A new structural and functional role of Pdlim7 in mouse vasculature

Pdlim7, an actin-associated protein of the PDZ-LIM protein family, is found in many actin-rich structures such as vascular smooth muscle and platelets, and has diverse biological functions. In the mouse, the absence of Pdlim7 leads to a hyperthrombotic phenotype in which the mice form blood clots very quickly. Studies of the platelets and the blood coagulation cascade of Pdlim7-deficient mice have not generated a complete explanation for this phenotype (Krcmery et al., 2013). The aim of this project is to determine the extent to which the loss of Pdlim7 alters the structure and resulting function of the mouse blood vessels, in order to better understand how the blood vessels may contribute to the hyperthrombotic phenotype. A variety of vessel types will be analyzed using immunohistochemistry and confocal microscopy to make this qualitative determination.

When a blood vessel is cut, it will bleed until a blood clot is formed and seals off the damaged area of the vessel. The three main contributors to blood clot formation are the blood coagulation cascade, the platelets, and the blood vessels. Platelets normally circulate through the bloodstream in a small, inactive form. However, upon vessel damage, nearby platelets are activated, they spread and form a network with fibrin that ultimately leads to clot formation. A critical component in platelet activation is the structural protein collagen, which is found within the vessel wall, but is normally separated from the platelets by the inner lining of the vessel. However, when injury causes damage of the vessel lining, the collagen becomes exposed and activates the platelets. The vessels also contribute to clot formation by constricting at the site of the cut, thus decreasing the cross-sectional area through which the blood can flow and increasing the contact between inactive platelets and collagen (Clemetson, 2012).

The ability of vessels to constrict or dilate is a function of the smooth muscle action in the vessel wall. A major component of smooth muscle is actin, a key protein of the cell's cytoskeleton, which controls cell shape and mobility. Associated with this actin cytoskeleton are many proteins, including Pdlim7. Previous cellular work has shown that Pdlim7 binds to polymerized actin and is involved in actin dynamics, but a detailed functional role remained unclear (Camarata et al., 2006). Suprizingly, mice lacking Pdlim7 are hyperthrombotic and have about a 60% lethality rate in the first two days after birth due to pre-mortem blood clots found in multiple types of vessels. These mice clot so quickly in fact, that when the ends of their tails are clipped, most do not bleed at all, whereas wild-type mice stop bleeding after an average of about one and a half minutes (Krcmery et al., 2013).

The Simon laboratory has been investigating each of the three major contributors to blood clot formation listed above. They have determined that the coagulation cascade is unaffected by the loss of Pdlim7, but that the platelets have an aberrant actin cytoskeleton. However, the functionality of these platelets appears normal enough that the mutant platelets alone cannot explain the hyperthrombotic phenotype. Furthermore, developmental studies with mouse embryos suggested that the vessels were unaffected by the loss of Pdlim7. However, my research in the Simon lab with adult mice has provided new evidence that the filamentous actin is less organized in the ascending aortas of Pdlim7-deficient than wild-type control mice (See Appendix 1). I have only ever examined the aorta, though, so the extent to which the entire vasculature differs and how this may contribute to the hyperthrombotic phenotype has yet to be determined. A

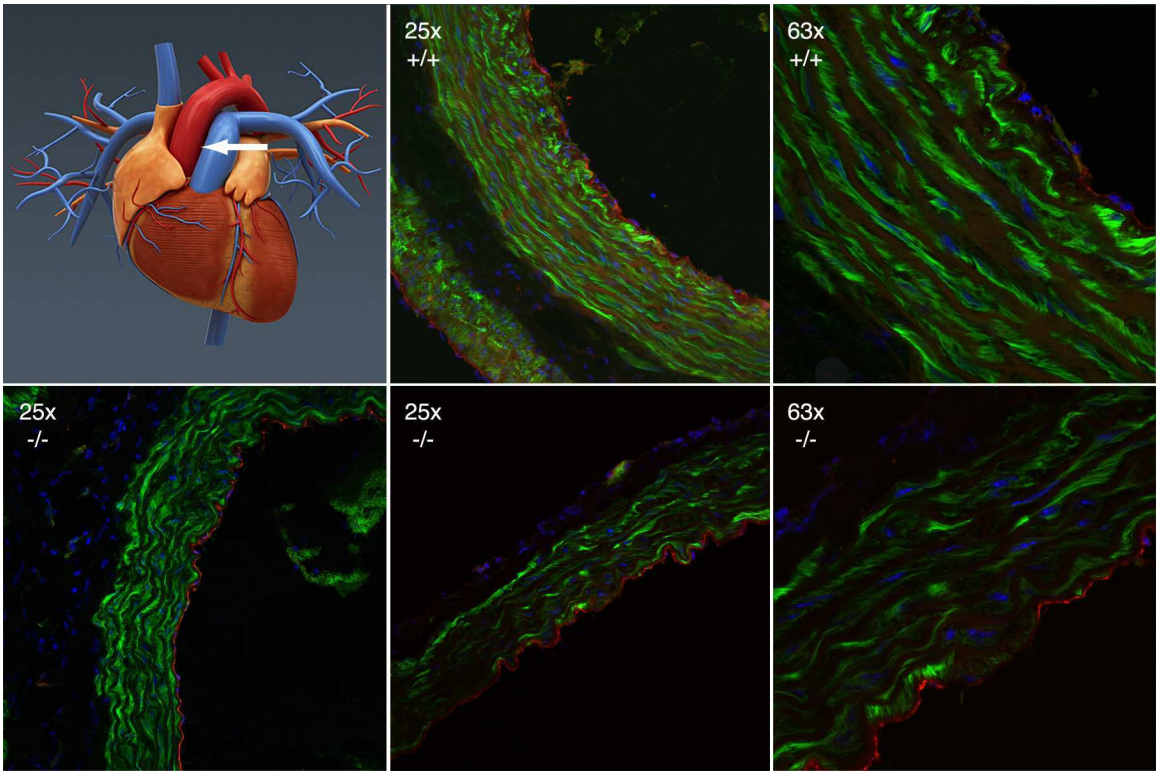
possible combinatorial defect in platelet and vessel function may explain the unusual rapid clotting observed in these mutant mice.

The objective of this project is to determine the extent to which the entire vasculature differs between normal mice and those lacking Pdlim7. Having already examined the ascending aorta, I must now examine different types of vessels. Specifically, I will examine the F-actin organization of the Superior Vena Cava, the tail vein, aortic arch, and thoracic aorta. In doing so, I will examine multiple types of vessels, those with lower blood pressure (veins), and those with higher blood pressure (arteries). Additionally, I plan to examine the aortic wall structure at different positions that each contain different blood pressures. These examinations will allow me to determine if the loss of Pdlim7 leads to disorganized actin structures in veins as well as arteries, and the role that blood pressure may play in this disorganization. Because embryonic aortas displayed a similar actin organization between wild-type and Pdlim7-deficient mice, which is in contrast to my observations in adult mice, I hypothesize that the vasculature of mice lacking Pdlim7 essentially ages faster, evidenced by poor actin organization, and that this accelerated aging will be more pronounced in vessels containing high blood pressures.

The procedure I will use for this project is the same I was trained in when I joined the Simon lab last summer. All materials will be provided by Dr. Simon. The first step is to sacrifice the mice using CO_2 euthanasia and cervical dislocation. The heart, tip of the tail, and entirety of the aorta are then harvested. Each tissue is then placed with identical orientation in separate O.C.T. blocks and snap-frozen using dry ice. I use a cryostat to cut 10 μm "sections" of the frozen O.C.T. blocks containing the tissue. I then perform immunohistochemistry on the sections, which have been mounted on glass slides. I will use DAPI as a nuclear stain, a specific antibody to F-actin, and another to PECAM to identify the inner vessel endothelial layer. I will also use a specific elastin antibody to stain elastic fibers that are sandwiched between the actin filaments. Using a Zeiss 510 confocal microscope, I can then take pictures of the vessel sections in which the different cell layers and proteins, which are stained by the specific antibodies, fluoresce in different colors for clear identification. This allows for the direct observation of the macrostructure of the vessels. This entire procedure will be repeated between 5-10 times to account for biological variation and to ensure a statistically relevant n value. The final images will be used to qualitatively determine the level of F-actin organization in each vessel type and to compare these levels of organization between vessel types. These comparisons will determine if the loss of Pdlim7 affects certain vessel types more than others and if there is any correlation to the blood pressure within those vessels.

Thanks to my coursework at Northwestern University and to having worked in the Simon lab for the past nine months, I am very proficient in the procedures outlined above and have a strong understanding of this project and the overall goals. Upon the completion of this summer project, I plan to continue my studies throughout my senior year and write a senior thesis about Pdlim7's role in the vasculature of mice. Dr. Simon has provided extensive training concerning the various methodologies but also given me substantial freedom to design the experiments my way. I believe that this approach has and will continue to help me to become a more independent thinker, problem-solver, and scientist. The application of these strengths to my coursework and extra-curricular activities will help me reach my ultimate goal of attending medical school.

Appendix 1:



Transverse section through the aorta of 6-month old WT and Pdlim7 null mice. Vessels were stained for F-actin (green) using Phalloidin, nuclei (blue) using DAPI, and endothelium (red) using PECAM. In contrast to normal organization of smooth muscle actin filaments in WT mice, Pdlim7-/- vessels display disorganization of actin.

Literature Cited

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