Lecture IV

29th March 2010

Mechanisms of regulation of VEGF expression
Regulation of VEGF expression
Structure of VEGF human promoter
### Cytokines That Cause Upregulation of VEGF

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Cell Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>Blood mononuclear cells, umbilical endothelial cells</td>
</tr>
<tr>
<td>IL-1β</td>
<td>A549, SRC-3, PC-14, RERF-LC-AI, synovial cells, smooth muscle cells, visceral glomerular epithelial cells</td>
</tr>
<tr>
<td>IL-3</td>
<td>Vascular endothelial cells</td>
</tr>
<tr>
<td>IL-15</td>
<td>Blood mononuclear cells</td>
</tr>
<tr>
<td>IL-18</td>
<td>Blood mononuclear cells in vitro, rheumatoid arthritis, synovial fibroblasts</td>
</tr>
<tr>
<td>TPO</td>
<td>Hematopoietic stem cells</td>
</tr>
<tr>
<td>bFGF</td>
<td>Vascular smooth muscle cells upregulation</td>
</tr>
<tr>
<td>EGF</td>
<td>Gastric cancer cells, endometrial stromal cells, prostate cancer cells</td>
</tr>
<tr>
<td>HGF</td>
<td>Breast cancer and leiomyosarcoma, endothelial cells, papillary carcinoma cells, HaCaT cells</td>
</tr>
<tr>
<td>KGF</td>
<td>Keratinocytes</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Thyroid carcinoma or pancreatic cancer cells, mesangial cells, endothelial cells</td>
</tr>
<tr>
<td>PDGF</td>
<td>Vascular smooth muscle and endothelial cells</td>
</tr>
<tr>
<td>TGF</td>
<td>Epidermoid carcinoma cells, vascular smooth muscle cells, endothelial cells, cholangiocarcinoma cells</td>
</tr>
<tr>
<td>TNF</td>
<td>Glioma cells</td>
</tr>
<tr>
<td>MIF</td>
<td>Hepatocellular carcinoma cells</td>
</tr>
</tbody>
</table>
Effect of IL-1β on VEGF mRNA expression in rat vascular smooth muscle cells

Li e et al., JBC 1995
## Cytokines That Cause Downregulation of VEGF

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Cell Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>Visceral glomerular epithelial cells, blood mononuclear cells</td>
</tr>
<tr>
<td>IL-10</td>
<td>Blood mononuclear cells, visceral glomerular epithelial cells</td>
</tr>
<tr>
<td>IFN-a</td>
<td>Smooth muscle cells, blood mononuclear cells, brain tumor</td>
</tr>
<tr>
<td>IFN-b</td>
<td>Melanoma cells</td>
</tr>
</tbody>
</table>
## CYTOKINES

### Cytokines That Cause BOTH Upregulation and Downregulation of VEGF

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Cell Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ</td>
<td>Melanoma cells, ovarian cancer cells, endometrial stromal cells</td>
</tr>
<tr>
<td>IL-12</td>
<td>Blood mononuclear cells in vitro, breast cancer animal model</td>
</tr>
<tr>
<td>IL-13</td>
<td>Smooth muscle cells, visceral glomerular epithelial cells</td>
</tr>
</tbody>
</table>
VEGF

- hypoxia
- reactive oxygen species
- nitric oxide
- cytokines
- heme oxygenase-1

growth factors
GROWTH FACTORS

• Adenoviral FGF-4 overexpression upregulates endogenous VEGF production and increases vascular permeability, therapeutic angiogenesis, and arteriogenesis.

• Non-viral liposomal KGF cDNA gene transfer improved neovascularization, as well as dermal and epidermal regeneration through stimulation of epithelial (VEGF) and mesenchymal (insulin-like growth factor-I, IGF-I) factors expression in skin cells.

• Stimulation of the production of VEGF as well as plasminogen activator inhibitor (PAI-I) after TGF-β treatment leads to vascular remodeling during angiogenesis. Numerous data indicate that blocking of TGFβ action inhibits tumor viability, migration, and metastases in mammary cancer, melanoma and prostate cancer model probably due to inhibition of VEGF synthesis. Therefore, reduction of TGFβ production and activity may be a promising target of therapeutic strategies to control tumor growth.
GROWTH FACTORS

KGF A

<table>
<thead>
<tr>
<th>KGF (50 ng/ml)</th>
<th>6h</th>
<th>12h</th>
<th>24h</th>
<th>48h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

VEGF -

Niu et al., JBC 2007

FGF-2

Malabanan et al., Circ Res 2008

Vascular SMCs
Reactive Oxygen Species (ROS)

- \( \text{O}_2^- \) (superoxide)
- \( \text{OH}^- \) (hydroxy radical)
- \( \text{H}_2\text{O}_2 \) (hydrogen peroxide)
- \( \text{O}_3 \) (ozone)
- \( ^1\text{O}_2 \) (singlet oxygen)
- \( \text{LOOH} \) (lipid peroxides)
- \( \text{LOO}^- \) (lipid peroxy radical)
- \( \text{LO}^- \) (lipid alkosyl radical)
Hydrogen peroxide increases VEGF production in different cell lines

acting via Sp1 transcription factor

Cisowski, Loboda et al. BBRC 2005
Reactive oxygen species

ROS

Transcription factors (NF-κB, HSF-1)

Protein kinase cascades (PI3/Akt, ERK1/2, JNK, p38)

Transcription factors (NF-κB, Sp-1, Sp-3)

VEGF transcription
hypoxia

VEGF

nitric oxide

reactive oxygen species

cytokines

heme oxygenase-1

growth factors
HYPOXIA

A state where O₂ availability/delivery is below the level necessary to maintain physiological O₂ tensions for a particular tissue.

- When tissue demand exceeds its O₂ supply.
- Different tissues have different oxygenation levels

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>O₂ Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atmospheric</td>
<td>21% O₂</td>
</tr>
<tr>
<td>Lung capillaries</td>
<td>13% O₂</td>
</tr>
<tr>
<td>Healthy tissues</td>
<td>2.5-9% O₂</td>
</tr>
<tr>
<td>Diseased tissue</td>
<td>&lt; 1% O₂</td>
</tr>
</tbody>
</table>
Tumors are hypoxic

Fig. 10.2. The $P_{O_2}$ gradient from inspired air to normal and tumour tissues. A significant drop in $P_{O_2}$ is observed as inspired air flows from the lungs into tissues. An even greater decrease is observed in tumour tissue, in which a low level of oxygen (hypoxia) has been correlated to the expression of a transcription factor termed the hypoxia-inducible factor.

Brahimi-Horn et al., 2007
Tissue hypoxia

1. A part of physiological event, in particular in embryogenesis

2. In pathological situations – in ischemic diseases and cancer
HYPOXIA

Tumor growth is dependent on angiogenesis
Cancer cells require the process of angiogenesis and metastasis to form distant tumors

• **Angiogenesis**
  When tumors become greater than 1 mm in size, the host supplies blood vessels to deliver oxygen and nutrients required for further growth

• **Metastasis**
  Cancer cells can be disseminated from a primary to distant site by blood vessels or lymphatics
Hypoxic area is formed inside growing tumor
HYPOXIA

Ischemia in cardiovascular diseases

Coronary artery disease

Peripheral vascular disease

Impaired blood flow, hypoxic simulation of angiogenic factors,
How do cells sense the changes in the oxygen level?
The studies of hypoxia responsive element (HRE) of the erythropoietin gene lead to the discovery of HIF-1 by Semenza and Wang in 1992.


HIF-1 is a protein with DNA binding activity. It is composed of two subunits: HIF-1α and HIF-1β

Epo- glycoprotein produced by the kidney that promotes the formation of red blood cells in the bone marrow. It controls erythropoiesis.
What does HIF-1 do?

- Helps normal tissues and tumors survive hypoxic conditions
- Is a transcription factor which turns on over 100 genes needed for survival of hypoxic conditions

Group 1 genes such as transferrin and VEGF deliver $O_2$. Group 2 genes such as enolase 1 and hexokinase 1 provide sugars which generate energy. Group 3 genes such as IGF-2 and IGF binding proteins 1 and 3 increase proliferation and viability.
Hypoxia inducible factor – a master regulator of oxygen homeostasis

- Erythropoiesis & iron metabolism
  - Erythropoietin
  - Transferrin
  - Transferrin receptor
  - Ceruloplasmin
  - Heme oxygenase-1 (rodents)

- Vasomotor control
  - NOS II

- Angiogenesis
  - VEGF
  - VEGFR-1
  - PlGF
  - PDGF
  - TGFβ

- Energy metabolism
  - GLUT1, 2 & 3
  - PEPCK
  - LDH A
  - PGK3
  - Aldolase A & C
  - PFK L & C
  - Pyruvate kinase
  - Enolase

- Cell proliferation & viability
  - IGF-1
  - IGFBP-1 & 3
  - TGFβ3
  - NOS II
Hypoxia induce Epo production

- In HepG2 cell, hypoxia induces marked elevation of Epo mRNA and protein expression

Table 1. Effect of hypoxia on Epo production by HepG2 cells

<table>
<thead>
<tr>
<th>Epo assay</th>
<th>21% O₂</th>
<th>5% O₂</th>
<th>2% O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioassay</td>
<td>8.6 ± 3.0 (n = 3)</td>
<td>24.7 ± 2.5 (n = 3)</td>
<td>30.5 ± 6.2 (n = 4)</td>
</tr>
<tr>
<td>RIA</td>
<td>10.5 ± 2.2 (n = 2)</td>
<td>22.4 ± 14.8 (n = 3)</td>
<td>29.8 ± 9.9 (n = 4)</td>
</tr>
</tbody>
</table>

PNAS 1987;84:7972-6
• A heterodimeric member of basic helix-loop-helix (bHLH) family, containing PAS domain
• Built of HIF-1α and HIF-1β subunits
• Persistent expression of HIF-1β, identical to aryl hydrocarbon receptor (AHR) nuclear translocator (ARNT)
• Binds to DNA sequence 5’-(A/G)CGTG-3’ called HRE – hypoxia responsive element

- bHLH - basic Helix-loop-helix domain required for dimerization
- PAS - domain identified to be required for dimerization
- ODDD – O2-dependent degradation domain
- TAD – transactivation domain
Activation and degradation of HIF-1α

Mole et al., IUBM, 2001
Stabilisation of HIF-1α protein in hypoxia

Hammond et al., Mol Cell Biol 2002
HYPOXIA

\[ O_2 \downarrow \]

HYPOXIA

PROLYL HYDROXYLASES

HIF-1α

stabilization

e.g. VEGF

NORMOXIA

HIF-1α

proteasomal degradation

2-OXOGLUTARATE

\[ O_2 \]

\[ Fe^{2+} \]

\[ CO_2 \]

Succinate

OH OH

pVHL
Activation and degradation of HIF-1α

Zagórska & Dulak, Acta Biochimica Polonica, 2004
Prolyl hydroxylases are iron-dependent dioxygenases involved in HIF-1α degradation

PHD1 – hydroxylates P402 & P564

PHD2 - hydroxylates P402 & P564

PHD3 - hydroxylates P564

Co-factors of PHDs: iron, oxygen, 2-oxoglutarate, ascorbic acid
Asparaginyl hydroxylase (FIH) modifies HIF-1α and prevents binding of p300 co-activator

Zagórska & Dulak, Acta Biochimica Polonica, 2004
HYPOXIA

**Activation and degradation of HIF-1α**

*Brahimi-Horn et al., 2007*
HIF-1α protein is stabilized in hypoxia

Acker & Plate 2007
In hypoxia HIF-1α localizes in the nuclei

Immunofluorescent detection of nuclear HIF-1α in cells exposed to hypoxia. Cultured HeLa cells were incubated in normoxic or hypoxic conditions and stained for nuclei (DAPI) or for HIF-1α.

Brahimi-Horn et al., 2007
More on HIF transcription factors

- Both $\alpha$ and $\beta$ subunits are members of basic helix/loop/helix (Per/Arnt/Sim) (PAS) family

- there are three HIF$\alpha$ family members: HIF-1$\alpha$, HIF-2$\alpha$, HIF-3$\alpha$ and three HIF$\beta$ members: HIF-1$\beta$/ARNT1, HIF-2$\beta$/ARNT2, HIF-3$\beta$/ARNT3,

- over 100 HIF-dependent genes

- canonical HIF biding site: 5’-CGTG – HRE – hypoxia responsive element
Various HRE

**HYPOXIA**

- **CATACGTGGGCTCCAACAGGTCTCT**  
  **HBS**  
  **HAS**  
  VEGF

- **CCTACGTGCTGTCTCACACAGCCT**  
  **HBS**  
  **HAS**  
  EPO

- **CAGGCGTGCCGTCTGACACGCATC**  
  **HBS**  
  **HAS**  
  GLUT-1

- **CACACGTGGGTCCCGCCGACGTAATC**  
  **HBS**  
  **HAS**  
  LDHA

*Zagórska & Dulak Acta Biochim Pol 2004*
HIF-1 binds to hypoxia responsive element present in regulating regions of many genes.
HIF-1 transcription factors

HIF-1α – knockouts die at E 9.0

HIF-2α – knockouts die in utero at days 12.5-16 – adrenal insufficiency
Protective physiological mechanisms against hypoxia

1. Increased production of tyrosine hydroxylase – controls the ventilation through the carotid body

2. Increased expression of glycolytic enzymes

3. Increased synthesis of erythropoietin

4. Increased production of VEGF – stimulation of new blood vessels
HYPOXIA

Hypoxia – one of the strongest inducers of VEGF expression

Three ways of increasing VEGF expression

1. VEGF transcription

2. VEGF mRNA stabilisation

3. VEGF translation
VEGF gene
Increased VEGF mRNA
Increased VEGF proteins
Increased angiogenesis

HYPOXIA

HIF
RNA-binding proteins
Increased transcription
mRNA stabilization
IRES mediated translation

Increased transcription
mRNA stabilization
IRES mediated translation

HYPOXIA
VEGF mRNA stabilization

Half-life of endogenous VEGF mRNA is about 65 min - stability increases ~ 3 times in hypoxia

HuR - a member of Elav-like family of binding proteins; binds to distal AU-rich region in the VEGF 3’UTR. Stabilizes VEGF mRNA
An alternative mechanisms of translation initiation, independent of cap – using IRES – internal ribosomal entry site

VEGF mRNA has two IRES

IRES A is located within the 300 nucleotides upstream from the AUG start codon. RNA secondary structure prediction and site-directed mutagenesis allowed the identification of a 49-nucleotide structural domain (D4) essential to IRES A activity. UV cross-linking experiments revealed that IRES A activity was correlated with binding of a 100-kDa protein to the D4 domain. IRES B is located in the first half of the 5' UTR. An element between nucleotides 379 and 483 is required for its activity. Immunoprecipitation experiments demonstrated that a main IRES B-bound protein was the polypyrimidine tract binding protein (PTB), a well-known regulator of picornavirus IRESs.

IRES initiates translation independent of cap
Regulation of VEGF expression

Nat Clin Pract Oncol 4: 536–550 doi:10.1038/ncponc0905
Hypoxia – 0.5-2%O₂

Hypoxia is created using a Modular Incubator Chamber (Billups-Rothenberg Inc., Del Mar, CA, USA) by putting the cells into the chambers which are tightly closed and aired for 20 min with the gas mixture containing 1% O₂, 5% CO₂ and 94% N₂.
Activation of VEGF promoter and HRE part by hypoxia

**HRE sequence**

<table>
<thead>
<tr>
<th>Luciferase activity [Relative units]</th>
</tr>
</thead>
<tbody>
<tr>
<td>normoxia</td>
</tr>
<tr>
<td>0</td>
</tr>
</tbody>
</table>

* Significant difference

**VEGF promoter**

<table>
<thead>
<tr>
<th>Luciferase activity [Relative units]</th>
</tr>
</thead>
<tbody>
<tr>
<td>normoxia</td>
</tr>
<tr>
<td>0</td>
</tr>
</tbody>
</table>

* Significant difference

*Loboda et al., 2006*
HIF-1 activation increases VEGF expression in human microvascular endothelial cells

**Loboda et al., 2006**

* *p<0.05 vs normoksysja **p<0.01 vs normoksysja*
Regulation of VEGF expression by hypoxia is cell-type dependent

**HMEC-1**

**NIH 3T3 fibroblasts**

*V. Loboda A et al., 2006*
Other ways of induction of HIF-1

HYPOXIA

HIF-1α

stabilization

PROLYL HYDROXYLASES

2-OXOGLUTARATE

Fe²⁺

CO₂

SUCINATE

DMOG

Desferrioxamine

CoCl₂

e.g. VEGF

NORMOXIA

HIF-1α

proteasomal degradation

OH• OH•
pVHL

HIF inducers

Desferrioxamine
HIF inducers

How to modulate HIF-1 activity?

• Hypoxia
• Iron chelators: desferrioxamine, $\text{CoCl}_2$
• Dominant positive/negative forms, siRNA, oligonucleotide decoys
• Chemical compounds: DMOG (dimethyloxallyl glycine)
DMOG (Dimethyloxallyl glycine)

- Competitive inhibitor of the oxygen-sensing enzymes - prolyl hydroxylases (PHDs), which destruct HIF-1α when hydroxylated at a specific proline residues.

- Stabilizes HIF-1α expression at normal oxygen tensions at concentrations between 0.1 and 1 mM.
DMOG (Dimethyloxallyl glycine)

2-oxoglutarate
DMOG activates HIF-1 in human microvascular endothelial cells

HIF1 binding activity

Luciferase activity [relative units]

control 250 1000 hypoxia

HIF inducers

EMSA assay

Western blot

Loboda A et al., ARS 2009
HIF-1 activation increases VEGF expression in human microvascular endothelial cells

**RT-PCR**

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>DMOG*</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**ELISA**

- **VEGF [% of control]**
- **DMOG**
  - control
  - 250 µM
  - 500 µM
  - 1000 µM

- **ELISA**
  - **VEGF [% of control]**
  - **DMOG**
    - control
    - DMOG* 6 h
    - DMOG* 12 h
    - DMOG* 24 h

* DMG – 250 µM

**p < 0.01 vs control, ### p < 0.001 vs control"
HYPOXIA

Activation and degradation of HIF-1α

Mole et al., IUBM, 2001
Cofactors are needed for HIF-1 signaling

Chetomin – an inhibitor of p300 binding
Other ways of induction of HIF-1

HYPOXIA

HIF-1α

stabilization

PROLYL HYDROXYLASES

2-OXOGLUTARATE

O₂

Fe²⁺

CO₂

SUCCINATE

Desferrioxamine

CoCl₂

NORMOXIA

HIF-1α

proteasomal degradation

OH OH

pVHL

e.g. VEGF

HIF inducers
CoCl$_2$ is known to potently activate HIF-1

Increase in HIF- protein levels by cobalt stimulation in osteoblast-like cells

Kim at al., Cytokine. 2002 Jan 7;17(1):14-27.
CoCl$_2$ activates VEGF promoter in its HRE site

Loboda A et al., 2006
Desferrioxamine activates HIF-1 binding
Are all pro-angiogenic factors induced by hypoxia?
HIF-1 activation down-regulates IL-8 expression in HMEC-1 cells

PCR

<table>
<thead>
<tr>
<th>DMOG (µM)</th>
<th>IL-8</th>
<th>EF-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td></td>
<td></td>
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</table>

Real-time PCR

<table>
<thead>
<tr>
<th>DMOG (µM)</th>
<th>IL-8 mRNA [rel.expression]</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>1.0</td>
</tr>
<tr>
<td>500</td>
<td>#</td>
</tr>
<tr>
<td>1000</td>
<td>#</td>
</tr>
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</table>

ELISA

<table>
<thead>
<tr>
<th>DMOG (µM)</th>
<th>IL-8 protein [% of control]</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>100</td>
</tr>
<tr>
<td>250</td>
<td>#</td>
</tr>
<tr>
<td>500</td>
<td>#</td>
</tr>
<tr>
<td>1000</td>
<td>#</td>
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</table>

<table>
<thead>
<tr>
<th>AdGFP (MOI)</th>
<th>IL-8 protein [% of control]</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
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<tr>
<td>50</td>
<td>80</td>
</tr>
<tr>
<td>100</td>
<td>60</td>
</tr>
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<table>
<thead>
<tr>
<th>AdHIF-1α (MOI)</th>
<th>IL-8 protein [% of control]</th>
</tr>
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<tbody>
<tr>
<td>control</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>50</td>
<td>80</td>
</tr>
<tr>
<td>100</td>
<td>60</td>
</tr>
</tbody>
</table>

Loboda et al., ARS 2009
HIF-1 signal pathway and cancer angiogenesis
HIF-1 as a master regulator of physiological responses to hypoxia

Acker & Plate, 2007
Mutation in VHL gene causes disruption in HIF-1 degradation process.
Diseases caused by disturbances in HIF-1 signaling

- von Hippel Lindau disease

Diseases dependent on enhanced HIF-1 activity

- cancer
- atherosclerosis
- wound healing
VHL disease

Von Hippel-Lindau disease is a hereditary cancer syndrome characterized by a variety of tumors, including clear cell renal carcinoma, hemangioblastomas, and pheochromocytomas (28–30). Individuals with this disorder carry one wild-type \( VHL \) allele and one mutated \( VHL \) allele. Tumors develop when the remaining wild-type allele is somatically mutated or lost in a susceptible cell, thereby leading to loss of functional pVHL. VHL-associated tumors are highly vascular, owing partly to overproduction of VEGF, and occasionally produce marked increases in red blood cell production (polycythemia) because of tumor-derived erythropoietin production. In 1999, Maxwell and coworkers (31) showed that tumor cells lacking pVHL do not degrade HIF\( \alpha \) subunits in the presence of oxygen, thus accounting for the observed overproduction of HIF-responsive mRNAs.
Von Hippel Lindau disease

- rare (1:36 000 live births) dominantly inherited cancer syndrome

- multiple hemangioblastomas of CNS and retina, renal cell carcinomas pancreatic islet cell tumors and others

Described in 1911 by Eugene von Hippel and further studied in 1926 by Arvid Lindau

Defect in VHL tumor suppressor gene in chromosome 3p25-p26

Mutations in VHL occur also in the majority of kidney cancers

Brigham and Women’s Hospital, Boston
HIF-1 and tumor angiogenesis

- In HIF(+) xerograft, tumor bleed profusely and severely

- HIF(-) xerograft’s vasculature is more uniform, with fewer large vessel and more avascular zone

Embryonic stem cell tumor
EC: endothelial cell

Nature 1998;394:485-90
Disruption of HIF inhibit tumor growth

- Block HIF-1 binding to transcriptional cofactor p300/CBP by fragmented HIF-1 (TAD-C)

- Block HIF-1 increase cell death in HepG2 cell during hypoxia

- Other pathway contribute to apoptosis during hypoxia

Nat Med 2000;6:1335-40
Is hypoxia the only one activator of HIF-1?

\[
\text{L-Arg + O}_2 \xrightarrow{\text{NOSI, NOSII, NOSIII cofactors}} \text{NO}^\cdot + \text{L-Cit}
\]
Effect of IL-1b on VEGF mRNA expression in rat vascular smooth muscle cells

Li e et al., JBC 1995
IL-1b induces also the expression of inducible nitric oxide synthase

Dulak et al., ATVB 2000: 20: 656-666
Nitric oxide induces VEGF synthesis

L-Arg + O₂ \xrightarrow{NOS II (iNOS)} NO⁻ + L-Cit

\xrightarrow{NOS III (eNOS)} IL-1β \xrightarrow{VEGF}

Dulak et al., ATVB, 2000; Atherosclerosis 2001; JACC 2001; Jozkowicz et al., Cardiovasc Res 2001
NO induces VEGF synthesis

\[ \text{L-Arg + O}_2 \xrightarrow{\text{NOS II}} \text{NO} \xrightarrow{\text{L-Cit}} \text{VEGF} \]

\[ \text{IL-1}\beta \xrightarrow{\text{NAME}} \text{VEGF} \]

Dulak et al., ATVB, 2000
Interleukin-1β induces VEGF through NO

VEGF synthesis is enhanced by NO
- generated by NOS II after cytokine stimulation
- derived from NOS II or NOS III after gene transfer
- released from NO-donors

VEGF promoter activation

*Dulak et al., ATVB, 2000; Atherosclerosis 2001; JACC 2001; Jozkowicz et al., Cardiovasc Res 2001*
Enhancement of VEGF synthesis by gene transfer of nitric oxide synthases

Role of NO in regulation of VEGF synthesis in pathological conditions

1. Induction of iNOS expression in tumors and other inflammatory diseases

2. Inhibition of NO production in cardiovascular diseases
Role of nitric oxide in tumors
Overexpression of eNOS enhances angiogenesis in hind limb ischemia

Namba K et al., Circulation 2003: 108:2250-2257
VEGF

- hypoxia
- reactive oxygen species
- nitric oxide
- cytokines
- growth factors
- heme oxygenase-1
Take-home messages

1. VEGF expression is regulated by many stimulus, including cytokines, growth factors, ROS, hypoxia, NO

2. Hypoxic regulation of VEGF expression occurs on different levels

3. Enhancement of VEGF mRNA expression
   - activation of VEGF promoter
   - increased stability of VEGF mRNA

4. Enhanced VEGF translation through internal ribosome entry site (IRES)

5. HIF-1 signaling is important for cancer angiogenesis