International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Angiogenic Biomarkers of Dysfunctional Uterine Bleeding

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Abstract: ***Disturbed endometrial angiogenesis plays a role in the mechanism of Dysfunctional uterine bleeding (DUB).Under hypoxic conditions, Hypoxia inducible factor 1 alpha (HIF-1α) rapidly accumulates and activates angiogenic growth factors, receptors and extracellular proteases such as matrix metalloproteinases (MMPs) like MMP-9. Copper influences the production of number of angiogenic factors including HIF-1α.***

Keywords: Angiogenesis, Dysfunctional uterine bleeding(DUB), Hypoxia inducible factor 1 alpha (HIF-1α), Matrix metalloproteinase -9(MMP-9),Copper, Vascular endothelial growth factor (VEGF)

1. Introduction

Dysfunctional uterine bleeding (DUB) is characterized by abnormal bleeding that is unrelated to pharmacological agents, pregnancy, and identifiable pelvic or systemic disease.DUB have an underlying etiology of chronic anovulation with unopposed estrogen stimulation of the endometrium. Approximately 10% to 15% of all gynecologic patients have DUB.[1]

It has been demonstrated that biochemical disturbances, including increased endometrial vascular fragility, disturbed endometrial angiogenesis, and inconsistency of the endothelial, epithelial, and stromal supporting structures in the local endometrial environment, may play an important role in the mechanism of DUB.[2,3]

Vascularization is essential for endometrial repair and growth, and the formation of new vessels depends on interactions between various hormones and growth factors. Although many growth factors can induce endometrial angiogenesis, vascular endothelial growth factor (VEGF) is essential in controlling endometrial angiogenesis and has been considered as a key factor in the occurrence of abnormal uterine bleeding in previous studies.[2,3]

Copper is one of the essential trace elements. It is part of a number of enzymes. Copper has been shown to influence the bioactivity or production of a number of angiogenic factors including VEGF-A and shares some of the pathways utilized by hypoxia to regulate VEGF-A expression.

Hypoxic pathways other than VEGF may also regulate matrix metalloproteinases(MMP).Reduced oxygen levels stimulate MMP-9 expression in a highly invasive, metastatic breast cancer cell line, but did not have an effect on MMP-2 expression.[4]
HIF-1α rapidly accumulates and transactivates hundreds of genes under hypoxic conditions, including angiogenic growth factors and receptors, and extracellular proteases such as matrix metalloproteinases (MMPs).[5]

MMPs are endopeptidases that degrade most of the components of the extracellular matrix (ECM), including the basement membrane. MMPs are also involved in the regulation of cell proliferation, release of growth factors and angiogenesis.

MMP degradation happens in endometrial extracellular matrix when the endometrium is deprived of progesterone.MMP play an important role in triggering endometrial bleeding. Similar changes in the expression and activity of MMP-2 and -9 (gelatinase A and B) are found,MMP-2 and VEGF expressions and increased endometrial micro-vascular density in women with anovulatory DUB were only seen in hyperplasia endometrium. Although increased MMP-9 expression in women with anovulatory DUB was also seen in proliferative endometrium, hyperplasia endometrium still had more MMP-9 expression than the proliferative endometrium.[6]

It has been demonstrated that chronic unopposed estrogen can directly cause the production of MMP-2 and -9 and can also indirectly cause the production of MMP-2 and -9 by inducing the production of VEGF, interleukin-8, monocyte chemoattractant protein-1, cyclooxygenase-2, and the influx of leukocytes.

2. Dysfunctional uterine bleeding (DUB)

DUB is a diagnosis of exclusion and hence one must rule out organic causes for the abnormal bleeding (after excluding pregnancy). Organic causes can be classified into three major categories: pelvic pathology, systemic diseases, and iatrogenic causes. (Defination by the European society for human reproduction and embryology)

The local pelvic pathology category includes, benign pelvic lesions, such as myomas, adenomyosis, endometriosis, endometrial or cervical polyps, cervicitis, and severe vaginal infection. Malignant causes of menorrhagia include carcinoma of the reproductive tract and premalignant changes of the endometrium such as hyperplasia. Systemic diseases are another important group to consider, including coagulation disorders (thrombocytopathies, Von

Willebrand's disease, and leukemia), hypothyroidism, systemic lupus erythematosus, and cirrhosis. Iatrogenic etiologies include use of hormone therapy, contraceptive injections and devices, and medications including tranquilizers, antidepressants, anticoagulants, and corticosteroids. When all the above have been excluded, the diagnosis of dysfunctional uterine bleeding can be given. DUB is a diagnosis that does not apply to menorrhagia only, but also includes excessively prolonged and frequent bleeding (menometrorrhagia). DUB occurs more frequently in anovulatory than ovulatory cycles. Anovulatory DUB is the end result of unopposed estrogen effects on the endometrium, leading to proliferative, disordered proliferative, hyperplastic, or neoplastic changes.[7]

3. Angiogenesis and DUB

Angiogenesis

Angiogenesis consist in the formation of new blood vessels from proliferation of new capillary from pre existing vessels, playing a very important role in the uncontrolled proliferation of cells. The formation of new vessels depends on the interaction between different hormones and growth factors. The endometrium expresses several growth factors involved in angiogenesis, including epidermal growth factor (EGF), transforming growth factor (TGF-β) and vascular endothelial growth factor (VEGF) etc.[8]Angiogenesis is a fine balance between angiogenic factors (growth factors) and interaction with extra cellular matrix as ECM is a storage place of angiogenesis promoters and other biologically active molecules and is a rich source of angiogenesis inhibitors.

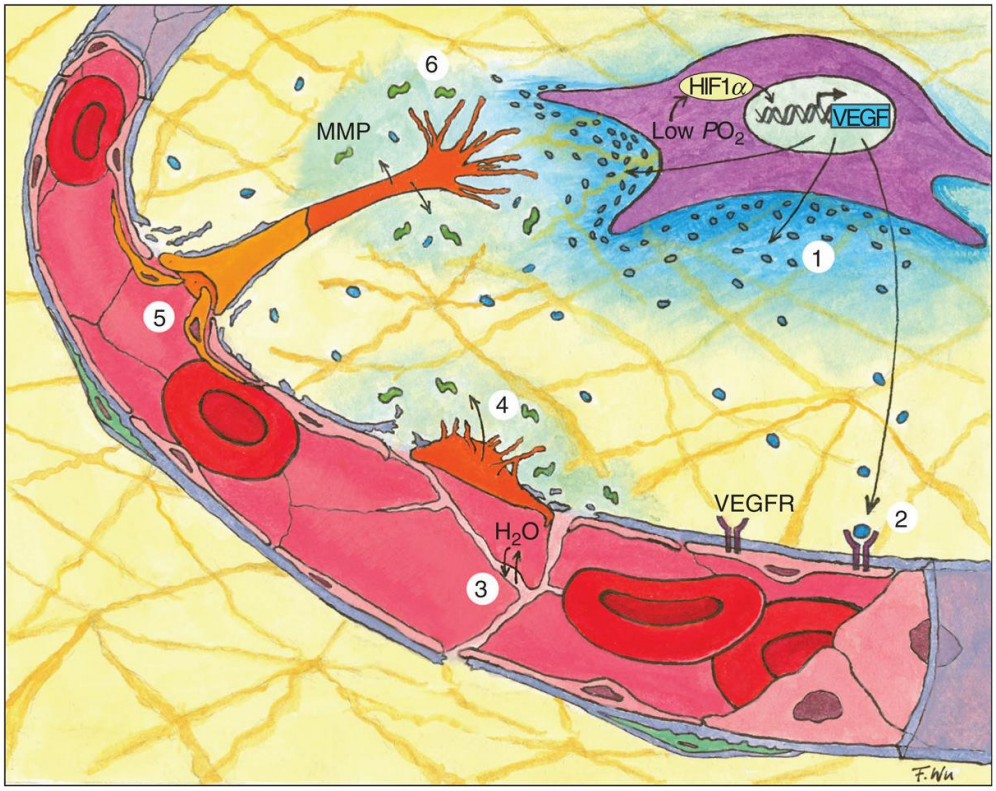

Figure 3.3: Schematic of processes involved in angiogenesis. 1) Hypoxia-inducible factor 1 (HIF1) is upregulated in a hypoxic cell. HIF1 activates the transcription of vascular endothelial growth factor (VEGF), which is then secreted by the cell. 2) VEGF-VEGFR binding on the capillary surface. 3) Vessel permeability changes. 4) An activated EC (the tip cell) starts to break down the basement membrane. 5) Stalk cells proliferate behind the tip cell. 6) The leading edge of the moving sprout releases MMPs which proteolyze the surrounding extracellular matrix, allowing the cell to migrate.[10]

HIF 1 α

HIF-1 was an αβ heterodimer, both subunits of which belonged to the rapidly growing family of basic helix-loop-helix (bHLH) transcription factors.[11]

HIF 1α functions and their expression in endometrium

The presence of HIF and some of its major target genes has been demonstrated in the uterus. It has been shown that both HIF1α and HIF1β are expressed in the endometrium, and expression levels varied throughout the menstrual cycle.[12,13]

HIF1 α appeared to be most strongly expressed during the perimenstrual phase in those endometrial layers that would be sloughed off at menstruation

L. Erichaung et al presented the evidence that unstable HIF-1α was controlled by an oxygen-dependent degradation (ODD) domain within HIF-1α and the degradation was catabolized through the ubiquitin-proteasome pathway (Fig 3.4). Removal of the entire ODD domain rendered HIF-1α stable even in oxygenated cells, resulting in autonomous heterodimerization, DNA binding, and transactivation independent of hypoxia signaling.[14]

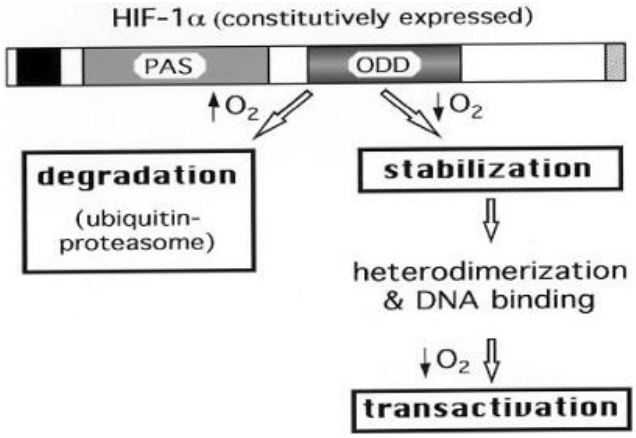

Figure 3.4: A model for hypoxia-induced activation of HIF-1. A schematic drawing of HIF-1α is illustrated. The bHLH domain ismarked by a black box and the C terminus transactivation domain bya hatched box. PAS and ODD domains are indicated. Steps regulated by changes of oxygen tension are boxed with bold lines.[15]

The interaction between pVHL and a specific ODD domain of the HIF-1α subunit was regulated through hydroxylation of a proline residue (Pro^{564}in HIF-1α) by prolyl-4-hydroxylase, which required molecular oxygen iron and 2 oxoglutarate for its activity (Fig 3.5).

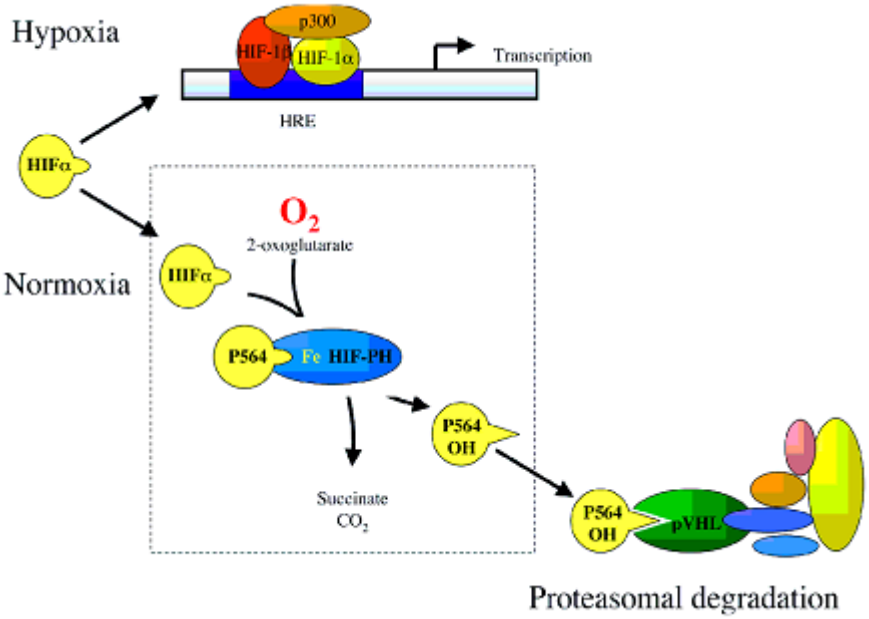

Figure 3.5: Recognition of HIF-1α bypVHL is regulated, at least in part, through an oxygen, iron, and 2-oxoglutarate dependent enzymatic hydroxylation of its proline 564 residue, by an enzyme termed HIF prolyl hydroxylase (HIF-PH). The key position of this hydroxylation in the degradation pathway of HIF-α, together with its requirement for molecular dioxygen as a cosubstrate, provides the potential for HIF-PH to function directly as a cellular oxygen sensor.[16]

HIF-1α was scarcely detectable because it was targeted for rapid destruction by an E3 ubiquitin ligase containing pVHL (von Hippel–Lindau tumour suppressor protein). pVHL(which functions as an E3 ubiquitin ligase) bound directly, through the β domain, to a region of HIF called the oxygen-dependent degradation domain (ODD), which had previously been shown to render HIF unstable in the presence of oxygen. The binding of pVHL to HIF1-α directed the polyubiquitination[17] and this in turn led to proteasomal degradation of HIF.[18]Under hypoxic conditions, HIF-1α expression increased as a result of suppressed prolyl hydroxylation of HIF-1α and decreased ubiquitination and degradation. As a result, HIF accumulated in the cell and was available to activate transcription.

4. HIF 1 α and angiogenic growth factor

HIF-1α over-expression has been associated with up-regulation of the VEGF pathway, with increased micro vascular density and with a poor prognosis. In addition, HIF-1α over-expression was related significantly with well-differentiated endometrial neoplasms and with a rich Estrogen receptor (ER) status, although the latter relation was only marginal. A similar association between HIF-1α and ER also was noted in patients with breast carcinoma.[19]Several other growth factors and growth factor receptors, such as epidermal growth factor and insulin-like growth factor up-regulate HIF-1 α protein expression. It was possible that ER stimulated pathways induce HIF-1α expression, and it has been shown that estradiol stimulated VEGF in ER positive smooth muscle cell lines.[20,21]

Copper and Neovascularization

Copper has been shown to influence the bioactivity or production of a number of angiogenic factors[22,23] and shared some of the pathways utilized by hypoxia to regulate VEGF-Aexpression.[24]

Copper increased VEGF expression through regulation of HIF1α activity

Copper shared some of the pathways utilized by hypoxia to regulate VEGF-Aexpression. Studies, have suggested that copper was required for HIF-1 transcription activity. Copper chelation in cultured cells blocked insulin-like growth factor-1 (IGF-1)-induced HIF-1 binding to hormone responsive element (HRE) and VEGF expression. This inhibitory effect could be relieved by the addition of excess copper in cultures.[25]There were multiple sites that potentially required copper for activation of HIF-1, including HIF-1α synthesis, stabilization, translocation from cytosol to nucleus, binding to the HRE sequence of target genes, and HIF-1 transcriptional complex formation. In this study, they reported that copper was required for HIF-1 binding to the HRE sequence of target genes and for the HIF-1 transcriptional complex formation in a HepG2 human hepatoma cell line.

Li Q, Chen H et al have demonstrated that copper was required for VEGF expression and cell growth, through its regulation of hypoxia-inducible factor (HIF) activity, a transcription factor that was required for VEGF expression. This copper requirement for VEGF expression did not explain the effect of high levels of copper on VEGF over-production.[26] High levels of copper stabilized HIF-1α, leading to HIF-1α accumulation and enhanced HIF transcriptional activity. However, the mechanisms underlying these two actions were different. It has been known that HIF-1α was the critical transcription factor for VEGF expression.[27] The activity of HIF-1α mainly regulated by its intracellular stability and accumulation. Metals such as cobalt, nickel and copper could inhibit the process of HIF-1α degradation, thus leading to its accumulation and activation. Therefore, cells exposed to high levels of cobalt, nickel or copper led to over-production of VEGF. On the other hand, the activation of HIF-1α transcription activity required copper. It's depletion resulted in suppression of HIF-1α activation as well as inhibition of VEGF expression. Most studies have used high levels of copper to examine its effect on VEGF production and cell growth, led to a conclusion that copper stimulation of cell growth was mediated by VEGF production. However, the present study made a clear distinction between stimulation of cell growth by physiologically relevant levels of copper and that by copper overload. Under the present experimental condition, copper did not increase VEGF expression, but copper was clearly required for VEGF expression.[28]

Matrix metalloproteinases

The first matrix metalloproteinase activity discovered was a collagenase in the tail of a tadpole undergoing metamorphosis. To date, 24 different vertebrate MMPs have been identified, of which 23 were found in humans. As shown in fig 3.7, MMPs generally consisted of a prodomain, a catalytic domain, a hinge region, and a hemopexin domain .They were either secreted from the cell or anchored to the plasma membrane.[29]

MMPs expression in endometrium

Many MMPs were found in the human endometrium, and most of them were preferentially expressed at menstruation .Collagenase-1 (MMP-1) and stromelysin-1 (MMP-3) and -2 (MMP-10) were almost exclusively expressed during the menstrual phase, whereas gelatinases A (MMP-2) and B (MMP-9) occured throughout the cycle, but were more abundant during the menstrual phase.[30]

In the human endometrium, expression of MMP-1, MMP-3, MMP-10, and MMP-9 was almost exclusively restricted to the perimenstrual period, as shown by Northern blotting[31] and in situ hybridization studies.[32]The expression of MMP-1 and MMP-9 was focal and limited to the functionalis layer, which was subsequently shed. A role of MMPs in endometrial matrix breakdown, in particular of MMP-1 and MMP-9, was also strongly suggested by the tight control sex steroids exert on their expression[33], secretion, and activation.

MMP-9 mRNA was expressed in the human endometrium only during menstruation, being absent in proliferative, secretory and late secretory phases of normal menstrual cycle.[34]

MMPs being involved in angiogenic and anti-angiogenic activities:

The regulation of angiogenesis was a very delicate balance between pro- and antiangiogenic factors, and it was observed that MMP-9 played a dual role in this process. It could act as a proangiogenic factor via VEGF regulation; as Hiratsuka et al. demonstrated, primary tumors in premetastatic lungs induced MMP-9 expression in a VEGFR-1-dependent manner, which enhanced the invasion of cancer cells and facilitated metastasis. MMP-9 also triggered the angiogenic switch by releasing VEGF. MMP-9 and VEGF were expressed during invasive oral squamous cell carcinoma (OSCCs) of the tongue and in metastatic tumors that tend to express higher levels of VEGF and MMP-9, than non metastatic tumors. In another study, increased VEGF expression was associated with a poor prognosis in OSCC patients, whereas MMP-9 expression levels had no correlation with patient outcomes.[35]

To conclude, from all the mentioned review that serum copper was required for VEGF A expreesion in endometrium,which could lead to angiogenesis in endometrium. And in hypoxic condition, elevated HIF 1 α expression could lead to elevated MMP-9 , which caused extracellular matrix breakdown & proliferation of cells leading to angiogenesis. Copper was required to increase activity of HIF 1 α in the endometrium. All these parameters serum Copper, HIF 1 α & MMP-9 led to angiogenesis which further led to dysfunctional uterine bleeding.

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