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Role of estrogen in angiogenesis in cardiovascular diseases

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Abstract

The formation of new blood vessels from existing ones is a major process of angiogenesis and it is most effective in the vascular systems. The physiological process like hypoxia inducible factors involved in the regeneration of damaged tissues varies within the vascular systems in the endothelium and could be limited due to some major angiogenic growth factors like vascular endothelial growth factor, fibroblast growth factors and epidermal growth factor among others which bring about this cellular vascular regrowth. These physiological processes leading to cellular vascular regrowth could be a major function for the treatment of cardiovascular diseases such as ischemia and atherosclerosis. Estrogens are one of the known factors within the cellular mechanisms that could initiate repairs to the damaged vascular tissues, since estrogens are known inducers of angiogenesis leading to this cellular regrowth. Research has also shown that this cellular regrowth is induced by vascular angiogenic growth factors via the estrogen receptors. In this review we will attempt to summarize the main angiogenic growth factors involved in these physiological processes leading to angiogenesis and possible new mechanisms that could lead to this vascular regrowth. And also we will try to summarize some reports on the effect of estrogen on these physiological processes leading to angiogenesis in cardiovascular diseases.

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1 Introduction

The formation of new blood vessels from existing ones is referred to as Angiogenesis.[1] The proliferation of blood vessels could be aided by the mechanism of estrogens which are essential for the normal growth in adults mostly for the function of female reproductive organs. A form of angiogenesis referred to as physiological angiogenesis is suppressed in the adult tissues, thereby restraining capillary growth but could only occur in the state of cell abnormalities that results from cell damage in the physiological processes leading to wound healing, tissue re-growth, *etc*. These physiological processes are been regulated by some angiogenic growth factors under pathological conditions, but when lacking or insufficient, it could lead to various forms of cardiovascular diseases like atherosclerosis, menopause and other related diseases.[2]

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2 Physiological processes leading to angiogenesis

Oxygen among other nutrients is essential in maintaining homeostasis in adults and it plays a pivotal role in the physiology and pathological growth of blood vessels. Thus oxygen is constantly being regulated by the circulatory system through some factors that lead to the regeneration of blood vessels, these processes are regarded as angiogenesis.[3] Low oxygen (hypoxia) could facilitate the angiogenic stimuli, which develops from tip cells on the endothelial cells which form vascular endothelial growth factor receptors (VEGFR) with notch signaling that induce high concentrated VEGF leading to formation of new capillaries. Hypoxia inducible factor (HIF-1) is also another physiological process responsible for the regulation of angiogenic growth factors like VEGF for the processes of angiogenesis in cardiovascular diseases.[4,5]

Other angiogenic growth factors responsible for angiogenesis include transforming growth factors-beta (TGF-β), fibroblast growth factors (FGFs), epidermal growth factor (EGF) and angiogenin. They are capable of altering the physiological processes of endothelial cells. Ang II (angio-

tensin II) is among the main bioactive peptide of the rennin-angiotensin system, it acts in controlling cardiovascular homeostasis, with effects on cardiovascular diseases.[6]

Estrogen plays a critical role in some physiological and pathological factors that lead to cardiovascular diseases in women mostly in the stage of pre-menopause.[7] Estrogen may act in modulating the inflammatory response system within vascular cells, aiding its metabolism, insulin sensitivity, hypertrophy development and stem cell death.[8] Estrogens activates gene regulation via estrogen receptors (ERs),[9-11] and when expressed enhances the pathophysiological processes of angiogenesis in endothelial cells. The role of estrogens is in the regulation of lipid and cholesterol levels, estrogens could directly affect the growth of vascular cells, and the recovery from vascular damage leading to cardiovascular diseases.[12] ERs may be involved in this protective effect. Estrogen is also known to increase HDL plasma levels [13] which response varies largely among. women and may be part of genetic factors.[14]

3 Mechanisms of angiogenesis

Angiogenesis is the process of forming new blood vessels through sprouting and budding of new capillaries from existing blood vessels.[15] Endothelial cells (ECs) form the inner layer of blood vessels, however, the vascular endothelial cells and circulating endothelial cells play a very important role in the physiological and pathological processes leading to angiogenesis. Angiogenesis is essential for the functions of the circulatory system, including growth responses, responses to sustained exercise, estrus cycle, wound healing and ageing. Furthermore, the decrease in angiogenesis could act as natural and therapeutic mechanistic effects on some cardiovascular disease conditions like hypertension, coronary heart disease, tumor growth and menopause.[16] Some key factors could aid the mechanism that leads to the generation of angiogenesis within the endothelium. The stimulation of VEGF and its receptors is a key factor that leads to the formation of new blood vessels in ECs.[17] ECs release some enzymes matrix metallo-proteinases (MMPs) which degrade the vascular basement membrane to form a sheath-like covering of blood vessels. This is followed by the initiation of intracellular signaling cascade that stimulates the formation of other building blocks of endothelial cell formation. The endothelial cells divide and pass through the dissolved openings of the basement membrane to where the new vessel will form. The sprouting of the newly developing vessel is aided by adhesion molecules known as integrin that bind and help pull the vessel into place. Meanwhile, other enzymes such as matrix metallo-proteinases degrade the surrounding extracellular matrix to make room for the growing vessel.[18] The endothelial cells join together to form individual capillary sprouts which are stabilized by smooth muscle cells and pericyte muscle cells, which develops into new capillaries. Many studies have also shown that estrogen play a significant role via angiogenesis in ischemic cardiovascular diseases.[19,20] In ovariectomy and estrogen treatment of female rabbits, estrogen mediated an increase in the density of blood vessels within two weeks of its supplementation period.

Further mechanisms of therapeutic angiogenesis using angiogenic growth factors and inhibitors in the treatment of cardiovascular diseases are still not fully understood. Therefore, it is most important to study more physiological and pathological mechanisms within ECs. High density lipoprotein (HDL) development in ECs is a major physiological factor leading to cardiovascular disease. HDL functions by binding to cholesterol in the peripheral tissues and moving the cholesterol to the liver. This action is induced by high affinity HDL receptor, scavenger receptor B type I (SR-BI). SR-BI could mediate angiogenesis by initiating signals in the endothelium through search which could promote endothelial NO synthase activity and cell migration.[21] Studies also suggest that endothelial cells function effectively by inducing cell migration via an adaptor protein PDZK1 which mediates the HDL and SR-BI.[22]

Others studies show that the mechanism of ARIA (Apoptosis regulator through modulating IAP expression) acts like a protein which functions in regulating apoptosis and angiogenesis in ECs.[11] ARIA through inhibitor of apoptosis (IAP) expression regulates PTEN/ phosphatidylinositol 3-kinase (P13K) pathways in ECs. It also mediates angiogenic growth factors in endothelial progenitor cells (EPCs) via P13K/Akt/endothelial nitric oxide synthase (eNOS) signaling. Akt may function as a mechanism used in inducing cell survival and growth through the parallel pathways leading to cell proliferation via Erk, therefore Akt activation can mediate cell proliferation.

4 Angiogenic growth factors and its signaling pathways

4.1 VEGF signaling pathway in angiogenesis

Estrogen could play a vital role in the mediating of this angiogenesis via VEGF/eNOS/Akt [23,24] signaling pathway which is responsive through both ER-α and ER-β present in the endothelium. The activation of VEGF is critical in angiogenesis processes and it is dependent on the availability of ligands.[25] VEGFR2 serves as a critical signal transducing VEGF receptor for angiogenesis in endothelial cells.[16]

VEGF binds the receptor tyrosine kinase, VEGFR2, leading to different signaling pathways resulting in the up-regulation of genes involved in mediating the proliferation and migration of endothelial cells. VEGFR2 binding by homodimerization results in kinase activation and auto phosphorylation involving various isoforms of tyrosine. The signal transduction could be conducted by activation of some molecules like SRc and phosphatidylinositol 3-kinase (PI3k). PI3k activation stimulates the phosphorylation of Akt/PKB (Protein Kinase-B) which is actually downstream targets for inhibition.

Other growth factors involved in the processes of angiogenesis could be FGFs, which are small polypeptide growth factors with some structural characteristics of binding heparin in the ECs. FGFs functions by signaling peptides for secretion that binds to the heparin-lie glycosaminoglycan's (HLGAGs) of the extracellular matrix (ECM). FGFs could act on target cells, or may be released via active carrier proteins. FGFs may bind receptor tyrosine kinases in the presence of HLGAGs. The binding of FGFs at this level induces receptor dimerization and stimulates the activation of various signal transductions to downstream signaling cascades.[26] FGFs signaling play a critical role with estrogens in development of angiogenesis.[27] In some other research reported,[28] the use of fibril gel assay Knockdown of PCOLCE, Col1A1, SPARC, IGFBP7, and βig-h3 in pairs had little or no effect on EC sprouting, but the double-siRNA combinations of SPARC/Col1A1, IGFBP7/PCOLCE, IGFBP7/βig-h3, and PCOLCE/βig-h3 all significantly reduced EC lumen formation.

4.2 Role of estrogen in cardiovascular diseases

Many risk factors lead to cardiovascular diseases like unhealthy dietary habits, aging and smoking which bring about increasing blood lipid levels and inflammation in the arterial wall. Prevalence in the occurrence of these cardiovascular diseases in premenopausal women is low, including ischemic heart disease and heart failure. Consequently, the incidence increases with menopause have suggested an essential protective role of estrogens. Studies have shown that estrogen is essential for the regulation of vascular tone and in the pathophysiology of cardiovascular disease.[29,30] Physiological effects of estrogen are mediated through estrogen receptors α (ERα) and β (ERβ), both expressed by different genes,[31] which possess a similar domain structure and are stimulated by ER agonist 17β-estradiol in a variety of cell types, including vascular smooth muscle cells and endothelial cells.[10] ERβ plays a role in mediating systemic blood pressure by modulating endothelial independent vascular smooth muscles.[32] In order to effectively understand the role of estrogen in cardiovascular disease, it is critical to examine the molecular mechanisms by which estrogen mediates the ERα & ERβ receptors in the vascular physiological and gene-regulatory pathways.

Recent research has also shown that endogenous estradiol (E2) could mediate physiological effects in G-protein-membrane coupled receptor 30 (GPR30) suggesting that GPR30 could act as a new form of the estrogen membrane receptor in ECs.[33–35] GPR30 is a seven trans-membrane-associated G protein-coupled receptor that is located at the plasma membrane and endoplasmic reticulum. GPR30 has been reported to induce physiological vasorelaxation effects by E2 through its receptors in rat aorta,[36] and it mediates the actions of E2 in human epithelial cells.[35]

Using wild type mouse exposed aorta, Jazbutyte, et al.[32] and Zhai, *et al*.[37] observed that gene up-regulated by estrogen is largely mediated by ERα. ERα stimulates endothelial dysfunction and diminished basal NO-release as observed in mice but does not reduce blood pressure in a spontaneous hypertensive rat model.

Although ERα and ERβ are both involved in stimulating the cells within the cardiovascular system, the mechanisms of their operation are of different forms. ERβ mediates E2 induced Cardio-protection through modulation of ion channel expression and calcium-handling protein. E2 and its isoform 8β-VE2 treated in rats showed increased expression of ERβ that led to the stimulation of receptor subtype in 8β-VE2. Also the stimulation of ERα in 17β-estradiol treated in spontaneous hypertensive rats reduced the up-regulation of ERβ with 8β-VE2.[32] Therefore, ERβ agonist 8β-VE2 could lower elevated blood pressure in ovariectomized SHR.

Wang, *et al*.[38] also reported that ERβ mediates myocardial protection by upregulation of PI3K/Akt stimulation with other factors in female hearts following ischemia-reperfusion. Therefore, it suggests that ERβ-mediated PI3K/Akt and anti-apoptotic signaling in the myocardium and may indicate insight on mechanistic pathways of cardiovascular diseases in females.

4.3 Mechanism of estrogen in angiogenesis

Estrogens are a group of steroid compounds, named for their function in the estrous cycle, and as primary female sex hormones. Estrogens are in three forms: E2, estrone (E1) and estriol (E3). E2 is the most essential compound among the estrogens and it stimulates endothelial cell migration, proliferation and survival in ECs.[39,40] In the cardiovascular system, estrogen is activated by binding to estrogen receptors (ER) in the ECs. There are basically two classical estrogen receptor subtypes which are ERα and ERβ and a non-classical estrogen receptor GPR30. Estrogen receptors

functions in regulating the transcriptional processes in cells by binding to the ER in the nucleus which mediates dimerization and binding to specific response elements (ERE) to promote its target gene.[41] Estrogen mediates protein reactions within the cell by binding to the ER through protein-protein interactions with ERE in the nucleus. This stimulates the activator protein 1 (AP1) or SP1 sites in the promoter region of estrogen-responsive genes to activate coregulator proteins to the promoter, regulate mRNA levels and other protein production.

ER mediates the action of estrogens and the regulation of its gene expression by acting as ligand transcription factors. Estrogen is mediated selectively via ERα and ERβ in uterine artery (UA) and uterine artery endothelium (UAE); this was determined by immunoblotting and RT-PCR analysis of mRNA expression carried out *in vivo* and cultured UAECs *in-vitro*.[11,42] Physiological studies showed that ERα is more potent in stimulation of estrogen than ERβ ligands in vascular endothelial cells. ERα up-regulation in human dilated cardiomyopathy brings about increase in mRNA which was higher in women than men.[43] The transcription factors of ER can interact with cytoplasmic proteins and activate signaling pathways.

E2 stimulates ERs and HIF to the VEGF gene promoter in the endothelium. It was observed that when the VEGF mRNA levels decline, ERα persists in its activity. HIF-1 mediates E2 expression by binding to the upstream medium of the endothelium that contains the hypoxia response elements and ERα to the proximal GC-rich region that contains several Sp protein sites.[44]

Estradiol stimulates the activation of phosphatidylinositol 3-kinase (PI3K) and MAPK signaling pathway. ER mediates PI3K pathway in estrogen induction of VEGF expression in the endometrium.[17] Another example of an estrogen mediated signaling pathway is tissue factory pathway inhibitor-1 (TFPI) which is regarded within the endothelium as a physiological inhibitor[45] of the tissue factor pathway of blood coagulation. TFPI may be involved in angiogenesis due to its stimulation with ER ligands. The regulation of TFPI involves post-transcriptional effects mediated by the amino-terminally truncated 45 kDa version of ERα.[46] TFPI may affect angiogenesis through peptides within its carboxyl terminus which may directly block VEGF2 activation, thereby hindering the migration of endothelial cells. [29]

Estrogen could elicit its cardioprotective effect via ER-mediated non-genomic signaling pathways.[46] Membrane ER binding results in rapid, non-genomic actions and are mediated by several pathways, such as receptor tyrosine kinases and protein kinases including PI3K, Akt, mitogen-activated protein kinase (MAPK), Src protein kinase A and C and by increasing the concentration of intracellular calcium.[47] With regard to cardiovascular events, direct membrane signaling causes vasodilatation through nitric oxide release and opening of the calcium-activated potassium channels through a NO and cyclic GMP pathway.[47] A number of studies have suggested that acute addition of 17β-estradiol to either ovary-intact females or ovariectomized females reduces ischemic reperfusion. Some studies suggest location of ERs at the plasma membrane, where they could elicit rapid protective effects via the activation of non-genomic signaling pathways.[48]

Estrogen critically can bind at different receptors to initiate acute signaling pathway.[8] It alters different levels of proteins and signaling pathways, leading to posttranslational modifications that alter protein activities. A direct protein-protein interaction between ligand-activated ERα and the regulatory subunit p85 of PI3K in endothelial cells through a non-genomic mechanism by which E2 rapidly stimulates eNOS via the activation of PI3K/Akt would lead to downstream activation of NOS/NO/SNO signaling. This clearly suggested that ERα activation of PI3K might play a role in cardio-protection.[49]

NOS can be signaled via S-nitrosylation (SNO) and SNO levels are mediated by activation of estrogen. Some research work also suggested that ERβ agonist could increase the SNO of a series of proteins in ovariectomized females.[50]

5 Conclusions

Angiogenesis plays an important role in the physiological and pathological process in cardiovascular diseases. ECs is a major physiological factor leading to these cardiovascular diseases which are most prominent in menopausal women. Therefore the understanding of the molecular mechanisms within the ECs is very important. The processes of cell migration and cell proliferation within the ECs determine the development of new vessels. Angiogenic factors like VEGF and FGF play very critical roles in the development of these new vessels. Some proteins like ARIA regulates P13K pathways within the ECs and SR-B1 activates signals in the endothelium through which promotes angiogenesis but more studies are required for better understanding of the molecular pathway leading to cell proliferation in ECs.

The role of estrogens has been fully established in the proliferation of cells, which as we have shown is a key factor for angiogenesis. The two estrogen receptors, ERα and ERβ, play critical roles in the binding estradiol ligands which acts as agonists of several signaling pathways leading to angiogenesis. Studies have shown that the estrogen receptors are selective in their actions and ERα is more receptive to the binding of Estradiol.

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