, the endothelial cells have mechanosensors that transduce signals from haemodynamic fluctuations to induce changes in its vessel caliber or formation. Therefore, it is postulated that there must be a mechanotransducing channel in the arterial vessels of *alk1*-deficient zebrafish that dictates both the formation of shunts and the spatiotemporal location of their development. Literature analysis has indicated that Piezo1, a calcium gated ion channel and biological transducer of mechanical force (Moroni et. al., 2018), is required for endothelial cells to respond to shear stress (Davies et. al., 2019). The global and endothelial-specific loss of the channel results in embryonic lethality, as it regulates vascular development and remodeling (Wang et.al. 2016). Piezo1 therefore acts as a sensor of the blood flow, which it does by regulating ion influx of calcium into the

epithelial cells (Li et. al., 2014). The regulation of this ion flux has been shown to be critical, as repeated mechanostimulation of cell membranes has shown to cause Piezo1 to become desensitized and lose its inactivation properties. It is hypothesized that disruption of the cortical cytoskeletal support and/or membrane domain structure are the main factors in the loss of inactivation properties of the channels (Suchyna et. al. 2018). If the inactivation gate is lost, voltage-gating of the channel is hindered. With outward permeation of the channel, depolarizing voltage from a positive electrochemical gradient is induced, and the channels can recover. Keeping that in mind, it still takes considerable voltage change (+100 mv in zebrafish), to open inactivation gates. Therefore, voltage modulation of Piezo1 channels is needed to bring channels to a certain range, wherein mechanical pressure can release an appropriate amount of channels to respond to the haemodynamic changes on the cell surface, without developing desensitization (Moroni et. al. 2018).

As mentioned, although Piezo1 is flow and force induced, the ion flux plays an important role especially after the channel is stimulated. The Piezo1 Flow Induced Shear Stress Pathway is a series of mechanotransduction signaling cascades shown in mice models (Wang et.al. 2016). The cascade follows as such: shear stress in vascular structure induces the Piezo1 channel activation which in turn signals for calcium ions to enter the endothelial cell. This influx triggers some currently unspecified mechanism wherein ATP is released. The ATP then activates G_q/G_{11}-coupled purinergic $P2Y_2$ receptor that in turn is an upstream regulator for the PECAM-1/VE-Cadherin/VEGFR mechanosensory complex. This complex is thought to cause pathway AKT to phosphorylate eNOS which triggers sustained NO release, which is a key vasoactive

mediator to maintain vascular tone and blood pressure. NO is also regarded as the primary endothelial relaxing and vasodilation factor. It is important to note that acute increase in flow still leads to NO formation, but it happens through transient intracellular calcium elevation which is activated in calcium/calmodulin-dependent manner (Wang et. al. 2016). To test Piezo1's abilities, researchers used Yoda1, a selective agonist on Piezo1 channels. They found that Yoda1, mimicked fluid shear stress on Piezo1, and dose-dependently induced vasodilation of isolated arteries (Wang et.al. 2016). It did so following the Flow Induced Cascade Model, and was strongly reduced after Piezo1 knockdown (Wang et.al. 2016). The researchers also found that it dilates pre-contracted arteries at increasing concentrations as well. These results, as mentioned before, could be due to dose-dependent increase of Yoda1, or due to its role as a broader differential pathway effector. The second option could be plausible, as recent research indicates that Yoda1 can still activate Akt and Erk1 in the absence of Piezo1 and stimulates intracellular calcium ion elevation through other calcium ion channels (Dela Paz & Frangos, 2018). It has also been shown that Yoda1 causes dramatic changes in kinetic response of the Piezo1 channel, notably slowing the activation phase of transient currents and also causing increased sensitivity of the channel by pressure, even reducing its half maximal activation pressure (Syeda et.al. 2015). This all supports the idea that the gating of the Piezo1 channel is not solely due to mechanical force, but rather is a combined with voltage gating. Furthermore, although all this is true, it is critical to note that Piezo1 channel intracellular calcium increase is not the main factor that induces the greatest calcium concentrations, rather it is the downstream amplification system. This is indicated by the loss of the G_q/G_{11}-coupled purinergic $P2Y_2$

receptor causing a strong decrease in flow-induced release calcium concentrations. This is further corroborated with research that shows loss of Piezo1 but continued activation of the aforementioned G-coupled receptor does not decrease levels of intracellular calcium levels (Wang et.al. 2016).

With investigations into a Piezo1 channel agonist, analysis of the channel in response to antagonists is necessary, specifically GsMTx4. This molecule has been found to have an inhibitory effect on Piezo1 by blocking of channel. The inhibition causes similar effects to the Piezo1 knockdown experiments, as it has been found to suppress shear stress evoked calcium entry into HUVEC cells, and also disrupted HUVEC cell alignment (Li et.al. 2014). It is noted that GsMTx4 does not bind to the channel directly, but rather it changes the local membrane properties near the channel, which it does by its deep bilayer connection in the membrane (Suchyna et. al. 2018). Further studies also show GsMTx4 inhibits up-regulation of Akt kinase, critical in the aforementioned flow-dependent pathway. It has also been shown that GsMTx4 causes vascular smooth muscle relaxation by both decreasing mechanically activated exterior cation currents and down-regulation of Endothelin-1 stimulated arterial resistance increase (Suchyna et. al. 2018).

All of this research is imperative for understanding the Roman Lab's AVM models of zebrafish. Yoda1 appears to increase sensitivity of the channel to flow-induced force, while GsMTx4 appears to decrease it. These Piezo1 channel influencers could be important in modulating the response of endothelial cells to haemodynamic forces within vasculature, particularly in understanding the differential spatiotemporal locations of the shunts within the zebrafish brain. All of this information outlined in this literature

analysis, gives the foundational background necessary to understand the molecular basis of the cell signaling pathway that impacts the Piezo1 channel function in endothelial cells. Literature results of both the antagonist and agonist's effects on the channel gives good reason to suspect it will play a critical role in AVM formation within *alk1*-deficient zebrafish embryos. As detailed earlier, the increase of channel sensitivity to shear stress imposed on the endothelial cells by Yoda1 stands to reason that it could lead to worsening of the AVM phenotypes. Introduction of increased Yoda1 concentrations within vessels should lead to amplified signaling cascades of the downstream signaling components, specifically with the G-coupled receptor activation resulting in high concentrations of intracellular calcium concentrations which leads to vasodilation. This supports the idea that the AVM phenotype could get worse, as in normally pruned, primitive, and transient AVM vessels can end up constantly dilated, and would remain large. This also stands to reason that if the vessels are constantly dilated, this would mean more blood volume—hence greater haemodynamic forces—will be processed through them. This should in turn lead to a feedback loop that maintains the increased diameter of the vessels which supports more blood volume and force against a larger cross-sectional area of endothelial cell wall to continually stimulate other Piezo1 channels, increase intracellular calcium concentrations, and hence lead to exacerbated AVM phenotypes.

On the other hand, with GsMTx4, the expectation is that this antagonist would be able to rescue AVM phenotypes observed in mutant zebrafish. As previously mentioned, GsMTx4 has shown overall inhibitory effects on both the Piezo1 channel and Akt upregulation resulting in a lack of eNOS phosphorylation and NO release,

preventing vasodilation of blood vessels. This suggest that GsMTx4 has the potential to keep arteriovenous connections pruned or transient, and should also have the potential to return dilated vessels back to their primitive state. Therefore, connecting the molecular basis and the perceived effects together, it is reasonable to assume that inhibitory effects of GsMTx4 could either decrease the effect of the regular Piezo1 channel signaling that is stimulated by shear stress invoked calcium signaling, or it could possibly hinder the downstream cascade components and also restore the vessel to its previous state. In contrast to Yoda1, the pruned vessels should have less cross-dimensional area of the endothelial cell wall where blood volume can come into contact and create high shear stress situations; however, regardless of high shear stress exerted onto the vessel walls, the prevention of the signaling cascade and intracellular calcium buildup should be able to prevent AVM phenotypes or prevent them from getting worse.

In conclusion, based on all literature analysis, it seems highly likely that both Yoda1 and GsMTx4 can be used as treatments in *alk1*-deficient zebrafish embryos to differentially affect the AVM phenotypes. Yoda1 should exacerbate the phenotypes and GsMTx4 should alleviate or rescue some phenotypes. As for future direction, a lot of the literature analysis suggests the importance of not just high shear stress and its corresponding mechanical transduction pathway activation, but also ion transduction with voltage modulation having a great effect on Piezo1 channel activity. This seems to overlap with evidence regarding the differential cell polarization of cells movement in *alk1*-deficient embryos during vessel formation. It offers an interesting question as to whether the changes in ion flux and transduction could be causing these changes in

where the cells end up. This could possibly give an answer as to why the shunts form in specific regions. With this semester being cut short, I could not investigate personally into how administration of Yoda1 and GsMTx4 could change the spatiotemporal locations of the shunts, but I hope this literature analysis at least provides some supplementary information as to the mechanisms and pathways in the Piezo1 system's effects on AVMs and aids in any foreseeable experiments or papers.

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