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REVIEW ARTICLE

Hypoxia inducible factor-1 alpha and multiple myeloma

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Abstract

Rapid tumor growth creates a state of hypoxia in the tumor microenvironment and results in release of hypoxia inducible factor-1 alpha (HiF-1α) in the local milieu. Hypoxia inducible factor activity is deregulated in many human cancers, especially those that are highly hypoxic. In multiple myeloma (MM) in initial stages of disease establishment, the hypoxic bone marrow microenvironment supports the initial survival and growth of the myeloma cells. Hypoxic tumour cells are usually resistant to radiotherapy and most conventional chemotherapeutic agents, rendering them highly aggressive and metastatic. Therefore, HIF is an attractive, although challenging, therapeutic target in MM directly or indirectly in recent years.

Introduction

Angiogenesis in multiple myeloma:-

Multiple myeloma (MM) is a hematological malignancies characterized by presence of a monoclonal protein in the serum, urine or both, osteolytic lesions and accumulation of malignant plasma cells (PC) into a hypoxia bone marrow (BM) microenvironment that supports their growth and survival. Bone marrow is the site of the origin in nearly all myelomas and the microenvironmental interactions releases different cytokines that regulates neoangiogenesis which is thought to have a governing role in pathogenesis and progression of MM (Vacca *et al*, 1994, Carmelite and Jain, 2000). Response to treatment with anti-angiogenic agents has further underscored the importance of angiogenesis in pathogenesis of MM. New pharmacological agents which act by inhibiting angiogenesis are being increasingly investigated in current clinical trials for treatment of MM (Giatromanolaki *et al*, 2010, Borsi *et al*, 2014, Wang *et al*, 2014, Storti P 2013).

Angiogenesis plays an important role in the pathogenesis and progression of MM. Bone marrow angiogenesis progressively increases along the spectrum of PC disorder from the monoclonal gammopathy of undetermined significance (MGUS) to smoldering MM (SMM) to MM, indicating that angiogenesis is related to disease progression (Vacca *et al*, 1994). Degree of BM angiogenesis also correlates with overall survival (OS), disease severity and tumor burden: BMPC infiltration, PC labelling index and serum β2-microglobulin levels in MM (Vacca *et al*, 1994; Rajkumar *et al*, 2000; Ria *et al*, 2011). All this evidence indicates that angiogenesis is critically involved in the pathophysiology of MM.

The MM BM microenvironment is characterized by the presence of PC, extracellular matrix proteins, hematopoietic stem cells (HSC) and BM stromal cells, including fibroblasts, osteoblasts, osteoclasts, chondrocytes, endothelial cell (EC), endothelial progenitor cell (EPC), T- lymphocytes, macrophages and mast cells, which are intimately involved in all biological stages of intramedullary growth (Klein and Battallie, 1992; Klien *et al*, 1995; Zhang *et al*, 2005, Nowak *et al*, 2010; Bhaskar *et al*, 2012).

The molecular mechanisms underlying the increased angiogenesis in MM are complex. Numerous autocrine and paracrine interactions between tumor cells and stromal cells within the BM microenvironment stimulate the secretion of chemokines, cytokines, growth factors and matrix metallo proteins which collectively orchestrate angiogenesis. Myeloma cells also produces numerous angiogenic regulators including hypoxia inducible factor (HIF)-1α, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), platlet drived growth factor (PDGF), angiopoitein (Ang) -1, Ang-2, osteopontin (OPN) and metalloproteinases MMP-2 or MMP-9 which leads to EC proliferation, matrix degradation and tube formation (Carmelite and Jain, 2000; Yata *et al*, 2003; Vacca *et al*, 2005). The number of cytokines produced by both the MM cells and the BM stromal cells result in the proliferation of MM cells, the extravasation of the MM cells to secondary sites, and the stimulation of angiogenesis to generate blood vessels that provide nutrients and other factors for the growing tumor. Furthermore this network of cytokines mediates myeloma cell growth, proliferation, survival, drug resistance and migration.

Hypoxia and angiogenesis:-

Angiogenesis is a multistep process characterized by the formation of new blood vessels from the preexisting vasculature that is maintained by the dynamic balance between the pro-angiogenic and anti-angiogenic factors. The initiation of angiogenesis, the angiogenic switch is recognized as a critical step for tumor progression and depends on the induction of several positive angiogenic regulators released by tumor cells or induced in the microenvironment of tumor cells. Among them hypoxia and growth factors like VEGF, Ang-1, Ang-2, and bFGF play a central role.

Hypoxia means a reduction in the physiological oxygen level. It is caused by vascular and pulmonary diseases or by triggering of cancerous-tissue growth and leads to cellular dysfunction. One of the most well-studied and predominant hypoxic responses is the induction of angiogenic and growth factors, which lead to the formation and growth of new blood vessels. When tissues grow beyond the physiological oxygen diffusion limit, the relative hypoxia triggers expansion of vascular beds by inducing angiogenic factors in the cells of the vascular beds, which are physiologically oxygenated by simple diffusion of oxygen. One of the angiogenic factors, VEGF, has been reported to be the most remarkable one stimulating angiogenesis in a strictly dose-dependent manner (Ferrara *et al*, 2003). Other factors such as Ang-2/Ang-1 (Graham *et al*, 1998; Phelan *et al*, 1998), tyrosine kinase with immunoglobulin and epidermal growth factor homology domain (Tie-2) receptor (Kuwabara *et al*, 1995), PDGF (Negus *et al*, 1998; Wykoff *et al*, 2000), bFGF (Sakuda *et al*, 1992) and monocyte chemoattractant protein 1 (MCP-1) (Phillips *et al*, 1995) have also been reported as indispensable factors responsible not only for increasing vascular permeability, endothelial sprouting, maintenance, differentiation and remodeling but also cell proliferation, migration, enhancement of endothelial assembly, and lumen acquisition. Under hypoxia conditions, angiogenesis is modulated because of the concomitant inflammation and by several factors secreted from immune cells, because leukocyte subtypes produce a myriad of angiogenic factors, various interleukins such as tumour growth factor (TGF)-β1, MCP-1, and proteinases (Vacca *et al*, 1998; Norrby, 2002). Thus, hypoxia provides an important environment not only for angiogenesis but also for related phenomena in the hypoxic or surrounding area; this implies that hypoxia is more than simply a regulator of angiogenesis (Paleolog, 2004).

The first step in the process of angiogenesis is activation of the endothelial cells in response to hypoxic condition. Tumor hypoxia is associated with poor prognosis, tumor aggressiveness, and metastases, recurrence following treatment and resistance to radiation therapy (Theodoropoulos *et al*, 2004; Isobe *et al*, 2012). Hypoxia in tumor is mediated by the up regulation of transcription factor HIF-1 complex, which is composed of heterodimer of HIF-1α and HIF-1β, the genes associated with angiogenesis, pH adaptation, glucose transport and apoptosis.

Hypoxia-inducible factors (HiF)-1α:-

A vast number of reports have shown that HiF-1 is the key regulatory of transcription factor in these hypoxia-induced processes. Hypoxia-inducible factors-1 is a heterodimer comprising HiF-1α and HiF- 1β subunits, both of which are basic helix-loop-helix transcription factors. Hypoxia-inducible factors -1β (ARNT) is a nuclear protein that is constitutively expressed and is independent of O_2 tension. Hypoxia-inducible factors -**1**α, in contrast to HiF-1β, is a cytoplasmic protein responsive to O_2 levels (Pauyssegur *et al*, 2006; Semenza *et al*, 2009).

Regulation of the HiF transcription factors:-

Under normoxia (sufficient oxygen levels) the α subunit is hydroxylated on specific proline residues allowing the von Hippel-lindau protein (pVHL tumor suppressor protein) and the E3 ligase complex to bind (Maxwell *et al*,

1997). This consequently leads to ubiquitination, which target the α subunit to proteosomal degradation. In addition, hydroxylation of an asparagine residue in HiF-α disrupts the interaction between HiF-α and the coactivator p300 through a process that is independent of proteasomal degradation, which leads to reduced HiF transcriptional activity (Salceda *et al*, 1997; Cook and Figg, 2010). Under hypoxia (absence of oxygen) condition, prolyl hydroxylase cannot modify HiF-1α and the protein remains stable and translocates to the nucleus where they heterodimerizes with the HiF-1β subunit. The activated HiF transcription factor binds specifically to consensus sequence in the target genes known as the hypoxia response elements (HREs) associated with HiF-regulated genes (Pouyssegur *et al*, 2006; Enholm *et al*, 1997; Cook and Figg, 2010). By this mechanism, HiFs transactivates a number of pro-angiogenic factors, including VEGF, Ang, FGF, PDGF etc, erythropoietin, various glycolytic enzymes, transferrin, and a variety of other proteins essential for systemic, local, and intracellular homeostasis (Figure 1).

HiF-1α in cancers:-

Hypoxia is an important selective force in the clonal evolution of tumor cells (Graeber *et al*, 1996). Using oxygen electrodes to study tumor oxygen supply, Vaupel and colleagues have detected hypoxia in solid tumors (Hockel *et al*, 1999; Hockel *et al*, 2001). Elevated expression levels of HiF have been observed in early stages of tumor development before histological evidence of angiogenesis and invasion, and this is thought to contribute to the angiogenic switch (Zhong *et al*, 1999). Increased HiF-1α and HiF-2α expression is a key clinical feature of a number of human malignancies, including cancers of the brain, colon, lung, breast, prostate, kidney, pancreas, cervix, bladder, and ovary (Bos *et al*, 2001, Zhong *et al*, 1999; Turner *et al*, 2002; Hofmann *et al*, 2008; Dales *et al*, 2010; Wu *et al*, 2010; Birner *et al*, 2001). In these tumors, HiF over-expression arises from both the hypoxic nature of the tumors and aberrant HiF-1α activation induced by oncogenes. Hypoxia-inducible factors-1α is over-expressed in >70% of human cancers which denotes a highly aggressive disease phenotype and is associated with poor prognosis and resistance to treatment in many cancers, including non-small cell lung carcinoma (Giatromanolaki *et al*, 2001), oligodendroglioma (Birner *et al*, 2001), breast carcinoma (Bos *et al*, 2001), and bladder cancer (Theodoropoulous *et al*, 2004). It has also been proven through the method of gene knockout that the loss of HiF-1α may significantly suppress the growth of tumors and most importantly decrease the neovascularization (Zhang *et al*, 2009; Jiang *et al*, 1997). Overexpression of HiF-1α is related to progression free survival (PFS) and OS in several human cancers (Theodoropoulos *et al*, 2004; Isobe *et al*, 2012; Hoffmann *et al*, 2008; Zhong *et al*, 1999).

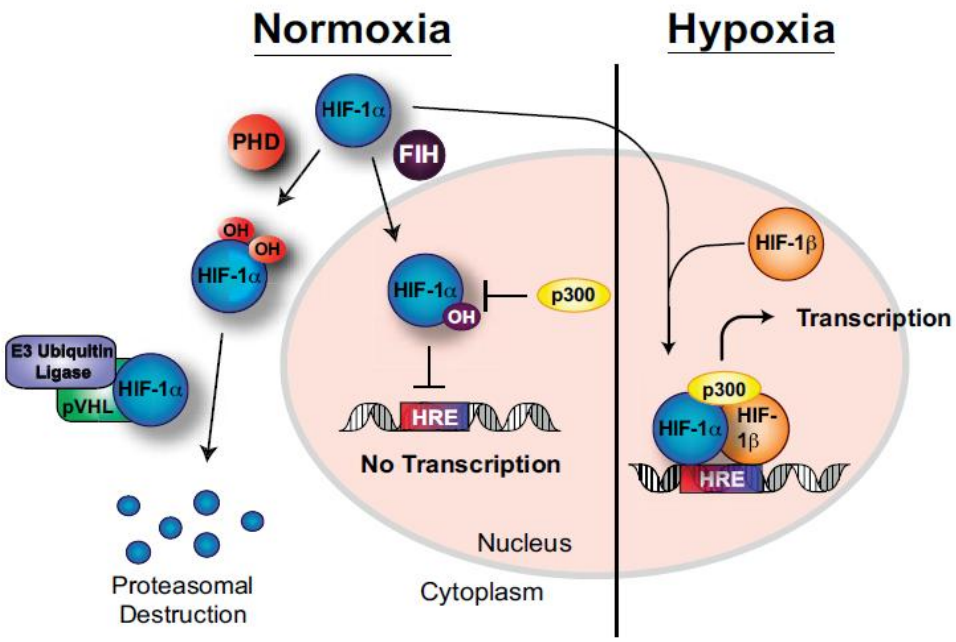

Figure 1: The oxygen-dependent regulation of the HIF complex (adapted from Cook and Fig 2010). PHD, prolyl hydroxylase; FIH, factor inhibiting HIF; OH, hydroxylated amino acid residues

HiF-1α in multiple myeloma:-

Multiple myeloma is characterized by the accumulation of malignant PC in the BM environment. The normal BM microenvironment is physiologically hypoxic, which is crucial for the support of normal BM haematopoiesis. *In vitro* studies have shown that myelomatous BM environment is actually more hypoxic than the normal BM microenvironment; therefore, MM PC has to survive and grow in an environment which is naturally hypoxic. In the initial stages of disease establishment, the hypoxic BM microenvironment supports the initial survival and growth of the MM cells (Asosingh *et al*, 2005; Martin *et al*, 2011; Colla *et al*, 2007). As the MM PC establish themselves within the endosteal niche of the BM, they become exposed to even greater levels of hypoxia, which activates HiF-1 and/or HiF-2, and stimulates the production of angiogenic factors and angiogenesis to increase oxygen delivery to the tumor cells, thereby facilitating tumor growth. How BM hypoxia and MM cells affect each other is unknown.

Angiogenesis is required for tumor growth and metastasis and thus constitutes an important target for the control of tumor progression (Carmelite and Jain, 2000; Sato, 2003; Quach *et al*, 2010). *In vitro* and *in vivo* studies have shown that the angiogenic cytokines such as VEGF, bFGF, Ang-1, Ang-2 and HIF-1α are important in the neovascularization, growth and development of tumor in MM (Yata *et al*, 2003; Vacca *et al*, 2005; Dmoszynska *et al*, 2002; Neben *et al*, 2001; Terpos *et al*, 2012; Zhang *et al*, 2009). Hypoxia inducible factor-1α is an important regulator of VEGF and VEGF is associated with poor prognosis in MM patients. HiF-1α have been reported to be increased in MM as compared to controls and correlated significantly with serum β2-microglobulin levels and increases from stage I to III. The expression level of HiF-1 α was also correlated with serum levels of VEGF, bFGF and Ang-2. (Vacca *et al*, 2005; Dmoszynska *et al*, 2002; Neben *et al*, 2001; Terpos *et al*, 2012; Zhang *et al*, 2009; Zhang *et al*, 2005; Bhaskar *et al*, 2012; Pour *et al*, 2010; Colla *et al*, 2007).

Rapid tumor growth creates a state of hypoxia in the tumor microenvironment and results in release of HiF-1α in the local milieu which in turn leads to secretion of other angiogenic cytokines and increased tumor angiogenesis (Carmelite and Jain, 2000; Pouyssegur *et al*, 2006). Higher expression of HiF-1α was found to be related with inferior PFS in MM patients, although this was not substantiated in multivariate analysis (Bhasker *et al*, 2013).

By using anti-immunomodulatory drugs in *in-vitro* and *in vivo* model of MM in the BM milieu decreases the HiF-1α expression indicating HiF-1α as a novel molecular target in MM and adds another facet to anti-MM drug activity (Zhang *et al*, 2009; Vacca *et al*, 2006; Asosingh *et al*, 2005; Giatromanolaki *et al*, 2010, Borsi *et al*, 2014, Wang *et al*, 2014, Storti P 2013). Recent studies have shown that the many anti-immunomodulatory drugs, antisense oligonucleotide and Gambogic acid suppress the HiF-1α which decreases the induced angiogenesis in MM. However the role of HiF-1α in MM induced angiogenesis are not completely elucidated. An overview of the emerging studies on HiF-1α in MM is summarized in Table 1.

Table 1: Hypoxia inducible factor (HiF)-1α in cancers

Reference	Study	Method	Outcome
Abd-Aziz N *et al*, 2015	MM	qRT-PCR	bortezomib attenuates the transcriptional activity only of HIF-1
Borsi *et al*, 2014	MM cell lines (MM1.S, RPMI8226, U266 and OPM-2)	qRT-PCR and Western Blotting	it is suppressed by the effect of EZN-2968, a small 3rd generation antisense oligonucleotide
Wang *et al*, 2014	Human MM U266	IHC, western blot	Gambogic acid suppresses hypoxia-induced hypoxia-inducible factor-1α/vascular endothelial growth factor expression via inhibiting phosphatidylinositol 3-kinase/Akt/mammalian target protein of rapamycin pathway in multiple myeloma cells
Storti P 2013	MM	In-vivo	HIF-1α suppression in MM cells significantly blocks MM-induced angiogenesis and reduces MM tumor burden and bone destruction in vivo
Bhaskar *et al*, 2013	MM (n=71), HC (n=50)	RT-PCR	increased as compared to controls and were found to have poor PFS
Giatromanolaki *et al*, 2010	MM (n=106)	IHC	upregulated and significantly linked with high VEGF & vascular density
Dales *et al*, 2010	Breast cancer (n=53)	qRT-PCR	mRNA expression of Hif-1α^{Taq} splice variant reflects a stage and progression is associated with a worse prognosis
Wu *et al*, 2010	Colon cancer (n=68) undergoing curative intent surgery	IHC	is highly expressed and provide a possible basis for DFS of all patients after curative to predict tumor recurrence & metastasis
Cao *et al*, 2009	Colorectal cancer (n=71)	IHC	can be used as biomarkers indicating tumors in advanced stage, correlated with VEGF & independently implied poor prognosis
Zhang *et al*, 2009	in vitro and in vivo Zebrafish MM Model	Western Blot	elevated in MM cells, which is associated with oncogene c-Myc mediating VEGF and decrease with bortizomib based therapy
Hoffmann *et al*, 2008	Pancreatic adeno carcinoma (parafin embedded tissue samples (n=41)	Laser capture microdissection qRTPCR	significantly correlated to bFGF, VEGF, PDGFA and expression had a significant expression survival
Yang *et al*, 2007	Osteosarcoma (n=39)	IHC	predictive of poor outcome
Colla *et al*, 2007	Human myeloma cell lines, MM (n=50)	ELISA, qRT-PCR	hypoxia induces HiF-1α and suppression of HiF-1α reduced the production of IL-8, OPN which indicates that it regulates angiogenic related molecule expression
Sumiyoshi *et al*, 2006	Gastric cancer (n=216)	IHC	is independent prognostic factor
Lidgren *et al*, 2005	Renal cell carcinoma (n=92)	western blot analysis	independent prognostic factor for favorable prognosis, although no association to tumor stage and VEGF
Asosingh *et al*, 2005	5T2 MM murine model	FCM	higher in CD45- as compared to CD45 + and CD45- secretes VEGF

Huang *et al*, 2005	Hepatocellular Carcinoma (n=36), Control (n=6)	IHC	expression is higher than normal tissue and correlated with expression of VEGF & MVD
Theodoropoulos *et a,l* 2004	Bladder cancer (n=93)	IHC	correlated with VEGF expression & important predictive & prognostic markers
Bos *et al*, 2003	Lymph node negative breast carcinoma (n=150)	IHC	correlated with VEGF expression and associated independently with shortened survival of patients
Kurakawa *et al*, 2003	Oesophageal squamous cell carcinoma (OSCC) (n=130)	IHC	high expression may predict an unfavourable prognosis
Birner *et al*, 2000	Early stage invasive cervival cancer (n=91)	IHC	is strong independent prognostic marker
Zhang *et al*, 1999	Tumor specimen human cancers and metastasis (n=179)	IHC	correlated with cancer proliferation and is biomarker of metastatic potential

MM, multiple myeloma; HC, healthy control; pl, plasma; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; Ang, angiopoietin; HiF-1α, hypoxia inducible factor; ELISA, enzyme linked immunosorbent assay; RT-PCR, reverse transcriptase polymerase chain raection; FCM, flow cytometer; IHC, immune histochmistry; qRT-PCR, Real-time polymerase chain reaction; BMPC, bone marrow plasma cells; MVD, microvessel density; PFS, progression free survival; DFS, disease free survival; PDGF, platelet derived growth factor; IL, interleukin; OPN, osteopontin.

Conclusion:-

This review highlights that, HiF-1α plays important roles in tumor pathology which may, thus, be evaluated as potential targets for anti-angiogenic therapy in future

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