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Review

Endothelial cells in the context of brain arteriovenous malformations

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ABSTRACT

A subset of brain arteriovenous malformations (AVM) cannot be treated using today's treatment paradigms. Novel therapies may be developed, however, as the underlying pathophysiology of these lesions becomes better understood. Endothelial cells (EC) are the subject of new biological therapies, such as radiosensitisation and vascular targeting. This work reviews the current research surrounding EC in the context of brain AVM, including both *in vitro* and AVM specimen analysis, with a particular focus on the effect of radiation on EC. EC are heterogeneous with no recognised common phenotype, which leads to difficulties in applying the results of the common studies using human umbilical vein endothelial cells to AVM research. Human brain EC are observed to have a high rate of proliferation and also have a reduced apoptotic response to inflammatory mediators such as transforming growth factor-beta. The angiogenic factors vascular endothelial growth factor and endothelin-1 (ET-1) are not normally produced by quiescent brain vasculature, but are produced by AVM EC. Radiation causes EC to separate and become disrupted. Leucocyte and platelet adherence is increased for several days post-irradiation due to increased E-selectin and P-selectin and intercellular adhesion molecule-1 expression. ET-1 is highly expressed in irradiated AVM EC. Radiosurgery produces local radiation-induced changes in EC, which may allow these changes to be harnessed in conjunction with other techniques such as vascular targeting.

1. Introduction

Brain arteriovenous malformations (AVM) consist of vessels that shunt blood from the arterial to the venous systems. The major cause of morbidity and mortality associated with AVM is haemorrhage; the major goal of AVM treatment is to reduce the risk of rupture. Treatment of AVM consists of surgery, radiosurgery, or embolisation, alone or in combination. There does, however, remain a subset of AVM where treatment carries an unacceptably high morbidity or mortality risk. These are typically large AVM located in eloquent brain. Surgical techniques are unlikely to improve sufficiently to render large deep AVM resectable. Radiosurgery is constrained by size and dose and is also unlikely to improve to the point where large AVM are effectively treated. Embolisation as a curative treatment is limited to small AVM with few feeding vessels.

Development of biological therapies may offer a new treatment option. A detailed understanding of AVM pathophysiology and molecular biology will be necessary before such therapies can be developed. The endothelium has great theoretical potential as a therapeutic target in AVM. This review provides an overview of the molecular biology of AVM endothelium, with particular reference to the effect of radiation on AVM endothelial cells (EC).

2. Arteriovenous malformations

AVM are characterised by a conglomerate of tortuous vessels, with feeding arteries and draining veins. The vessels range from relatively normal arteries and veins to highly abnormal vessels that are difficult to distinguish as either arterial or venous in origin. Although the normal intervening capillary network is absent, abnormal capillary proliferation may occur in adjacent parenchyma.[1] The endothelial cell layer of an AVM is structurally different to the normal cerebrovascular endothelium (Fig. 1a–d).[2]

3. Endothelium

EC form a highly metabolically active monolayer, and are important in the control of vasomotor tone, cell movement, vascular permeability and immunity. They regulate thrombosis and thrombolysis, platelet adherence and leucocyte interactions with the vessel wall. The endothelium is involved either in initiation of the disease process or is damaged as a result of disease in almost all pathological states.[3]

3.1. Endothelial cell heterogeneity

Although there are factors common to the function of the endothelium as a whole, marked heterogeneity is observed from one organ to another and in different parts of the vasculature.[4–6] Thus,

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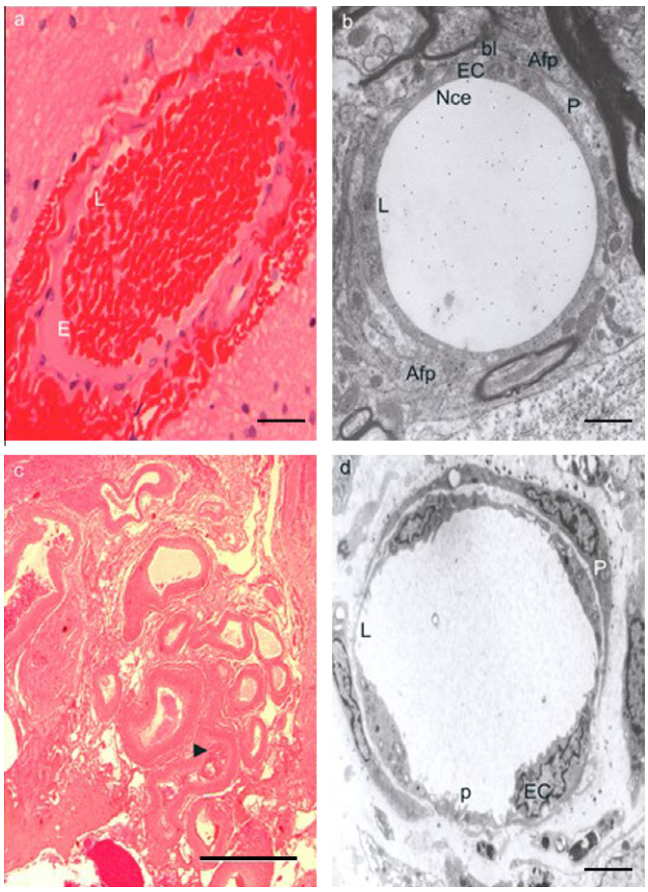

Fig. 1. Haematoxylin and eosin (H&E) stained sections (a, c) and electron micrographs (EM) (b, d) of normal human capillaries and arteriovenous malformation (AVM) vessels showing: (a) normal capillaries in the cortex, lumen (L) filled with fresh erythrocytes and endothelium (E) (H&E, bar = 80 μm); (b) a transverse section of a normal capillary with non-fenestrated, continuous endothelium (Nce) – the capillary vessel consists of flat endothelial cells (EC) coated with a relatively thick basal lamina (bl), pericytes (P) covered by a single basement membrane; Afp = perivascular foot process of astrocytes (EM, bar = 2 μm); (c) gelfoam within an AVM vessel (arrowhead) (H&E, bar = 620 μm); (d) AVM vascular wall lined by a discontinuous layer of fenestrated EC that are hyperactive with filopodia (p) towards the interior side (EM, bar = 10 μm). These images have been re-used with permission of Wolters Kluwer Health (No. 100216-000149) from the following article: Tu J, Stoodley MA, Morgan MK, et al. Responses of arteriovenous malformations to radiosurgery: ultrastructural changes. *Neurosurgery* 2006;**58**:749–58.

a characteristic phenotype has not been defined. Some characteristic structures are not specific to EC (e.g. caveolae), and some are not present in all EC (e.g. Weibel-Palade bodies).[5] There are few, if any, protein/mRNA markers that are both specifically and uniformly expressed by EC.[5] Endothelium may be continuous, such as in brain, skin, heart and lung, or discontinuous, as in mucosal glands, glomeruli and choroid plexus. EC may have tight junctions, as in the blood–brain barrier, or adherens junctions, as in venules. Recognition of the heterogeneity of EC is fundamental to interpreting research.

3.2. Endothelial response to stimulation

Pober and Gimbrone[7] first demonstrated that a well-defined stimulus can induce the expression of E-selectin, an EC marker. EC express pro-adhesive and procoagulant antigens on the cell surface in response to inflammatory stimuli, such as interleukin 1 (IL-1) and tumour necrosis factor-alpha (TNF-α).[8–11] Weibel-Palade bodies are versatile storage vesicles that are stimulated to release their contents in response to vascular injury, releasing thrombotic and fibrinolytic mediators (von Willebrand factor [vWF], Factor XIIIa, tissue plasminogen activator), inflammation (P-selectin, IL-8, eotaxin, α-1,3-fucosyltransferase VI), vasoconstriction and vasodilatation (endothelin-1 [ET-1], endothelin-converting enzyme, calcitonin gene-related peptide), cell adhesion and migration (CD63/Lamp3), membrane glycosylation (α-1,3-fucosyltransferase VI), vascular calcification (osteoprotegerin), and angiogenesis (angiopoietin-2).[12,13] A goal of using endothelium for targeted treatment may be to manipulate or "prime" the cell to exhibit certain molecular characteristics, which can be targeted as a result of these unique characteristics.

3.3. Brain endothelial cells

The neuroendothelial inflammatory response results in increased expression of EC adhesion molecules and the recruitment of rolling and adherent leucocytes and platelets. In the cerebral vasculature, transforming growth factor-beta (TGF-β) down-regulates adhesion molecule expression of the EC,[14] whereas other cytokines such as TNF-α, exert the opposite effect.[15] Brain EC are characterised by a delicate balance between pro- and anti-inflammatory effects. The cells adapt and respond; there is increasing evidence that the structural properties of the endothelium are modulated in disease.[16]

4. Angiogenesis

AVM are inherently angiogenic.[17] Both clinical studies and molecular research support the argument that AVM cannot be completely treated unless all tissue is resected and/or the angiogenic characteristics rendered inactive.[17] The first phase of angiogenesis is proliferation and migration of EC, and vascular endothelial growth factor (VEGF) is a key regulator of this process. Brain angiogenesis is activated in response to chronic hypoxia,[18] shear stress and certain hormones.[19,20] Altered angiogenic factor expression may be important in the vascular remodelling and continued angiogenesis that is thought to occur in AVM.[21]

Integrins are key regulators of angiogenesis by controlling signal transduction.[22] In conjunction with other pro-angiogenic factors, they are key to migration, adhesion, proliferation and differentiation of EC. Tumour research has identified two integrins important in tumour angiogenesis, αvβ3 and αvβ5, that are highly expressed not only on some tumour cells, but also on neurovascular EC.[23] Ligation of αvβ3 activates pathways that stimulate endothelial migration, proliferation and adhesion, but unligated integrin αvβ3 stimulates apoptosis and activates a cell death pathway to inhibit angiogenesis.[24] Ionising radiation can kill EC, but it can also induce pro-angiogenic survival factors. The αvβ3 signalling that occurs after irradiation can be interrupted by coadministration of the integrin binding cyclic Arg–Gly–Asp (cRGD) peptide;[25] blocking the interaction between αvβ3 and its natural ligands results in adhesion disruption, detachment of EC from the matrix and increased levels of apoptosis.[22]

5. Cell culture (*in vitro*) models

In the early 1970s, Jaffe et al.[26] and Gimbrone et al.[27] independently reported the isolation of human umbilical vein endothelial cells (HUVEC), allowing the study of cell biology in great detail and in a controlled manner. Cultured HUVEC have been the most extensively studied EC but due to EC heterogeneity, they cannot be considered representative of all EC. EC of microvascular origin are still difficult to purify; this is reflected in the number of isolation methods that have been described.