www.nature.com/nature Vol 438 Issue no. 7070 15 December 2005

Cover Illustration
Developing mouse retinal blood vessels grow towards areas of low oxygen concentration. (Courtesy of M. Fruttiger, Kings College London/*Development*.)

ANGIOGENESIS

Blood vessels are a complex network of tubes that carry oxygenated blood and nutrients throughout our bodies. If laid end to end, the vessels from a typical adult would circle the Earth twice. It comes as no surprise, then, that the process of growing new blood vessels — angiogenesis — is a fundamental biological mechanism that results in serious disease when it goes awry. Indeed, more than US$4 billion has been invested in the research and development of medicines to promote or reduce angiogenesis, making it one of the most heavily funded areas of medical research today.

Angiogenesis is an essential process during development — growth of a vascular system is one of the earliest events in organogenesis. Nonetheless it also occurs in adulthood, during wound healing and restoration of blood flow to injured tissues. Angiogenesis is regulated by a very sensitive interplay of growth factors and inhibitors, and their imbalance can lead to disease. In cancer, diabetic eye disease and rheumatoid arthritis, excessive angiogenesis feeds diseased tissue and destroys normal tissue. Conversely, insufficient angiogenesis underlies conditions such as coronary heart disease, stroke and delayed wound healing, where inadequate blood-vessel growth leads to poor circulation and tissue death.

This Insight describes many of these physiological and pathophysiological processes of angiogenesis and lymphangiogenesis (the development of new lymph vessels) from development through to the immune response and nervous system function. In addition, it introduces some exciting therapeutic applications that have recently been made available. We are indebted to all our authors.

We are pleased to acknowledge the support from our corporate sponsor Genentech and academic sponsors the NIH and the JDRF in producing this Insight. As always, *Nature* retains sole responsibility for editorial content and peer review.

Natalie DeWitt, Senior Editor

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nature insight

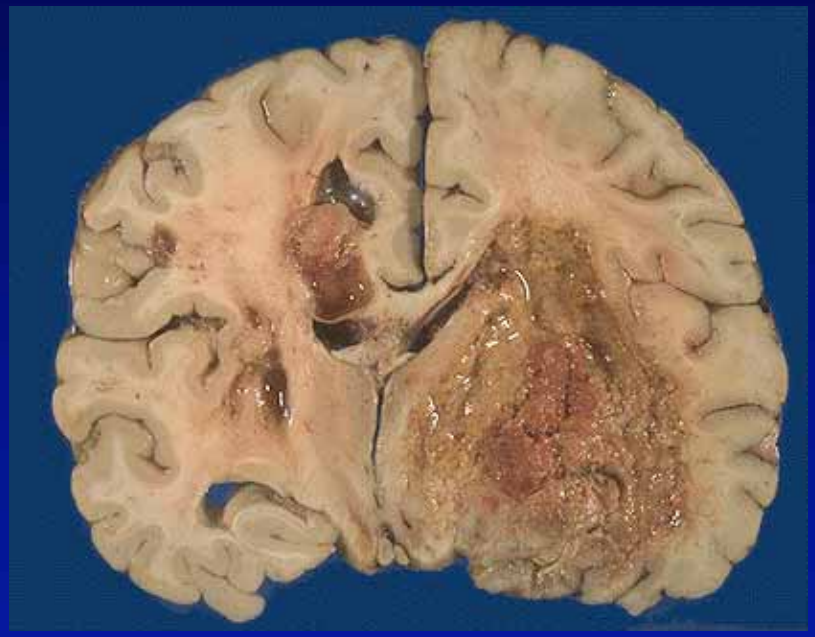
Angiogenesis

Topics:

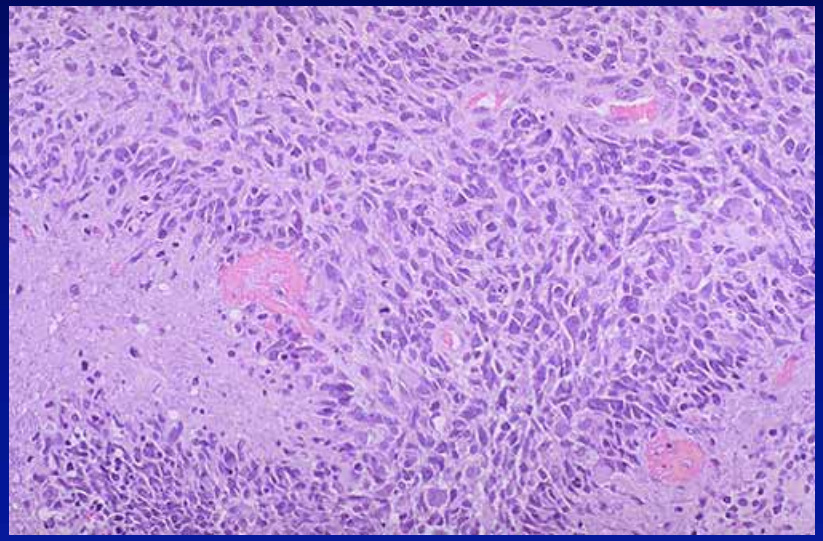
1. Vasculogeneis & Angiogenesis
2. Tumor angiogenesis
3. VEGF & VEGFRs - structure & function
4. Physiological roles for VEGF, VEGFR
5. VEGF, VEGFR in tumor angiogenesis
6. Anti-angiogenic therapy

Glioblastoma multiforme

- most common form (50-60%) of primary brain cancers of all ages
- few pts survive more than 3 yrs
- prognosis has not changed in the last 20 yrs



- vascular
- necrotic
- hemorrhagic



- marked cellularity
- vascular
- necrotic

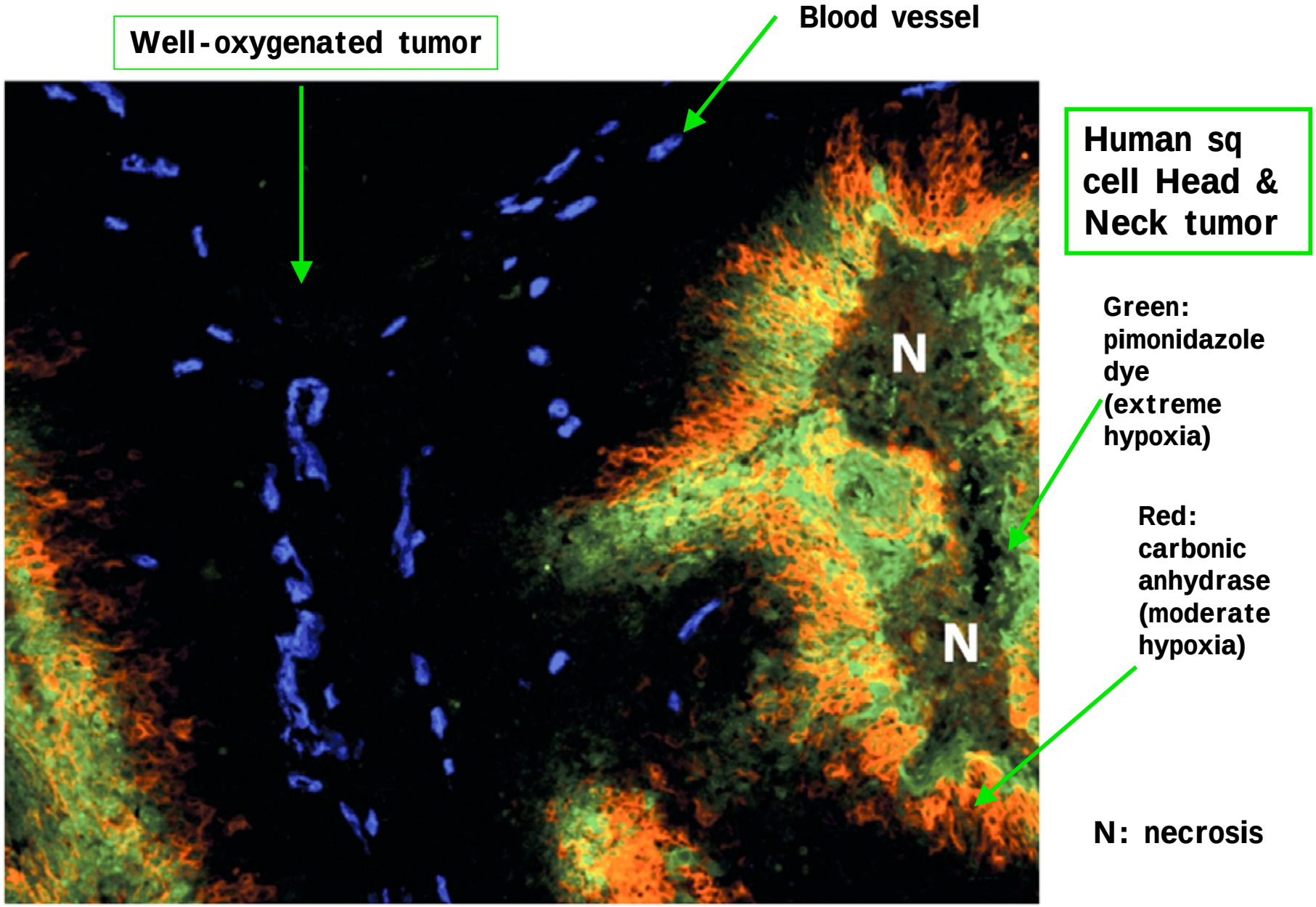


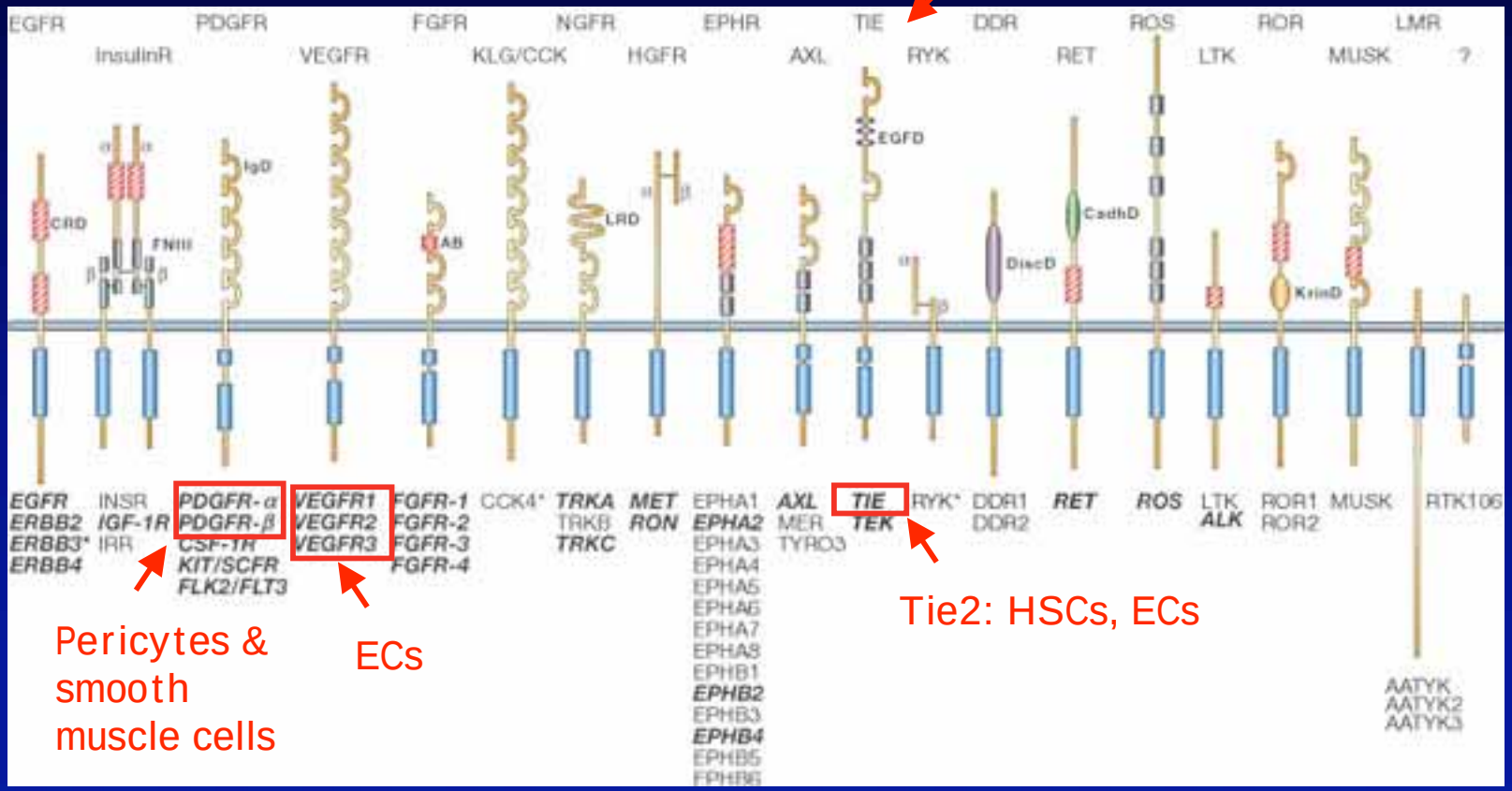
Figure 13.27c *The Biology of Cancer* (© Garland Science 2007)

Can we target the neovascularization in GBM and other cancers?

Receptor tyrosine kinases

Blume-Jensen & Hunter, Nature 411:355, 2001

Angiopoietins:Ang1, Ang2



Selected signal transduction inhibitor drugs

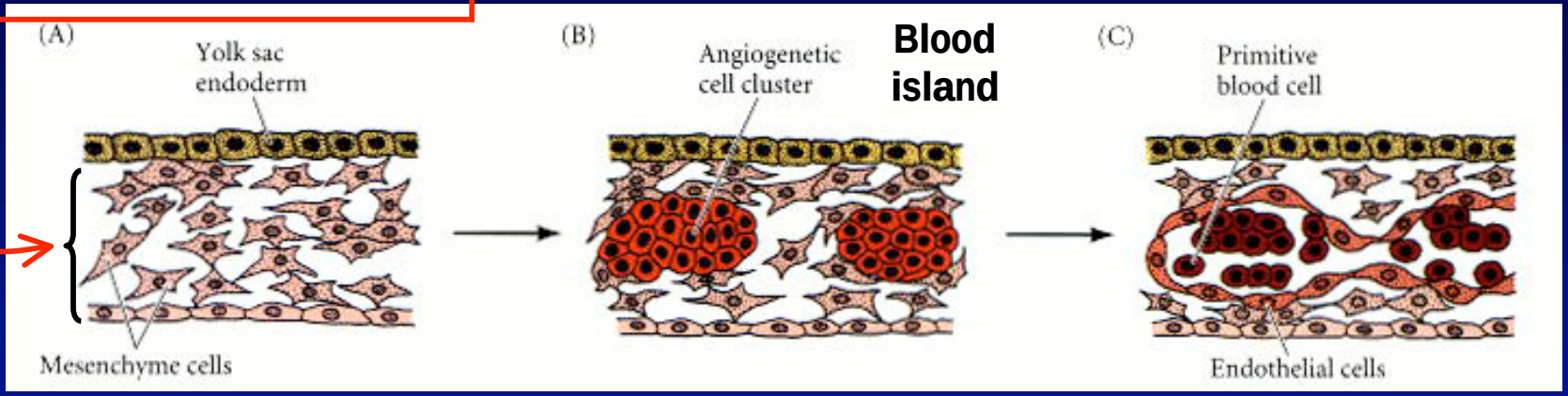
Target	Agent	Indication	Company	Stage
Bcr-Abl	Gleevec, sm	CML, GISTs	Novartis	Registered
Her2	Herceptin, Mab	Breast CA	Genentech	Registered
EGFR	Iressa, sm	NSCLC	AstraZeneca	Reversed
EGFR	Erbitux, Mab	Head and Neck	Imclone/BMS	Phase 3
EGFR	Tarceva, sm	NSCLC, pancreatic	Genetech/OSI/Roche	Approved
Ras	R11577, FTI	Lung Cancer	Johnson & Johnson	Phase 3
mTOR	Rapamycin	Immunosuppression	Wyeth	Registered
PKC	Affinitak, AS	NSCLC	Isis/Lilly	Phase 3
PKC-beta	Ruboxistaurin, sm	Diabetic Retinopathy	Lilly	Phase 3
VEGF	Avastin, Mab	Colorectal Cancer	Genetech	Approved
VEGF	PTK787/ZK222584	Metastatic Cancer	Novartis/Schering	Phase 3
VEGFR (multi-kinase	SU11248-Sutent	Renal cell, GIST	Pfizer	Approved
VEGF	Neovastat	NSCLC	Aeterna	Phase 3
EGF	ABX-EGF. Mab	Colorectal, NSCLC	Abgenix	Phase 2
EGFR	TheraCIM. Mab	Head and neck	YM Biosciences	Phase 2
MLK	CEP1347, sm	Parkinson disease	Cephalon	Phase 2
TK	CEP701, sm	Pancreatic	Cephalon	Phase 2
CDK	Flavopiridol, sm	Solid tumors	Aventis	Phase 2
VEGF	VEGF Trap (decoy)	non-Hodgin's lymphoma	Regeneron/Aventis	Phase 1
VEGFR, multi-kinase	BAY4309006 (sorafen	renal cell cancer	Onyx-Bayer	Phase 3

Blood vessel development

- Vasculogenesis
  - the de novo formation of blood vessels
  - first organ system to develop in the embryo
  - many different cell types are involved:
    - ECs, smooth muscle cells, pericytes, basement membrane components
- Angiogenesis
  - process of remodelling an established primitive network of blood vessels
  - still contributes to organ growth after birth but quiescent in adults except in the cycling ovary and in placenta during pregnancy
  - normal physiological stimuli: tissue regeneration and inflammation
  - tumor angiogenesis may involve recruitment of bone marrow-derived endothelial precursors (co-expression of CD34 & VEGFR2)
  - implicated in various pathophysiological conditions e.g. tumors, rheumatoid arthritis and proliferative retinopathies

Vasculogenesis

Vascular progenitors migrating from the primitive streak of the embryo proper into extraembryonic tissues

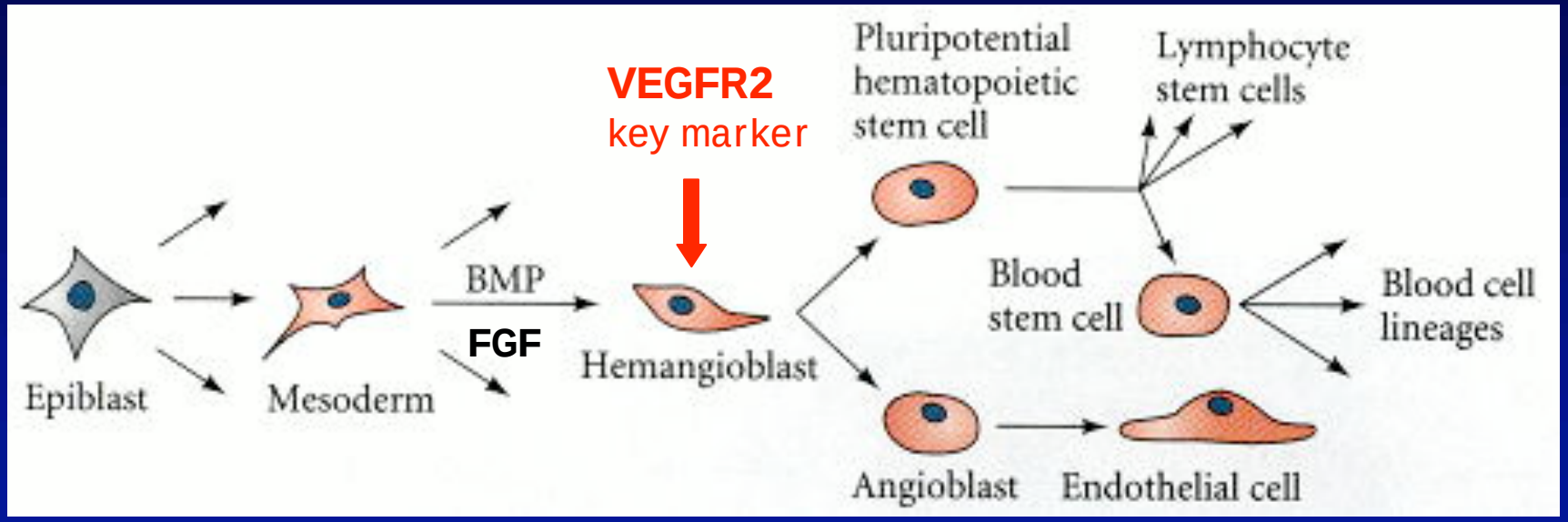


From "Developmental Biology" by S. Gilbert, 6th edition, online through PubMed

Vasculogenesis also occurs in the embryo proper and gives rise to the dorsal aorta and cardinal veins. These fuse with those derived from the blood islands.

Additional reading: Ferkowicz & Yoder, Exp Hematol 33:1041, 2005

Common origin of the blood and vascular systems: the hemangioblast

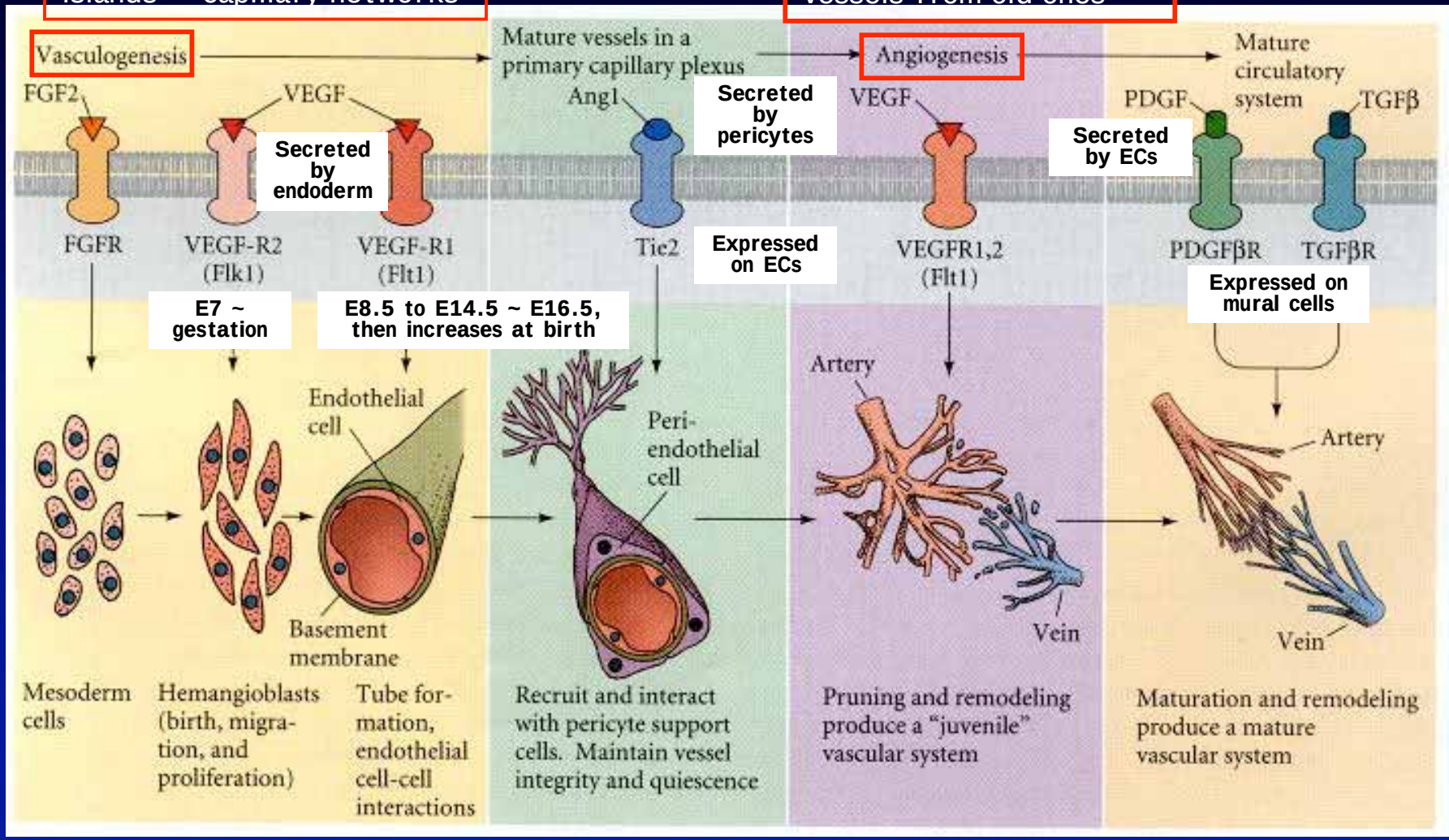


From "Developmental Biology" by S. Gilbert, 6th edition, online through PubMed

Vasculogenesis & angiogenesis

Hemangioblastic blood islands => capillary networks

Formation of new blood vessels from old ones



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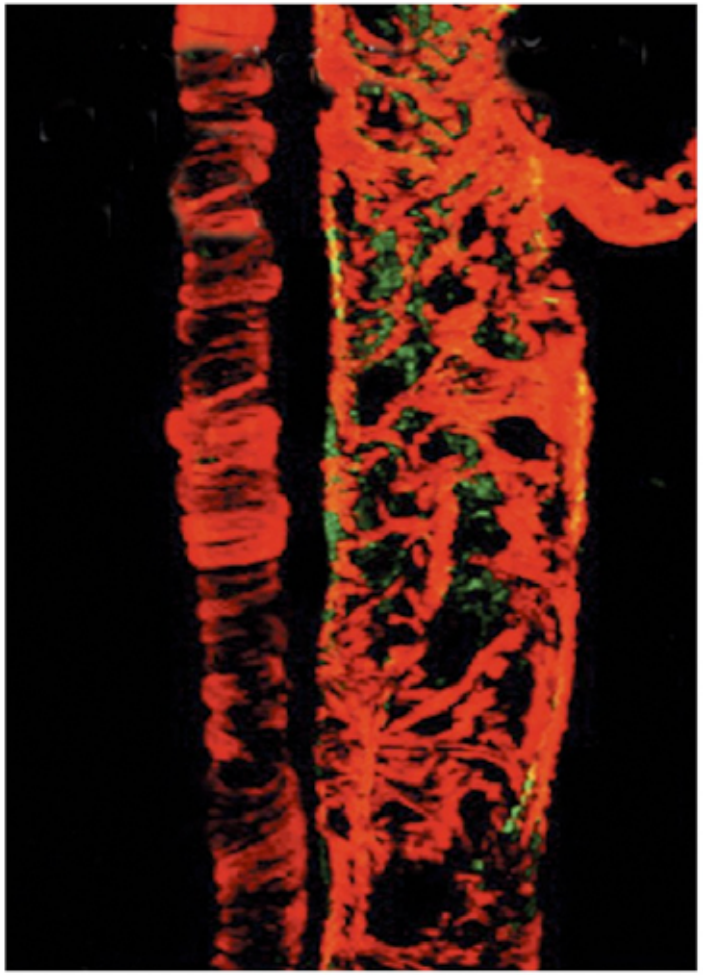
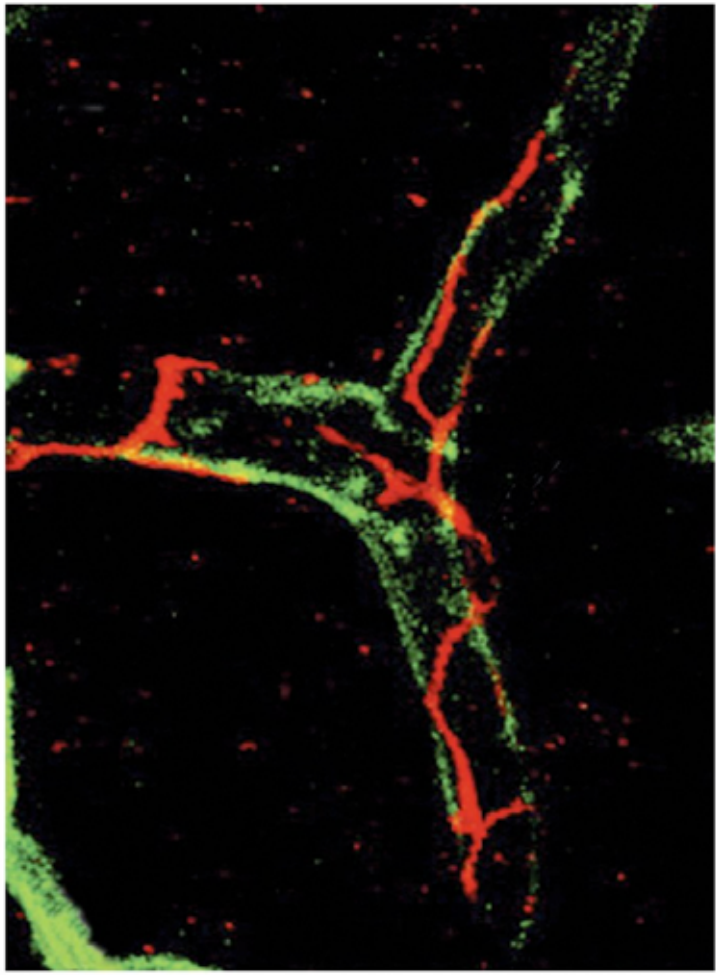




Red: pericytes & SMC

Green:

ECs

Arterioles & venules

capillaries

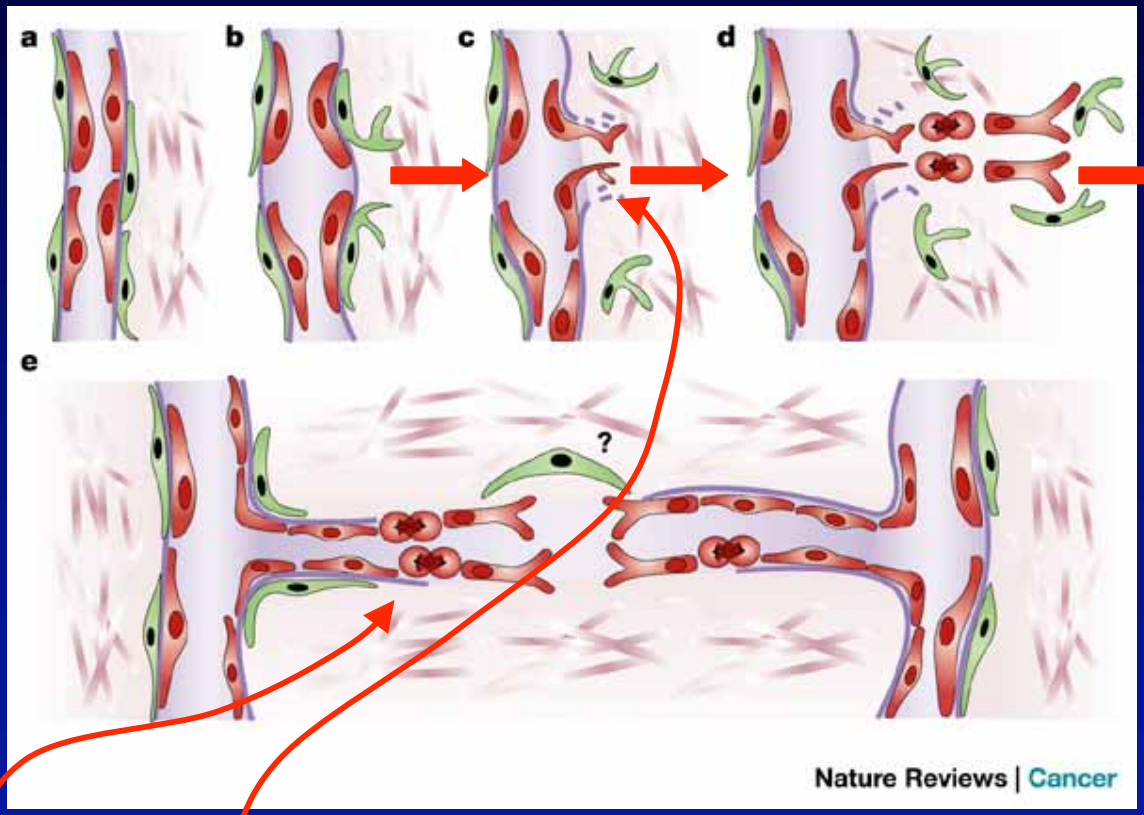
Figure 13.6b *The Biology of Cancer* (© Garland Science 2007)

Endothelial cells

- high proliferative capacity in the embryo
- > 1000 days cycling time in the adult (i.e. vasculature is quiescent under normal conditions)
- retain their high proliferative capacity so that they can resume rapid division when called upon (e.g. tumor angiogenesis -- ECs may cycle as rapidly as a week)

Formation of new blood vessels from existing capillaries or venules

Bergers & Benjamin Nat. Rev. Cancer 3:401, 2003



b: pericytes detach and BVs dilate

c: EC cells secrete proteases (e.g. MMPs) to degrade BM and ECM

d: ECs migrate into the perivascular space towards angiogenic stimuli (e.g. tumor cells)

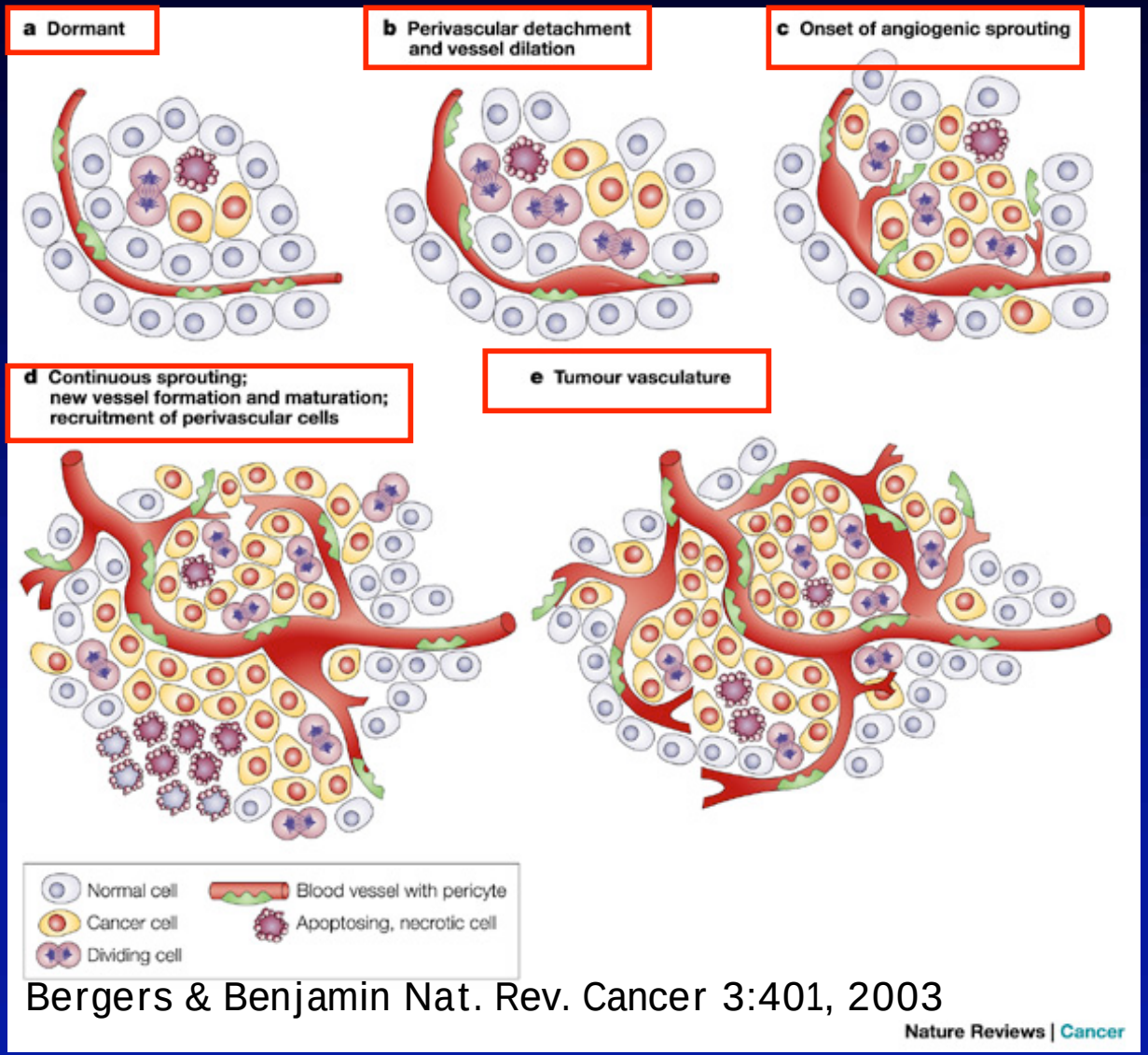
e: Behind the migrating column, ECs adhere to each other to create a lumen, w/ BM formation and pericyte attachment

BV sprouts fuse with each other to build new BVs => blood flows

ECs secrete PDGF, TGF to attract pericytes

new basement membrane (BM) is highly specialized: collagen IV, laminin, fibronectin

Tumor angiogenesis - the angiogenic switch



Bergers & Benjamin Nat. Rev. Cancer 3:401, 2003

- growth of solid tumors beyond 1-2 mm requires a new blood supply
- angiogenic switch occurs
  - when there is a steady state of proliferating and dying cells
  - when the balance between inducers and inhibitors of angiogenesis is offset by secretion of angiogenic factors
- Rip-Tag mouse
  - makes large amts of VEGF before switch but sequestered by ECM;
  - Switch coincides w/ secretion of MMP, degrading ECM

Tumor angiogenesis - timeline

1800's - Virchow reported an association between tumor growth and vascularization

1927 - Lewis (Johns Hopkins) observed that different tumors in rats have different levels of vascularization

1939 - Ide (Rochester) postulated that tumors secrete factors that stimulate blood vessel growth

1945 - Algire (NCI) proposed that rapid growth of tumor explants is dependent on the development of a rich blood supply

1971 - Folkman (Harvard) proposed angiogenesis inhibitors for treatng cancer

1973 - first successful culture of human ECs in vitro

1980 - Folkman reported the first endogenous angiogenesis inhibitor (IFNα/β)

1983 - Senger (Beth Israel) identified and partially purified a vascular permeability factor made by tumor cells (later shown to be VEGF)

1989 - VEGF purified and cloned (several groups)

1992 - first VEGF receptor (Flt1) was cloned

Earliest in vivo image of tumor angiogenesis. A rabbit epithelioma was transplanted into a rabbit's ear using a transparent chamber. This picture was taken 8 days after transplant.

Ide et al. Am. J. Roentgenol. 42:891, 1939.

Judah Folkman

At age 34, he became chief of surgery at Boston's Children's Hospital. As he observed hundreds of bloody tumors in juvenile cancer patients, he began to wonder if there was a way to stop the growth of tumors by blocking the process, called angiogenesis, by which new blood vessels develop to feed the cancerous growth.

Folkman's hypothesis met with indifference and ridicule for 20 years.....

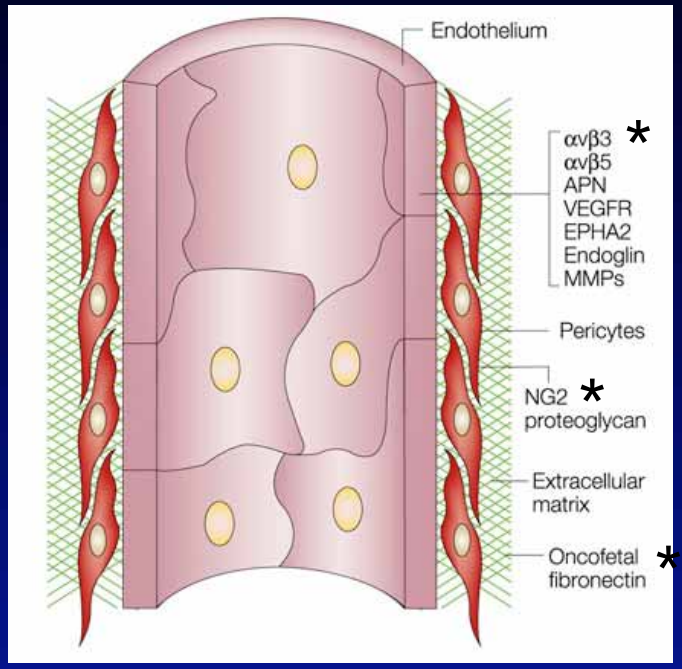
"Rarely in the history of modern biomedical research has a major advance been attributable directly to the energies and vision of a single individual. This is such a story, about one man's vision, drive, indeed obsession with an idea that will one day dramatically change cancer therapy." ROBERT A. WEINBERG, WHITEHEAD INSTITUTE FOR BIOMEDICAL RESEARCH AND MIT

FYI: **http://www.pbs.org/wgbh/nova/cancer/folkman.html**

Tumor angiogenesis - characteristics

- cannot grow w/o a blood supply; induces "angiogenic switch"
  - respond to hypoxia, induces expression of VEGF
- tumor blood vessels are abnormal: tortuous, leaky and surrounded by too few pericytes
  - "wounds that do not heal"
- have distinct markers
  - elevated VEGFR1 and VEGFR2 in ECs of tumors
  - upregulated αVβ3 and αVβ5
  - selective expression of α5β1 (fibronectin receptor)
  - many other differences
- lymphatics are an important conduit of metastasis; present in some but not all tumors
- differences between normal & tumor vasculature can be therapeutically exploited

Ruoslahti Nat. Rev. Cancer 2:83, 2002



Wall of a blood vessel; markers (*) indicated are selectively expressed in angiogenic blood vessels of tumors

- APN - aminopeptidase N
- Endoglin - cell surface protein that binds TGFβ and MMPs
- NG2- proteoglycan, marker of angiogenic pericytes
- Oncofetal fibronectin - selectively expressed in ECM of angiogenic vessels

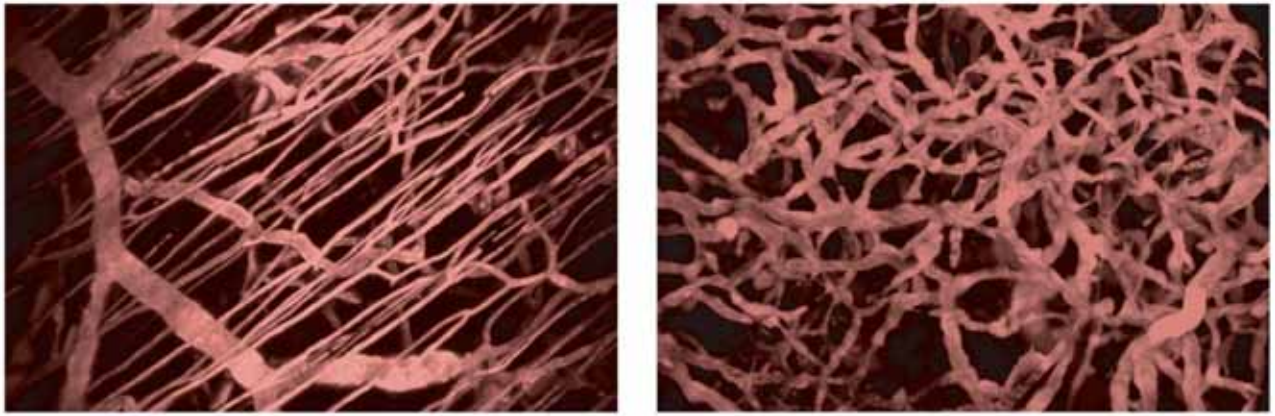


Figure 13.34b *The Biology of Cancer* (© Garland Science 2007)

Microvessel in a lung cancer in a mouse:

ECs (green)

Pericytes, SMCs (red)

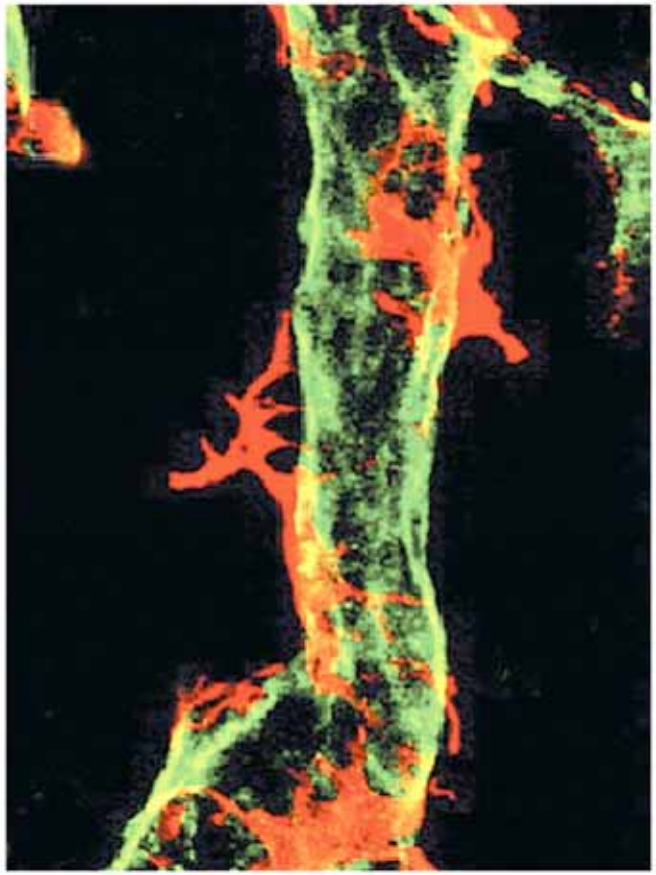
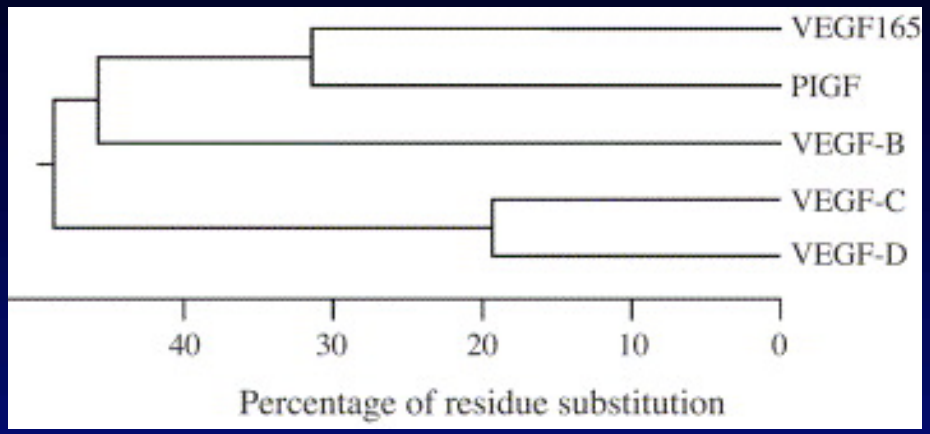


Figure 13.33 *The Biology of Cancer* (© Garland Science 2007)

VEGF isoforms



VEGF family members (different genes)

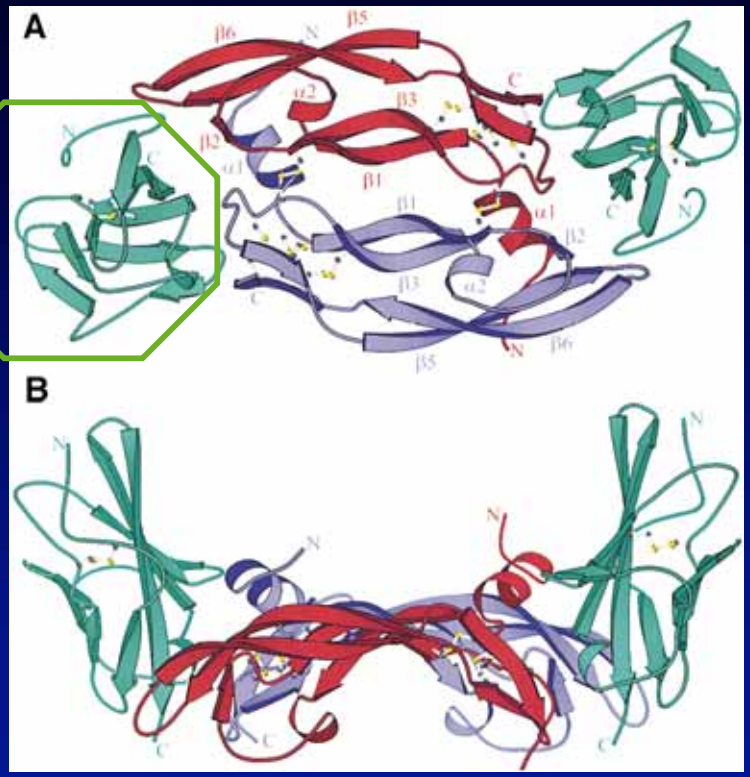
- VEGF (VEGF-A): main hypoxia-inducible angiogenic factor
  - 6 alternative splice isoforms
- VEGF-B: function is not clear; ko mice have smaller hearts
- PlGF (placental growth factor): mediates arteriogenesis
- VEGF-C
- VEGF-D

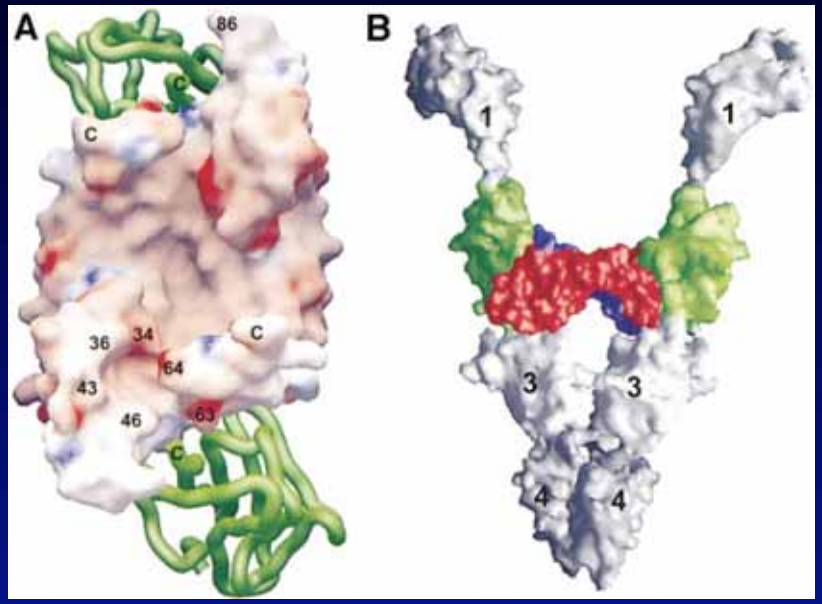
} primarily lymphangiogenic factors

- VEGF-E (from a parapoxvirus Orf, most similar to $VEGF_{121}$)

The VEGF-VEGFR complex

VEGFR1





Wiesmann et al.
Cell 91:695, 1997

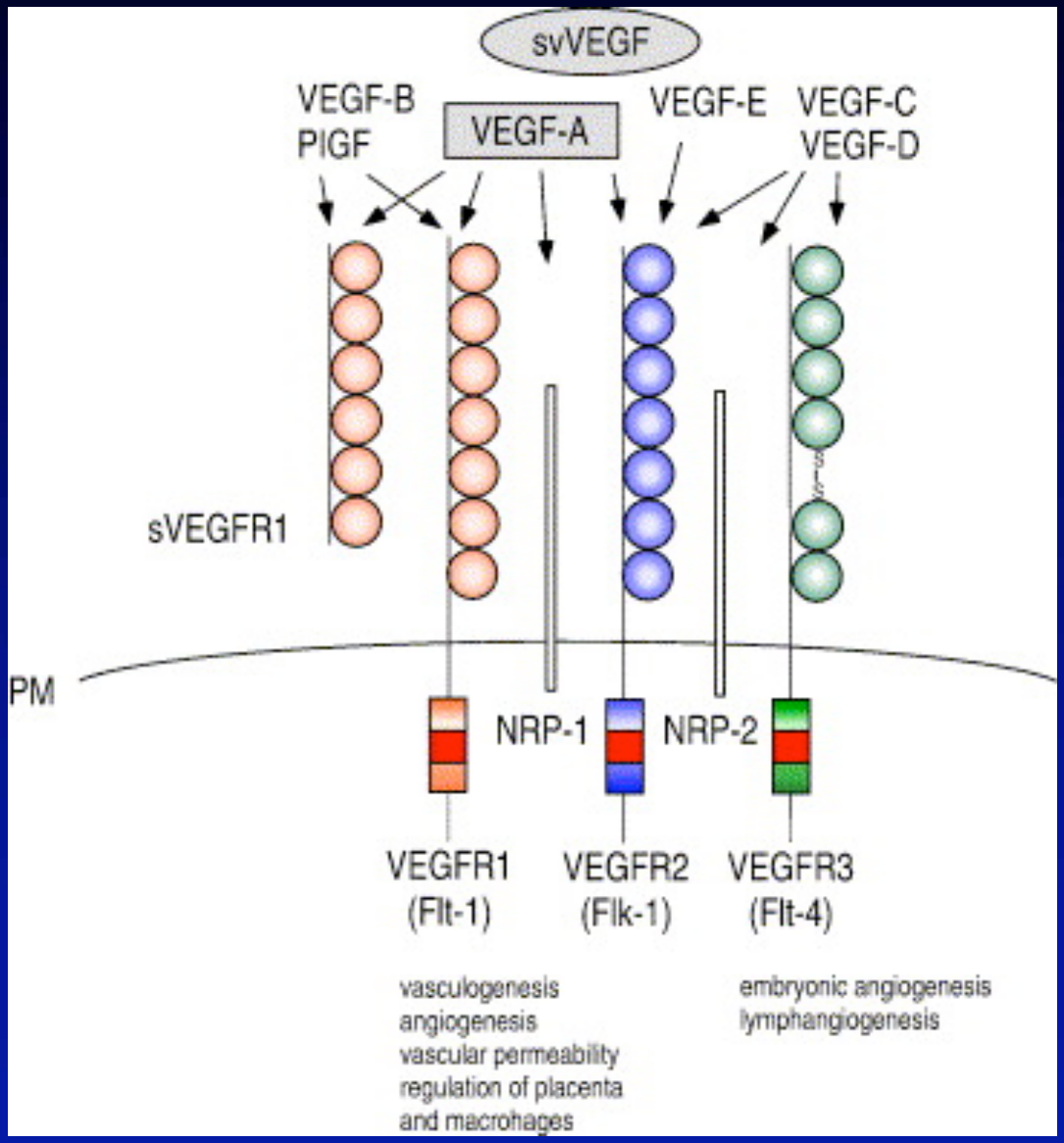
VEGF monomer 1

VEGF monomer 2

VEGFR domain 2

- complex of VEGF (8-109) bound to domain 2 of VEGFR1
- ligand binding involves primarily domains 2 and 3 (D2 contributing the most)
- each receptor binds both ligands
- modeling puts adjacent D3 and D4 very close together suggesting the possibility of receptor-receptor interactions

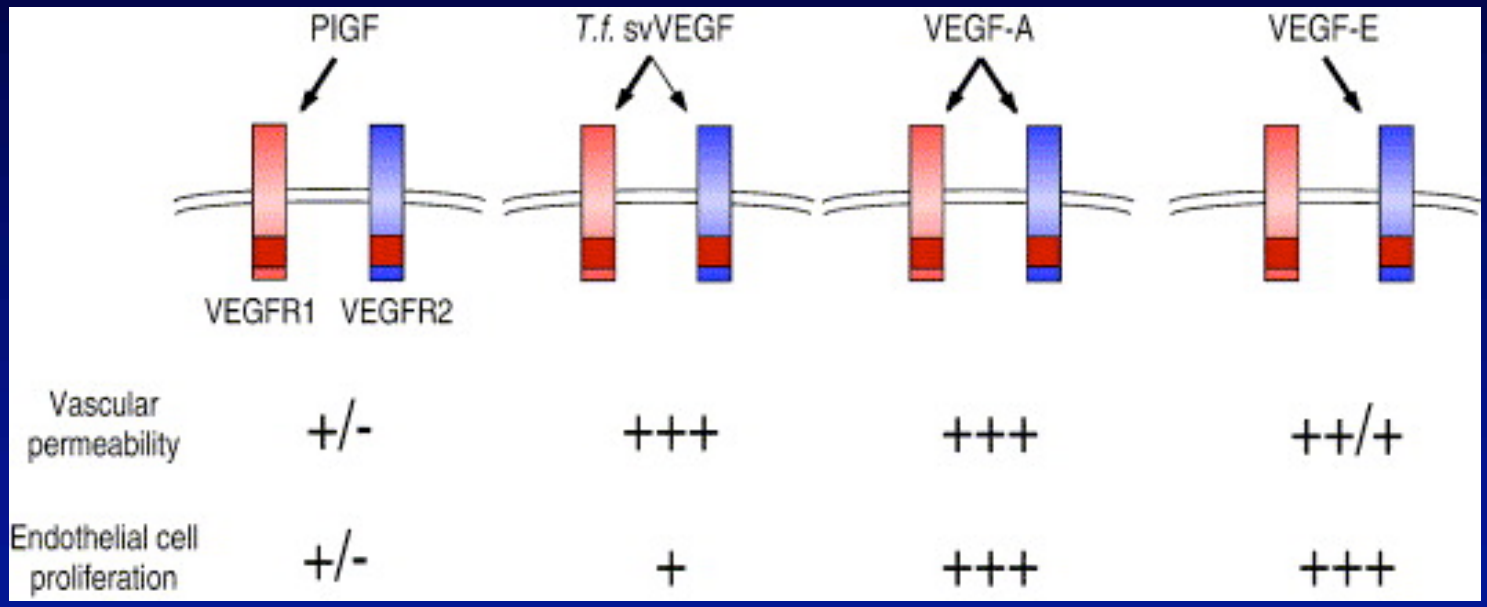
VEGF and VEGFR interactions



- 7 Ig domains (except for VEGFR3)
- kinase insert
- VEGF-A binds VEGFR1, 2
- VEGF-B & PIGF selective for VEGFR1
- VEGF-C, D selective for VEGFR3 (processed forms also bind VEGFR2)
- VEGF-E selective for VEGFR2
- svVEGF - snake venom VEGF, binds VEGFR1 primarily and to VEGFR2 w/ lower affinity

Shibuya & Claesson-Welsh, Exp Cell Res 312:546, 2006

VEGFR1 & VEGFR2 cooperate to generate maximal vascular permeability



Shibuya & Claesson-Welsh, Exp Cell Res 312:546, 2006

- All 3 VEGFRs are expressed to different levels on vascular ECs
- VEGFR2 is highly expressed on EC progenitors in early embryogenesis

VEGF receptors - developmental aspects

- VEGFR1 = Flt-1 (fms-like tyrosine kinase)
  - binds VEGF-A, B and PlGF
  - expressed on vascular ECs, osteoblasts, HSCs, myeloid cells, renal mesangial cells
  - expression upregulated by hypoxia
  - homodimerizes and heterodimerizes w/ VEGFR2
  - ko mice: die at E8.5-9, xs of hemangioblasts, overgrowth of ECs, abn. vasculature
- VEGFR2 = KDR (human) or Flk-1 (mouse, fetal liver kinase-1)
  - binds VEGF-A, C, D
  - expressed on vascular and lymphatic ECs, HSCs, megakaryocytes, neuronal cells, osteoblasts, retinal progenitors
  - key marker of the hemangioblast
  - expression not upregulated by hypoxia
  - ko mice: die by E9.5, defects in both hematopoietic and endothelial lineages, no organized blood vessels, impaired liver morphogenesis
- VEGFR3 = Flt-4
  - binds VEGF-C, D
  - expressed on all ECs during development, on lymphatic ECs in adults
  - expression not upregulated by hypoxia
  - ko mice: die by E9.5 due to cardiovascular failure, lots of diff. problems

Neurophilins: co-receptors for VEGFR - 1

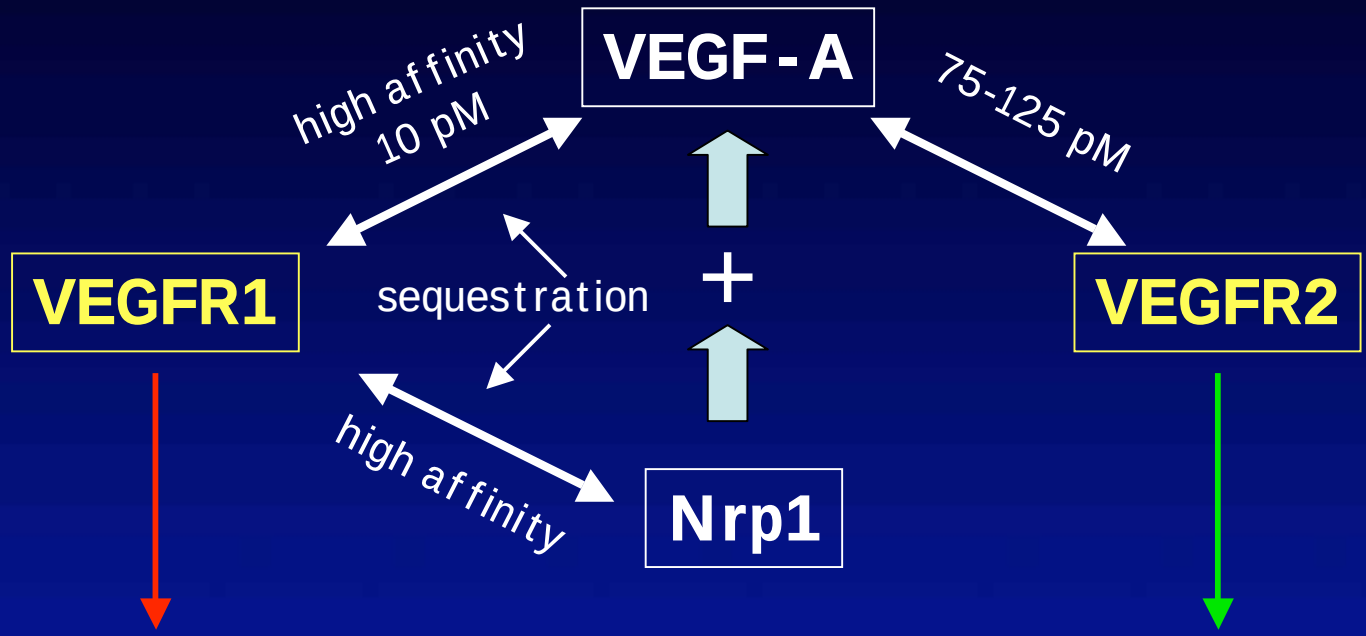
- Nrp1, Nrp2 widely expressed
- Nrp1 is artery-specific and Nrp2 is vein-specific
- cell surface molecules w/ short cytoplasmic tails
- best characterized as binding partners for semaphorins (axonal guidance)
- bind VEGF-A (but not $VEGF_{121}$), VEGF-B, PlGF & needs a part of the heparin binding region in VEGF
- present VEGF to VEGFR and increase affinity of binding and downstream signaling
- naturally occurring soluble Nrp1 can act as tumor antagonist (sequestering away VEGF)

Neurophilins: co-receptors for VEGFR - 2

during embryogenesis, VEGFR1 exerts a negative regulatory effect on VEGFR2 (could explain xs hemangioblast production in VEGFR1-/- mice)

- VEGFR1 can directly bind Nrp1 and sequester it away from VEGFR2
- a soluble form of VEGFR1 can sequester away VEGF-A

VEGFR1 & VEGFR2



inhibitory signals

weak kinase activity
no A-loop autoP
antagonizes VEGFR2
(PI3K dependent, mechanism unknown,
does not require
Heterodimerization)

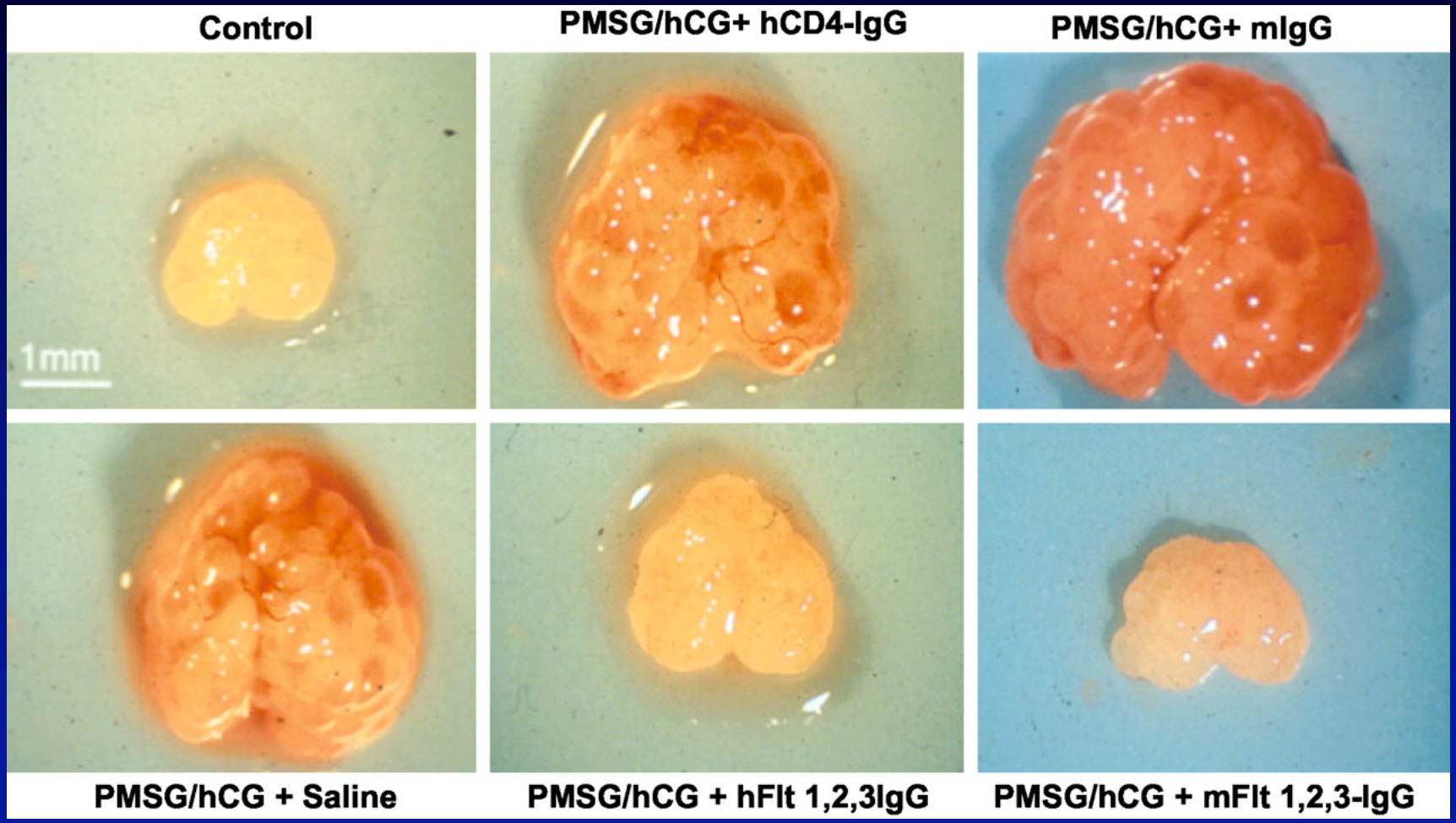
activating signals

strong kinase activity
A-loop autoP
migration
proliferation
survival
differentiation

VEGFs as angiogenic factors in normal physiology: what's the evidence?

- correlation between VEGF expression and vascularization of the corpus luteum
  - pre-ovulation: granulosa cells are avascular, low VEGF expression
  - post-ovulation: granulosa cells differentiate into the progesterone-producing luteal cells (corpus luteum) accompanied by extensive angiogenesis; high VEGF expression
  - corpus luteum angiogenesis blocked by a dominant-negative VEGFR
- temporal & spatial correlation between VEGF expression and blood vessel growth during embryogenesis
- high affinity VEGF binding sites restricted to vascular endothelium of large/small vessel
- VEGF-A +/- and -/- mice are embryonic lethal
  - impaired angiogenesis and blood island formation, growth retardation & developmental abormalities

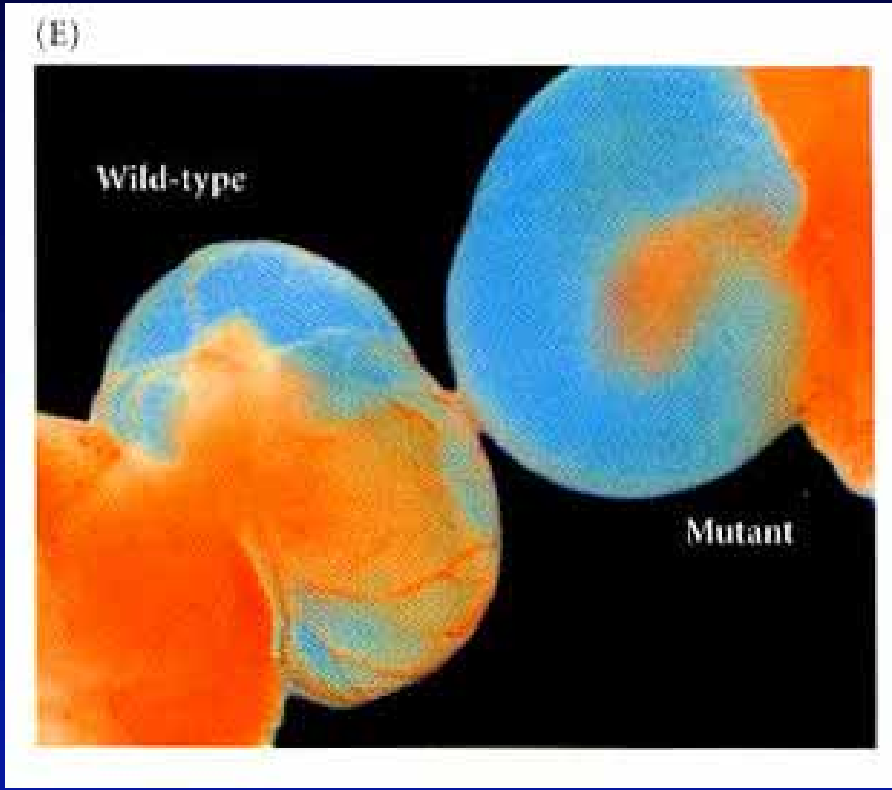
VEGF is essential for corpus luteum angiogenesis



PMSG = pregnant mare's serum gonadotropin

Ferrara, Endo Rev 25:581, 2004

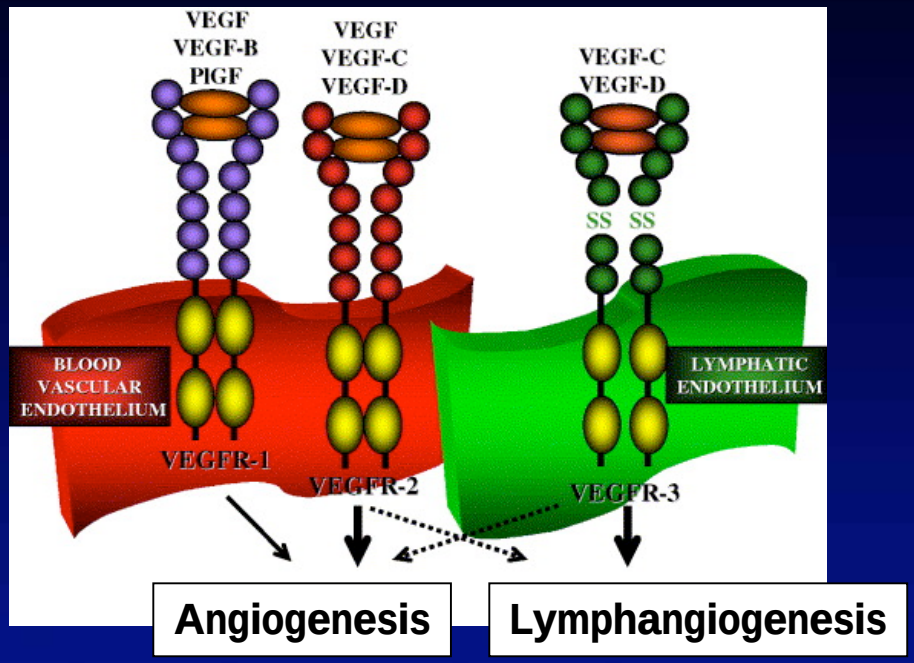
Yolk sac of E10.5 VEGF +/+ and VEGF +/- mouse embryos



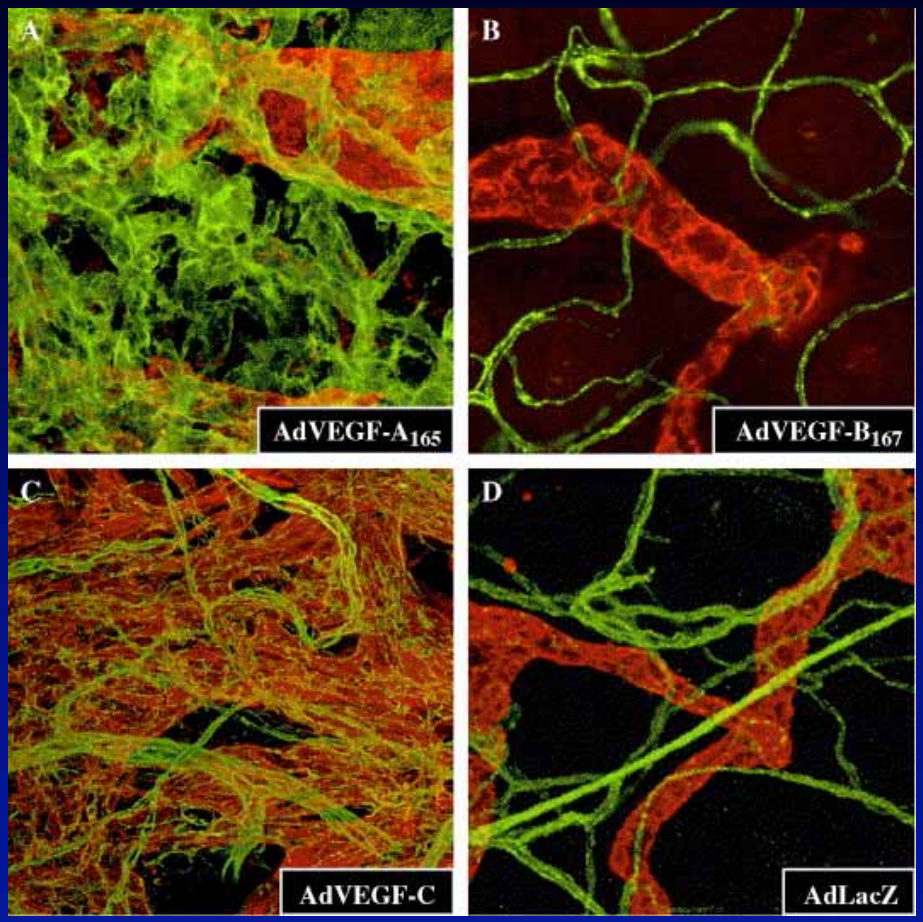
haploinsufficiency

Ferrara & Alitalo Nat. Med. 5:1359, 1999

VEGFs & their actions



Tammela et al. Cardiovasc. Res. 65:550, 2005

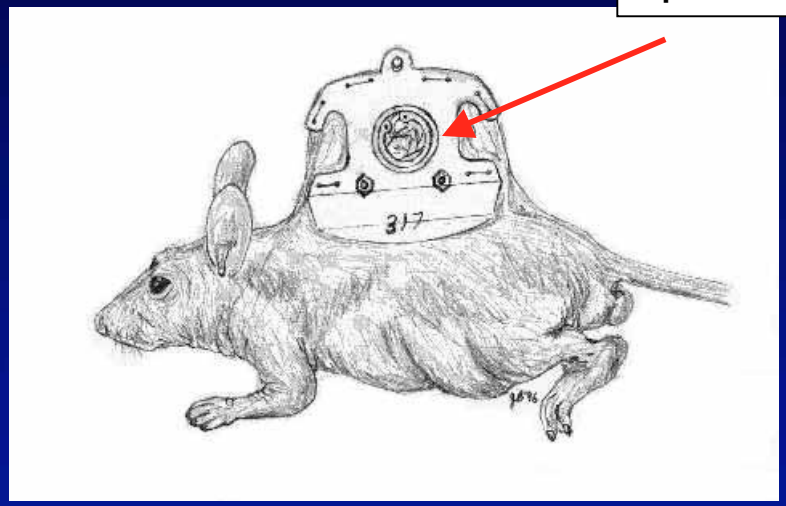


- adenoviruses expressing various VEGFs were injected into the skin of mice and examined after 2 weeks
  - blood vessels: green; lymphatics: red

VEGF as a tumor angiogenesis factor - what's the evidence?

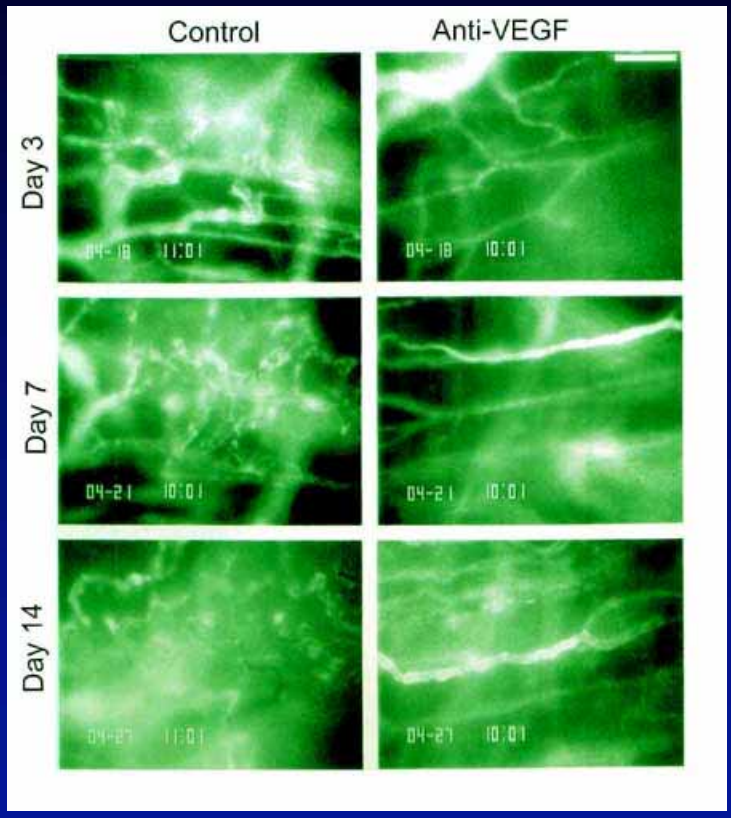
- most if not all tumors upregulate VEGF mRNA and protein
- GBM and other tumors assoc. w/ significant necrosis:
  - VEGF is highest in hypoxic areas adjacent to necrotic areas
- stroma can also secrete VEGF
- effect of VEGFR2 inhibitor PTK 787
  - 41% reduction of tumor volume in a nude mouse model for high grade thyroid CA
- effect of neutralizing anti-VEGF antibodies (next slide)
  - no effect on growth rates of tumor cells in vitro
  - inhibited tumor growth when injected into mice: tumors show decreased blood vessel density
  - inhibited tumor spheroid growth using the transparent chamber model

Inhibition of tumor angiogenesis with anti-VEGF Abs



Cartoon of the dorsal skinfold chamber adapted to nude mice

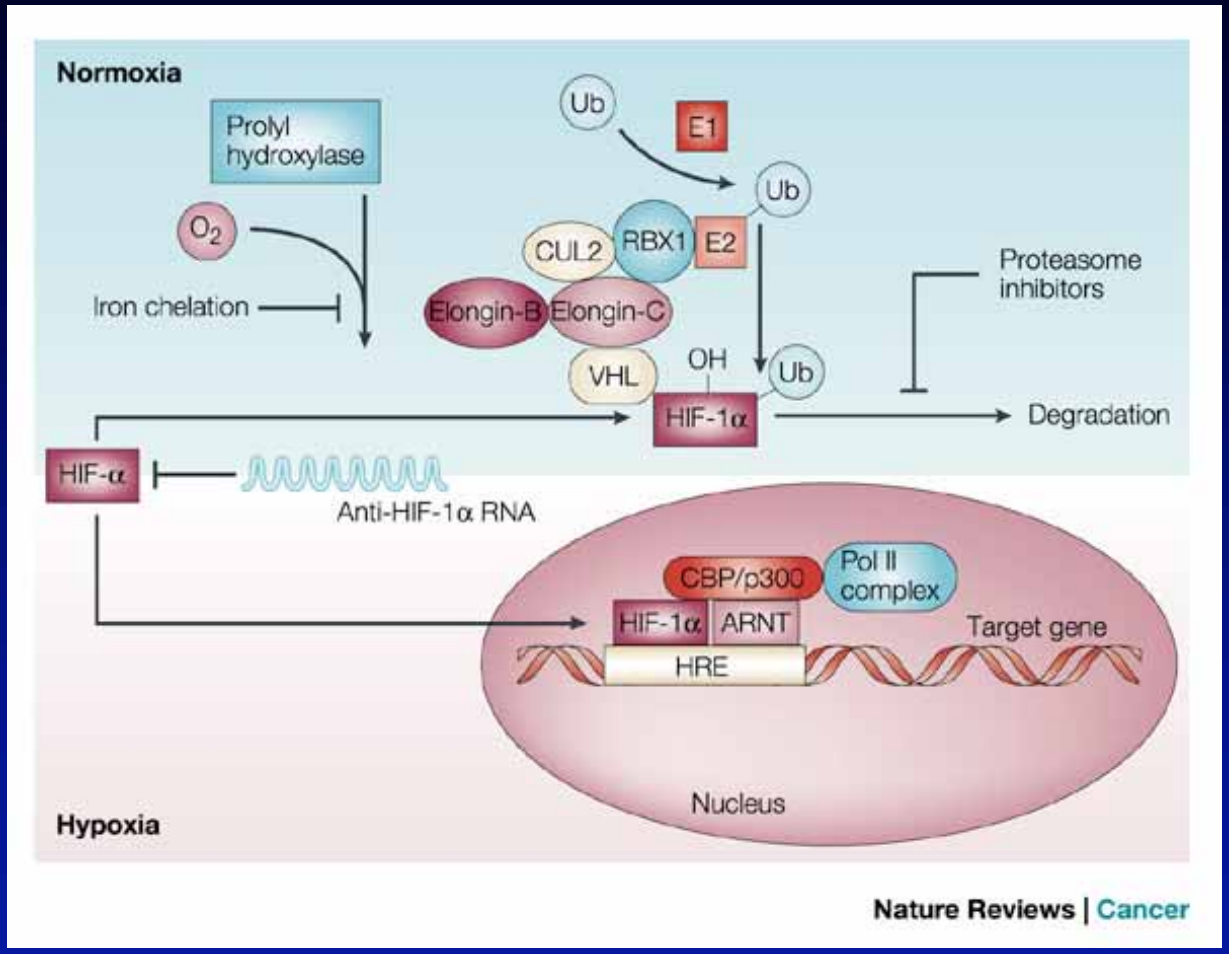
The dorsal skinfold chamber technique was developed already in 1943 by Algire. (Algire, G.H. (1943). An adaptation of the transparent chamber technique to the mouse. J. Natl. Cancer Inst. 4, 1-11.).



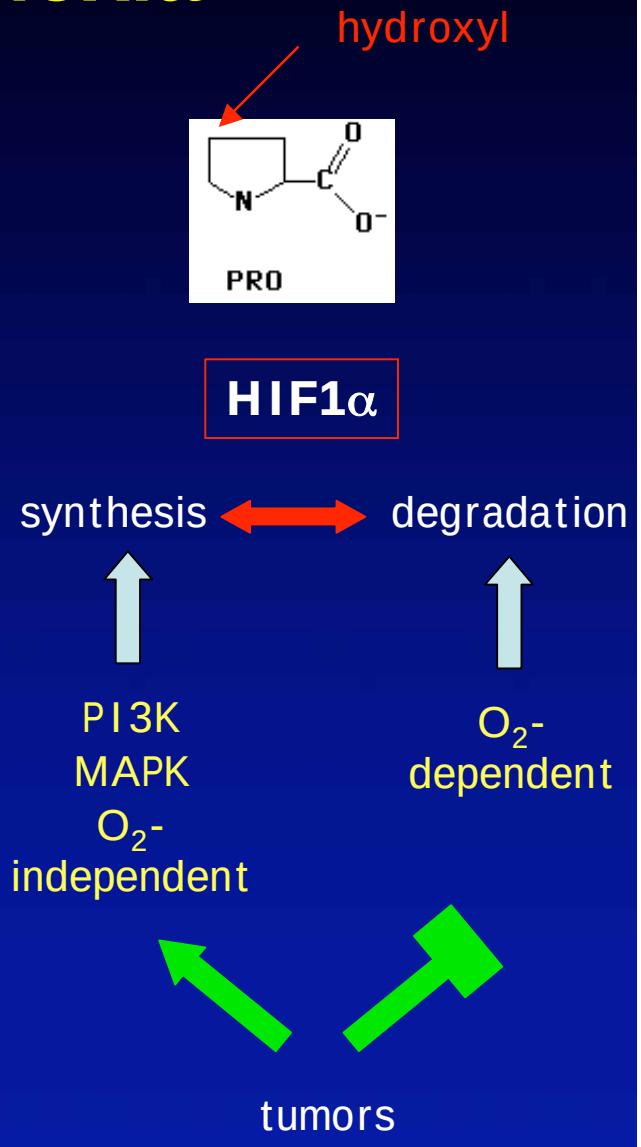
Per Borgstrom, Sidney Kimmel Cancer Center

- Implantation of DU 145 prostate cancer cells using the transparent chamber model
- visualization by intravital videomicroscopy

HIF1α pathway in normoxia

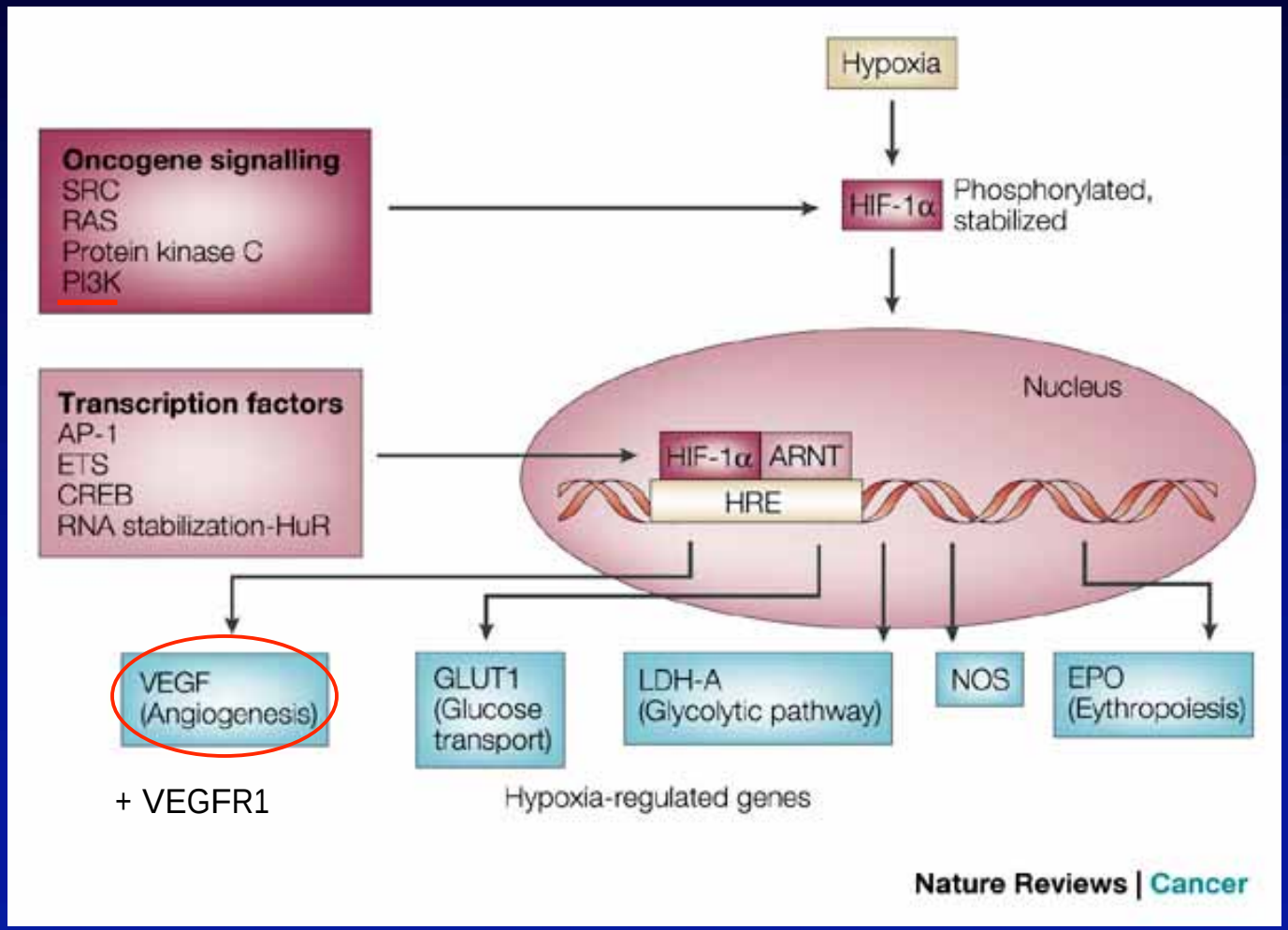


Harris Nat. Rev. Cancer 2:38, 2002



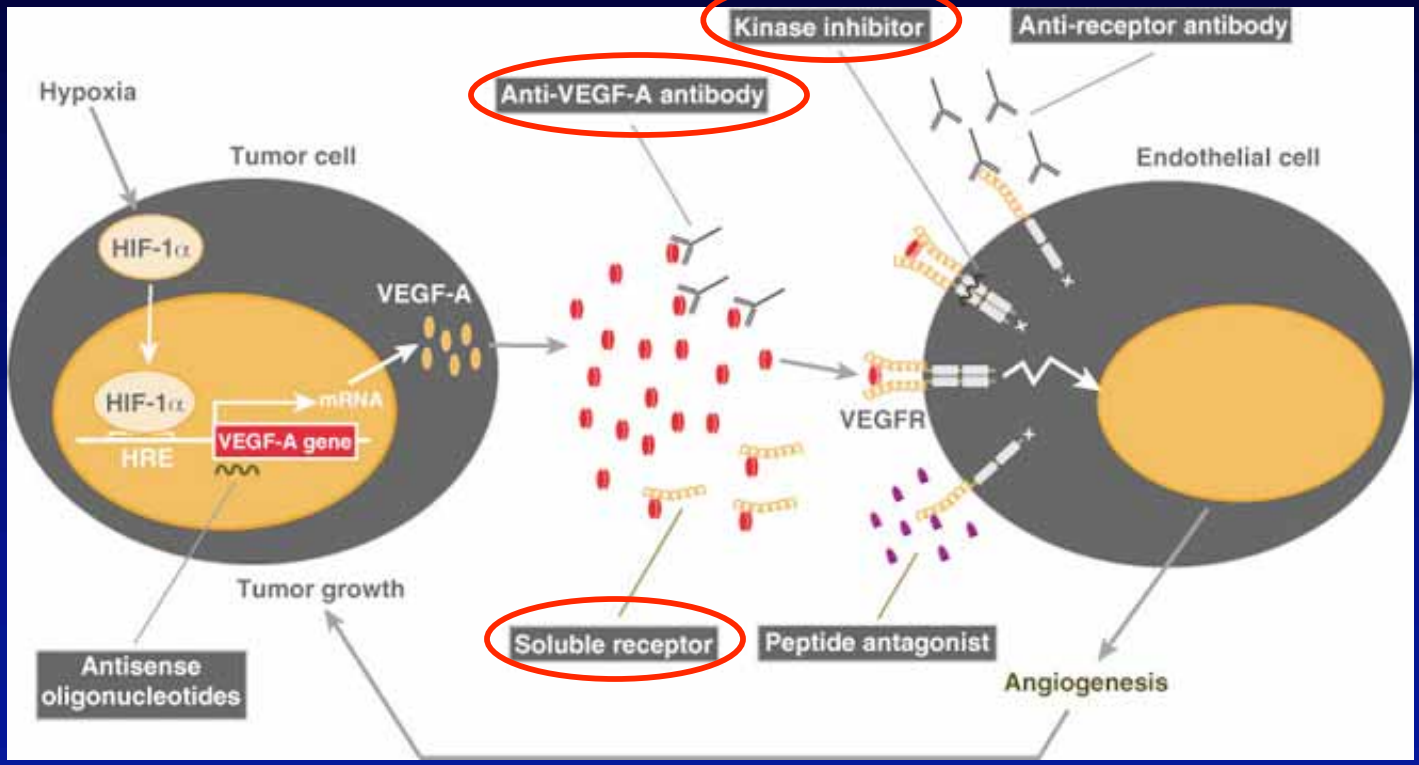
VEGF expression under hypoxic conditions

Harris Nat. Rev. Cancer 2:38, 2002



HIF1α is also a therapeutic target

How to target tumor angiogenesis via the VEGFs?



Matsumoto & Claesson-Welsh, STKE 2001

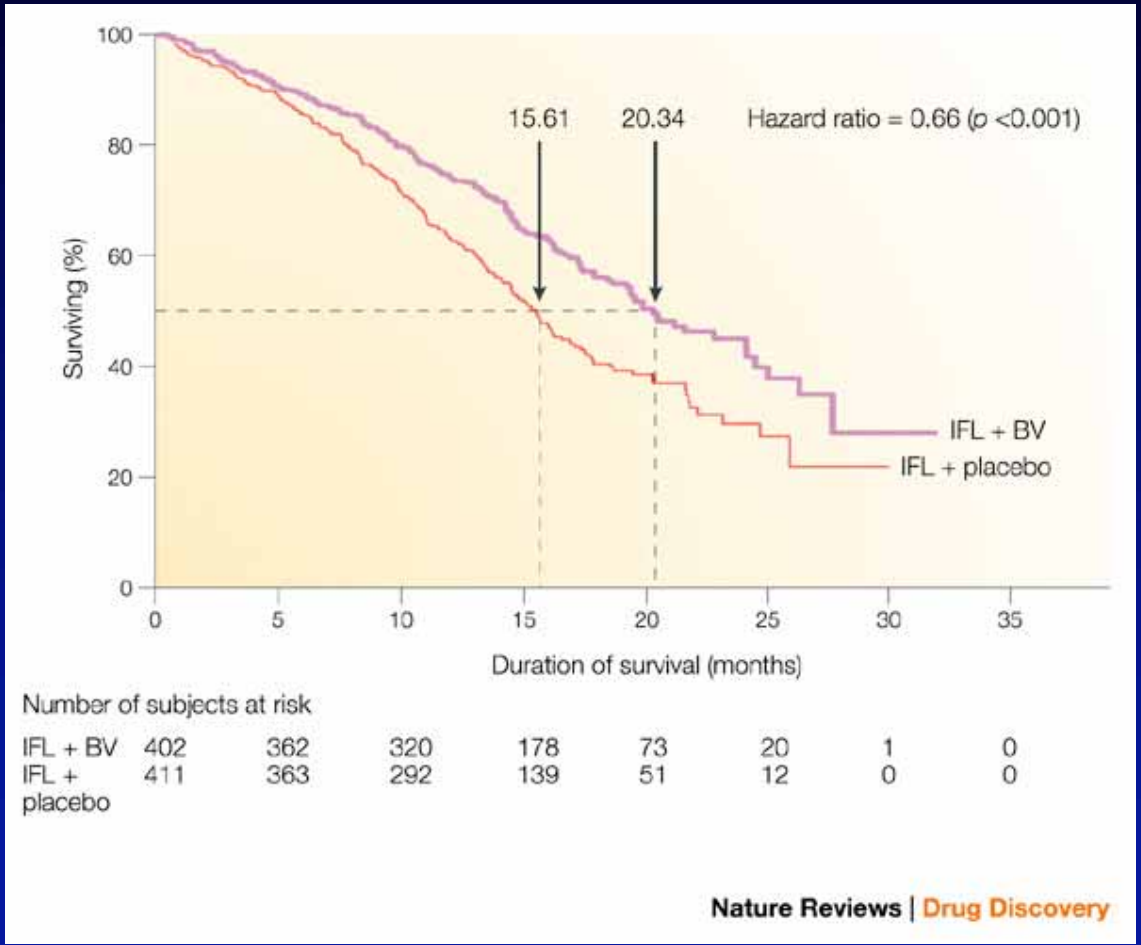
Bevacizumab (Avastin) - anti-VEGF Mab

- first developed in 1993 as a mouse anti-human Mab
- markedly inhibited proliferation of cancer cell lines and tumors using the nude mouse model
- humanized version developed in 1997
  - does not induce antibody response in pts
- neutralizes all human VEGF-A isoforms but not VEGF-B, C, D, E
- long half life = 17-21 days (dosing every 2 weeks)
- side effects in animal models
  - in young adult monkeys: growth plate dysplasia (inhibition of vascular invasion of growth plate)
  - in females: decreased ovarian function, absence of corpus luteum
- side effects in pts
  - well tolerated overall
  - may be an increased incidence of arterial thrombosis
  - significant side effect: 2% incidence of GI perforation when used with chemo
- IND filed in 1997, FDA approval in 2004
- estimated market: $US 3.5-4 billion (Nat. Rev. Drug Discovery 3:95, 2004)

Bevacizumab - clinical trials (1)

- **metastatic colorectal CA**
  - rationale
    - correlation between angiogenesis (as measured by microvessel counts) and worse prognosis
    - correlation between expression of pro-angiogenic factors (e.g. VEGF) with increased vascularity, advanced disease, worse prognosis
  - results of a phase 3 trial (other phase 3 trials ongoing)
    - compared standard therapy (5-FU + Leucovorin) +/- Bevacizumab
    - randomized, double-blinded, placebo-controlled
    - overall survival increased from 15.6 to 20.3 months ($p<0.001$)
  - mechanism of action
    - inhibition of blood vessel formation
    - normalization of vasculature - decrease in interstitial pressure, better cytotoxic drug delivery (may explain why there is lack of activity as single agent)

Bevacizumab in metastatic colorectal CA

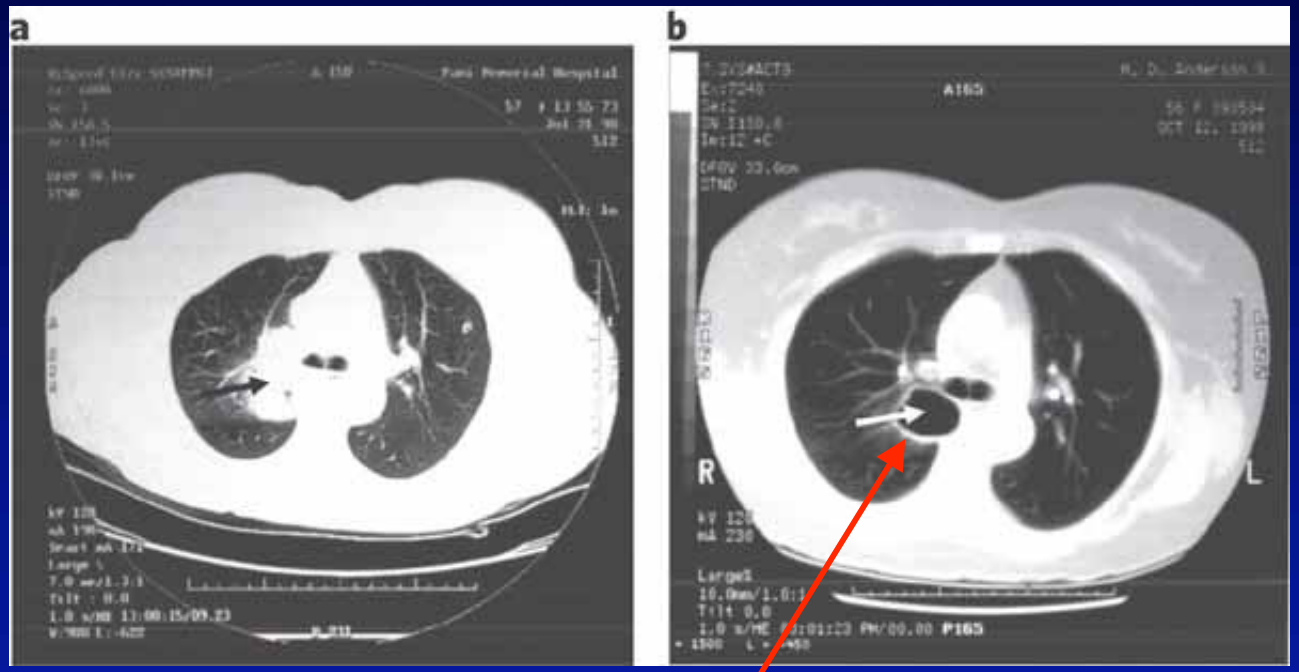


Bevacizumab - clinical trials (2)

- metastatic renal cell carcinoma
  - frequently (50%) have VHL mutations, high VEGF levels
  - Phase 2 data:
    - as single agent, limited benefits: see sig. increase in time to progression but no increased survival
    - in combo w/ Tarceva, are seeing promising results (Hainsworth, J, Sosman J, Spigel D , et al. Phase II trial of bevacizumab and erlotinib in patients with metastatic renal carcinoma (RCC). Proceedings from the 40th annual meeting of the American Society of Clinical Oncology. New Orleans, LA. June 2004. Abstract #4502)
  - Phase 3 trials: ongoing

Sutent (multikinase inhibitor) was recently approved for renal cell carcinoma

Bevacizumab + Chemo in NSCLCs



Tumor mass underwent extensive necrosis & cavitation

Outlook

- progression occurs in pts w/ colorectal CA while on bevacizumab - understand the mechanism of angiogenesis escape
- develop biomarkers (none currently)
- other VEGF pathway inhibitors in trial or under development
- investigate the role of VEGF inhibitors in different tumor types
- determine the most effective combination therapy
- other anti-angiogenic therapy - make use of endogenous inhibitors (e.g. the statins)

Strategies to inhibit tumor angiogenesis

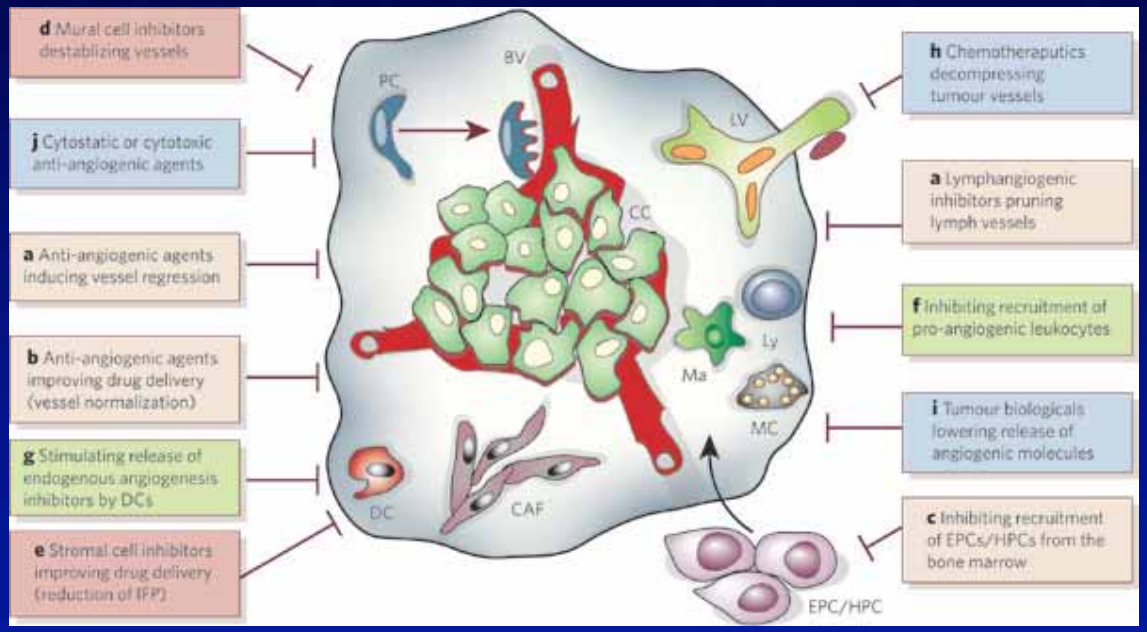
Mural+stroma

Cancer cells

Endothelial cells

hematopoietic cells

Mural+stroma



Cancer cells

Endothelial cells

hematopoietic cells

Cancer cells

Endothelial cells

Express VEGFR1

Carmeliet Nature 438:932, 2005