Microenvironmental influence on angiogenesis and tumor cell survival

Mark W. Dewhirst, DVM, PhD
Duke University Medical Center
Lecture Outline

• Angiogenic Switch
• Tumor-host cell interactions
  – Endothelial cell
  – Macrophage
• Effects of Rx and microenvironment on angiogenesis
Lecture Outline

- Angiogenic Switch
- Tumor-host cell interactions
  - Endothelial cell
  - Macrophage,
- Effects of Rx and microenvironment on angiogenesis
What causes the angiogenic switch in tumors?

- Hypoxia
- Oncogene mutations or upregulation
  - Ras, myc, epidermal growth factor, Her2 upregulation
- Loss of suppressor gene function
  - PTEN via PI3K
    - Brader and Eccles, Tumori 90:2, 2004
  - P53
    - Bardos and Ashcroft, Bioessays 26:262, 2004
HIF-1α Protein Stability is Regulated by O₂ and by VHL Tumor Suppressor

EGF ↓
PI3-K ↓
AKT ↓
FRAP ↓

von Hippel-Lindau/
Ubiquitin-protein Ligase
Complex

HIF-1α

Elongin B

Cul2

Rbx1

E2

Ub

HIF-1α

ODD

Elongin C

Proteasome

Courtesy of M. Brown
Key tyrosine kinase receptors involved in angiogenesis regulation

- **Flk/flt**
  - Receptor for VEGF
- **Tie-2/TEK**
  - Receptor for Angiopoietin 1 and 2
- **FGFR2**
  - Cooperates with VEGFR
Functions of VEGF and Tie2 Receptors

• VEGF binding
  – Hyperpermeability
  – Endothelial cell
    • Proliferation, migration, survival

• Ang 1 to Tie2
  – Maintain vessel maturity

• Ang 2 to Tie2
  – Endothelial cell de-differentiation
  – Loss of pericyte, SMC association with vessels
  – Increased receptivity to VEGF
Ang-1 contributes to vascular maturity

- Pericytes, smooth muscle cells associate with endothelial cell
  - Arrest of endothelial proliferation
  - Endothelial cell survival
  - Vasoreactivity

Figure courtesy of M. Neeman
Ang 2 contributing to vessel immaturity

- Pericytes, smooth muscle cells disassociate with endothelial cell
  - Facilitates reactivity to VEGF
  - Increases permeability
  - Loss of vasoreactivity

VEGF now becomes survival factor
Pericyte Structure: Normal vs. Tumor Microvessels

Ang / Tie2

Morikawa et al., Am J Path 160: 985, 2002
**VEGF vs. Tie 2 signaling for vessel growth / maturation**

<table>
<thead>
<tr>
<th>Conditions</th>
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<th>Flow</th>
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<tr>
<td><strong>Angiogenic Factors</strong></td>
<td>Normoxia</td>
<td>Normal</td>
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<tr>
<td><strong>Receptors</strong></td>
<td>VEGF (-)</td>
<td>Ang 2 (-) Ang 1 (+)</td>
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<tr>
<td><strong>Outcome</strong></td>
<td>VEGFR (-)</td>
<td>Tie2(+)</td>
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- No Vessel Growth
# VEGF vs. Tie 2 signaling for vessel growth / maturation

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Angiogenesis
Vascular adaptation in response to changes in shear stress

Variation in shear stress in mesenteric vascular network

Studying vascular adaptation in tumor microvasculature

Dreher, Dewhirst, unpublished
Segmented model for vascular adaptation simulations
Lecture Outline

- Angiogenic Switch
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  - Endothelial cell
  - Macrophage, fibroblast
- Effects of Rx and microenvironment on angiogenesis
Truncated receptor proteins added to window tissue at time of surgery and tumor cell transplant

GFP$_{cmv}$ reporter cells

Serially monitor daily post transplant
Chemotactic behavior of 4T1 tumor cells toward host vessels

CY Li et al, JNCI, 2000
Pre-angiogenic tumor and vessel behavior

Day 0

Day 2

Day 4

C.Y. Li et al., JNCI, 2000

Bar = 300µm

Proliferation / Chemotaxis

Angiogenesis

C.Y. Li et al., JNCI, 2000
exFlk (VEGF blockade) inhibits proliferation/migration toward host vasculature

BSA

a (day1)

b (day5)

c (day10)

All tumors engraft

exFlk

d (day1)

e (day5)

2/6 tumors engraft

Cells die before Angiogenesis onset

f (day10)

C.Y. Li et al., JNCI, 2000
Effects of Tie2 blockade vs bFGF on tumor cell survival post transplant

From: Shan et al, FASEB J 18:326, 2004
Working model for paracrine survival signaling

Pro-Survival Signal

VEGF
Ang 2
bFGF

Tumor Cell

Endothelial Cells
### VEGF vs. Tie 2 signaling for vessel growth / maturation

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#### Angiogenesis

- **Conditions:** Hypoxia, Low flow
- **Angiogenic Factors:** VEGF (+), Ang 2 (++) Ang 1 (+)
- **Receptors:** VEGFR (+), Tie2(+)*
At what point during tumor growth does hypoxia influence angiogenesis?

Dewhirst, Cao and Li, unpublished
4T1 Mouse Mammary Carcinoma

Day 1  Day 2  Day 3

%Hb_{sat}

Red = Tumor
Green = HIF-1 Activity
4T1 Mouse Mammary Carcinoma

Day 4

Day 5

Day 7

%Hb_{sat}

= Tumor

= HIF-1 activity
Intussceptive angiogenesis examples

Observation in CAM

FaDu xenograft

Burri et al, Dev Dyn, 2004

Dreher, Dewhirst, unpublished
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Liver hypoxia caused by acute alcohol (2 h post ethanol administration)

Blockade of Kupffer cell activation reduces hepatic hypoxia post ethanol ingestion

Arteel et al, AJP 271, G494-G500
Dietary glycine reduces angiogenesis and tumor growth - fibrin gel chamber

Glycine blocks Chloride channels in macrophages; prevents macrophage activation

Control 10% Glycine in diet

Amin et al, Can Biol Ther 2; 173, 2003
10% dietary glycine reduces tissue iNOS levels

Amin et al, Can Biol Ther 2; 173, 2003
Hif-2α and macrophages colocalize in human breast cancer

CD68

HIF-2α

Breast Ca

Histology of Wound Healing Reaction

Normal Rat Skin (25X)

Day 1 (25X)

Prov. Fibrin Matrix

Hypoxia in Provisional Fibrin Matrix on day 1 (-) vs. at day 4 (++++)

Day 1

Day 4

(25X)
Proliferation and Apoptosis are Maximum at Day 4

Ki67

TUNEL

Hypoxic Induction of P53 / Apoptosis?
Wound → Tissue Injury → Platelets → ↑VEGF, TGF β → ↑Hyperpermeability → Fibrin Formation → Influx of Inflammatory & Endothelial cells → Angiogenesis and Fibrinolysis → Tissue Remodeling & Scar formation → HYPOXIA

Inflammation

Proliferation

Remodeling
Examples of vascular remodeling and regression or collapse

HCT 116
Colon Carcinoma Xenograft

Bar: 200 μm

Dewhirst et al, Sem in Hematol Oncol, in press, 2004
Demonstration of Static Flow

QuickTime™ and a YUV420 codec decompressor are needed to see this picture
Red cell flux relates to perivascular pO2

From Kimura et al., 1996
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Supervascularized state of irradiated tumors

- First described by Rubin and Casarett, Clin Radiol, 17:346-355, 1966
- Qualitative assessment - done using microangiography

Irradiated Sham Irradiated

5Gy x 3, daily Fx, Walker carcinosarcoma
Paradoxical HIF-1 Signaling During Tumor Reoxygenation: The Role of Free Radicals and Stress Granules

Mark W. Dewhirst
Ben Moeller
Yiting Cao
Chuan Li

Moeller et al., Cancer Cell, May 2004, page 429
Two HIF-1 - mechanisms protect against endothelial death post RT

- Hypoxic tumor cell
- Stress Granules Protect HIF-1 transcripts
- Reoxygenation
- Stress Granules Depolymerize Releasing HIF-1 regulated mRNAs
- ROS
- HIF
- HIF-1α Stabilized Enters nucleus
Overlap between Hypoxia Marker & HRE-GFP pre/48hr post RT

Sham = 92% overlap HRE-GFP with Pimo
Irradiated = 18% Overlap

**Red** = Pimonidazole - hypoxia marker drug
**Blue** = Hoechst - perfusion marker
**Green** = HRE-GFP reporter gene

HRE-GFP expressed in Aerobic cells post RT

Methods to test whether RT induces reoxygenation → free radicals → HIF-1

4T1

radiation (reoxygenation) vs. sham-RT

4T1 HRE-GFP
HIF-1 mediated GFP
Tumor cells

antioxidant vs. PBS

H$_2$DCFA or DCFA microscopy (change ROS)

microscopy (change HIF-1 GFP)
Reoxygenation post RT increases free radicals

2 x 5Gy
24hr after 2nd RT dose

ROS (H2DCFDA)
PBS
white light

SOD
white light

pre-RT post-RT

bar = 300µm
Free radicals post RT increase HIF-1α levels

RT = 5Gy x 2

bar = 300µm
Significance of Reoxygenation-Mediated HIF-1 Activation

RT (reoxygenation)

RT + YC-1 (HIF-1 inhibitor)
HIF-1 Activation blockade increases RT response

4T1
10 Gy x 1

YC-1
5mg/kg
Tying the pieces together

Promote tumor cell survival, angiogenesis and growth

Pro-angiogenic Signaling via HIF-1 (tumor / host cells)

Vascular Remodeling

Cell Killing

Treatment

Hypoxia

Stress Granule

Reoxygenation

ROS
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- G. Arteel