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Junctional adhesion molecules in angiogenesis

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1. ABSTRACT

The process of new blood vessel formation from pre-existing vessels is known as angiogenesis. This process is important, both during physiological processes such as development and during wound healing, as well as during pathological processes, such as cancer and arthritis. Understanding the molecular mechanism of the regulation of angiogenesis and the identification of the key players involved in this process may help identify new therapeutic targets to combat and control angiogenesis and hence arthritis and cancer. This review focuses on a recently identified novel cell adhesion molecule, Junctional Adhesion Molecule A, and its role in the process of regulating angiogenesis.

2. INTRODUCTION

The process of angiogenesis involves activation of quiescent endothelial cells that line the existing blood vessels by proangiogenic factors. The activated endothelial cells then proliferate, migrate through the basement membrane and form tube-like structures, which form new capillaries (1). The process of the creation of new blood vessels from pre-existing blood vessels is tagged as angiogenesis (2). Angiogenesis are characterized into three types: sprouting, intussusceptive, and therapeutic angiogenesis. In sprouting angiogenesis, new blood vessels sprout from existing ones due to splitting of the original vessel. Intussusceptive angiogenesis is similar to sprouting angiogenesis, but it involves the splitting of existing blood

vessels into new blood vessels. Therapeutic angiogenesis is the use of chemical compounds to induce the creation of new blood vessels, which may be used to treat diseases or enhance recovery of a patient (3, 4).

3. ANGIOGENESIS

Angiogenesis, a multi-step process, is influenced by soluble angiogenic factors and a close interaction between adhesive proteins of the extracellular matrix and their integrin receptors (5, 6). Recent studies have also implicated transmembrane proteins present in adherens and tight junctions (TJs) as intermediaries through which these angiogenic signaling pathways are regulated (7, 8). In this review, we will discuss the role of a TJ protein in FGF2-induced angiogenesis and its contribution to further understand the complex regulation of blood vessel formation.

3.1. Growth factors

Factors that induce the process of angiogenesis are termed proangiogenic factors. Among these, vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF), and fibroblast growth factor 2 (FGF2), also known as basic FGF, are the most important and well studied (9). Each of these growth factors is normally expressed during development and regulates several physiological processes. These factors are also highly expressed by tumors and hence play a role in the process of angiogenesis. Interestingly, it has been shown that these growth factors induce angiogenesis via two distinct pathways (10, 11). FGF2 induced angiogenesis is the integrin alpha v beta 3 dependent. Whereas, angiogenesis induced by VEGF is integrin alpha v beta 5 dependent. Both these integrins are receptor for vitronectin, the extracellular matrix component.

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3.2. Junctional Adhesion Molecules

Cell adhesion molecules have been implicated for long to be involved in the regulation of angiogenesis (12). Most of these belong to the immunoglobulin (Ig) superfamily. These proteins seem to negatively regulate the process of angiogenesis and are downregulated during the process. Interestingly, a novel adhesion molecule belonging to the Ig superfamily has been identified and named as junctional adhesion molecule (JAM) due to it's unique localization at the TJs of epithelial and endothelial cells (13, 14). Subsequently, four more proteins having similarities to JAM have been identified, extending this family to five, JAM-A, JAM-B, JAM-C, JAM-D and JAM-L (15-17).

3.2.1. Nomenclature of JAM-A

In the beginning, JAM-A was published under an assortment of names and characterizations, the first being a human platelet antigen F11R, a receptor for stimulatory monoclonal antibody mAbF11 (18). Initial cloning of cDNA of the mouse orthologue of the F11 antigen was then tagged as Junctional Adhesion Molecule, or JAM (13). Subsequent cloning of JAM from different organisms was then reported with numerous names such as, platelet adhesion molecule, PAM, human JAM-1 (14), bovine JAM (19), hJAM (20), F11R (21), and huJAM (22). In order to resolve any confusion, the current nomenclature of JAM family members as JAM-A, B, C, D and L have been suggested and practiced (23).

3.2.2. Structure and chromosomal localization

The cDNA of JAM-A codes for 299-amino acid, which adds up to a 32.6 kDa precursor protein with a 27-amino acid plasma membrane signal peptide and 29.7 kDa mature protein backbone. JAM-A is about 75-80% similar in sequence to JAM-B and JAM-C (24). As a single-span transmembrane protein, JAM-A is part of the immunoglobulin superfamily (IgSF) and the CTX family. Thus, JAM-A is labeled to be between antigen-specific receptors and adhesion molecules. JAM-A has two Ig domains in its extracellular domain: a VH-type membrane distal variable Ig domain and a C2-type proximal constant Ig domain (25, 26). The extracellular domain consists of 235 amino acids, leaving the transmembrane domain at 19 hydrophobic residues in length and the cytoplasmic tail at 45-amino acids long. Due to glycosylation, the molecular weight of the protein is found to range from 32-35 kDa. The JAM-A gene is 25.3 kb in size, contains 11 exons, with the coding region starting at exon II and ending in exon XI. JAM-A is localized on chromosome 1 regions q21.1 to q21.3, between D1S2705 (175.1 cM) and D1S2768 (176.8 cM), leaving a 1.7 cM interval between the two regions (14). Several human genetic disorders, including Gaucher's disease, glaucoma, prostate cancer, and Alzheimer's disease, are located near the region of JAM-A on human chromosome 1 (14).

3.3. Embryonic expression of JAM-A

JAM-A was initially reported as being expressed only postnately (27). However, using transgenic mouse expressing beta -galactosidase enzyme under the control of a JAM-A promoter, it was demonstrated that JAM-A is expressed as early as the blastocyst stage (28). Further, it was shown that by 8.5 days postcoitum, JAM-A expression was detected in the endoderm and part of the ectoderm. Later the expression of JAM-A was detected in early vasculature and epithelial component of the inner ear as well as the epithelium of the developing skin, olfactory system, lungs and kidney (28).

3.4. JAM-A induces endothelial cell proliferation and migration

JAM-A is a TJ protein and has been shown to be required for integrity and permeability of the tight junctions (29). It was therefore believed to play an inhibitory role in the process of angiogenesis. Interestingly, however it has been shown that JAM-A overexpression regulates endothelial cell morphology and induce both cell proliferation and migration on vitronectin (30, 31). Further it has been demonstrated this induced endothelial cell proliferation and migration on vitronectin is integrin alphavbeta3 dependent and can be completely inhibited by function-blocking antibodies specific to JAM-A as well as integrin alphavbeta3. It has been shown that JAM-A and integrin alphavbeta3 form an inactive complex at the TJ and growth factors dissociate this complex rendering each protein to participate in the angiogenic process (30, 31).

3.5. JAM-A is required for FGF2-induced angiogenesis

Angiogenic process consists of three parts: Endothelial cell proliferation, migration, and tube formation. All of these three processes are induced by proangiogenic molecules, including FGF2 and VEGF. Now FGF2 angiogenesis entails the endothelial cell migration on vitronectin through the integrin alpha v beta 3. Since JAM-A also induces endothelial cell proliferation and migration dependent on integrin alpha v beta 3 an in-depth assessment of the correlation between JAM-A and FGF2-induced angiogenesis provided mechanistic understanding of the growth of new blood vessels whether in cancer or vascular repair. Consequently, it was found that JAM-A specifically regulates FGF2-induced angiogenesis (30). When the JAM-A component was blocked using function blocking antibody, FGF2-induced angiogenesis was virtually nonexistent as seen in chick chorioallantoic membrane assays (30, 32).

It was found that JAM-A and alpha v beta 3 form a complex in quiescent endothelial cells (30). In response to FGF2 treatment, the JAM-A and alpha v beta 3 complex tends to separate and redistribute across the cell membrane. Further, FGF2 treatment and overexpression of JAM-A are nearly identical in terms of cell morphology, thus hinting that JAM-A overexpression imitates FGF2-induced morphology in human umbilical cord vein endothelial cells (HUVECs). Overexpression of JAM-A to FGF2 treatment, JAM-A also induces *in vitro* tube formation as determined by the use of tube formation assays. JAM-A is also shown to regulate FGF2 alpha v beta 3 -induced ERK1/2 activation thus regulating the signaling. This is further supported by the fact that JAM-A overexpression upregulates ERK1/2 activity in HUVECs that were plated on vitronectin. Results also suggests that FGF2-induced ERK1/2 activation is dependent on JAM-A and alpha v beta 3 and that blocking the integrin or JAM-A would block that signaling (30, 32).

3.6. JAM-A is required for FGF2-induced migration

Due to the fact that JAM-A is redistributed across the cell membrane with FGF2-treatment and involved in the signaling pathways of FGF2, the role of JAM-A in FGF2-induced endothelial cell migration was further investigated. The endogenous JAM-A was knocked down in HUVECs using RNA interference (RNAi) technology and its effect on HUVEC migration was assessed (32). Knockdown of JAM-A significantly blocked HUVEC migration on vitronectin without affecting the migration on fibronectin. This was not due to its effect on the expression or the localization of VE-cadherin, ZO-1, beta3 or beta -catenin as shown through Western blot analysis and immunofluorescence staining. This was further confirmed by more quantitative analysis such as haptotactic transwell motility assays (32).

3.7. *In vivo* role of JAM-A in angiogenesis

The most conclusive evidence for the role of JAM-A in FGF2-induced angiogenesis came from the use of JAM-A knockout mouse (33). JAM-A gene in a mouse was disrupted by inserting a large insert containing β-galactosidase between exons 4 and 5. The resultant homozygous mice did not make any JAM-A protein. Although, these mice were viable and fertile, they failed to show angiogenesis in response to FGF2 as shown by aortic ring assay as well as Matrigel® plug assay (33). These results unequivocally confirm important essential role for JAM-A in FGF2-induced angiogenesis both *in vitro* and *in vivo*. It appears that other members of the JAM-A family also play a role in angiogenesis. For example, anti-JAM-C specifically decreases angiogenesis in the model of hypoxia-induced retinal neovascularization *in vivo* and vessel outgrowth from aortic rings *in vitro* (34). The role of JAM-B, JAM-D and JAM-L in angiogenesis is not been tested yet.

4. SUMMARY

JAM-A is a member of the Ig superfamily of cell adhesion molecules. On quiescent endothelial cells, JAM-A is sequestered at the tight junction in association with integrin alphavbeta3 Upon stimulation with FGF2, the JAM-A and alphavbeta3 complex dissociates and relocalizes diffusely along the cell membrane, where it participates in the signaling process leading to the activation of ERK1/2, which leads to angiogenesis.

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