Tumor angiogenesis

BACKGROUND READING:

**Tumor Angiogenesis- general**

“Angiogenesis in Cancer and other Diseases”

**Notch in Tumor Angiogenesis**

‘Notch signaling in developmental and tumor angiogenesis’
Kofler NM, Shawber CJ, Kangsamaksin T, Reed HO, Galatioto J, **Kitajewski** J.

**Tumor Angiogenesis - therapeutics**

“Vascular Endothelial Growth Factor Signaling Pathways: Therapeutic Perspective”
Blood Vessel Development

• **Vasculogenesis** = de novo tube formation

• **Angiogenesis** = sprouting of new tubes off of pre-existing tubes

• **Cell types**
  - **Endothelial Cell** = cell type that makes up and lines blood vessels
  - **Mural Cells** = specialized cells that surround blood vessels
    - Pericytes
    - Smooth muscle cells

• **Angiogenic Factors**
  - Vascular Endothelial Growth Factor (VEGF-A, VEGF-B, PIGF, VEGF-C…..)
  - Angiopoietins (Ang 1, Ang2, ………..)
  - Notch ligands (Jagged1, Delta4)
Vessel structure

Blood Vessel

- Thin layer of endothelium with tight junctions and well developed basement membrane.
- Capillaries, venules, veins, arteries, arterioles
- Arteries with multiple layers of vascular smooth muscle cells. Capillaries with sparse, loosely attached pericytes

Lymphatic Vessel

- Thin layer of endothelium with a poorly developed basement membrane (BM) and lacking pericytes (PC)
- Endothelial cells (EC) overlap to form valves that can open with increases in pressure to let in fluid and immune cells
- Blood and lymphatic vessels develop in an overlapping pattern

Saharinen et al., Trends Immunol, 2004
pericyte

Endothelial cell
Vascular Development

**ANGIOGENESIS**

Cellular steps in Angiogenesis

1) Biochemical Response and Preparation
2) Sprout initiation
3) Migration
4) Proliferation
5) Survival
6) Tube Formation
7) Maturation
8) Completion
9) Blood Flow
Tip Cells

Stalk Cell → Lumen
VEGF and VEGF Receptors

Blood Endothelial Cell

VEGF-B

PIGF

VEGF-C

VEGF-D

BEC

NRP-1

VEGFR-1

VEGFR-2

LEC

NRP-2

VEGFR-2

VEGFR-3
VEGF-receptor signaling

Promotes:
- Proliferation
- Migration
- Survival
Angiogenesis - Basement Membrane Breakdown

Angiogenic Stimulus (VEGF)

Smooth Muscle Cells

Basement Membrane

Endothelium

Proteases
Angiogenesis - Endothelial Cell Migration

- VEGF
- Nascent Vascular Sprouts
- Smooth Muscle Cells
- Basement Membrane
- Endothelium
Notch drives cell fate determination

**Notch/Notch ligand interaction:**
- mechanism for setting and maintaining state of differentiation
- fates locked in via lateral inhibition

Notch signaling is a mechanism for defining tip versus tube cell during sprouting angiogenesis
Notch drives cell fate differentiation

**Notch/Notch ligand interaction:**
- mechanism for driving state of differentiation
- fates locked in via lateral inhibition
Notch drives cell fate differentiation

*Notch/Notch ligand interaction:*
- mechanism for driving state of differentiation
- fates locked in via lateral inhibition
Notch drives cell fate differentiation

*Notch/Notch ligand interaction:*
- mechanism for driving state of differentiation
- fates locked in via lateral inhibition
Notch drives cell fate differentiation

*Notch/Notch ligand interaction:*  
- mechanism for driving state of differentiation  
- fates locked in via lateral inhibition

*Notch blocks sprout initiation*
**Notch drives cell fate differentiation**

*Notch/Notch ligand interaction:*
- mechanism for driving state of differentiation
- fates locked in via lateral inhibition

*Notch blockade causes sprout initiation*
Notch functions to restrict sprouting in retinal angiogenesis.
Angiogenesis - Endothelial Cell Proliferation

- VEGF
- Sprout
- Elongation
- Smooth Muscle Cells
- Basement Membrane
- Endothelium
Angiogenesis - Capillary Morphogenesis

VEGF

Smooth Muscle Cells

Basement Membrane

Endothelium

New Lumen Formation
Angiogenesis - Vascular Maturation

VEGF

SMC, pericyte recruitment

Vascular Pruning (apoptosis?)

Smooth Muscle Cells

Basement Membrane

Endothelium
Angiogenesis - Vascular Maturation

VEGF

Endothelial cell-cell junctions

Negative Feedback

Smooth Muscle Cells

Basement Membrane

Endothelium
**Box 1 Figure** Cellular mechanisms of tumour (lymph) angiogenesis. Tumour vessels grow by various mechanisms: (1) the host vascular network expands by budding of endothelial sprouts or formation of bridges (angiogenesis); (2) tumour vessels remodel and expand by the insertion of interstitial tissue columns into the lumen of pre-existing vessels (intussusception); and (3) endothelial cell precursors (angioblasts) home from the bone marrow or peripheral blood into tumours and contribute to the endothelial lining of tumour vessels (vasculogenesis). Lymphatic vessels around tumours drain the interstitial fluid and provide a gateway for metastasizing tumour cells. (Adapted from ref. 38.)
(A) Breast cancers arise from the ductal epithelium of the breast; ductal lesions that are presumptive progenitors can be ordered into a pathway of increasing aberrancy, beginning with hyperplasia and progressing to dysplasia and CIS. Of these, a subset of CIS lesions have switched on angiogenesis, as evidenced by abundant new capillaries, suggesting that angiogenic-CIS is an intermediate stage between CIS and invasive cancer.
< 5 weeks
100% normal islets

5–7 weeks
~50% hyperplastic islets

7–12 weeks
10% angiogenic islets

12–14 weeks
2–4% tumors
necrosis

nests of adenocarcinoma
VEGF is a hypoxia induced gene

Figure 2: Role of hypoxia in tumour angiogenesis. Because of the irregular pattern and organization of the tumour vasculature, some cells in tumours are located more than 100 μm (the diffusion limit for oxygen) away from blood vessels and become hypoxic (red-to-blue gradient indicates progressive hypoxia). Tumour cells survive fluctuations in oxygen tensions, in part because clones are selected in hypoxic tumours that switch to a proangiogenic phenotype. HIFs increase transcription of several angiogenic genes (for example, genes encoding VEGF, PDGF-BB and NOS). HIFs also affect cellular survival/apoptosis pathways. Inset: relationship between the distance of tumour cells from nearby vessels and their degree of hypoxia (blue symbols) and acidosis (red symbols).
Models of Tumor Angiogenesis

Angiogenic sprouting

- Avascular tumour growth
- Hypoxic tumour: VEGF
- Angiogenic sprouting into tumour (VEGF & Ang2)

Vessel Cooption

- Vessel regression due to Ang2 leads to tumour regression & ↑VEGF
- Angiogenic sprouting (VEGF & Ang2)

Models of tumour angiogenesis. a, Model of avascular tumour initiation contrasted with b, tumour initiation involving host vessel co-option.
Multiple roles for VEGF when do you need and when not?
Lymphangiogenic VEGF Receptors
promote tumor lymphangiogenesis and lymph node metastasis
Structure of blood and lymphatic vasculature in dermis

Figure 2 Blood and lymphatic vessels in mouse ear. a, Whole mount immunohistochemical staining of lymphatic vessels (LYVE-1; red) and blood vessels (PECAM-1; green) in mouse ear. b, Transgenic overexpression of the VEGF-C mutant C156S in the skin leads to enlargement of the lymphatic vessels, whereas only a few lymphatic ECs are present in the ear of a Chy lymphoedema mouse\textsuperscript{12} (c). We thank G. Thurston for the stainings and D. Jackson for the LYVE-1 antibodies.
Tumor Lymphangiogenesis?

Expression of Tag + VEGF-C

Figure 1. The Angiogenic Switch Occurs Prior to Tumor Formation in Three Transgenic Mouse Models of Tumorigenesis

(A) Expression of the Tag oncogene in the pancreatic islets elicits four sequential stages in tumor development: normal, oncogene-expressing islets; hyperplastic islets, populated by proliferating cells with the histological hallmarks of CIS; angiogenic islets, in which new blood vessel growth has been activated; and solid tumors, which are islet cell carcinomas.
Table II. Tumour phenotypes and metastasis in Rip1Tag2 versus RipVEGF-C × Rip1Tag2 mice

<table>
<thead>
<tr>
<th></th>
<th>Rip1Tag2</th>
<th>RipVEGF-C × Rip1Tag2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour incidence(^a)</td>
<td>5.4 ± 1.4 (n = 27)</td>
<td>12.3 ± 2.9 (n = 33)</td>
</tr>
<tr>
<td>(per mouse)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumour volume(^b)</td>
<td>56.5 ± 35.8 (n = 27)</td>
<td>38.3 ± 29.1 (n = 33)</td>
</tr>
<tr>
<td>(per mouse)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenoma</td>
<td>36% (n = 17)</td>
<td>46% (n = 27)</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>64% (n = 17)</td>
<td>54% (n = 27)</td>
</tr>
<tr>
<td>Lymph node metastases(^c)</td>
<td>0% (0/17)</td>
<td>37% (10/27)</td>
</tr>
</tbody>
</table>

\(^a\)Tumour incidence per mouse was determined macroscopically by counting all apparent tumours >0.5 mm in the whole pancreas.
\(^b\)Tumour volume per mouse (in mm\(^3\)) was calculated from all macroscopically apparent tumours in the whole pancreas, with a diameter >0.5 mm.
\(^c\)As detected by haematoxylin and eosin staining.
Values are mean ± SD; n = number of mice.

Fig. 2. Expression of VEGF-C and VEGFR-3 in RipVEGF-C transgenic mice. Immunohistochemistry for (A and B) VEGF-C and (C) VEGFR-3. (A) Ten-month-old female wild-type littermate; (B and C) 11-month-old female RipVEGF-C mouse, family 24. (B) and (C)
Inhibition of VEGF and VEGF Receptors

Avastin - humanized anti-VEGF Ab

anti-VEGF-R1 Ab

anti-VEGF-R2 Ab

Soluble Flt-1

Chemical inhibitors Of tryosine kinases
<table>
<thead>
<tr>
<th>Name</th>
<th>Status</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Endogenous inhibitors of angiogenesis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endostatin</td>
<td>in clinical trial</td>
<td>scattered responses</td>
</tr>
<tr>
<td>Interferons-α and -β</td>
<td>effective in treating hemangioblastomas</td>
<td>Kaposi’s sarcomas; limited efficacy against most other types of tumors</td>
</tr>
<tr>
<td><strong>B. Agents that block VEGF and VEGF-R signaling</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avastin anti-VEGF MoAb</td>
<td>in clinical trial</td>
<td>delayed progression 1–3 months in lung, 3–4 months in colon</td>
</tr>
<tr>
<td>SU5416 inhibitor of VEGF-R2 (Flk-1)</td>
<td>trial abandoned</td>
<td>severe vascular toxicities</td>
</tr>
<tr>
<td>ZD6474 inhibitor of VEGF-R2</td>
<td>under clinical test</td>
<td></td>
</tr>
<tr>
<td>CP547, 632 inhibitor of VEGF-R2</td>
<td>in trial</td>
<td></td>
</tr>
<tr>
<td><strong>C. Miscellaneous other drugs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thalidomide</td>
<td>in trial</td>
<td>inhibits bFGF- and VEGF-dependent angiogenesis</td>
</tr>
<tr>
<td>Squalamine sterol from shark liver</td>
<td>in trial</td>
<td>strong anti-angiogenic activity</td>
</tr>
<tr>
<td>Celecoxib anti-inflammatory drug</td>
<td>in trial</td>
<td>multiple anti-neoplastic effects</td>
</tr>
<tr>
<td>ZD6126</td>
<td>in trial</td>
<td>antagonist of tubulin in endothelial cell cytoskeleton</td>
</tr>
<tr>
<td>Fumagillin and TNP-470</td>
<td>in trial; slowed tumor growth</td>
<td>antagonist of methionine aminopeptidase in endothelial cells</td>
</tr>
<tr>
<td><strong>D. Inhibitors of ECM breakdown—MMP inhibitors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marimastat</td>
<td>in clinical trial</td>
<td>no delay of tumor progression</td>
</tr>
<tr>
<td>Prinomastat</td>
<td>in clinical trial</td>
<td>no slowing of tumor progression</td>
</tr>
<tr>
<td>BMS275291</td>
<td>in clinical trial</td>
<td></td>
</tr>
<tr>
<td>BAY12-9566</td>
<td>in clinical trial</td>
<td></td>
</tr>
<tr>
<td>Neovastat (shark cartilage MMPI)</td>
<td>in clinical trial</td>
<td></td>
</tr>
</tbody>
</table>
VEGF Trap (Regeneron) case study

- Composite decoy receptor
- High affinity binding to all species of VEGF (Kd < 1 pM), half-life ~25 days
- Binds PlGF, VEGF-B
- Phase I clinical trials
Anti-VEGF blockade in experimental Wilms Tumor

Study conducted by Drs. Jessica Kandel (Surgery), Darrell Yamashiro (Pediatrics Oncology), Jay Huang (Surgery)
Tumor regression schema:

- Tumors are implanted and allowed to grow for 6 weeks, at which point they are large (> 1 gm) and metastatic.
- Treatment with VEGF Trap starts at Day 0.
VEGF-Trap regresses established tumors

Huang et al, PNAS June 2003
What happens if we continue to treat regressed tumors?

- Tumors regressed by ~80% at day 36
All tumors recur after initial regression by VEGF Trap
Block receptor activation via presenilin

GSI = gamma-secretase inhibitor
GSI inhibition of Notch is toxic to intestine

Gamma secretase inhibitor

Immunohistochemical studies of small intestines

DBZ treatment resulted in conversion of the transit-amplifying crypt cell population to goblet cells.

Nature 444, 1083-1087 (21 December 2006)
Dll4 blocking antibodies

Dll4 blockade induces excesses sprouting during retinal angiogenesis

Dll4 blockade promotes dysfunctional sprouting
Delta-like 4 as a therapeutic target in oncology

“The Dll4/Notch paradox”
DLL4 blockade promote sprouting while blocking tumor growth

Inhibits tumor growth
overgrowth
dysfunctional vasculature

2008 - Regeneron (Yancapoulous & Thurston) / Genentech / Adrian Harris (Oxford)
Case Study #2: Notch as a therapeutic target in Tumor Angiogenesis

*Notch decoy: inhibitor of Notch signaling*

Yasuhiro Funahashi, Carrie Shawber, Jan Kitajewski
Notch decoy blocks VEGF-induced dermal angiogenesis

control  VEGF-A  VEGF-A + N1ECD notch decoy

Notch decoy & VEGF^{121} secreted from dermally implanted chamber
- Notch-based fusion proteins and uses thereof  
  *Patent no. 7,662,919*

- Kitajewski

![Diagram of NOTCH1 protein structure with Fc antibodies](image)

**Anti-Jagged1/Dll4**

**Anti-Dll4**

**Anti-Jagged1**

![Graphs showing tumor weight vs time for different models](image)
ANTI-ANGIOGENIC THERAPY IN HUMANS
Bevacizumab (Avastin)

- Recombinant humanized monoclonal IgG1 antibody derived from the murine VEGF-A monoclonal antibody A4.6.1
- Murine VEGF-binding residues (7% of protein sequence)
- Human IgG1 framework (93%)
- Binds all isoforms of human VEGF
- Binding affinity: $K_d = 8 \times 10^{-10} \text{ M}$
- Half-life 20 days
- First FDA approved anti-angiogenic agent (approved in 2004)
Bevacizumab in Colorectal Cancer

402 Chemo alone (IFL) qw

411 Chemo (IFL) + bev 5mg/kg q2w

Outcome:
Overall Survival
Avastin = Bevacizamid = anti-VEGF-A Antibody (humanized)
Makes it way into the clinic - FDA approved 3/2004

Colorectal Cancer
• VEGF pathway

  – Approved drugs
    • Bevacizumab (Avastin)
    • Aflibercept (VEGF-Trap, Zaltrap)
    • Tyrosine Kinase Inhibitors

  – New agents in clinical trials
    • VEGF pathway
    • Other antiangiogenic agents
Bevacizumab and other tumor types

- Non-small cell lung adenocarcinoma
  - E4559: OS 12.3 vs. 10.3 m in pts w/ chemo + bev

- Renal cell carcinoma
  - AVOREN: PFS 10.2 vs. 5.4 m in pts w/ IFN + bev;
    - OS 2 month difference; attributed to crossover

- Glioblastoma multiforme
  - AVF3708g: 2nd line, 9.2 m OS in bev alone arm

Sandler A, et al. NEJM. 2006; 355: 2542-50
Friedman HS, et al. JCO 2009; 27:4733-40
WHAT ARE SOME OF THE POSSIBLE COMMON SIDE EFFECTS OF THERAPY?

Because targeted therapies like Avastin work in a different way than chemotherapy, there are different side effects.

Common side effects associated with chemotherapy:

- Diarrhea
- Nausea
- Vomiting
- Loss of appetite
- Loss of hair
- Tingling or numbness in fingers or toes
- Mouth sores
- Increased chance of infection
- Bleeding or bruising easily
- Fatigue
- Rash on the hands and feet

Avastin is used in combination with chemotherapy. There are additional common side effects associated with Avastin.

Common side effects associated with Avastin:

- Nosebleeds
- High blood pressure
- Too much protein in the urine
- Pain
- Diarrhea
- Reduced white blood cell count
- Weakness
Bevacizumab side effects & cost

• Common Side Effects:
  – Hypertension (23-34%)
  – Abdominal pain (50-61%), Constipation (29-40%)
  – Asthenia (up to 70%), Headache (24-50%)
  – Proteinuria (4-36%)
  – Upper respiratory infection (40-47%)

• Serious Side Effects
  – Thromboemboli (9%), Neutropenia (21-27%)
  – GI hemorrhage (19-24%), GI perforation (2.4%)

• Price in 2004: $2,750 per 400mg vial
  – One dose for 70kg pt (10mg/kg) = 2 vials ($5,500)
Bevacizumab in Breast Cancer

- Feb 2008: FDA grants accelerated approval
  - E2100: PFS 11.8 vs 5.9 (HR 0.6, P<0.001) paclitaxel/bev vs paclitaxel alone in 1st line
  - Accelerated approval program allows access to promising new drugs while confirmatory trials are conducted

Non-VEGF Antiangiogenic Targets

Hicklin D J, et al. 2005
Yancopoulos et al. 2006
Radtke et al. 2005
Ang/Tie Blockade

- **AMG 386**: First in class peptide-Fc peptibody
  - Ovarian ca phase II in combo with chemo – median PFS 7.2 vs 4.6 compared to pbo
  - Phase III TRINOVA-1 enrolling

- **MEDI3617**: Ang1, Ang2 mAb
  - 21 pts recruited; 4 continue (max response 6m)
  - 5 had stabilization of dz for 3m or greater

- **REGN910**: Humanized Ang2 mAb
  - Ongoing (NCT01271972)

---

Natale RB, et al. ASCO 2012 Abstract TPS2621
Papadopoulous KP et al. ASCO 2011 Abstract TPS159
Notch Inhibition: GSI (nonspecific)

– RO4929097:
  • Phase I: Prolonged stable disease (3-6m) in 30 of 92 pts; esp in melanoma, sarcoma and ovarian carcinoma
  • Toxicity: n/v/d, fatigue, low phosphate, skin rash
  • Phase I/II trials now recruiting for glioblastoma, RCC, pancreatic cancer, CRC, breast cancer

– MK-0752:
  • Phase I: 1 CR, 10 stable disease of 103 pts (most with brain tumors)
  • Toxicity: n/v/d, fatigue, rash
  • Phase I/II trials recruiting in breast, other advanced tumors

Clinicaltrials.gov
Notch Inhibition

• Dll4 mAb:
  – Demicizumab (OMP-21M18): humanized
    • Pancreatic cancer: with gemcitabine
    • NSCLC: with carboplatin and pemetrexed
  – REGN421: fully human mAb
    • Phase I in advanced malignancies
Complementary Angiogenic Signaling Pathways

Reviewed in: Thurston et al, Br J Cancer, 2008

Hypoxia

EC Proliferation
EC Migration

Vessel Architecture
Branching & Sprouting

VEGF, Dll4-Notch, and Ang2-Tie2 play distinct yet critical roles in angiogenesis:

- Blockade of single pathways can inhibit distinct tumors and different aspects of tumor angiogenesis; Blockade of pathways in combination has more potent effects than monotherapy
- Can we improve anti-tumor therapy by combined blockade of multiple angiogenic signaling pathways?

Vessel Stabilization
EC Survival
We Have Developed Multiple Therapeutic Agents for Targeting Critical Angiogenic Factors in Humans

- **VEGF, Dll4-Notch, and Ang2-Tie2** play distinct yet critical roles in angiogenesis
- **Regeneron** have developed multiple approaches for targeting key angiogenic factors

---

**REGN421**
- Fully human Dll4 monoclonal Antibody
- Phase 1 ongoing

**Aflibercept (VEGF Trap)**
- 3 ongoing pivotal Phase 3 trials

**REGN910**
- Fully human Ang2 monoclonal Antibody