Myxine glutinosa (Hagfish): a model for early endothelium

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The endothelium is the inner cell layer that lines the inside of all blood vessels. The endothelium participates in wide range of physiological processes, including hemostasis, growth and proliferation of other cells, antigen presentation, extravasation of immune cells, and metabolism of tissue- or blood-derived hormones. Endothelial cells display remarkable heterogeneity in structure and function. These cells can dynamically adapt to environmental insults such as shear stress, hypoxia, oxidative damage, or even frank denudation (1). There is growing awareness of the intricacy of the molecular machinery needed to mediate these multiple responses of endothelial cells in humans.

Hagfish are well placed in the evolutionary tree to call attention to certain heuristic difficulties that accompany the invertebrate/vertebrate divide. Several features of the hagfish body plan bridge (and blur) the morphologic transition from invertebrate to vertebrate. It shares several features common to vertebrates including segmental architecture with repeating myomeres, cellular blood, an endoskeleton, and mesoderm-derived endothelial cells. Unlike jawed vertebrates, however, hagfish lack a biting apparatus, a third semicircular canal in the inner ear, spleen and paired appendages; and they possess a large notochord that is retained into adulthood, horny teeth and internal gills. The uniqueness of hagfish is further evidenced by the existence of an aneural systemic heart, disseminated pancreatic tissue (within the submucosa of the gut), monomeric hemoglobin, slime gland pores and degenerate eyes. Research in hagfish biology may spark insights regarding the most basic features required for an endothelium in the vertebrate body plan.

The goal of the current ongoing study was to begin defining the form and function of the endothelium in hagfish. To that end, we examined the (1) gross vascular anatomy, (2) histology of local vascular beds, and (3) ultrastructure of endothelial cells in *Myxine glutinosa*.

With regards to the gross vascular anatomy, hagfish have a closed circulation. When injected intravenously, Evans blue dye remained within the blood vessels (Fig. 1). Hagfish maintain the lowest arterial blood pressures and highest relative blood volumes of any vertebrate. Consistent with these data, intra-aortic injection of Evans blue dye revealed little to no flow during diastole and a circulation time on the order of minutes (data not shown). Like other fish, hagfish have a double-chambered heart consisting of a single atrium and a single ventricle. Hagfish have a number of accessory hearts: the portal heart, two caudal hearts and two cardinal hearts. Most notably, the portal heart - a unique adaptation not shared by teleosts, elasmobranches, or lampreys - serves to increase blood pressure in the common portal vein. The single chamber of the portal heart beats asynchronously with the systemic heart, as it propels blood from the gut into the liver sinusoids. The hagfish circulation also possesses a series of blood sinuses which are in direct communication with systemic vessels, leading some to characterize the circulation as "semi-closed" (3). The most prominent of these is the large subcutaneous vascular sinus located between skeletal muscles and the skin, stretching from the tentacles of the snout to the caudal fin fold. Hagfish can hold up to 30% of their blood volume within the sinus system. It is believed that the caudal and cardinal hearts function to reintroduce sinus blood into the systemic circulation.

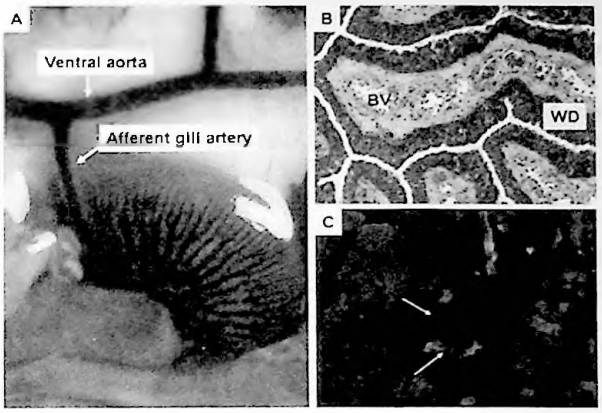

Fig. 1. A. Evans blue was injected into the posterior cardinal vein and appears in the ventral aorta, afferent gill artery and radial arteries of gill pouch. B. H&E staining of a 10 μm frozen section from the gill of hagfish. BV indicates one of several blood vessels cut in cross section. WD indicates water duct or channel. C. Immunofluorescent staining of 10 μm frozen section from the gill of hagfish using FITC-labeled UEA-1. Arrows point to UEA-1-positive endothelial cells.

Histological analyses were carried out using a combination of hematoxylin and eosin (H&E), and lectin staining (Fig. 1 shows gills). Previous studies have demonstrated that similar to large arteries of other vertebrates, the ventral aorta (and presumably other large arteries) of the hagfish contains three layers: the endothelium, media, and adventitia (2, 4). The presence of an endothelial layer and underlying smooth muscle cells was appreciated on H&E stains of the aorta. Immunofluorescent studies employing the lectin, UEA-1, revealed staining in the endothelial lining of the arteries and veins. Lectin binding was not uniform, but rather was observed in a minority of endothelial cells. Such heterogeneity has been described in studies of mammalian endothelium. Immunolocalization studies using other lectins are currently in progress.

Ultrastructural studies using electron microscopy have been initiated to examine the endothelium in various organs of the hagfish. These studies demonstrate structural heterogeneity in structure between endothelial cells from different sites of the vasculature. Endothelial cells from some vascular beds contain abundant caveolae. More detailed characterization of ultrastructural heterogeneity is ongoing.

In summary, hagfish possess endothelium identifiable by histochemistry, lectin binding and electron microscopy. The fact that the endothelium is shared by jawless and jawed vertebrates is evidence that the endothelium was present in the ancestor of these animals. The absence of endothelium in amphioxus indicates that this structure was not present in the common ancestor of cephalochordates and vertebrates, and must have evolved following the divergence of amphioxus from vertebrates, between 550 and 510 million years ago.

Further analysis of the hagfish endothelium – at both a physiological and molecular level - may provide important information about the evolutionary history of endothelial structure and function. The endothelium of higher vertebrates is involved in many aspects of homeostasis, including regulation of vasomotor tone, leukocyte trafficking, hemostasis, antigen presentation, barrier function, and wound healing. The endothelium is a mosaic of phenotypes, exhibiting site-specific differences in morphology and activity. Such complexity is likely to reflect the cell's ability to adapt to the varying needs of underlying tissues. We are interested in determining the extent to which the hagfish endothelium is

heterogeneous in structure and function and whether site-specific properties reflect local tissue function.

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