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Review Article

Molecular and hormonal regulation of angiogenesis in proliferative endometrium

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ABSTRACT

Angiogenesis is a hallmark of wound healing, the menstrual cycle, cancer, and various ischemic and inflammatory diseases. A rich variety of pro and anti-angiogenic molecules have already been identified. Vascular endothelial growth factor (VEGF) is an interesting inducer of angiogenesis and lymphangiogenesis, because it is a highly specific mitogen for endothelial cells. Signal transduction involves binding to tyrosine kinase receptors and results in endothelial cell proliferation, migration, and new vessel formation. In this article, the role of VEGF and other growth factors in the pathology of dysfunctional uterine bleeding is reviewed. We also discuss the role of VEGF expression and interaction with extracellular matrix that lead to possible inhibition or stimulation of Angiogenic factor on endometrium of dysfunctional uterine bleeding patients.

Keywords: Angiogenesis, Dysfunctional uterine bleeding, VEGF, HIF-1, Copper

INTRODUCTION

Angiogenesis is the process of formation of new blood vessels from the blood vessels that already exist. This process is an essential factor in each menstrual cycle. Neovascularisation or formation of new blood vessel is the process occurs often in endometrium during every menstrual cycle. Abnormal angiogenesis may have a profound effect on the pathogenesis of endometrial carcinoma and abnormal uterine bleeding.[1]

Heavy, prolong and frequent bleeding is often considered as dysfunctional uterine bleeding (DUB) which is devoid of any pregnancy causation or systemic disease[2] hormonal disturbance is considered to be one of the causes of excess endometrial proliferation[3] that rooted in capillaries and small vessels surrounding endometrium.[4] Among the elements, copper contributes more on regulation of angiogenesis that in trace amount is fundamental for living organisms[5] hence the excess amount of it may have a direct regulatory effect on angiogenesis.[6]

Several factors are known to have direct and indirect role in controlling the different part of multiple step of Angiogenic process. The mechanism of angiogenesis during normal menstrual cycle in human endometrium is markedly similar to pathological angiogenesis like endometriotic lesions, where synthesis of new blood vessel and regression during each menstrual cycle is under the control of estrogen and progesterone. However, regulation of endometrial angiogenesis in the endometrium is a complex process. Regulatory effect of estrogen added to the complexity of this process, due to both inhibitory and stimulatory effect of this hormone on vessel growth during different circumstances (Girling and Rogers, 2005). In addition, a large number of angiogenic factors and inhibitors have been identified in human

endometrium, but their exact role in regulation of vessel growth during normal menstrual cycle, pathological condition and during pregnancy is yet remain ambiguous. One of common cause of vaginal bleeding is dysfunctional uterine bleeding that may have a correlation with angiogenesis but the precise mechanism remains to be elucidated.

There are numerous factors identified which have a role in abnormal uterine bleeding, among them hormonal mechanism is considered to have a great impact on DUB which is the most common cause of abnormal vaginal bleeding during a woman's reproductive years. Any disturbance in the normal menstrual cycle mechanisms can lead to abnormal estrogen synthesis and DUB. Statistically about 30% of patients that referring to gynecologist have DUB.[7] This complication known to happen usually in the beginning and end of a woman's reproductive life, but could occur any time. Hypothalamic-pituitary axis during the first 18 months after menarche fail to respond to estrogen and progesterone due to immaturity and may lead to anovulation.[8,9] However, imbalance of steroid hormone and high steady-state estrogen with no secretion of progesterone is often seen in many cases of anovulatory DUB, this often occur by the decrease responsiveness of endometrium for hormone and decrease in hormone level when menopause approaches.[7,10,11] Normal menstrual cycles may be affected by other endogenous estrogen apart from the ovary that may be secreted in other condition like obesity.[10]

It is been demonstrated that biochemical disturbances, including disturbed endometrial angiogenesis, increased endometrial vascular fragility, and consistency of the epithelial, endothelial, and stromal supporting structures in the local endometrial environment, may play an important role in controlling the mechanism of DUB.[12,13]

The complex mechanism in human endometrium in each menstrual cycle requires an endothelial cell specific angiogenic peptide that orchestrate vascular and glandular proliferation, differentiation and regeneration in order to prepare implantation of an embryo. VEGF and other angiogenic protein appear to play a fundamental role in both physiological and pathological neovascularisation. The synthesis of new blood vessels depends solely on the interaction between different growth factors and hormones. Several growth factors like, transforming growth factor (TGF-beta) and vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) have been found to have a direct effect on endometrial angiogenesis.[14]

Secretion of growth factors leads to hypervascularization of quiescent endothelial cell phenotype and cause conversion of these quiescent cells into its active form that are then able to respond to mitogenic signals. Persistence of growth factor secretion switches the activated phenotype to an angiogenic phenotype that by interaction of growth factors with cell surface receptors or cytoplasmic receptors causes the activation of a cascade of signals for cell migration, proliferation and differentiation into new capillaries. The hypothesis that tumoral progression was dependent on angiogenesis also led to the concept of 'tumor angiogenic growth factors' (Folkman, 1971). There are similarities between physiological angiogenesis and vasculogenesis during embryogenesis but angiogenic growth proteins which are up regulated during pathological state is more persistent.

HORMONAL REGULATION

There is strong correlation has been noticed between anovulation and DUB. Studies conducted on adolescent female for a duration of 5 years have shown that levels of estrogen, progesterone, LH and FSH are below normal value in the first year of anovulation but the level of estrogen increased to almost normal value in the second year, within 5 years after anovulation the level of FSH, LH has returned to normal adult value, but serum progesterone levels are still remaining at a low percentage of ovulatory cycles (0-63%). During puberty, maturation of the HPO axis is characterized by an increase in the frequency and amplitude of pulsatile GnRH, which initiates and regulates secretion of pituitary gonadotropins.[15]

During a time right before puberty, secretion of LH is frequent during the night but during the early puberty, secretion of LH is increased so that the value of LH in circulation is essential for determining the normal ovulatory cycle. Timing of LH secretion is essential for differentiation between ovulatory cycles, as an increase in basal LH and immature timing would result in anovulatory cycles. Induce follicular development that is a requirement for the ovulation has been characterized by the regular cycle and the level of secretion of LH and FSH and ultimately the estrogen and progesterone are fundamental factors for the physiology of endometrium. Over secretion of estrogen causes out growth of blood vessels and change in endometrial architecture and ultimately endometrial growth that lead to partially break down and shedding in an irregular manner. Increase unopposed estrogen activate the negative feedback mechanism on hypothalamus and pituitary gland, result in decrease secretion of GnRH, FSH, LH. This mechanism result in vasoconstriction and collapse of endometrial vasculature that lead to heavy and often prolonged bleeding. With no ovulation and subsequent progesterone production, results in unopposed estrogen occurs, causing dilatation of the arterial supply in the endometrium, and lead to proliferation of endometrium and would associate with abnormal thickening of the endometrium without proper architectural integrity.[16]

Large, thin-walled, tortuous, superficial endometrial vessels often can be demonstrated on the surface of the endometrial hyperplasia; increase of blood loss is due to fragility of blood vessels. Vascular tone would reduce by

an unopposed estrogen and has a direct effect by inhibiting vasopressin release, that causes vasodilatation and increase blood.[17,18] Formation of blood vessels on endometrium are require for growth factor like VEGF that could be stimulated by unopposed estrogen, which may contribute to imbalance angiogenesis.[19,20] In addition, when estrogen synthesis is uneven and unopposed , synthesis of prostaglandin (PG) in endometrium would be less and in this condition synthesis of prostaglandin E is higher than PGF.[21] The layer of endometrium often sheds unevenly although when the circulating estrogen level is high this could be manifested with scattered red patches as seen hysteroscopy, corresponding to thrombotic foci of necrotic disintegration, adjacent to the abnormally proliferated endometrium.[22,23] Multiple factor involves in endometrial break down due to unopposed estrogen synthesis involving VEGF and increase production of nitric oxide in the endometrium. This has been postulated as another factor contributing excessive blood loss in anovulatory menstruation.[24]

Although anovulation is the most common finding associated with DUB, a number of ovulatory patients have abnormal menstrual bleeding. The mechanism of this particular disorder is unclear. In addition, conditions of prolonged progesterone excretion after ovulation, as a result of a persistent corpus luteum cyst (Halban's disease), can result in 6 to 8 weeks of amenorrhea followed by irregular menstrual flow. Hence a multi factorial mechanism is involved in pathogenesis of DUB.

EXTRACELLULAR MATRIX

Multiple mechanisms involve in the accuracy of the angiogenic balance between angiogenic factors and interaction with a compound of extracellular matrix. ECM is considered to be the storage place for angiogenic stimulator and inhibitors. These molecules are found to bind to component of ECM and they have been released via cleavage by protease, i.e. matrix metalloproteases (MMPs) which proteolytically cleave and activate precursors of angiogenesis promoters. One of the compounds that growth factors bind with, is a heparin sulfate proteoglycans in the extracellular matrix. MMPs generates growth-promoting signals by releasing growth factors bound to them and causing a generation of activating ligands for integrin signalling.[25] Among all the growth factors, vascular endothelial growth factor (VEGF), is consider to be a potent and best studied angiogenic peptide that is released from ECM by proteolytic action of MMPs and plasmin.[26-28] The activity of MMPs on angiogenic growth factors, chemokines, growth factor receptors, apoptosis mediators, adhesion molecules is critical for the rapid cellular responses, and essential for angiogenesis and also involved in mediating tumor growth and progression.[29]

Many angiogenesis activators and inhibitors are stored as fragments of the compound within larger molecules in the extracellular matrix among the most studied are endostatin derived from collagen XVIII.[30,31] Angiostatin derived from plasminogen[32,33] and tumstatin derived from type IV collagen.[34] MMP-9 can be both a promoter of the angiogenic switch by releasing angiogenic stimulator from the ECM or it may act as angiogenic inhibitor by releasing angiogenesis inhibitors from their parent matrix molecules. Serine protease family is other ECM involved protease that has a direct role in tumor angiogenesis. This is a family of plasminogen activator-plasmin system. During active angiogenesis by angiogenic growth protein, especially bFGF and VEGF, activation of plasminogen activator inhibitor type I (PAI-I) and urokinase type plasminogen activator (uPA) expression are induced and this is being implicated in tumor invasion and metastasis.[35] Collegens, laminins and fibronectins are among many other proteins are present in the ECM and surrounding vasculature, have pro-angiogenic properties and they capable of promoting endothelial cell proliferation, survival, migration and blood vessel formation.

Many of the angiogenic growth factors are processed with regard to activation or inactivation after binding to heparan-like glycosaminoglycans in ECM such as VEGF, basic fibroblast growth factor (bFGF), and transforming growth factor-beta (TGF-b). These factors can be mobilized during ECM degradation by proteases expressed on endothelial cells during non-pathological angiogenesis and by proteases secreted by tumor or stromal cells under pathological conditions.[36] The ECM not only serves as a storage place for vascular growth factors, but also plays an important role in tumor angiogenesis. Matrix molecules demonstrated to possess pro-angiogenic properties include collagen I, III, XV, laminin-1 and -8, fibronectin, and perlecan.

In endometrium, MMPs are involved in endometrial ECM remodelling[37,38] and are synthesized in an inactive form that by the action of proteases is converted to an active form, MMPs have been divided into several subgroups: collagenases (MMP-1, -8, -13); gelatinases (MMP-2, -9); stromelysins (MMP-3, -7, -10, -11); and membrane-type MMPs (MT-MMP1 to 6). An additional miscellaneous group of MMPs includes MMP-12, MMP-18, and MMP-19 to -26.[39,40] A Study on the expression of mRNA from the endometrium of different phases of menstruation have shown that the expression of specific members of MMPs are prominent in different phases. mRNA for MMP-1, MMP-3, MMP-8, MMP-9 and MMP-12 have found to be at low levels of expression during proliferation and secretory phases but these MMPs has shown a marked increase in expression during the menstrual phase. Proliferative phase of MMPs expression involves, MMP-11 and most common MMP that shown have increased level was MMP-26.[41] This pattern of MMPs expression is indicative of the important role of each MMPs in different phase of menstruation and disturbance of this pattern due to the pathophysiological

stimulator of growth factors that if disturbed in normal menstruation cycle leads to abnormal menstruation.

Furthermore, the role of MMP in stimulating tumor angiogenesis and growth is by cleaving a sequence of collagen IV that causes exposure of the pro - angiogenic sequence of it.[42] Normal angiogenesis are finely tuned process and factors involve in controlling abnormal vessel growth like endogenous anti-angiogenesis that controls out growth of blood vessels by inhibiting the factor involve in their activation. The first angiogenesis inhibitor identified was thrombospodin-1 (TSP-1).[43] It is a protein family of five large extracellular glycoprotein. Thrombospodin-1 and -2 by binding to collagen and fibronectin can affect ECM structure and modulate its protease activity such as MMP-9 and plasmin. Cryptic fragments of ECM are among the other angiogenic inhibitors. Degradation of C-terminal fragment of collagen XVIII lead to formation of endostatin, is one of the most studied anti-angiogenic factor of ECM.

COPPER

Numerous exogenous factor has been identified as playing a role in regulating angiogenesis, copper has been shown to have an effect on production of number of angiogenic factors including VEGF-A (Harris, 2004; Sen et al., 2002). Several experiments on animal have shown the role of copper as a stimulatory compound of angiogenesis and in the same cases copper chelation causes inhibition of angiogenesis (Finney et al., 2007). Copper containing Intra Uterine Devices (IUD) increases inflammation and uterine action (Kulier et al., 2006).

It has been shown that copper acts as an agonist of VEGF receptor and increase its serum level along with VEGF consider to have a direct correlation with menorrhagia, which is excessive uterine bleeding in the absence of pelvic pathology such as adenomyosis, fibroids and polyps, this condition is often referred as a DUB.[44]

Biological activity and synthesis of a number of angiogenesis growth factors have influenced by copper among them is VEGF-A[45,46] and shares some of the pathways utilized by hypoxia to regulate VEGF-A expression.[45,47]

Copper activation of angiogenic factors is by acting as a cofactor to molecules such as β-FGF, VEGF, and angiogenin. Without it, they cannot function, and growth of new blood vessels stops. In other words, reduce concentration of copper stops angiogenesis by activating apoptosis (programmed cell death) mechanism, and cancer remains in dormancy.[48] It is been shown that copper is having anti-angiogenic activity along with its angiogenic activity.[49] Copper is an essential element in the physiological system by having a large scale of contribution in hemoglobin synthesis and in the catalysis of metabolic oxidation.[50,51] Copper or copper complexes have been shown to directly stimulate angiogenesis in several animal model systems while copper chelation has been shown to inhibit angiogenesis.[52] Copper containing intrauterine devices (IUD) increases inflammatory action and uterine bleeding.[53]

The studies have been conducted on serum, secretory endometrium and menstrual blood copper levels in women using copper-T IUD. There was no change in the serum copper levels, but the secretory endometrium and menstrual blood copper levels were significantly increased in the endometrial tissue samples which were taken after insertion of IUD when compared with samples taken before insertion. The result of the study was that copper is not stored in basal layers of the endometrium but is continually released from the copper IUDs.[54]

VEGF

Currently, the VEGF family includes VEGF-A, PlGF (placenta growth factor), VEGF-B, VEGF-C, VEGF-D, VEGF-E and svVEGF (snake venom VEGF). The molecular and biological functions of each of these growth factors have been well characterized.

The human VEGF-A gene is organized into eight exons, separated by seven introns and is located at 6p21.3.[55-57] Human VEGF-A has at least nine subtypes due to the alternative splicing of a single gene: VEGF121, VEGF145, VEGF148, VEGF162, VEGF165, VEGF165b, VEGF183, VEGF189 and VEGF206.[58] There is less information available about how VEGF isoform levels are regulated, most VEGF produces cells appear to preferentially expressVEGF121, VEGF165 and VEGF189. VEGF165, the predominant isoform, is secreted as an approx. 46 kDa homodimer, which has a basic character and moderate affinity for heparin, owing to the presence of 15 basic amino acids within the 44 residues encoded by exon 7.[59-61] Gene expression of VEGF is regulated by a variety of stimuli such as hypoxia, growth factors, exogenous growth factor stimulator, transformation, p53 mutation, estrogen, TSH (thyroid-stimulating hormone), tumor promoters and NO (nitric oxide). Although all of the stimuli responsible for the upregulation of the VEGF gene are quite important, hypoxia has been of particular interest because of its importance and the unique transcriptional regulation involved. It is now well established that HIF-1 (hypoxia inducible factor-1) is a key mediator of hypoxia responses. HIF-1 is a transcriptional activator composed of HIF-1α and HIF-1β subunits. Both HIF-1α and HIF-1β are constitutively expressed in various types of tumors. Under normal oxygenation conditions, HIF- 1α is scarcely detectable because it is targeted for rapid destruction by an E3 ubiquitin ligase containing pVHL (von Hippel–Lindau tumor suppressor protein). The protein encoded by this gene is a component of the protein complex that includes elongin B, elongin C, and cullin-2, and possesses ubiquitin ligase E3 activity. This complex is involved in the ubiquitination and degradation of a hypoxia-inducible factor (HIF), which is a

transcription factor that plays a central role in the regulation of gene expression by oxygen. The interaction between pVHL and a specific domain of the HIF-1α subunit is regulated through hydroxylation of a proline residue (Pro564 in HIF-1α) by prolyl- 4-hydroxylase, which requires molecular oxygen and iron for its activity.

Under hypoxic conditions, HIF- 1α expression increases as a result of suppressed prolyl hydroxylation of HIF-1α and decreased ubiquitination and degradation.[62,63] Consequently, HIF-1α protein accumulates under normoxic conditions, and the transcription of VEGF-A is increased.[64]

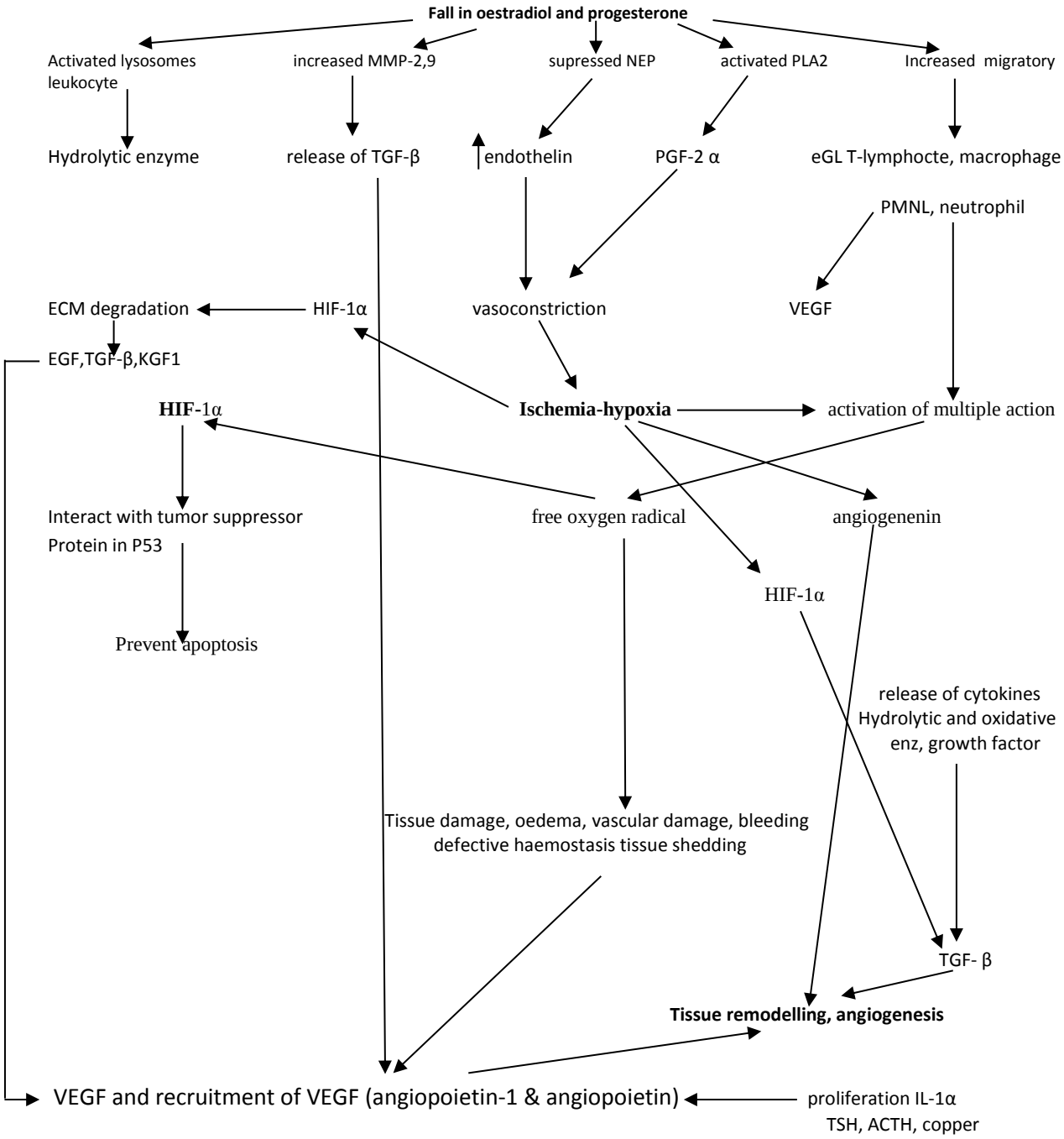

Figure 1: Schematic representation of interactions within endometrium during pathological angiogenesis. Disturbance of any of different clinical disorder can cause formation of abnormal blood vessel formation.

Key: MMP-matrix metalloproteinases; NEP-neutral endopeptidase; PLA2- phospholipase A2; PGF2-prostaglandin F2; VEGF-Vascular endothelial growth factor; TGF-β-transforming growth factor; IL-1α-Interleukin-1α.

HIF-1

HIFs and regulation by protein hydroxylation HIF-1 is an α,β-heterodimer that was first recognized as a DNA-binding factor that mediates hypoxia-inducible activity of the erythropoietin 3′enhancer.[65,66] After an extensive studies have been conducted on oxygen-dependent activity of the erythropoietin 3′enhancer in a wide variety of non-erythropoietin-producing cells.[67] It rapidly became clear that the HIF system is a key modulator of many other biological processes. These include angiogenesis among a wide range of cellular and systemic responses to hypoxia.[68,69] Both the HIF-α and HIF-β subunits exist as a series of isoforms encoded by distinct genetic loci.HIF-1βsubunits are constitutive nuclear proteins, whereas HIF-α subunits are inducible by hypoxia.

Among three HIF-α isoforms, HIF-1α and HIF-2α appear closely related and are each able to interact with hypoxia response elements (HREs) to induce transcriptional activity.[70,71] In contrast,HIF-3α appears to be involved in negative regulation of the response, through an alternately spliced transcript termed inhibitory PAS domain protein.[72] HIF-α subunits are regulated by a multistep process involving changes in activity, abundance, mRNA splicing and subcellular localization.[69] Recent analysis of post-translational modifications that mediate these processes has revealed an unexpectedly direct interface with the availability of oxygen, through a series of non-heme, iron-dependent oxygenases that hydroxylate specific HIF-α residues in an oxygen-dependent manner.[73,74]

Hydroxylation at two prolyl residues (Pro402 and Pro564 in human HIF-1α) mediates interactions with the von Hippel–Lindau (VHL) E3 ubiquitin ligase complex that targets HIF-α for proteasomal destruction.[73,75,76] Each site can interact independently with VHL E3,potentially contributing to the extremely rapid proteolysis of HIF-α that is observed in oxygenated cells .These sites contain a conserved motif and are targeted by a newly defined prolyl hydroxylase activity, that in mammalian cells is provided by three isoforms termed PHD (prolyl hydroxylase domain).[77,78] Determining the relative importance of different PHD isoforms in the regulation of HIF-α and other potential hydroxylation targets is require an extensive study. Figure 1 shows a schematic representation of interaction between different angiogenic factors during pathological formation of blood vessels.

CONCLUSION

Angiogenesis is a complex, seemingly multicellular process. For this reason, the understanding as well as the ability to manipulate an angiogenesis may depend on the attention given to these cellular collaborators that either promote or mitigate neovascularization. Among them, VEGF, HIF and hormonal disturbance play a key role. The soluble pro- and anti-angiogenic factors stored and process the ECM makes it more prone to capillary penetration or for the formation of the neovessels in the form of the epidermal cell (EPC) sub-population, or as cells potentially able to progress to an endothelial cell (EC) status.

Oxygen supply to tissue is a fundamental requirement of tissue for normal functioning that supply through blood vessels. Numerous mechanisms are activated to compensate the lack of oxygen (hypoxia) during oxygen tension. The mechanisms involved in this compensatory process are involved in synthesizing a factor known as hypoxia inducible factors HIF-1 and HIF-2. Prolyl and asparaginyl hydroxylases consider to be oxygen sensors allow the regulation of HIFs that are transcription factors that causes transcription of VEGF gene. VEGF plays a critical role in the formation of blood vessels during physiological processes such as embryogenesis or uterine endometrial lining during each menstrual cycle as well as during a numerous pathological condition such as tumor growth, retinopathy and ischemic disease (cerebral ischemia, myocardial infarction). There are various angiogenic factors additionally require in the process of formation of new blood vessel during pathological condition such as Angiopoietin TGF-β (transforming growth factor-β). The majority of these factors are synthesized and activated during hypoxic condition by HIF- β, although the binding site of HIF has yet been completely identified in the regulated sequence of these genes. Hypoxia-induced gene products that results in new vessel growth may be part of a self-regulated physiological protection mechanism preventing cell injury, especially under conditions of chronically reduced blood blow (chronic ischemia).

The signals that initiate angiogenesis vary with the condition that requires angiogenesis, and may be organ specific. Many numbers of cells may be the source of angiogenic signals, including tumor cell, fibroblast, endothelial cells, epithelial cells, or activated macrophages. Embryonic angiogenesis are activated by genes that are transcribed in response to hypoxia and hypoglycemia in the developing embryonic tissue. Importantly presence of inhibitory signals causes a decrease in the signal for angiogenesis, rather than simply requiring a positive stimulus.

Formations of new blood vessels are controlled by a balance between angiogenic stimulators and inhibitors. When this balance is lost, it causes outgrowth of blood vessels that is known as pathological angiogenesis and is the manifestation of tumor growth. There are many exogenous and endogenous angiogenic factors that involved in up and down regulation of the growth of blood vessels. The most studied angiogenic stimulators such as vascular endothelial growth factor (VEGF),), angiogenin, transforming growth factors (TGF-beta), fibroblast growth factors (FGF), epidermal growth factor (EGF), can induce the division of endothelial cells thus indicating a direct action on these cells.

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