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A Review on Chronic Stress Mediated Tumor Angiogenesis through IL-6 & VEGF

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ABSTRACT: Chronic Stress plays a significant role in the progression of tumor angiogenesis through the secretion of Catecholamines, which take part in increased production of IL-6 & VEGF. Chronic psychological stress induces secretion of catecolamines such as epinephrine (E) & noreepinephrine (NE) from adrenal medulla & sympathetic neurons which activate β-adrenergic receptors on tumor cells, which in turn enhances increased production of IL-6. IL-6 then plays a significant role in VEGF production through STAT3 activation. Il-6 follows a series of signaling cascades including cAMP/PKA & MAPK. NF-kB activation through MEK/ERK is crucial for IL-6 production. Angiogenesis, the formation of new blood vessels from preexisting vasculatures is mediated by VEGF. It also takes part in cell proliferation, cell migration & vasculogenesis, thus leading to tumor angiogenesis. The aim of this study was to know the relationship between chronic stress and tumor angiogenesis and to highlight the therapeutic point where further works may proceed on.

KEYWORDS: Angiogenesis, Therapeutic, Catecholamines, Epinephrine, Noreepinephrine, Cell Proliferation, Cell Migration and Vasculogenesis.

INTRODUCTION

In every sphere of life we are coming in contact with different type of stresses which are the ultimate source of depression and tension, affecting our mental and physical health. So called modern life living elevates the level of stress among us. There is hardly any sector where there is no stressful situation. The long lasting consequence of it is the progression of disease like cancer. Chronic psychological stress that persists for several hours a day for an extended period of time generally month to years (Dhabhar *et al.,* 1997) has a strong potential to induce tumor angiogenesis in the host body. Angiogenesis, the formation neo blood capillaries from existing vessels (Folkman *et al.,* 1992) is crucial for the supplement of nutrients to the endothelial cells for growth and survival which has utmost importance for the progression of tumor. Chronic stress, which results in increased induction of stress hormones such as norepinephrine (NE) and epinephrine (E) from adrenal medulla and sympathetic neurons has a significant role for the production of proangiogenic IL-6 and VEGF molecules. These are synthesized via a series of signaling pathways activation including cAMP/PKA and ERK/MEK. Experimentally it has been shown that ovarian carcinoma cells express elevated level of IL-6 and VEGF through the action of chronic stress mediated catecolamines. STAT3 pathway activation by JNK binding with IL-6 is crucial for VEGF transcription. On the other hand VEGF is the major modulator of angiogenesis, vasculogenesis, cell migration and proliferation. PLCγ binding with VEGFR2 activates PKC which leads to cell proliferation and vascular permeability through eNOS and MEK signaling. ERK/MEK are downstream pathways activated by IL-6 play a vital role for vascular endothelial growth factor activation can also participate in upstream regulation of IL-6 expression by activating NF-kB (Sano *et al.,* 2005). However, in this review it has been emphasized on how chronic stress induces tumor angiogenesis through VEGF and IL-6 production via stress hormone epinephrine and norepinephrine.

STRESS

Stress is a term that means different things to different people but generally has a negative connotation. Yet, stress is a familiar aspect of modern life, being a stimulant for some individuals, but a burden to many others. Stress is a constellation

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of events, consisting of stimulus (stressor) that precipitates a reaction in the brain (stress perception), which activates physiological fight-or-flight systems in the body (stress response) (Dhabhar *et al.*, 1997). Stress is of two types such as acute stress & chronic stress. Important distinguishing characteristics of stress include its duration & intensity. Acute stress lasts for a period of minutes to hour & chronic stress persists for several hours a day for extended period of time generally month to years. The magnitude of stress is gauged by the peak level of stress hormones, neurotransmitters & other physiological changes such as increases heart rate & blood pressure & by the amount of these changes persists during or following stressor exposure. Stress has long been suspected as a causal agent of many diseases, among them cancer is the most fatal one. A number of studies have been shown that stress can be detrimental & immunosuppressive to human health.

STRESS HORMONE CATECHOLAMINE THE MAJOR REGULATOR OF TUMOR ANGIOGENESIS

Catecholamines such as epinephrine (E) & norepinephrine (NE) play a significant role in the progression of tumor angiogenesis. Catecholamines are being secreted from the adrenal medulla & sympathetic neurons under the action of chronic stress which may adversely affect the health from various points of view (Kemp *et al.,* 1989). CAs is synthesized from the amino acid tyrosine. These neurotransmitters are also considered to be the physiological regulators of flight or fight response during stress and have both excitatory and inhibitory roles. NE and E act on their respective target cells through α and β adrenoceptors (Ganong *et al.,* 2005). These receptors are further subdivided into different subtypes; the α1 adrenoceptors act by increasing the intracellular calcium level, whereas the α2 adrenoceptors inhibit intracellular cyclic AMP (cAMP) by down-regulating adenylate cyclase (Ganong *et al.,* 2005). The β1 and β2 adrenoceptors increase intracellular cAMP by activating adenylate cyclase (Ganong *et al.,* 2005). NE & E have been implicated in stress –induced augmentation of tumor growth & progression. In an orthotropic model of ovarian carcinoma, the growth promoting effect was mimicked by a β- AR agonist. Similarly, activation of β-AR resulted in an increase in metastases in animal models of lung & breast cancer. Interestingly, recent reports have indicated that substantial amounts of NE and E are produced during chronic stress owing to the activation of sympathoadrenal medullary axis, and these CAs act through the β adrenoceptors to directly stimulate the growth of different types of malignant human tumors by up-regulating the synthesis of proangiogenic factors like VPF/VEGF (Thaker *et al.,* 2006, Lutgendorf *et al.,* 2003, Yang *et al.,* 2006, Yang *et al.,* 2008, Nilsson *et al.,* 2007 & Landen *et al.,* 2007). It is important to mention here that among the several proangiogenic factors, VPF/VEGF is the most critical cytokine required for the induction of tumor angiogenesis, and the action of VPF/VEGF is mediated mainly through its VEGFR 2receptors present in the tumor endothelial cells.

SIGNALING PATHWAYS ACTIVATED BY NE & E CRUCIAL FOR TUMOR ANGIOGENESIS

Stress induced signaling cascades are the key to tumor angiogenesis. It is proven in case of human malignant ovarian tumors (Hey-A8, SKOV3ip1) grown orthotopically in nude mice by acting through the β2 adrenoceptors present in these tumor cells (Thaker *et al.,* 2006). Thus, the underlying signaling pathway to promote angiogenesis in these malignant ovarian tumors can be summarized as βreceptor→ cAMP→ PKA→ VEGF. A similar result has been shown in the human pharyngeal carcinoma cell line HONE 1, in which NE by acting through β2 adrenoceptors stimulates synthesis of VPF/VEGF and matrix metalloproteases MMP-2 and MMP-9 (Lutgendorf *et al.,* 2003). Also, NE treatment significantly increases VPF/VEGF synthesis in several human multiple myeloma cell lines (NCI-H929, MM-M1, and FLAM-76) by acting through β_1 and β_2 adrenoceptors present in these cells (Yang *et al.,* 2008).

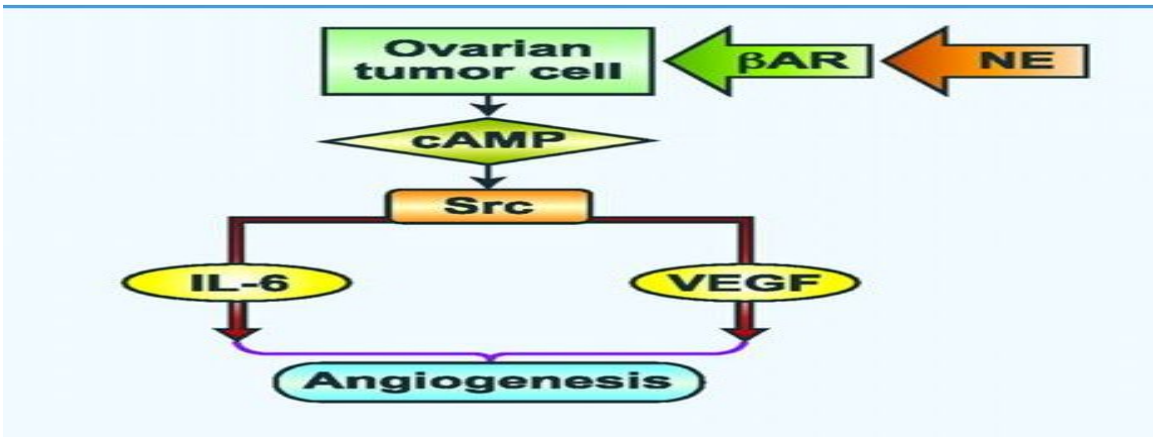

Figure 1. Schematic diagram of nor-epinephrine mediated signaling pathways in tumor cells, endothelial cells, & endothelial progenitor cells that regulate tumor angiogenesis (Chakroborty et al., 2009).

cAMP &PKA

Extracellular signals are often converted into an intracellular signal referred to as a second messenger. Adenosine 3', 5' cyclic monophosphate (cAMP) was one of the first secondary messengers identified in cells. Adenylyl cyclases, a large family of proteins that are regulated by trimeric G-proteins linked to G-protein coupled receptors, use ATP to generate cAMP (Beavo *et al.,* 2002 & Cooper *et al.,* 2005) which activates various signaling pathways involved in disease progression.

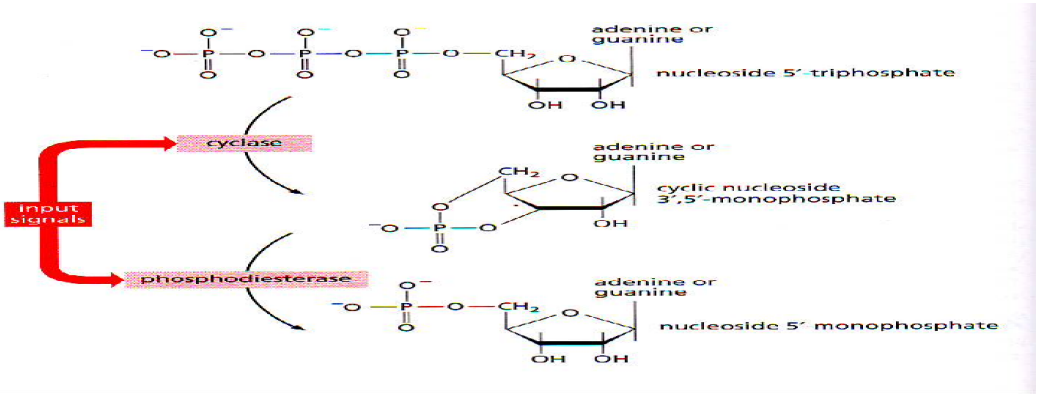

Figure 2. Generation & termination of cAMP/cGMP signal.

ACTIVATION OF PKA

PKA is a serine/threonine kinase that in its inactive form consists as a tetramer of two regulatory subunits (R) and two catalytic subunits (C). Each R subunit contains two binding sites for cAMP. Upon binding of cAMP to the R subunits, the PKA holoenzyme dissociates and the two C subunits, which possess the protein kinase activity, are released (Smith *et al.,* 2006, & Taylor *et al.,* 2007). Phosphodiesterases (PDE) hydrolyse cAMP into AMP. This represents one way to shut down the activated cAMP/PKA pathway (Taylor *et al.,* 2005 & 2007, Smith *et al.,* 2006, Skålhegg *et al.,* 2000 & Taskén *et al.,* 2004).

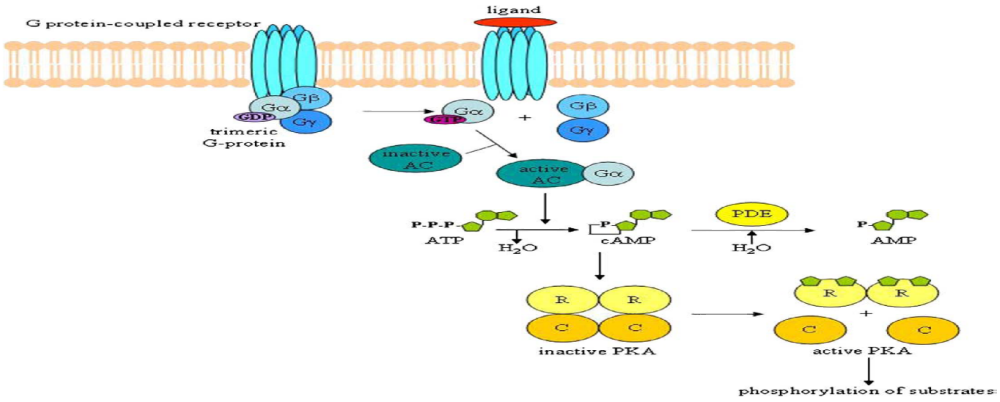

Figure 3. Schematic diagram of the cAMP/PKA signalling pathway. Upon activation of a G-protein coupled receptor, the trimeric Gαβγ protein dissociates into the active Gα subunit, loaded with GTP. The GTP-loaded Gα will activate adenylyl cyclase (AC), which generates cAMP from ATP. Next, cAMP binds to the regulatory subunits (R) of the PKA and induces dissociation of the holoenzyme. The catalytic subunits (C) can then phosphorylate their substrates.

SIGNALING PATHWAY LEADING TO MEK/ERK ACTIVATION

MEK/ERK activated by cAMP/PKA pathway is a strong up-regulator of NF-*k*b. How over NF-kB is crucial for IL-6 production which ultimately activates VEGF. Vascular endothelial growth factor induces angiogenesis & vasculogenesis in endothelial cell which is important for cell growth, metastasis & cancer progression.

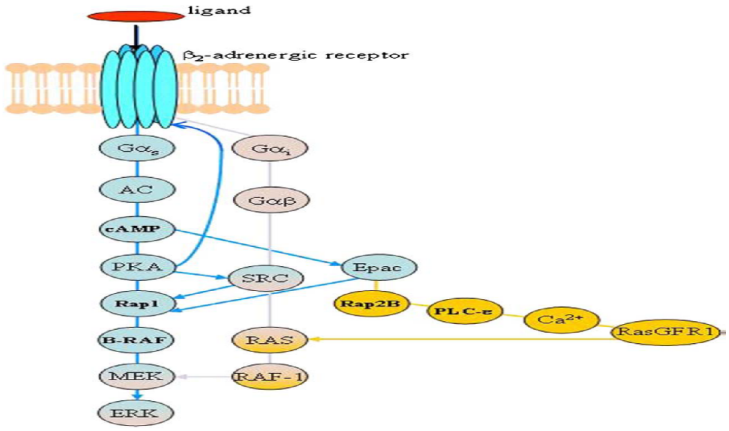

Figure 4. PKA-regulated MAPK signalling through G-coupled protein receptors. G-protein switching redirects the β2 adrenergic receptor-ERK signalling. The signalling from the β2 adrenergic receptor is mediated by Gαs, which through adenylyl cyclase (AC) increases cAMP levels and activates PKA. PKA can than modulate the MEK/ERK pathway. Activated PKA can also phosphorylate the β2 adrenergic receptor, which results in uncoupling of Gαs and increased coupling to Gαi. Next, Gαi regulates the MEK/ERK pathway via SRC, RAS, and RAF- 1. Alternatively, Epac can activate Rap2B and induce the RAS–RAF-1–MEK–ERK cascade in a Ca2+-dependent manner. The compounds of the signaling pathways that are only used by Gαs are shown in blue, while the transducing proteins solely engaged in the Gαi pathway are represented in light brown. The alternative Rap2B pathway is depicted in orange. Commonproteins used by the different pathways are indicated in both colours (Vossler et al., 1997).

NF-κB

NF-kB was first identified as a regulator of the expression of the kappa light-chain gene in murine B-lymphocytes, but has subsequently been found in many different cells. NF-kB represents a group of structurally related and evolutionarily conserved proteins that belong to the Rel family and are regulated via shuttling from the cytoplasm to the nucleus in response to cell stimulation (Birbach *et al.,* 2002). Mammals express 5 Rel (NF-kB) proteins that belong to two classes. The first class includes Rel A (p65), c-Rel and Rel-B proteins that are synthesized as mature products and do not require proteolytic processing. The second group is encoded by the *NF-kB1* and *NF-kB2* genes, whose products are first synthesized

as large precursors, p105 and p100, respectively, that require proteolytic processing to produce the mature p50 and p52 NF-kB proteins (Ghosh *et al.,* 1998).

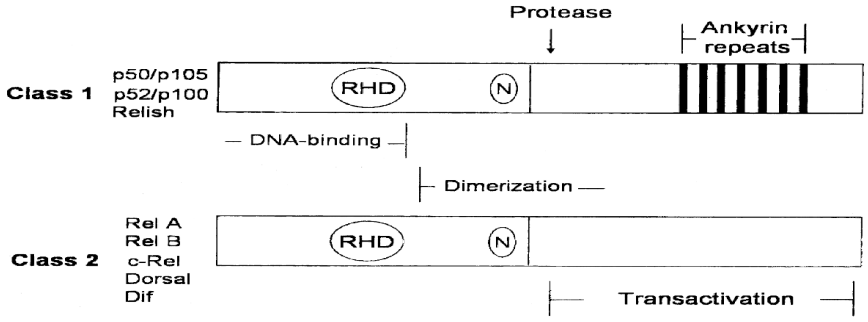

Figure 5. Structure of Rel family transcription factors

Adopted from www.ncbi.nlm.nih.gov/entrez/query.fcgi

NF-kB dimers containing Rel A or c-Rel are held in the cytoplasm through interaction with specific inhibitors, the IkBs. The IkBs are also members of a gene family that contains seven known proteins, IkB*a*, IkB*b*, IkB*e*, IkB*g*, Bcl-3 and the precursor Rel proteins p100 and p105. The IkBs are characterized by the presence of multiple ankyrin repeats and interact with NF-kB via Rel homology domain (RHD). The RHD serves several functions: it is the dimerization and DNA-binding domain for this class of proteins, it contains the nuclear localization sequence (NLS), and most important for its regulation it is the site for binding of NF-kB inhibitors the IkBs (Baldwin *et al.,* 1996, Jacobs *et al.,* 1998 & Ghosh *et al.,* 1998).

IL-6 PRODUCTION THROUGH NF-κB ACTIVATION BY THE IκB COMPLEX

Elevated level of IL-6 was reported with several tissue samples extracted from the cancer patients. So to say IL-6 is critical for tumor progression. Its transcription may be induced by different components. N-*k*B is one of the major inducers of IL-6 production. IkBs are a small family of related proteins with a core consisting of six or more ankyrin repeats, an N-terminal regulatory domain and a C-terminal domain that contains a PEST motif. The IkBs undergo rapid ubiquitin-dependent degradation after exposure to a variety of agonists, which activate the IkB (IKK) complex(Baldwin *et al.,* 1996, Jacobs *et al.,* 1998). IKK is composed of three subunits, IKK*a* (IKK1), IKK*b* (IKK2), and IKK*g* (also known as NF-kB essential modulator, NEMO) (Jacobs *et al.,* 1998). IKK*α* and IKK*β* are the catalytic subunits of the complex. The third subunit, IKK*g*/ NEMO, is the regulatory subunit and is not related to the catalytic subunits. IKK*a* and IKK*b* have similar primary structures and contain protein kinase domains at their Ntermini, and leucine zippers (LZ) and helix–loop–helix (HLH) motifs in their C-terminal portions. In addition to IKK*α,* IKK*β*, NIK, IKB*α* and NF-kB/RelA, IKK complex contains a fourth protein, a 150-kD IKK complex-associated protein called IKAP (IKK complex-associated protein). It is proposed to be involved in bIKK activation and functions as a scaffold protein due tobits ability to assemble IKK*α*, IKK*β*, NIK, and NF-kB :IkB. Stimulation by a diverse array of pathogens and other inducers, including viruses, cytokines, and stress-inducing agents lead to activation of signaling cascades that culminate with the activation of the IKK complex and phosphorylation of the IkB inhibitor. NF-kB DNA binding subunits are released and translocated to the nucleus, where they transactivate NF-kB responsive genes (Shimada *et al.,* 1999 & Thompson *et al.,* 1995).

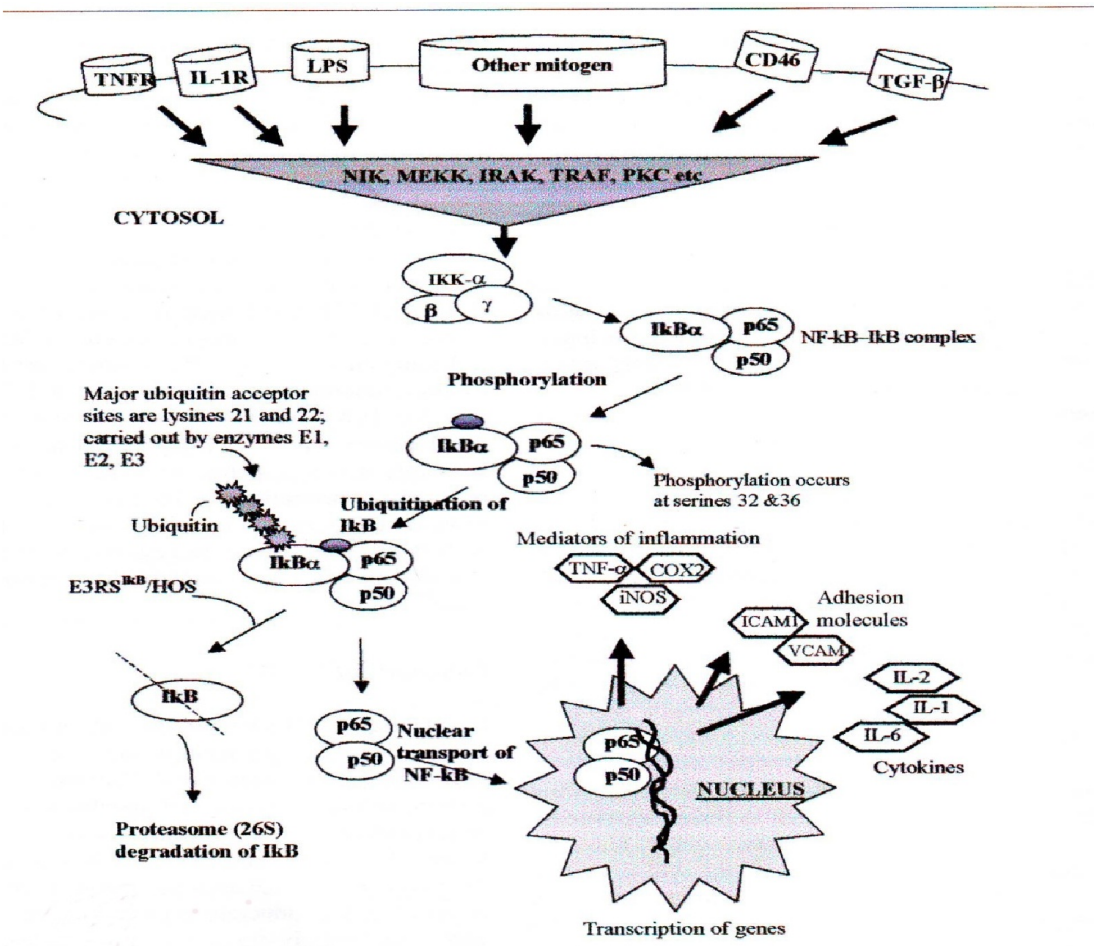

Figure 6. Schematic representation of pathways of activation and regulation of IkB & NF-kB & production of IL-6 (Ghosh et al., 2002, May et al., 1998, Baldwin et al., 2001 & Karin et al., 2000).

I*κ*B REGULATION

The mechanisms by which these highly diverse stimuli activate IKK are, however, poorly understood. Structure prediction programs suggest the presence of numerous docking sites forinteracting proteins on IKK*a*, IKK*b*, IKK*g* and IKAP, but the search for signaling molecules that directly dock to these sites is in its infancy. Mitogen-activated protein kinase/ERK kinase kinases (MAP3Ks), such as NIK and MEK kinase 1 (MEKK1), activate IKK when overexpressed (Tegethoff *et al.,* 2003 & Sun *et al.,* 1993).

THE MAJOR PRO-ANGIOGENIC MOLECULE

Interleukin-6 (IL-6) is a cytokine characterized by its diversified action. It modulates a variety of functions , such as cell proliferation & differentiation, & apoptosis. IL-6 has been implicated as an important molecule in tumor progression & angiogenesis. Human IL-6 is a protein with a molecular weight of 21kDa-28kDa (Noda *et al*., 1991). Crystallography X showed that IL-6 is formed by 4 a-helices, arranged as two couples of anti-parallel helices (Somers, *et al*., 1997), a common mode in the cytokine family. According to the length of the a-helices, IL-6 is part of the "long-chain" cytokine family, which also includes growth hormone (GH), erythropoietin and G-CSF factor (Brabo *et al.,* 2000).

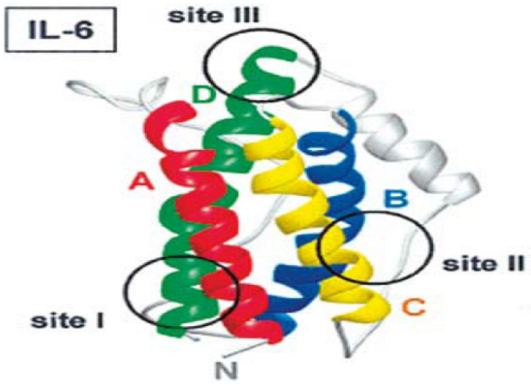

Figure 7. Tertiary Structure of IL-6. IL-6 is composed of four a-helices (colored) linked via connecting loops (grey). The figure also shows IL-6 receptor-binding sites, named site I, II & III, (Heinrich et al., 2003).

IL-6 RECEPTORS & THEIR BINDING PATTERN

IL-6 mediates its action through binding with the receptor molecules. It binds to two different membrane glycoproteins (receptors) that together form the common IL-6 receptor. These proteins are an 80 kDa protein (IL-6Rα, CD126) and a130 kDa protein (gp130, CD130). These receptors are type I membrane proteins, i.e. they contain a transmembrane domain and an extracellular N-terminal domain (Davis *et al.,* 1991) muscles, kidneys, etc (Hibi *et al.,* 1992). In contrast, IL-Rα expression is restricted and thus defines IL-6 target-cells. IL-6Rα is mainly expressed in hepatic cells and in subpopulations of leukocytes (monocytes, neutrophils, B- and T- cells), but also in neural, bone and skeletal tissue, etc. The relatively small intracellular part of IL-6Rα (82 amino acids) indicates that IL-6Rα plays a minor role in signal transmission (Taga *et al.,* 1989), but it is the intracellular domain of gp130 that contributes to the transmission of the Il-6 signal. Like other receptors, gp130 has no kinase activity (Hibi *et al.,* 1990); instead, gp130 binds through its intracellular part to JAK (cytoplasmic tyrosine kinases).

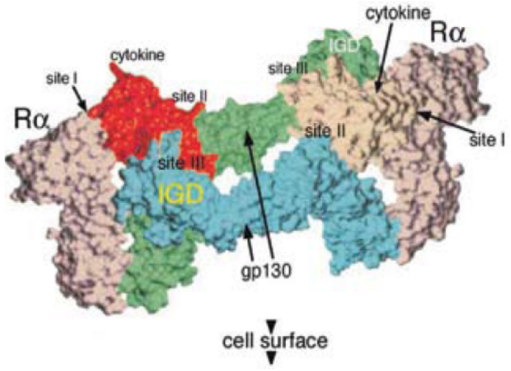

Figure 8. Space-filling model of the active hexamer protein complex produced through the binding of IL-6 to its receptors, Il-6Rα and gp130. The presence of an active membrane hexamer complex produced by the binding of IL-6 to its receptors, IL-6Rα and gp130, with 2:2:2 stoichiometry, is a prerequisite for IL-6 action. Initially, an IL-6 molecule (cytokine, red and brown) binds through site I to an IL-6Rα receptor (Rα, violet). The dimer is then connected through IL-6 site II with a gp130 receptor (gp130, blue and green). Finally, the two trimers bind through IL-6 site III and the gp130 Ig-like (IgD) domain, forming the active hexamer complex. (Bravo et al., 2002).

INTRACELLULAR SIGNALING CASCADES ACTIVATED BY IL-6

The gp130 receptor has not a kinase activity but following the formation of the active receptor complex, it binds to Janus kinase (JAK), which is a cytoplasmic (nonreceptor) kinase. At the active IL-6 receptor complex, the receptor gp130 forms homodimers (Murakami *et al.,* 1993). This leads to a close contact with JAK, which is followed by auto-activation of JAK. Activated JAKs phosphorylate tyrosine residues at the intracellular domain of gp130 and at other target molecules such as STATs In humans, receptor gp130 contains six tyrosine residues in its intracellular domain. Second tyrosine residue (Y759) is a part of the known motif Y759S(serine)T(Threonine)V(Valine), an amino acid sequence which is similar to the motif (amino acid sequence) that serves as a binding site for the protein tyrosine phosphatase SHP236. In detail, upon IL-6 activation, SHP2 (Nash *et al.,* 2002) is phosphorylated and tyrosine residue Y759 is essential for gp130-mediated SHP2 phosphorylation (Himbi

et al., 1996). The following tyrosine residues (Y767, Y814, Y905 and Y915) are part of YXXQ (glutamine) motifs, where X symbolizes any amino acid. These motifs mediate STAT activation (Stahl *et al.,* 1995, Hibi *et al.,* 1996) In short, IL-6-induced JAK activation leads to the activation of two mainly signaling pathways: the JAK/STAT pathway and the MAPK pathway (Figure 9).

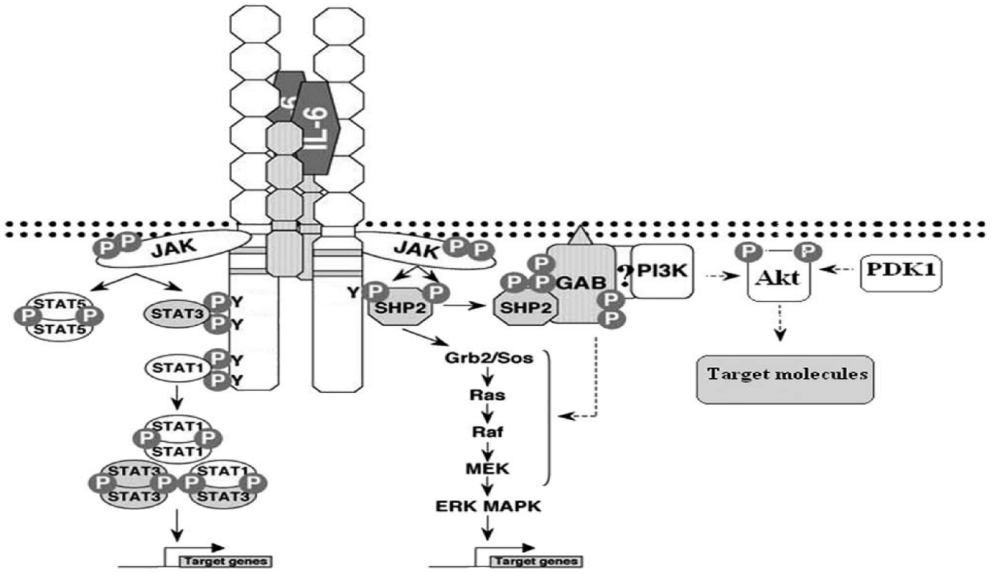

Figure 9. IL-6 regulated intracellular signal cascades. Following formation of the active hexamer complex, the intracellular domains of the two gp130 receptors and consequently the bonded JAK come in close contact. JAKs are activated, phosphorylate each other and certain tyrosine residues in the intracellular domain of gp130 receptor. As presented on the left, STAT factors bind to certain phosphotyrosines of gp130, leading to phosphorylation by JAK. Phosphorylated STAT factors form homo- and hetero- dimers, and translocate to the nucleus, where they induce gene expression. The mechanism that leads to the activation of MAPK cascade and PI3K cascade has not been specified. As presented at the right of the figure, SHP2 binding to the gp130 receptor and its phosphorylation by JAK, leads to the activation of the MAPK cascade. This is in part achieved through SHP2 binding to Gab1 and PI3K proteins. The mode of involvement of Gab1 and the activation of the PI3K cascade remain uncertain, as indicated by the symbol (?) in the figure. Activated PI3K modifies certain phospholipids on the plasma membrane, leading to the recruitment of Akt kinase on the plasma membrane and its subsequent activation through phosphorylation by PDK1 protein. Activated Akt phosphorylates certain target molecules. Y, tyrosine. (Hirano et al., 2003).

STAT3

Signal-transducer-and-activator-of-transcription (STAT) is a family of six different transcription factors, first discovered in 1993 by James Darnell,which play major roles in cytokine signaling (Shuai *et al.,* 1993 & Leavy *et al.,* 2002). A typical STAT protein consists of a coiled-coil domain, a DNA-binding domain, a linker, an SH2 domain, and a transactivation domain (TAD) (FIG. 1). The TAD contains tyrosine and serine phosphorylation sites that are needed for the activation of STAT.

ACTIVATION OF JAK/STAT PATHWAY

STAT proteins are transcription factors that bind to phosphotyrosines in the intracellular domain of gp130 receptor. This binding is followed by STAT phosphorylation by JAK and leads to the formation of STAT dimmers. STAT dimmers are translocated to the nucleus, where they induce gene expression. To date, seven STAT proteins have been recognized (STAT1-4, 5a, 5b and 6) in humans and mice (Bromberg *et al.,* 2000) . IL-6 mediates activation of STAT1, 3 and 5.

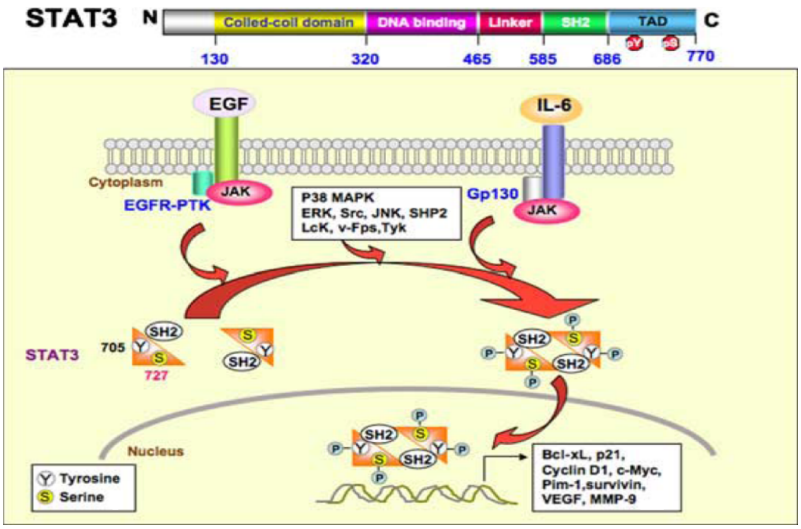

Figure 10. Signaling pathway leading to STAT3 activation.

JAKs INVOLVED IN IL-6 SIGNALLING

JAKs are intracellular tyrosine kinases (TYK) with a molecular weight of 120-140 kDa. In mammals, four JAKs have been recognized: JAK1, JAK2, TYK2 and JAK3. The latter is present mainly in cells from hemopoietic line. IL-6 activates JAK1, JAK2 and TYK2 (Yuan *et al.,* 1994, Farruggella *et al.,* 1994 & Yoshida *et al.,* 1994). Depending on the cell type, different JAKs are activated upon Il-6 stimulation and in different amounts44. The presence of a hierarchy among JAKs is unknown. In the presence of sIL-Rα, IL-6 acts mainly through JAK1 in fibrosarcoma cell types. It is also noted that activated JAK2 and TYK2 cannot replace JAK1 activity in cells with JAK1 deletion45. JAK binding to receptor gp130 is mediated by amino acid sequences of gp130, which are named Box1 and Box2 and are located in the gp130 domain near the plasma membrane. These sequences are also met in various cytokine receptors (Pellegrini *et al.,* 1997 & Hibi *et al.,* 1991) JAK1connection with gp130 is very steady and JAK1 is not diffused like a common cytoplasmic protein, but its motility follows the motility of gp130 receptor.

STAT REGULATION

STAT activity is predominantly regulated by post-translational modifications, such as tyrosine or serine phosphorylation. STAT activation requires a transient binding of STAT to the gp130 receptor and phosphorylation by JAK (Fu *et al.,* 1993 & Greenlund *et al.,* 1994). All IL-6 type cytokines activate mainly STAT3 and to a lesser ext ent STAT1 through the common receptor gp130. In addition to tyrosine phosphorylation, other posttranslational modifications have also been reported, including methylation and serine phosphorylation & so on.

NUCLEAR TRANSLOCATION OF STATs

STATs are activated in the cytoplasm but act on the nucleus. Because of the size of STATs, they have to be actively transported through nucleus pores inside the nucleus. Usually this translocation is determined by the presence of a sequence of nuclear localization (NLS). STAT accumulation in the nucleus is triggered by STAT1 dimerism in response to phosphorylation to tyrosine residues (Devgan *et al.,* 1999 & Rosen *et al.,* 1999).

STAT3 REGULATION OF GENES INVOLVED IN TUMORIGENESIS

STAT3 is one of the major mediators of tumorigenesis (GARCIA *et al.,* 1998 & Schlessinger *et al.,* 2005). The oncogenic significance of activated STAT3 molecules is due to their effects on numerous parameters of the development and progression of malignancy, such as apoptosis, cell proliferation, angiogenesis, and immune system evasion (Bowman *et al.,* 2000, Kortylewski *et al.,* 2005 & Gamero *et al.,* 2004). Constitutively active STAT3 has been implicated in the induction of resistance to apoptosis, (Catlett-Falcole, R. *et al*. 1999) possibly through the expression of Bcl-xL (Zushi *et al.,* 1998) and cyclin D1(Ahasn *et al.,* 2005). Its role in tumorigenesis is mediated through the expression of various genes that suppress apoptosis, proliferation, invasion, and angiogenesis. These include Mcl-1(Bai *et al.,* 2001, & Putheir *et al.,* 1999), Bcl-xL, and survivin

(Aoki *et al.,* 2003), all of which suppress apoptosis; c-myc (Kiuchi *et al.,* 1999) and cyclin D1, which mediate cell proliferation; matrix metalloproteinase-9 which mediates cellular invasion; and vascular endothelial growth factor (VEGF), which mediates angiogenesis(Niu *et al.,* 2002). (Other genes that have been shown to be regulated by STAT3 include p21 (Sinibaldi *et al.,* 2000), SOCS-3(Naka *et al.,* 1997), receptor activator of NF-_B ligand (RANKL) (Lin *et al*. 1999) tumor necrosis factor (TNF) (Miscia *et al.,* 2002), MyD interferon-regulatory factor 1, c-fos, β-macroglobulin,antichymotrypsin, 108 and angiotensinogen (Mascerano *et al.,* 1998) which also have been linked with tumorigenesis.

VEGF: THE KEY REGULATOR OF ANGIOGENESIS

VEGF (vascular endothelial growth factor, vascular permeability factor, vasculotropin) – homodimeric protein -42 kDa produced by many types of cells (e.g. macrophages, VSMC, fibroblasts, and cancer cells). Expression is induced in response to hypoxia and proinflammatory cytokines. Receptors (-R1 and -R2) are present mostly on endothelial cells, therefore VEGF acts specifically on endothelium (but also on neurons and Schwann cells). It protects endothelial cells from apoptosis and induces their proliferation, migration, and formation of capillaries. VEGF is required for the normal development of embryonic vasculature, the cyclic growth of blood vessels in the female reproductive tract, and the formation of capillaries during wound repair. However, VEGF is also involved in abnormal angiogenesis, as seen in proliferative retinopathies, rheumatoid arthritis, psoriasis, and malignancies. Vascular endothelial growth factor (VEGF) is one of the most potent and specific angiogenic factors of tumor induced angiogenesis. Originally identified for its ability to induce vascular permeability and stimulate endothelial cell growth, VEGF is now recognized as a key factor required for growth of tumors.

VEGFS AND VEGFRS

The VEGF family members are secreted, dimeric glycoproteins of approximately 40 kDa. In mammals, the VEGF family consists of five members, VEGFA, B, C, D and placenta growth factor (PLGF). In addition, proteins that are structurally related to the VEGFs exist in parapoxvirus1 (Takahashi *et al.,* 2005) (VEGFE) and snake venom (Suto *et al.,* 2005) (a group of proteins known as VEGFFs). VEGFA, B and PLGFbind to VEGFR1, VEGFA and E bind to VEGFR2, and VEGFC and D bind to VEGFR3. Proteolytic processingof the human VEGFC and D allows for binding toVEGFR2. Structurally, the VEGFs are related to the PDGF family of growth factors, with intrachain and interchain disulfide bonds between eight cysteine residues in conserved positions. The cry stal structure of VEGFA revealed two monomers that are organized in an anti-parallel fashion to form a dimer, with the receptor-binding sites located at each pole of the dimer (Muller *et al.,* 1997). The VEGFs preferentially form homodimers, although VEGFA and PLGF heterodimers have been identified (De Falco *et al.,* 2002). The VEGFRs are members of the RTK superfamily and they belong to the same subclass as receptors for PDGFs and fibroblast growth factors (FGFs). The VEGFRs are equipped with an approximately 750-amino-acid-residue extracellular domain, which is organized into seven immunoglobulin (Ig)-like folds. In VEGFR3, the fifth Ig domain is replaced by a disulfide bridge. The extracellular domainis followed by a single transmembrane region, a juxtamembrane domain, a split tyrosine-kinase domain that is interrupted by a 70-amino-acid kinase insert, and a C-terminal tail (FIG. 2). Structural and functional studies have yielded insights into how the distinct domains contribute to VEGFR activity. The crystal structure of part of the extracellular domain of VEGFR1, alone and in complex with ligand, shows that the Ig domain-2 constitutes the ligand-binding site on the receptor (Christinger *et al.,* 2004). In addition, biochemical analyses showed that the Ig domain-3 in VEGFR2 is important for the determination of ligand-binding specificity (Fuh *et al.,* 1998).

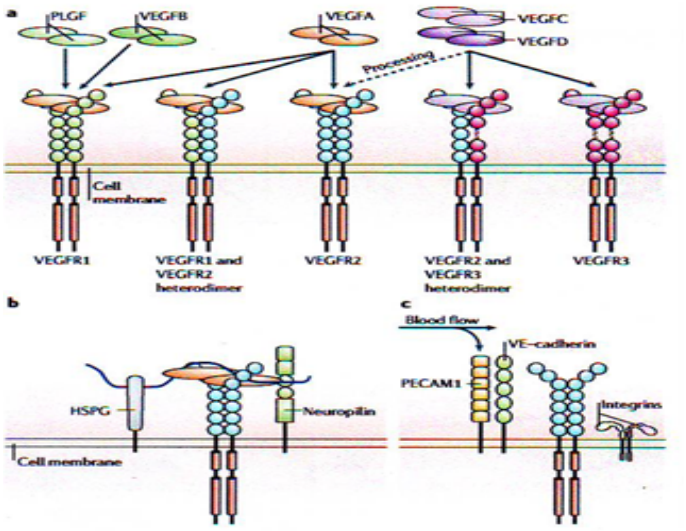

Figure 11. VEGF receptor-binding properties and signalling complexes. (a) Mammalian vascular endothelial growth factors (VEGFs) bind to the three VEGF receptor (VEGFR) tyrosine kinases, leading to the formation of VEGFR homodimers and heterodimers. Proteolytic processing of VEGFC and D allows for binding to VEGFR2. (b) VEGFR signalling is modulated by different co-receptors. VEGFs as well as VEGFRs bind to co-receptors such as heparan sulphate proteoglycans (HSPGs) and neuropilins. These interactions can influence VEGFR-mediated responses, for example, affecting the half-life of the receptor complex. (c) Mechanosensory complex formation. Blood flow might activate VEGFRs in a ligand-independent manner, by the formation of mechanosensory complexes that consist of platelet-endothelial-cell adhesion molecule-1 (PECAM1), vascular endothelial (VE)–cadherin, VEGFRs and integrins. PLGF, placenta growth factor.

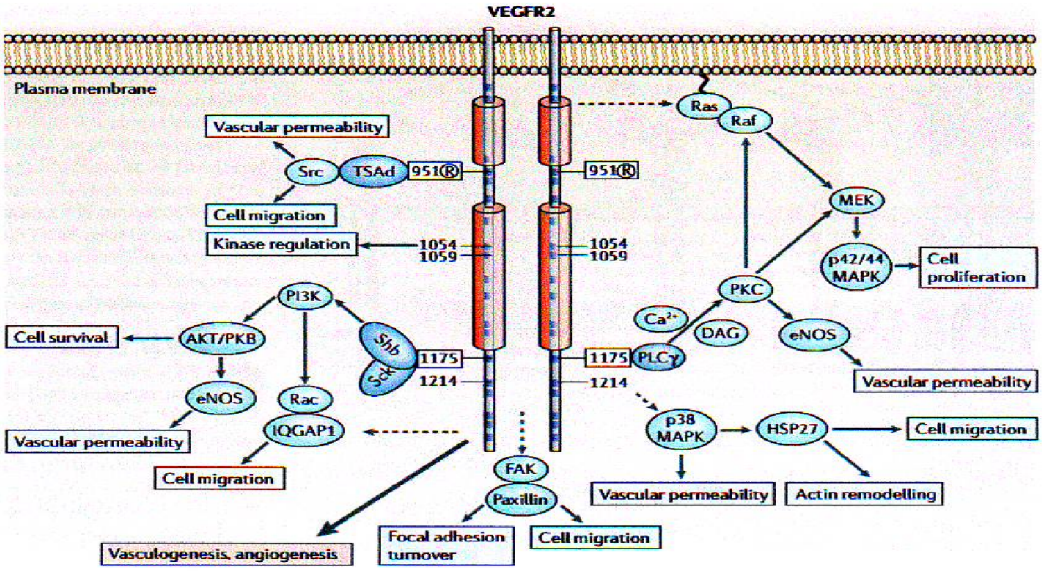

Figure 12. VEGFR phosphorylation sites and signal transduction. Intracellular domains of dimerized and activated vascular endothelial growth-factor receptor is shown with tyrosine-phosphorylation sites that are indicated by numbers. Circled R indicates that use of the phosphorylation site is regulated dependent on the angiogenic state of the endothelial cell (for VEGFR2) Dark blue squares in the receptor molecules indicate positions of tyrosine residues. Binding of signaling molecules (dark blue ovals) to certain phosphorylation sites (boxed numbers), initiates signalling cascades (light blue ovals), which leads to the establishment of specific biological responses (pale blue boxes). The mode of initiation of certain signalling chains is unclear (dashed arrows). Final biological outcomes that are coupled to the respective receptors are indicated in pink boxes. DAG, diacylglycerol; EC, endothelial cell; eNOS, endothelial nitric oxide synthase; FAK, focal adhesion kinase; HPC, haematopoietic progenitor cell; HSP27, heat-shock protein-27; MAPK, mitogen-activated protein kinase; MEK, MAPK and ERK kinase; PI3K, phosphatidylinositol 3′ kinase; PKC, protein kinase C; PLCγ, phospholipase C-γ; Shb, SH2 and ß-cells; TSAd, T-cell-specific adaptor.

ANGIOGENESIS

Angiogenesis, the formation of new blood capillaries from existing vessels, is an important mechanism for supplying nutrients to cells that are distant from existing blood vessels (Folkman *et al.,* 1992). Angiogenesis is critically important during embryonic development (Breier *et al.,* 2000)**.** Angiogenesis is a complex process that is mediated by the endothelial cells that line blood vessels (Daniel *et al.,* 2000). Unlike quiescent endothelial cells that rarely divide, angiogenic endothelial cells undergo a complex sequence of events that includes the secretion of metalloproteases and other matrix-degrading enzymes, cell migration into the newly created space, endothelial cell division and proliferation, and vessel formationThese are wellregulatedprocesses involving a number of stimulators such as fibroblast growth factor (FGF) (Nugent *et al.,* 2000), vascular endothelial growth factor (VEGF) (Petrova *et al.,* 1999), angiopoietins (Davis *et al.,* 1996), activators of integrins (Eliceiri *et al.,* 1999), and inhibitors such as thrombospondin (Roberts *et al.,* 1996), angiostatin (O'Reilly *et al.,* 1994), and endostatin (O'Reilly *et al.,* 1997).

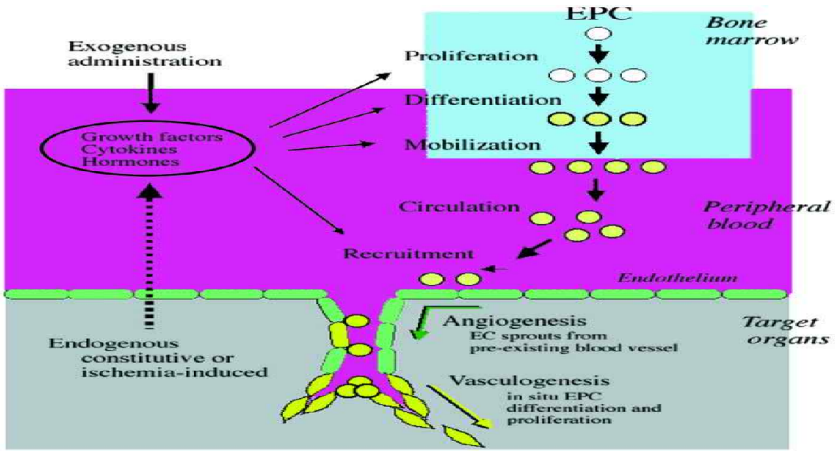

Figure 13. Vasculogenesis & Aangiogenesis

TUMORANGIOGENESIS

In addition to its important role in normal physiological processes, angiogenesis contributes to the pathology of a number of diseases (Patz *et al.,* 1980, McLaren *et al.,* 1996, Fava *et al.,* 1994), including tumor progression (Carmeliet *et al.,* 2000). This is because angiogenesis provides nutrients that maintain the viability of diseased tissue. Tu m o r-associated angiogenesis allows the tumor to maintain its growth advantage and also facilitates metastatic spreading by establishing connections to the existing vasculature.

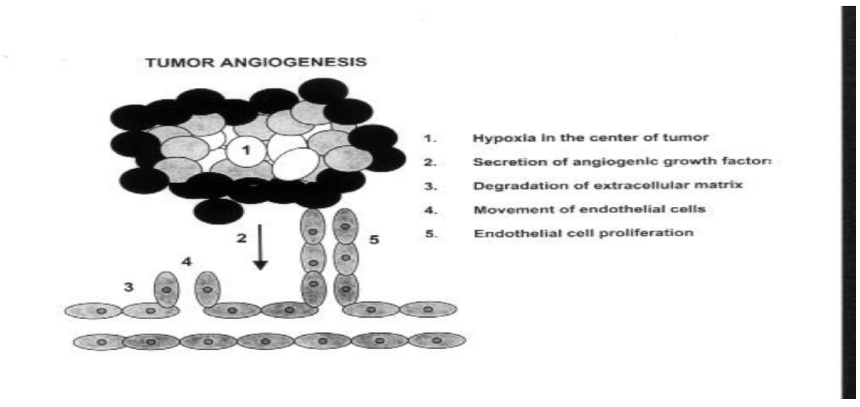

Figure 14. Tumor angiogenesis. Once a tumor grows to a certain size, the cells in the center are too far away from existing blood vessels to receive the necessary nutrients for cell survival. The lack of oxygen stimulates the production of VEGF, which is secreted from the starved cells. VEGF binds to receptors on endothelial cells of existing blood vessels, stimulating a series of events, including the secretion of matrix degrading enzymes, cellularmovement into the newly created space, and cell proliferation. The endothelial cells then form tubes, and provide the necessary nutrients to the tumor.

CONCLUSIONS AND FUTURE DIRECTIONS

Chronic stress and disease progression are the two opposite parts of a coin. Huge numbers of researches are being conducted, focusing these two correlated factors. Chronic stress persists a prolonged period and its long lasting consequence is dangerous for human health. Chronic stress upregulates catecholamines such as, epinephrine and nor-epinephrine secretion from adrenal medulla. Subsequently NE or E mediates tumor angiogenesis through a number of signaling cascades. Thus the underline signaling pathway to promote angiogenesis (the formation of new blood vessels) in tumor cells can be summarized as, β-adrenoceptor→ cAMP→PKA→VEGF. cAMP, the first second messenger actives PKA which is involved in activation of a variety of signaling pathways. MEK/ERK activated by cAMP/PKA through RAS/RAF pathway is a strong upregulator of NF-kB. NF-kB represents a group of proteins which are regulated via shuttling from cytoplasm to nucleus in response to cell stimulation. Active transport of NF-kB to the nucleus generates the major pro-angiogenic molecule IL-6. IL-6 activate target gene like VEGF through JAK/STAT or ERK/MEK. VEGF is the key regulator of angiogenesis & mediates its action through activated VEGFR2. Signaling molecules bind to the VEGFR2 receptor which phosphorylates second group of signaling molecules & ultimately leading to cell proliferation, cell migration, cell survival, vascular permeability & the final outcome of these signaling pathways is vasculogenesis & angiogenesis. Adhesion molecules such as FAK and paxillin are very important for cell migration but their mode of initiation of signaling chains are obscure through VEGF. VEGF mediated IQGAP1 and p38/MAPK are also unclear. Future research may be under taken through these directories. Targeting stress induced VEGF and Il-6 rather than tumor which is already developed would be a potential checkpoint for angiogenesis.

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