The G protein–coupled receptor (GPCR) APJ (X-msr, angiotensin receptor like 1b) was cloned in several laboratories by homology screening, with the goal of identifying new members of this class of cell surface receptor. This gene attracted attention because of its sequence similarity to the important angiotensin II type 1 receptor and its highly restricted pattern of expression in endothelial cells. Five years later, this GPCR was deorphanized with cloning of the APJ-endogenous ligand apelin. Apelin was found to be synthesized as a 77-aa preproprotein and cleaved to a number of active peptides, including those of 36, 17, and 13 amino acids. Interestingly, apelin was also found to be highly expressed in endothelial cells.

Detailed evaluation of APJ and apelin expression patterns in embryogenesis, and in the developing retina, suggested the hypothesis that autocrine signaling of this pathway in endothelial cells provides a mechanism for regulating new blood vessel growth, or angiogenesis. Loss of function experiments in frog embryos using morpholino knockdown experiments have consistently shown vascular developmental abnormalities, varying from perturbed intersomitic vessel branching to more fundamental developmental defects, including loss of the posterior cardinal vein, and decreased numbers of endothelial cells. Overexpression of Xapelin led to disorganized expression of endothelial precursor markers at the neurula stage. In zebrafish, knockout studies of one of the APJ homologues (angiotensin receptor like 1b) failed to perturb vascular development, but APJ receptor knockdown was found to decrease the hypoxia-induced vessel regeneration in the Fli-1 transgenic zebrafish model.

Recent studies with an apelin null mouse model have suggested that this pathway is downstream of the angiopoietin-tek/tie pathway, and regulates blood vessel caliber. Activation of this pathway in cultured endothelial cells has been shown to promote migration and proliferation, and blood vessel growth—promoting functions of apelin have been demonstrated in the Matrigel plug assay in the mouse and chick chorioallantoic membrane assay.

With this background, in this issue of ATVB, Kasai et al investigated the role of apelin-APJ signaling in retinal angiogenesis. Retinal vascular formation is of great interest because of the highly programmed pattern of development that allows easy study of angiogenesis in vivo in the mouse, and the relationship to vascular proliferation and visual impairment in human diseases such as diabetes. These investigators found Apelin-APJ mRNA expression in the retina to be transiently upregulated in the period of early postnatal vascular development, with expression disappearing in adult mice, after completion of angiogenesis. Retinal vascularization in apelin null mice (apelin-KO) appeared to be temporally delayed in the early stages of vascular development compared to that in wild-type mice, but the pattern and extent of vascularization was the same in adult mice. Interestingly, in apelin-KO mice, the angiogenic response to VEGF and FGF introduced in the corneal pocket assay was reduced. Although apelin alone had no effect on angiogenesis in this assay, apelin treatment of the apelin-KO mice restored the angiogenic response of VEGF and FGF, showing an angiogenic effect of apelin when working cooperatively with VEGF or FGF.

VEGF and FGF are well known angiogenic molecules involved in cell proliferation, survival, migration, and proteolysis. VEGF binds to tyrosine kinase receptors VEGFR-1 and VEGFR-2 on the endothelial cell surface. These two receptors have different functions, and VEGFR-2 mediates most of the VEGF-induced angiogenic functions such as chemotaxis, mitogenesis, and interaction with integrin αvβ3. VEGF is known to increase production of NO, an important regulator of angiogenesis, by increasing eNOS expression and phosphorylation through PKC, PI3kinase/Akt, or the calcium/calmodulin pathway in endothelial cells (Figure). FGF binds to FGF1 or FGF2 on endothelial cells and stimulates angiogenesis via the MAPK pathway. Whether increased NO release is an important feature of FGF-stimulated angiogenesis is unclear. Through studies reported by Kasai et al and others in the literature, apelin-APJ is clearly linked to these pathways in the context of angiogenesis. For instance, Kidoya et al have shown that VEGF and FGF increase APJ and apelin expression in endothelial cells respectively (see Figure). However, the molecular mechanisms by which the apelin/APJ pathway promotes angiogenesis is not clear. There is no evidence that apelin promotes VEGF or FGF expression. There were only minor differences in VEGF and FGF mRNA between apelin-KO and wild-type mice in the retinal vascularization data of Kasai. Cox et al showed that apelin did not act via upregulation of VEGF. Inhibition of VEGF and FGF receptor activity by specific tyrosine kinase inhibitors failed to inhibit apelin-induced cell proliferation, suggesting that the effect of apelin on angiogenesis is independent of VEGF and FGF receptors. It is well known that NO is a mediator of angiogenic processes. Apelin induces phosphorylation of eNOS and NO release from endothelial cells, and thus NO could mediate stimula-

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tion of angiogenesis by apelin. Both angiogenic and antiangiogenic effects have been attributed to NO, and Jones et al showed that low levels of NO stimulated tube formation but high concentration of NO inhibited this response. Further studies are needed to explore whether NO production in response to apelin is the mechanism by which apelin potentiates VEGF- and FGF-stimulated angiogenesis, whether apelin is capable of independently stimulating angiogenesis in nonretinal tissues, and what pathways in addition to NO may be involved in apelin-mediated angiogenesis.

It will be important to investigate the relationship of apelin signaling to pathological angiogenesis associated with disease states. There is reason to expect apelin signaling to increase retinopathy, given its responsiveness to hypoxia. Hypoxia is a potent stimulus for angiogenesis, primarily through activation of hypoxia-inducible factor–1α (HIF-1α), a heterodimeric transcription factor that activates a large number of angiogenesis-related genes, including VEGF. Recently, Eyries et al showed that apelin is a HIF-1α target gene, and hypoxia-induced apelin is involved in the proliferation of endothelial cells. Increased expression of VEGF is seen in the new vessels in retinopathy, is considered to be involved in the pathogenesis of disease progression, and upregulates APJ expression. In a mouse model of retinopathy of prematurity, APJ expression is selectively upregulated in newly formed vessels. However, there is also reason to expect apelin may block disease-related angiogenesis, through inhibitory interactions with the angiotensin II (Ang II) signaling pathway. Ang II is known to upregulate VEGF, its receptors, and other proangiogenic molecules in ocular retinopathy disease models. There is now ample evidence for antagonism between apelin and Ang II signaling pathways in disease settings in both the heart and vasculature. Importantly, recent data from this laboratory have shown that direct APJ–AT1 interactions can inhibit Ang II–mediated vascular disease, through inhibition of Ang II signaling to the nucleus, attributable in part to direct receptor–receptor interactions. Further studies will be required to determine how the balance of these various interactions of the apelin–APJ pathway affect pathological angiogenesis.

Disclosures

None.

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