Angiogenesis and Neoplasia

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Cross-section of retinal capillary

pericyte

Image removed for copyright reasons.
Source: Henkind, P. "The retinal vascular system of the domestic cat." Exp. Eye Res. 5 (1966): 10-20.

endothelial cell

scanning electron micrograph of capillary

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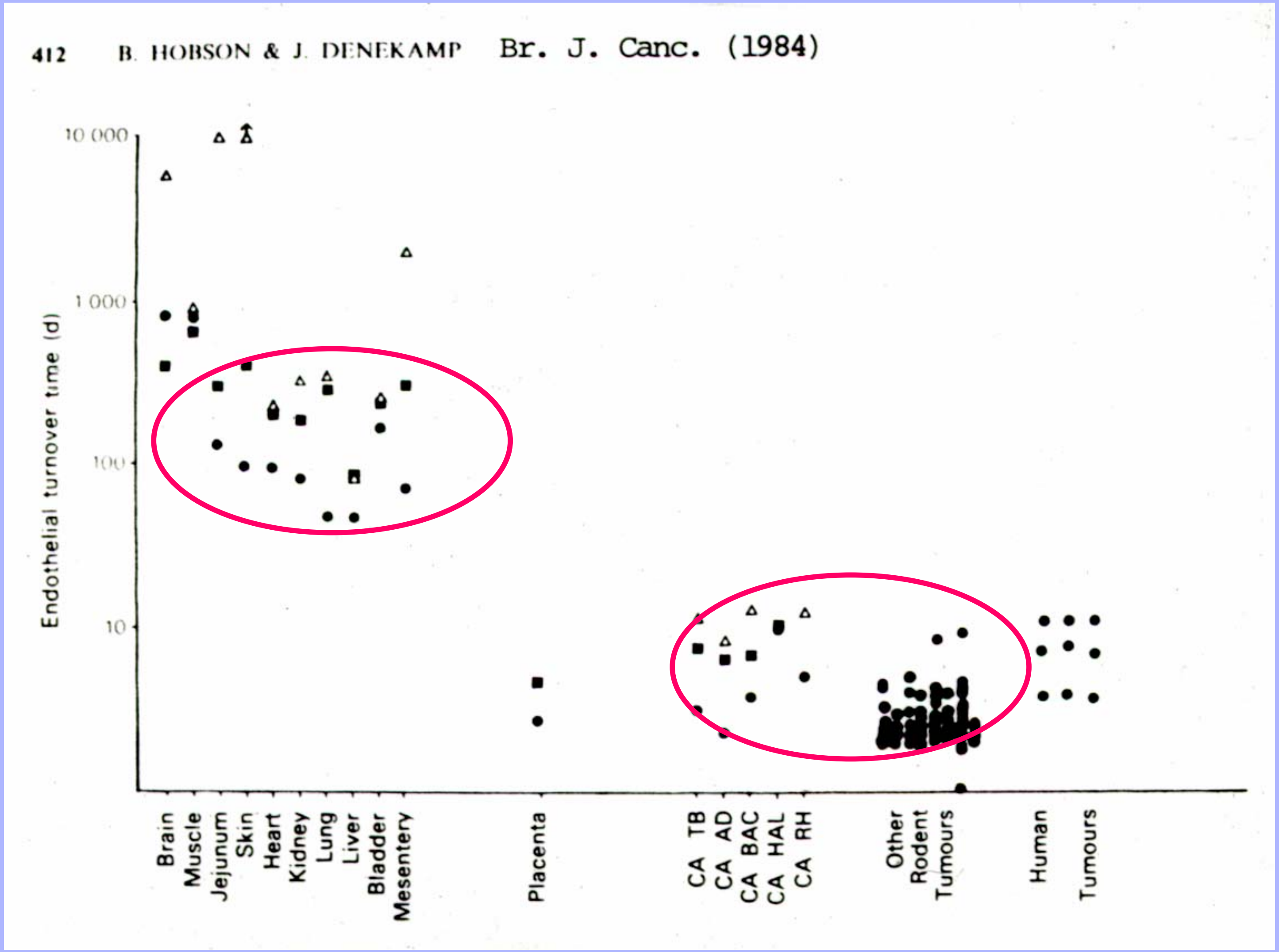
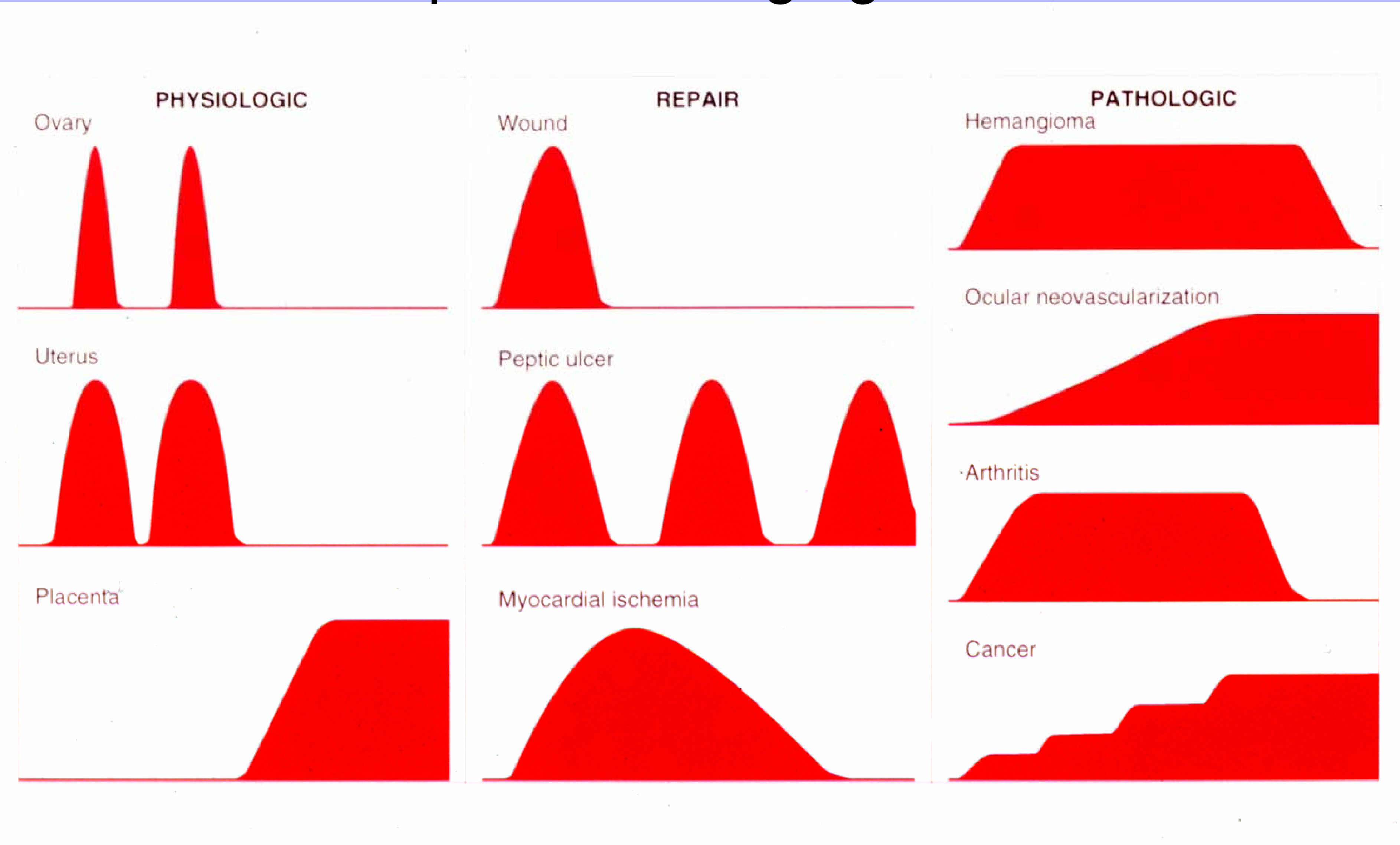
comparison of endothelial turnover in various microvascular beds



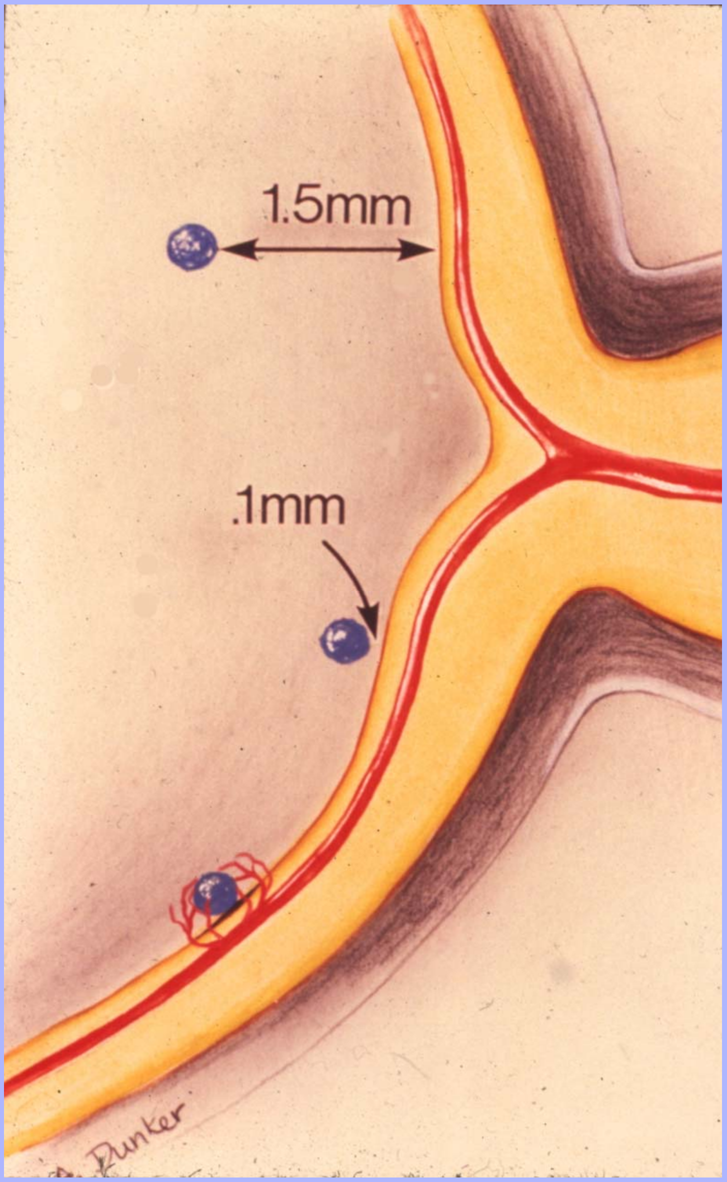
normal tissues

tumors

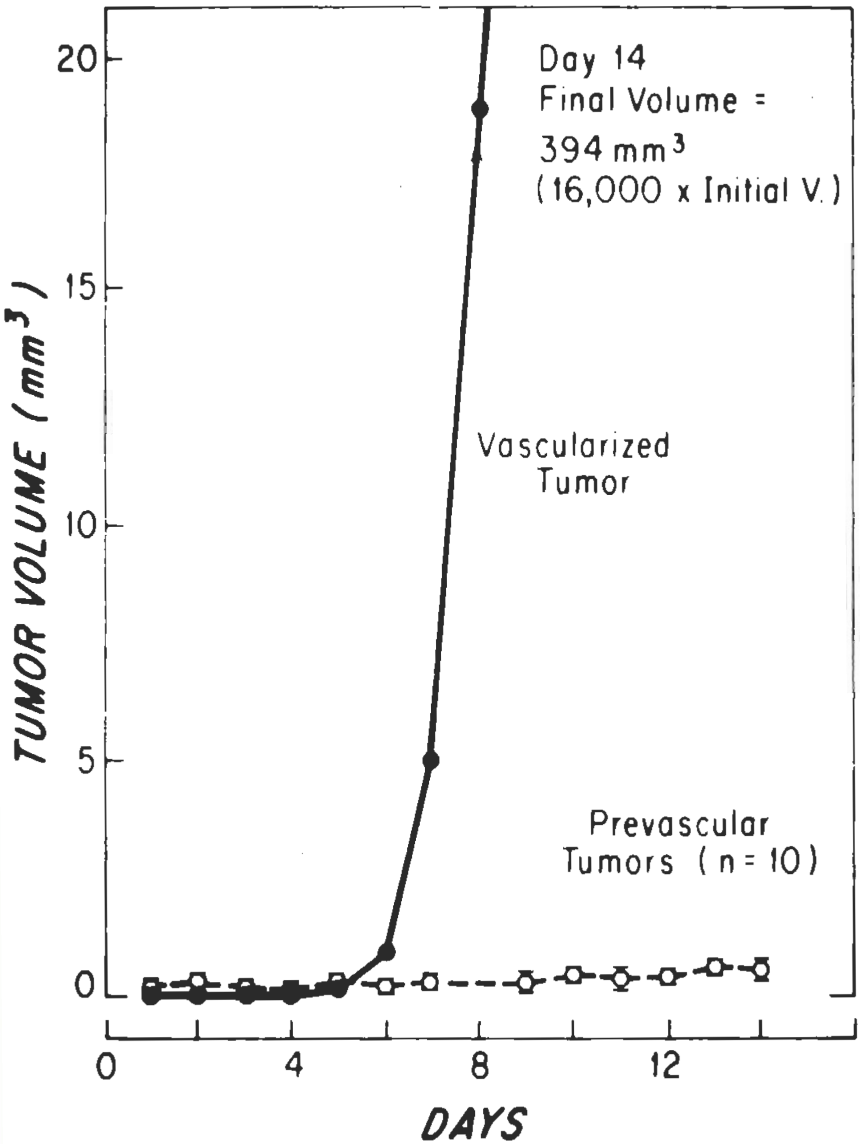
Circumstances and time frame of postnatal angiogenesis



tumor growth is angiogenesis-dependent

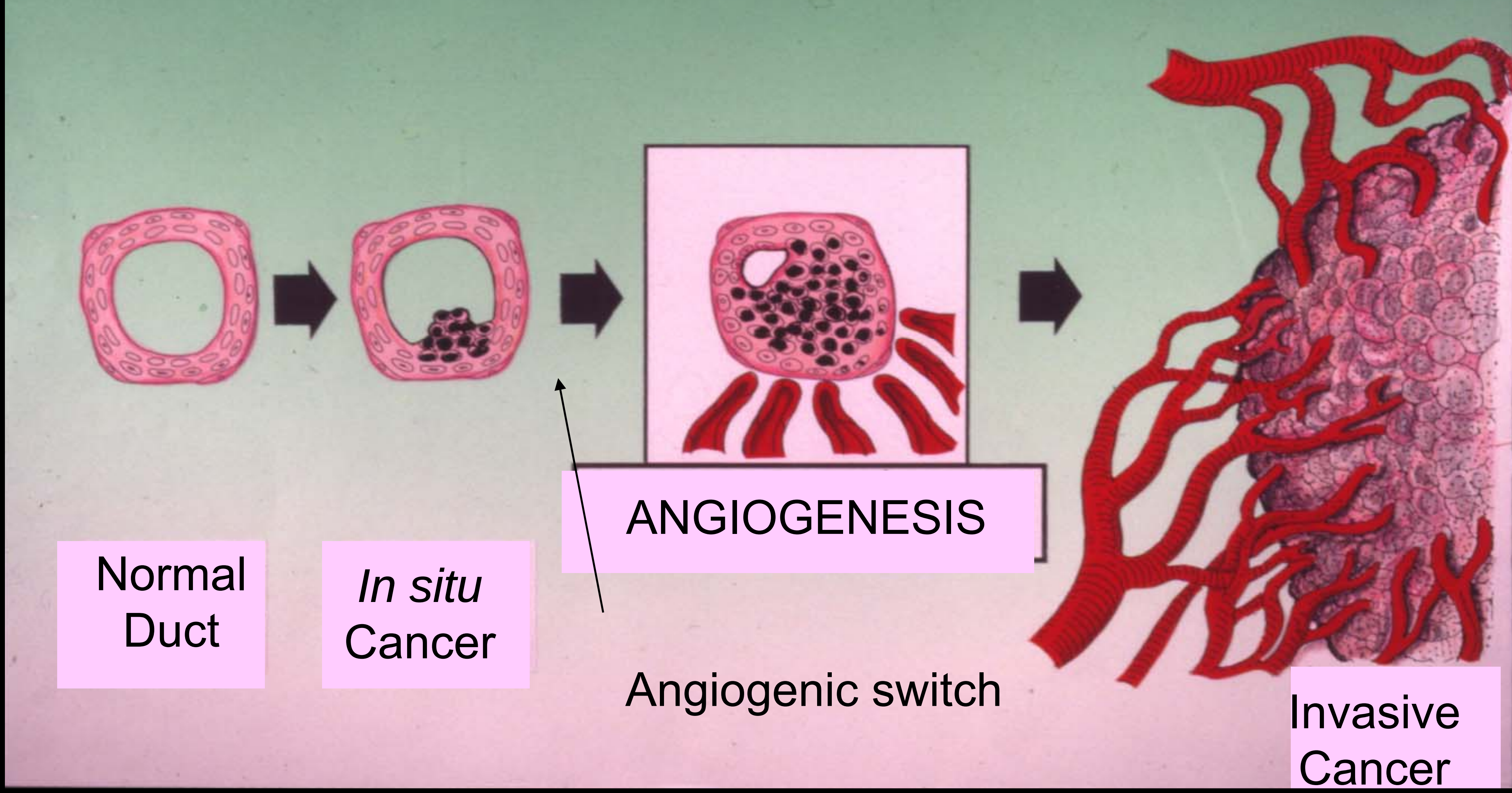


TUMOR GROWTH IN THE ANTERIOR CHAMBER OF THE RABBIT EYE



Gimbrone et al., JNCI, 1973

Role of Angiogenesis in Breast Cancer Progression



vessel density in normal vs invasive breast tissue

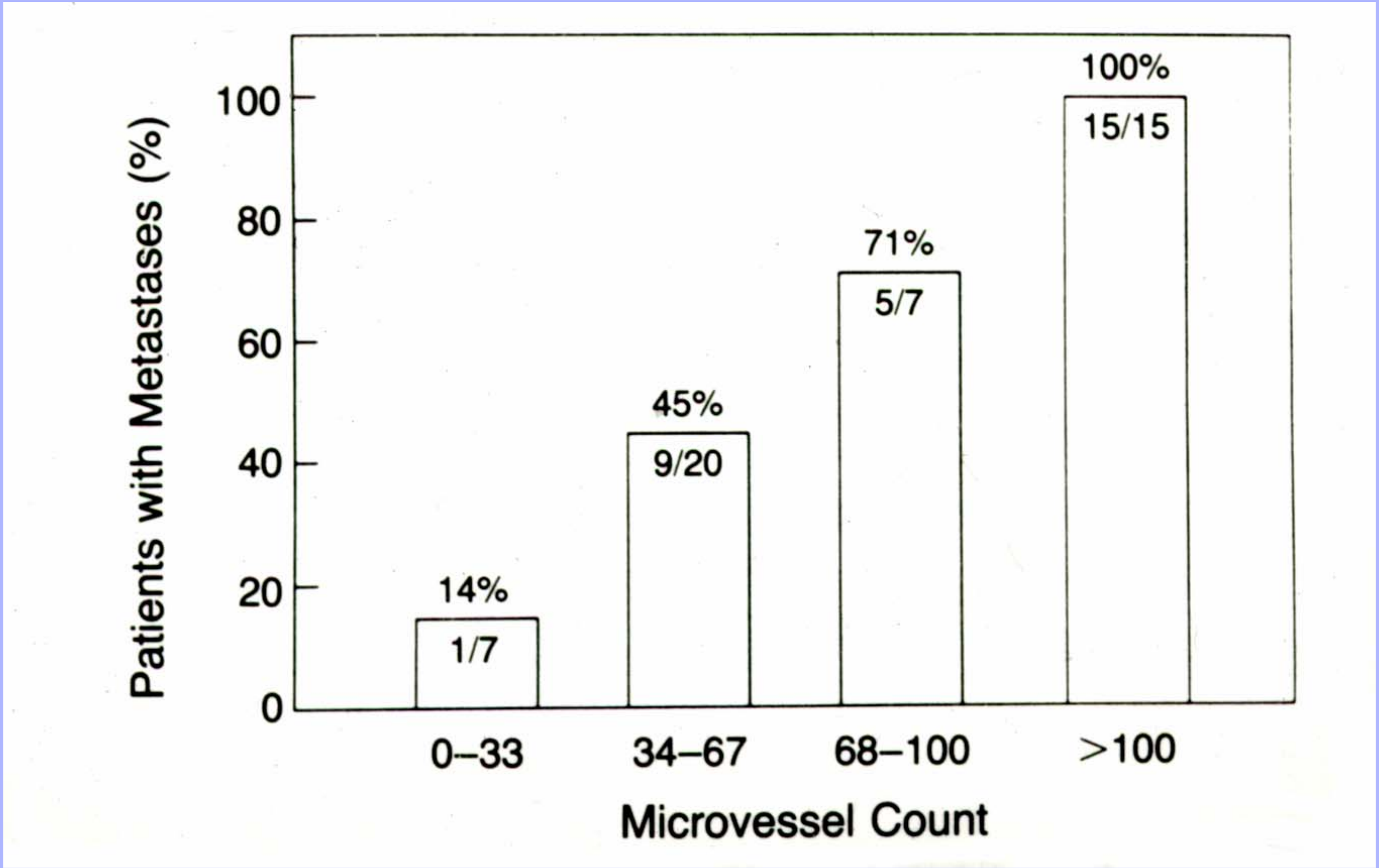
Image removed for copyright reasons.
Normal vessel density.

Image removed for copyright reasons.
Higher vessel density.

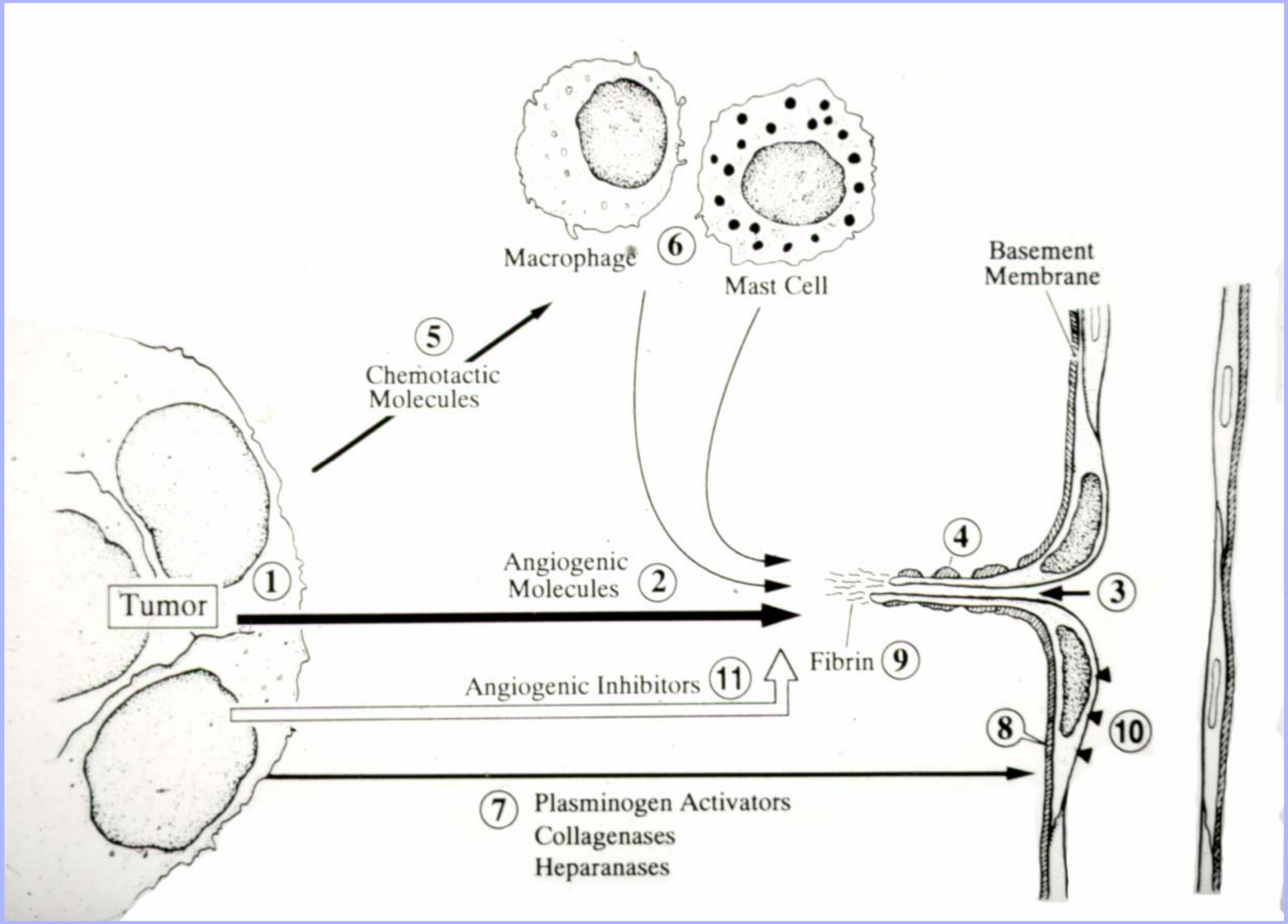
normal

invasive carcinoma

microvascular density vs metastasis



anti-angiogenic therapy: points of attack



Vascular Endothelial Growth Factor/ Vascular Permeability Factor

- Secreted 40-46 kd homodimer
- Three forms: 120, 164, and 188 amino acids produced by alternative splicing
- Produced by epithelial cells, tumor cells, smooth muscle cells and macrophages
- Specific endothelial cell mitogen
- Angiogenic factor
- Increases vascular permeability
- Regulated by hypoxia

Role of VEGF in Vasculogenesis

Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele.

Carmeliet *et al*., Nature 380:430-435, 1996

Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene.

Ferrara *et al*., Nature 380:439-442, 1996

Exogenous vascular endothelial growth factor induces malformed and hyperfused vessels during embryonic neovascularization.

Drake and Little, PNAS 92: 7657-7661, 1996

VEGF and endothelial cells in glioblastoma

VEGF

vWF

Images removed for copyright reasons.

VEGF

vWF

VEGF mRNA in pallisade cells of tumors

Image removed for copyright reasons.

VEGF mRNA

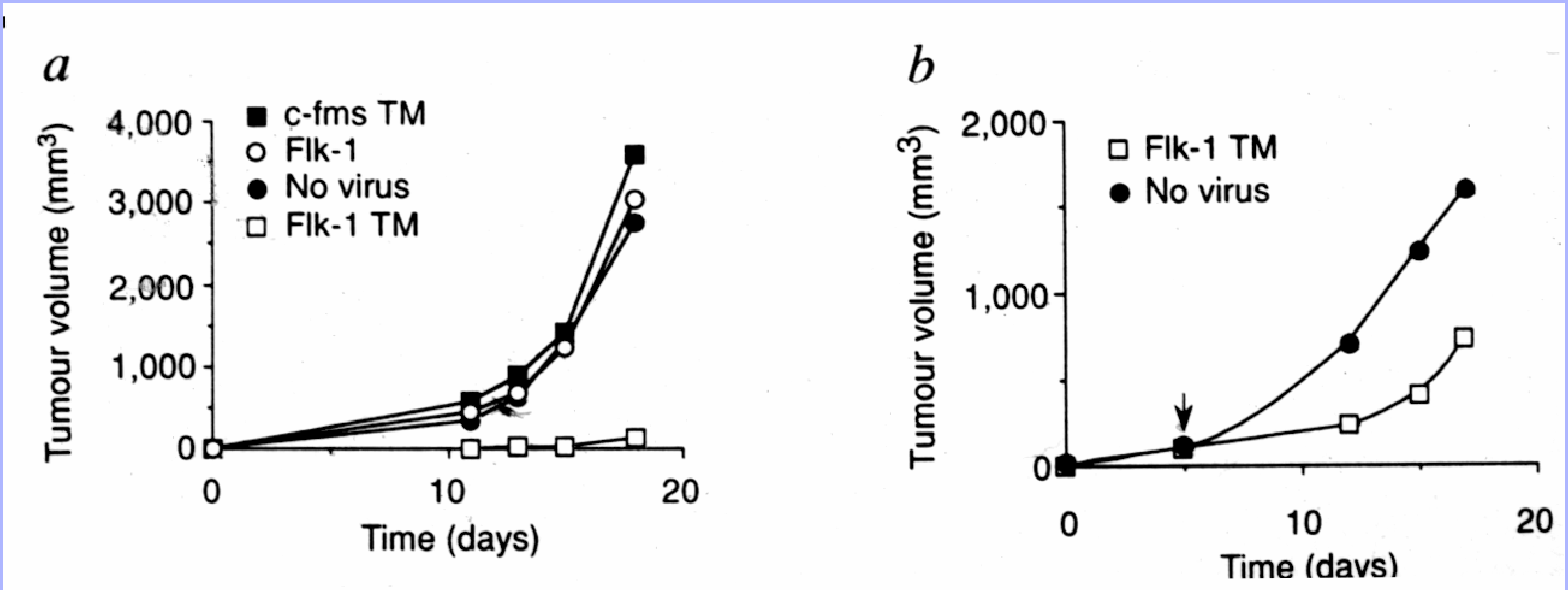
VEGFR2 mRNA in tumors but not normal brain capillaries

Image removed for copyright reasons.

Normal brain

Glioblastoma

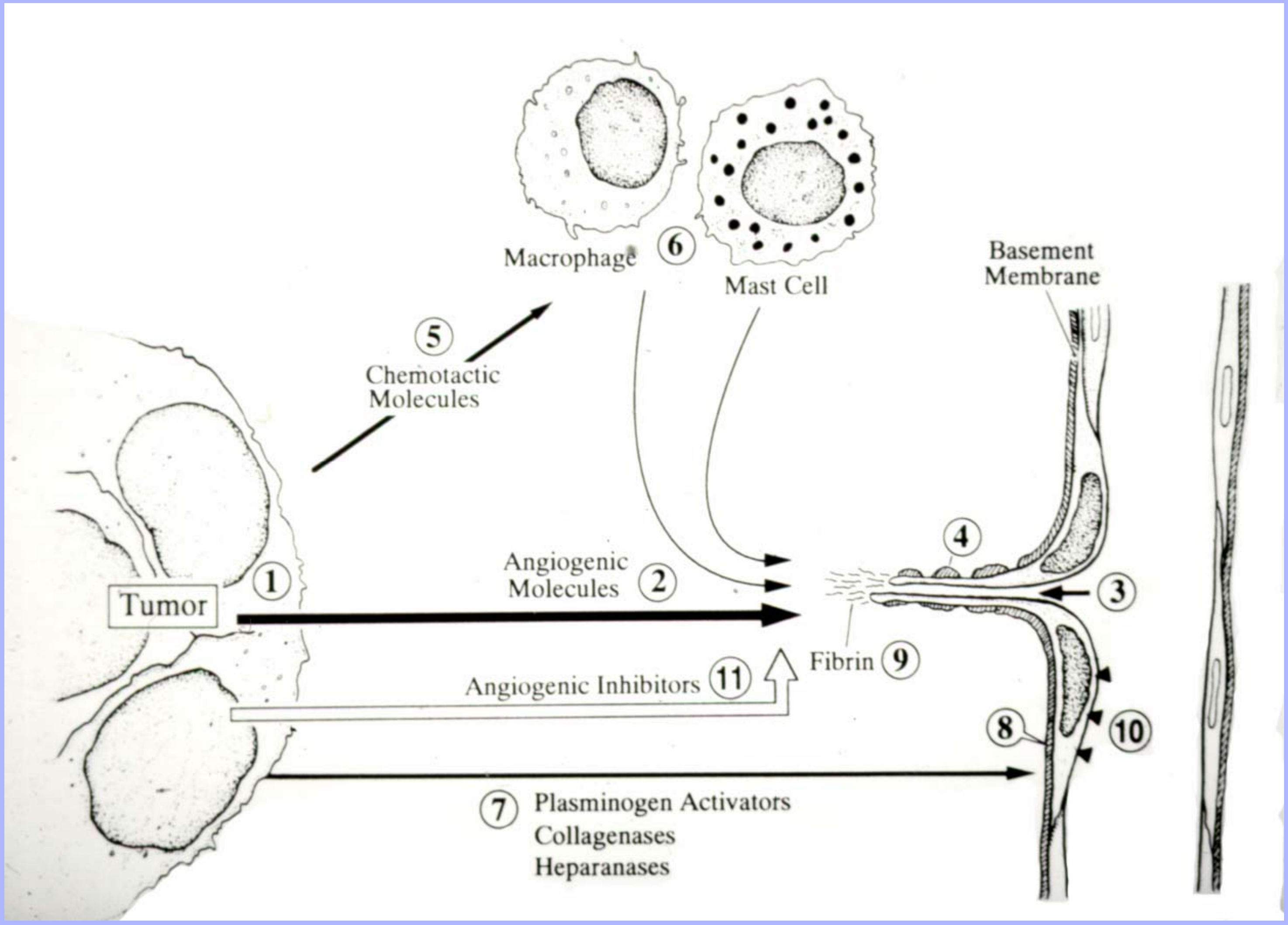
effect of blocking VEGFR2 signaling on tumor growth



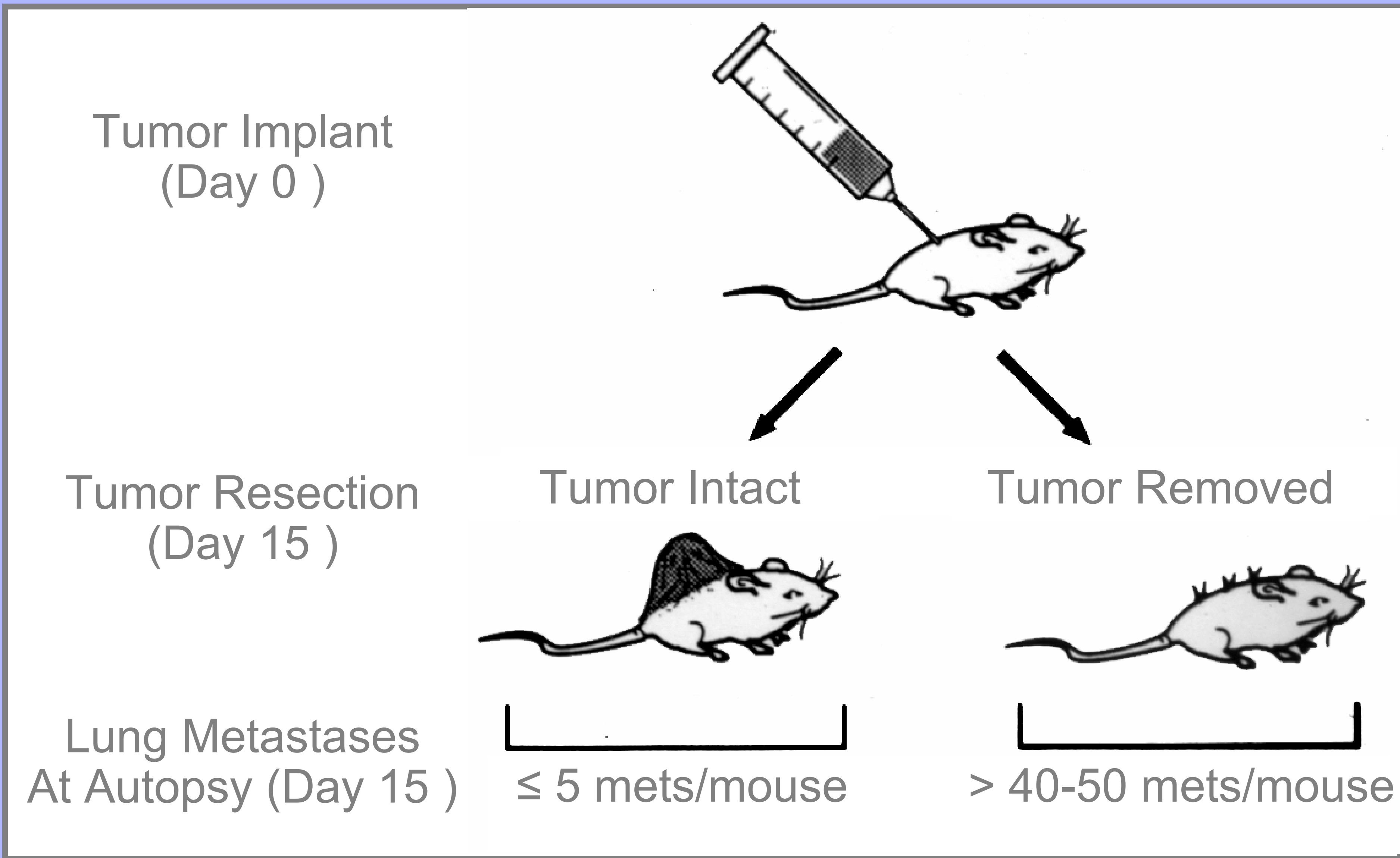
anti-VEGF approaches in clinical trials

- Anti-VEGF monoclonal antibodies
- VEGF-trap (soluble receptor + Fc)
- Aptamers
- VEGFR2 tyrosine kinase inhibitors

anti-angiogenic therapy: points of attack



effect of primary tumor on lung metastasis



lung metastases in the presence and absence of primary tumor

Primary
Tumor:

Present

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Absent

1 cm

histology of mouse lung metastases

Dormant	Growing (Angiogenic)

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200 µm

Primary tumor present	5 days after removal of primary tumor

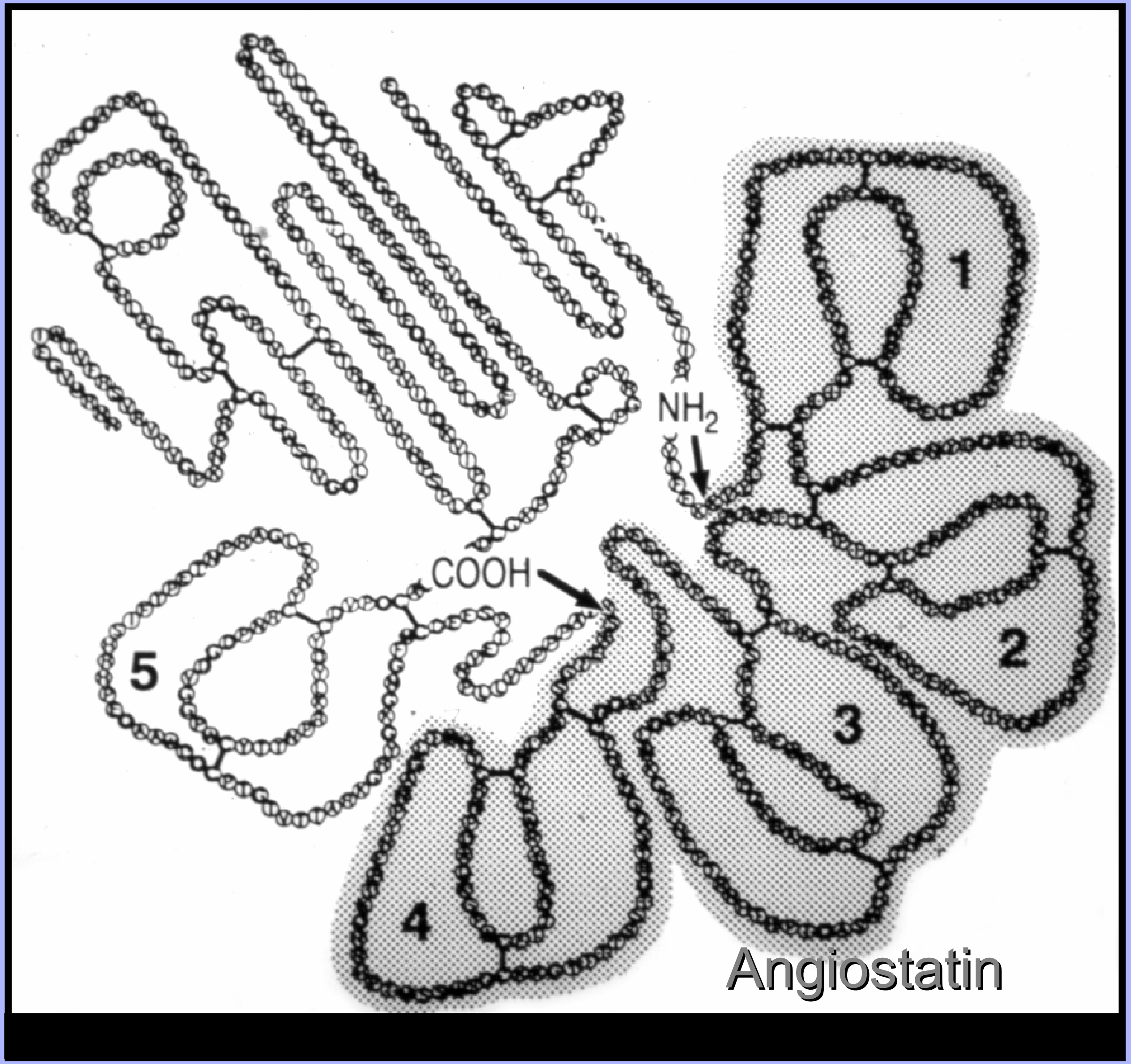
Angiogenesis Inhibition

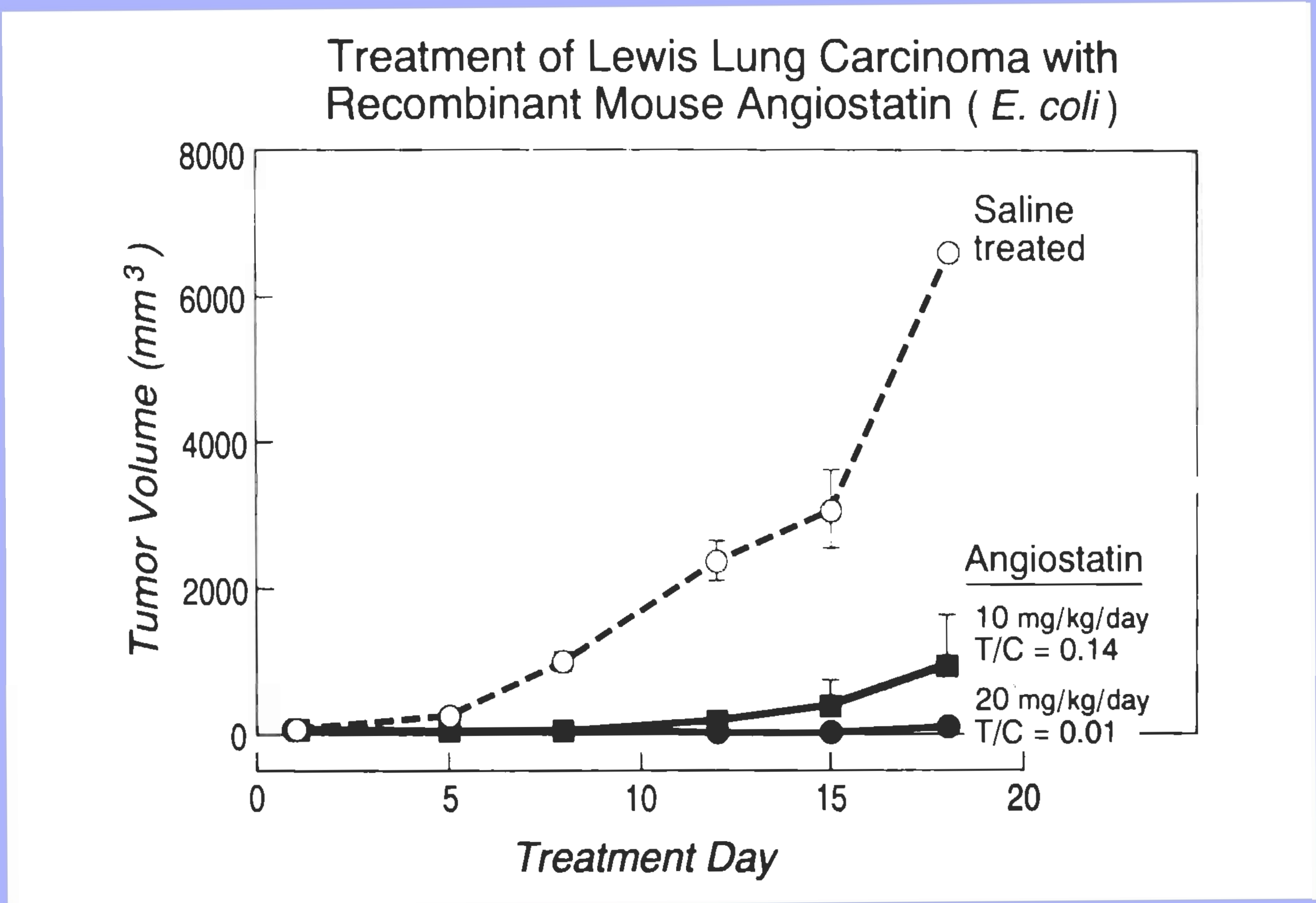
Maximum Diameter	Dormant micro-metastases (150 - 200 μm)	Growing macro-metastases (1000 - >5000 μm)
Proliferation Rate (BrdU)	40%	40%
Angiogenesis (Factor VIII)	0 or ±	++++
Apoptosis Rate (TUNEL)	7%	2%

Data: L. Holmgren et al, Nature Medicine 1:49,1995

Lewis Lung Carcinoma

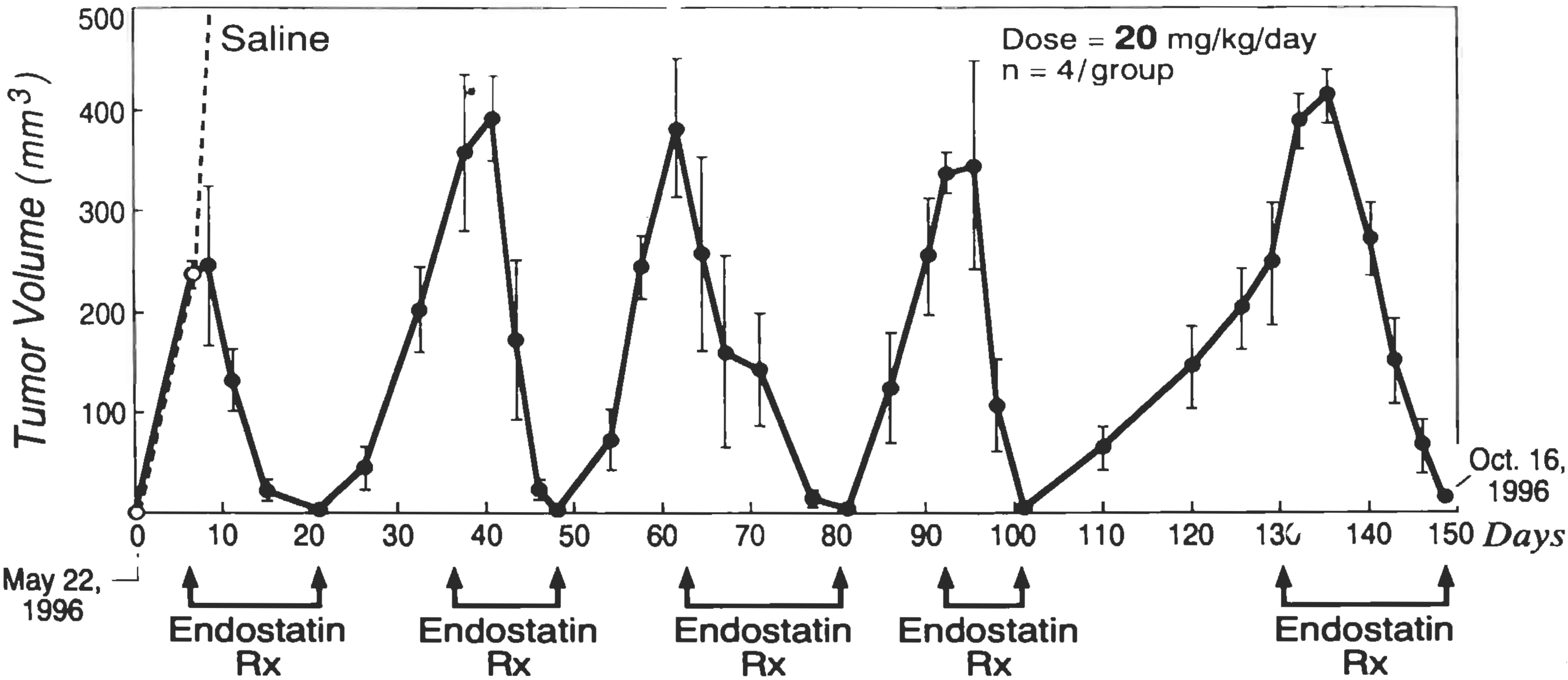
Plasminogen

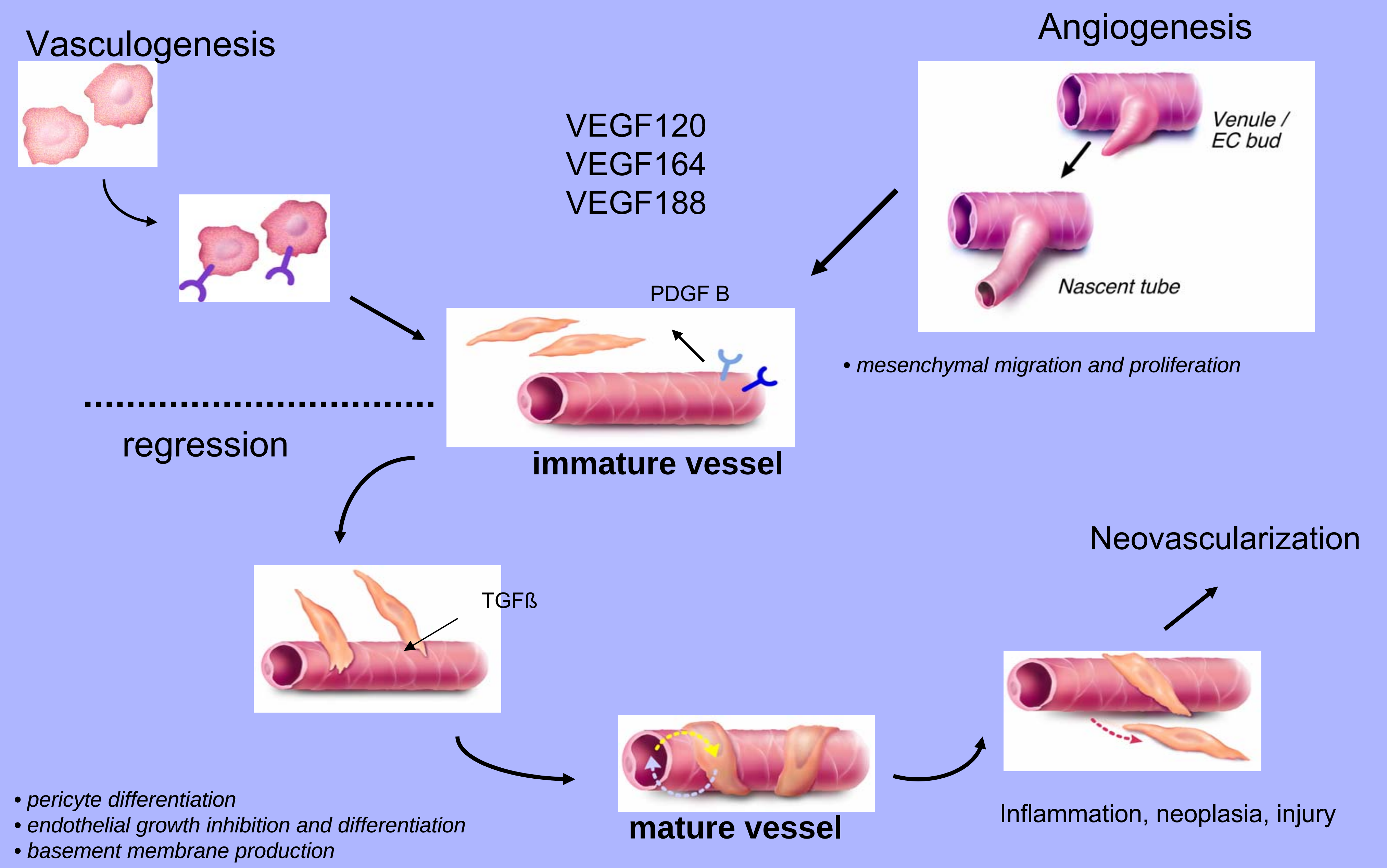




Treatment of Lewis Lung Carcinoma with
Recombinant Mouse Angiostatin (E. coli)
8000
6000
4000
2000
0
Tumor Volume (mm 3)
Saline
treated
Angiostatin
10 mg/kg/day
T/C = 0.14
20 mg/kg/day
T/C = 0.01
0
5
10
15
20
Treatment Day

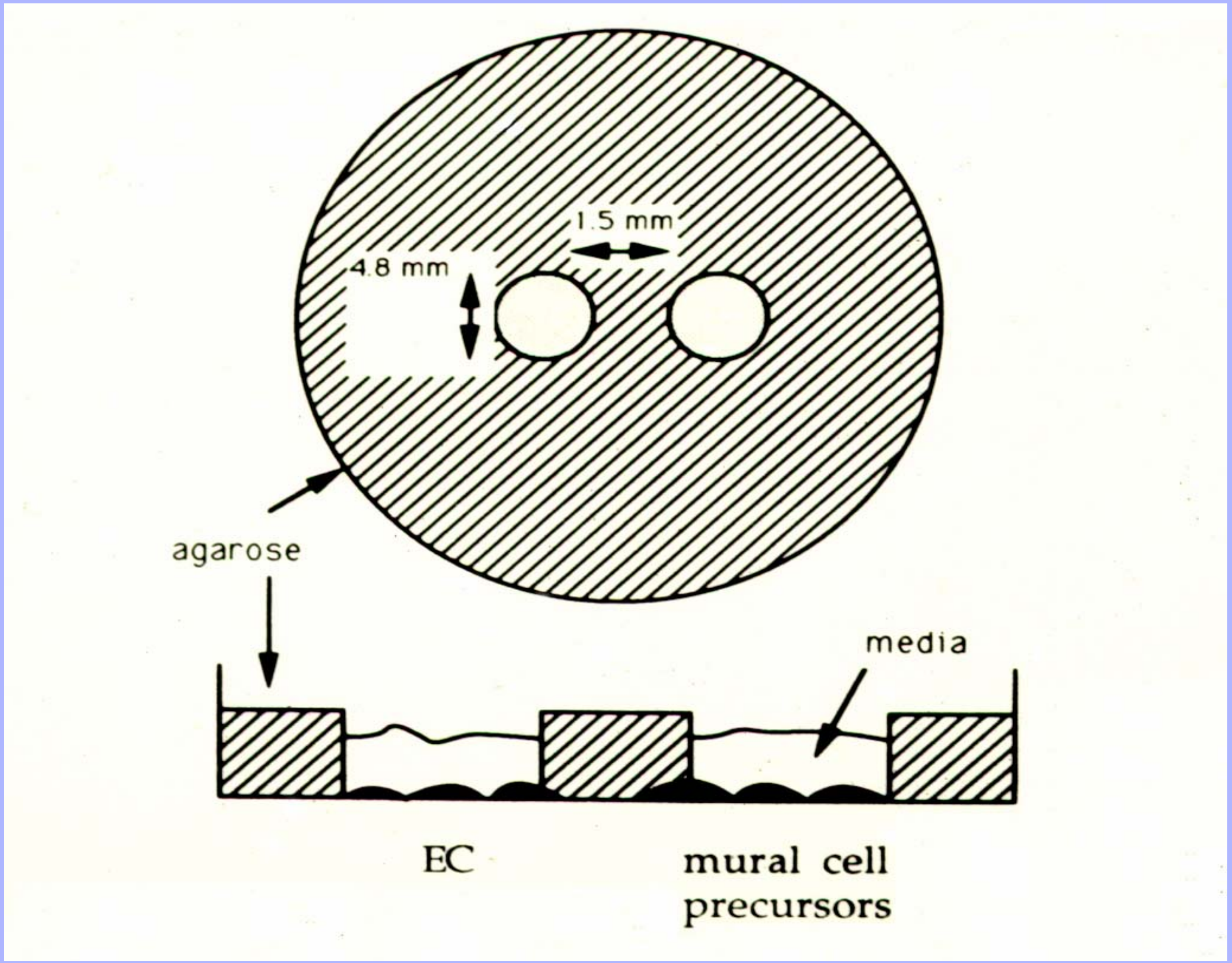
Cycled Dormancy Therapy of Lewis Lung Carcinoma with Recombinant Mouse Endostatin (*E. coli*)





Vasculogenesis
Angiogenesis
VEGF120
VEGF164
VEGF188
Venule /
EC bud
Nascent tube
PDGF B
• mesenchymal migration and proliferation
regression
immature vessel
Neovascularization
TGFß
• pericyte differentiation
• endothelial growth inhibition and differentiation
• basement membrane production
mature vessel
Inflammation, neoplasia, injury

Investigation of the Effects of Endothelial Cells on Mesenchymal Cell Migration



Endothelial Cells Induce the Directed Migration of Mesenchymal Cells

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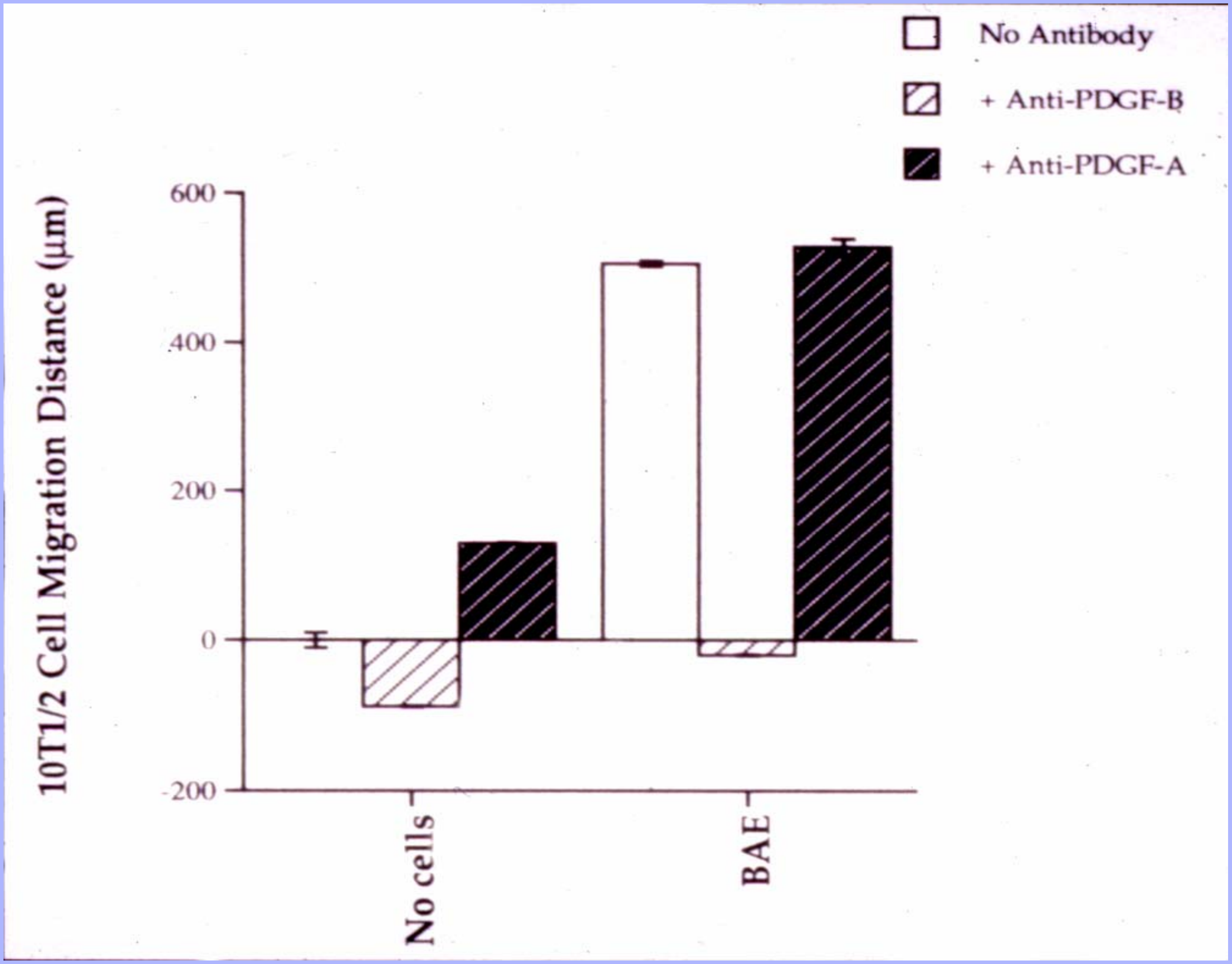
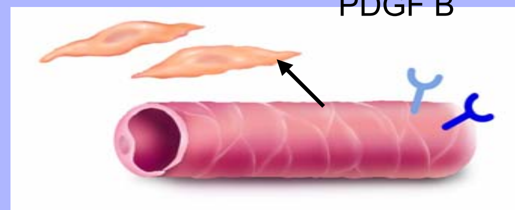
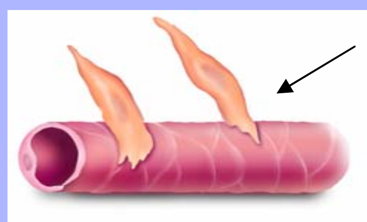
Endothelial Cells Induce Mesenchymal Migration via Secretion of PDGF BB





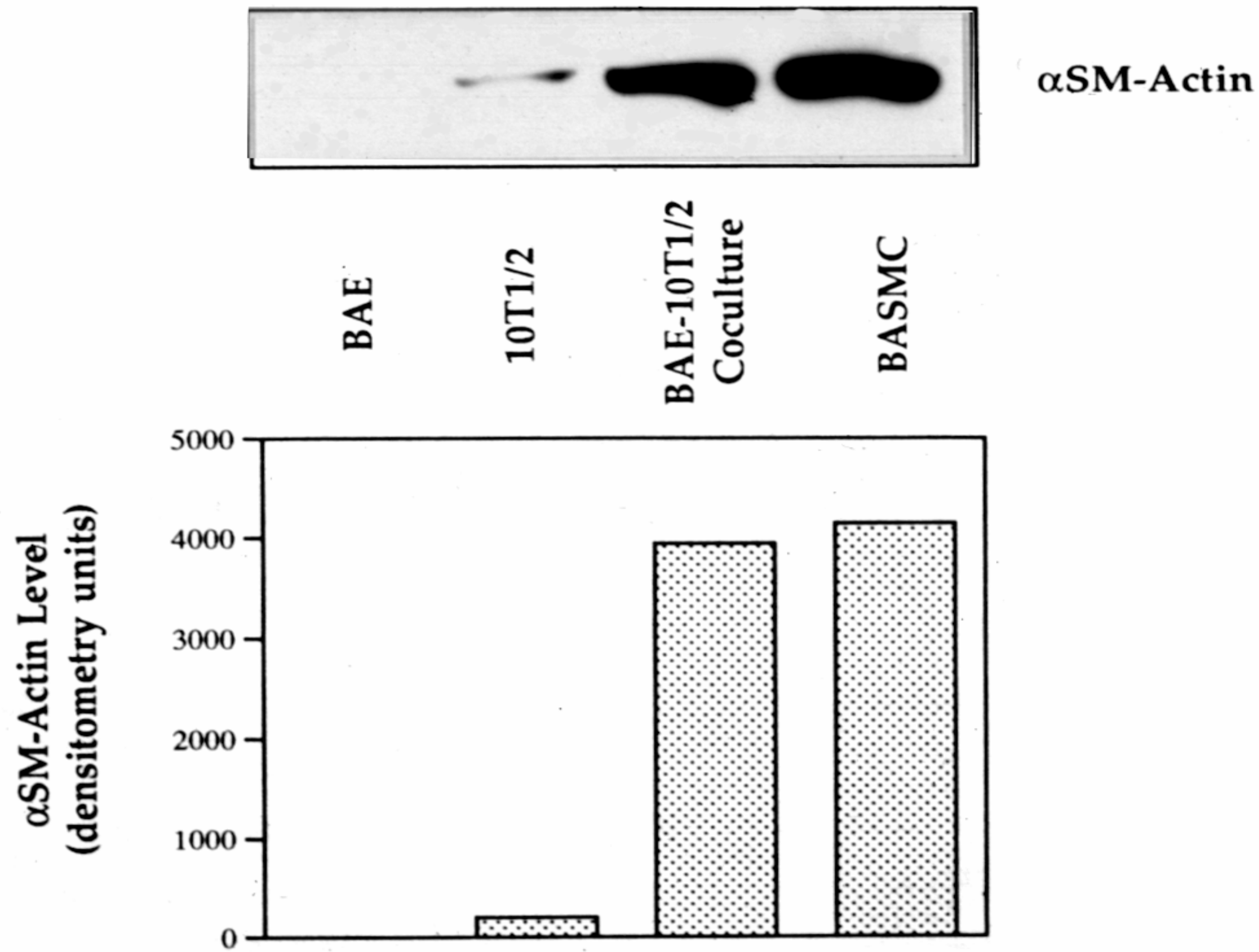
• *mesenchymal migration and proliferation*

immature vessel

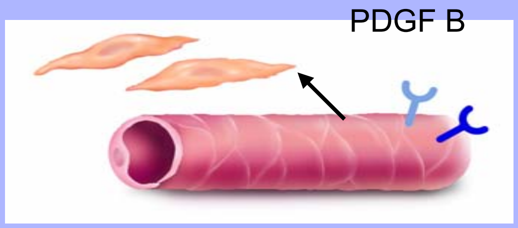


• *pericyte differentiation*
• *endothelial growth inhibition and differentiation*
• *basement membrane production*

Endothelial Cell-Mesenchymal Coculture Induces Mesenchymal Differentiation to SMC/Pericytes



regression



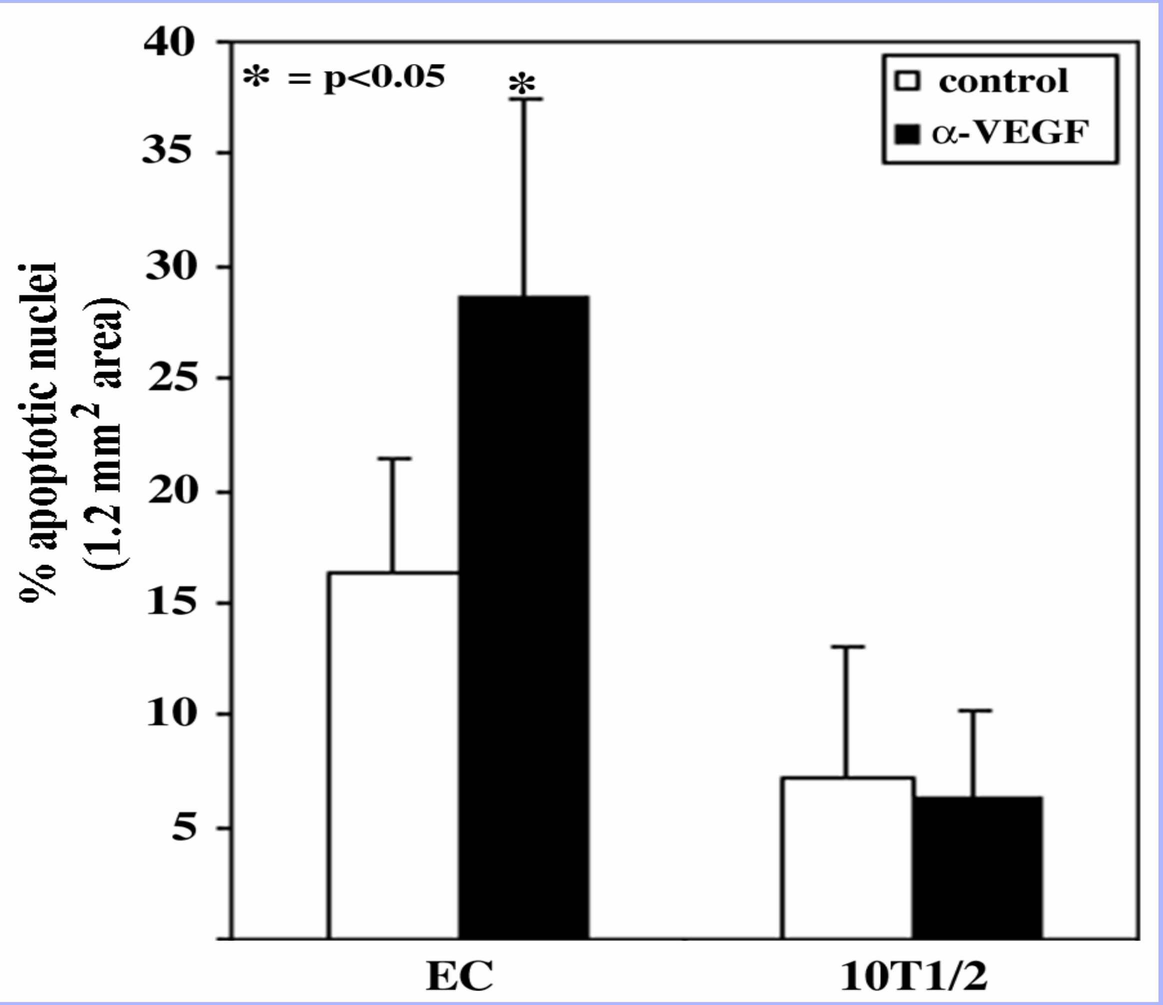
• mesenchymal migration and proliferation

immature vessel

mature, stable vessel

Does VEGF act as a survival factor for retinal vascular endothelial cells?

Effect of VEGF neutralization on EC survival in cocultures in vitro

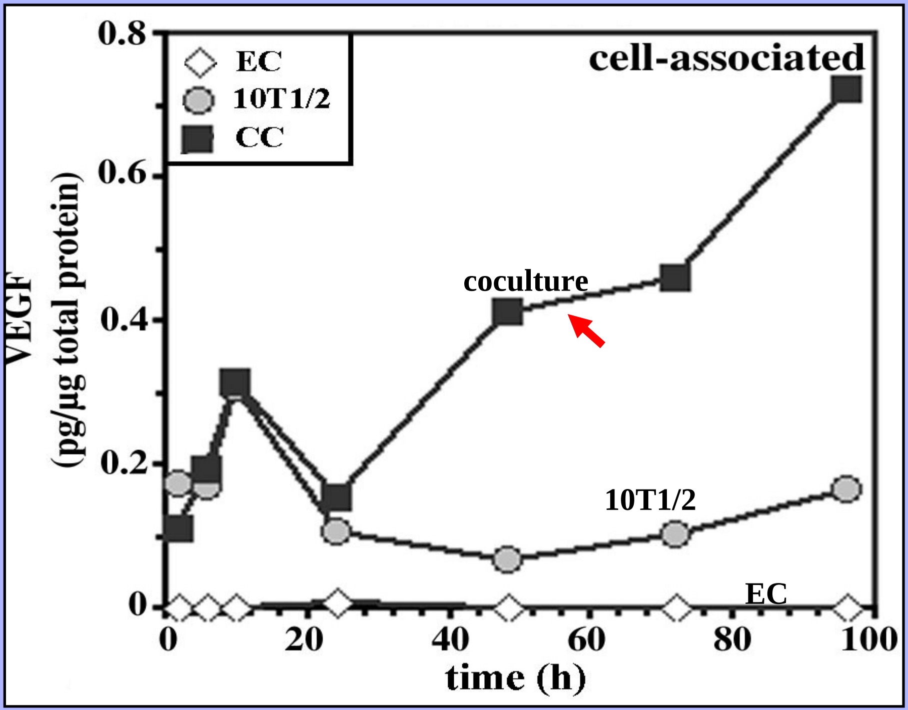


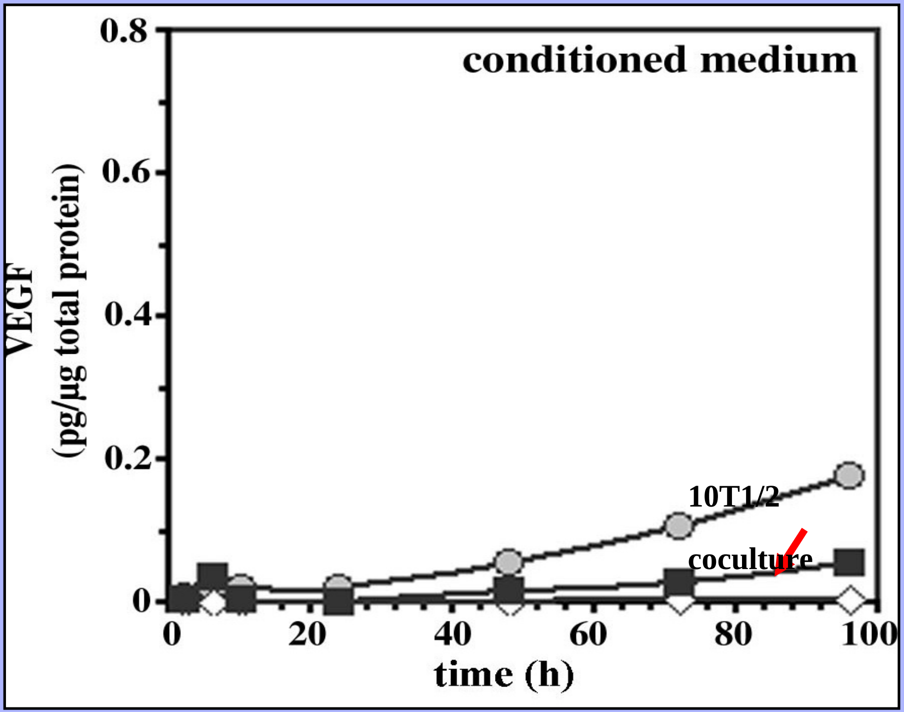
Hypothesis

Retinal endothelial cell survival and function is mediated by pericyte production of VEGF.

Is differentiation of mesenchymal cells to pericytes associated with induction of VEGF production?

EC-10T1/2 cell coculture lead to increased VEGF production





Summary of Coculture Studies

- EC-10T1/2 cell coculture leads to increased VEGF synthesis (and differentiation to pericytes)
- Most of the VEGF remains cell associated
- The pericytes are the major source of the VEGF
- Induction of VEGF synthesis is due to TGFß activation

VEGF localization in pericytes in retinal flat mounts of P10 VEGF-LacZ mice

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VEGF-LacZ Localization in Retinal Pericytes

PE-CAM

NG2

ß-gal

Image removed for copyright reasons.

VEGF-LacZ Localization to Retinal Pericytes in Trypsin Digests

NG2

ß-gal

Image removed for copyright reasons.

Pericytes in the Adult Retinal Vasculature Express VEGF

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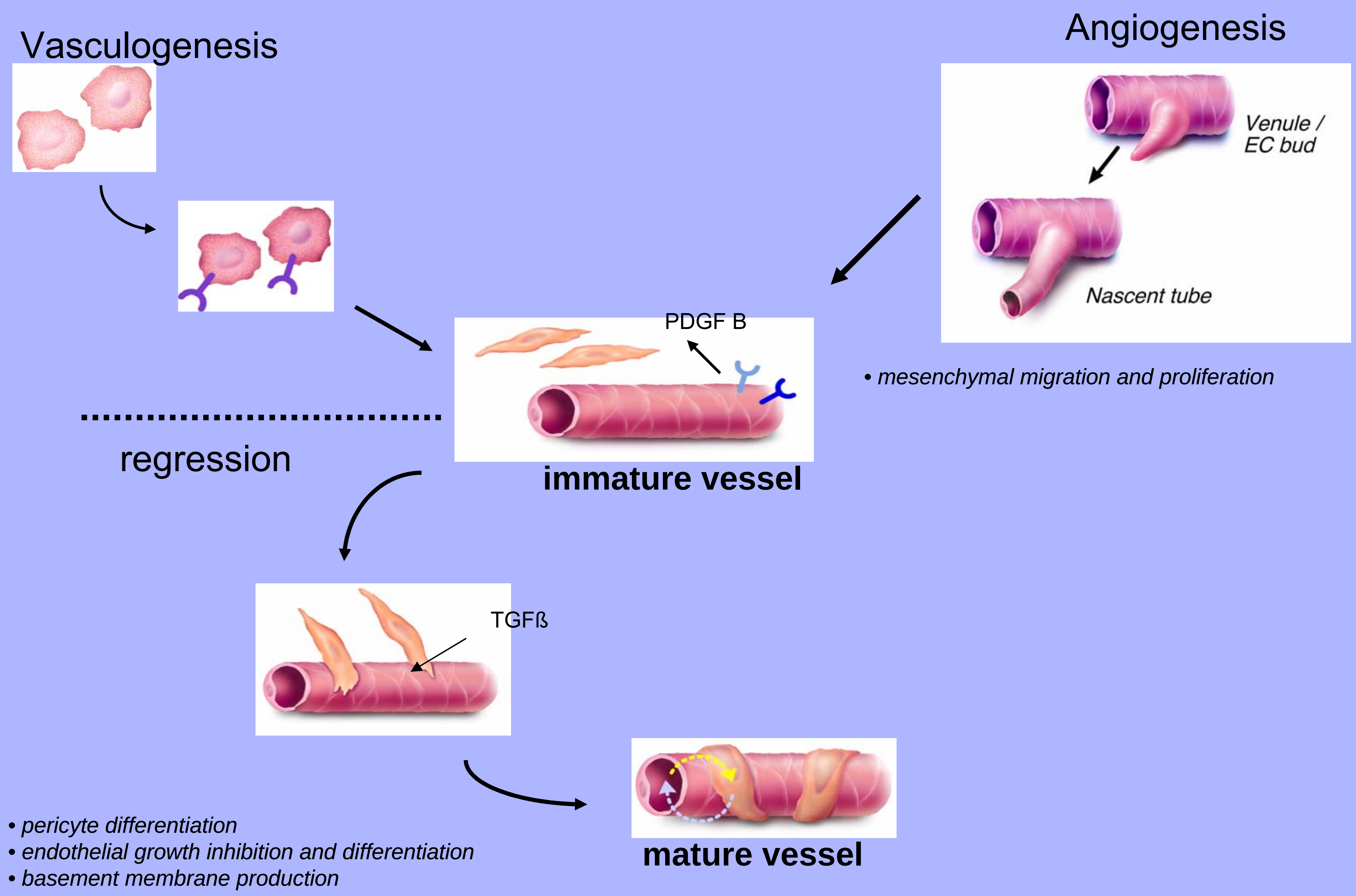
Vasculogenesis
Angiogenesis
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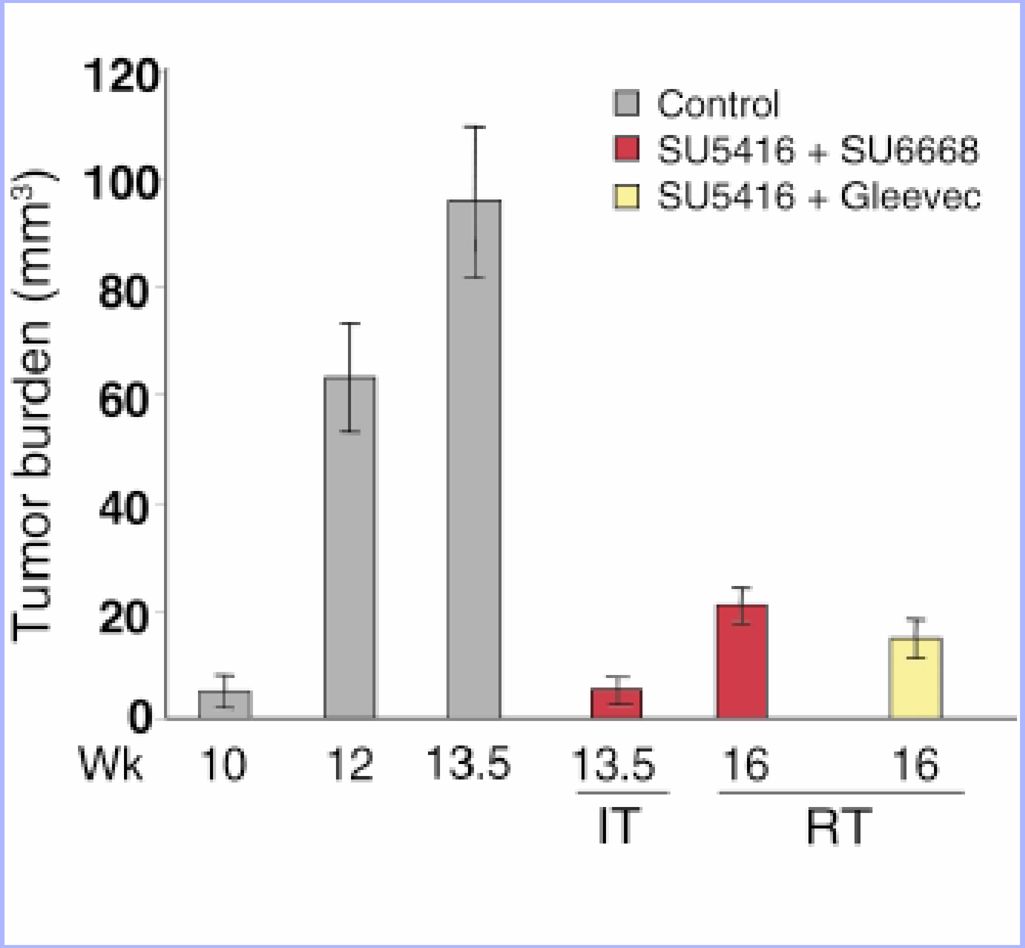
PDGF B
• mesenchymal migration and proliferation
regression
immature vessel
TGFß
mature vessel
• pericyte differentiation
• endothelial growth inhibition and differentiation
• basement membrane production

pericyte abnormalities in tumor vessels

See: Fig. 4 (f, g, h) in Morikawa S, Baluk P, Kaidoh T, Haskell A, Jain RK, McDonald DM. "Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors." *Am J Pathol* **160** (2002): 985-1000.

effect of blocking VEGF and PDGF on vessel growth



effect of blocking VEGF and PDGF on vessel morphology

Lectin = vessels

Desmin = pericytes

Lectin = vessels

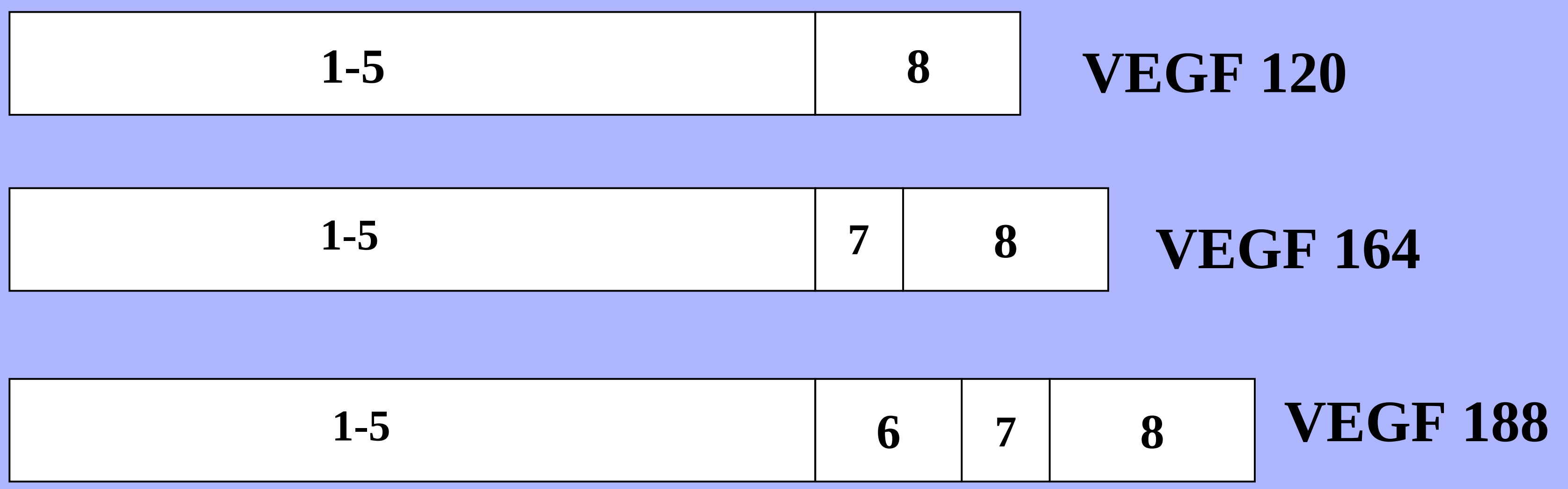
apoptosis

Images removed for copyright reasons.
See: Fig. 5 (g, h, i, j, k, l) in Bergers G, Song S, Meyer-Morse N, Bergsland E, Hanahan D. "Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors." *J Clin Invest* **111** (2003): 1277-80.

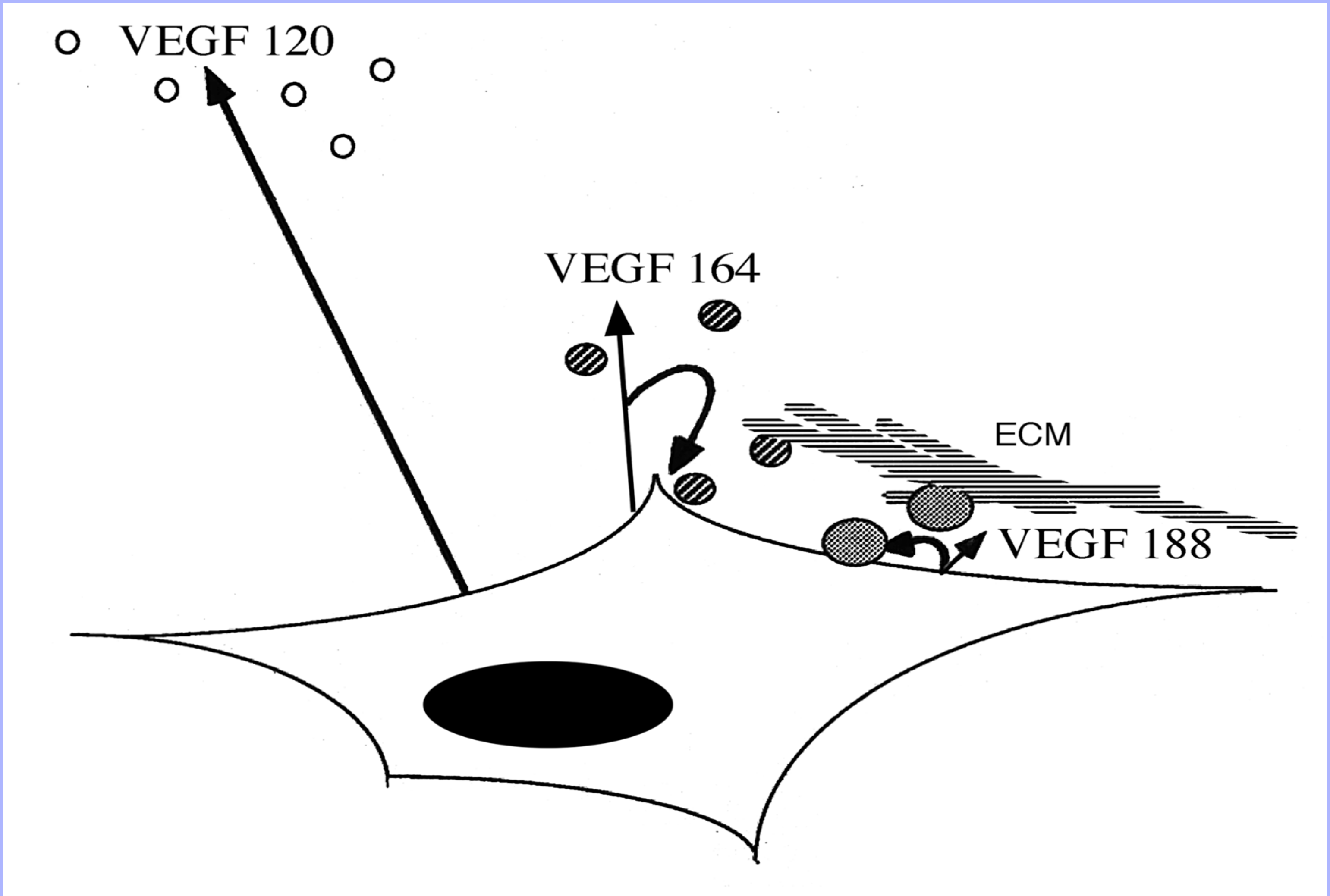
Lectin = vessels

Desmin = pericytes

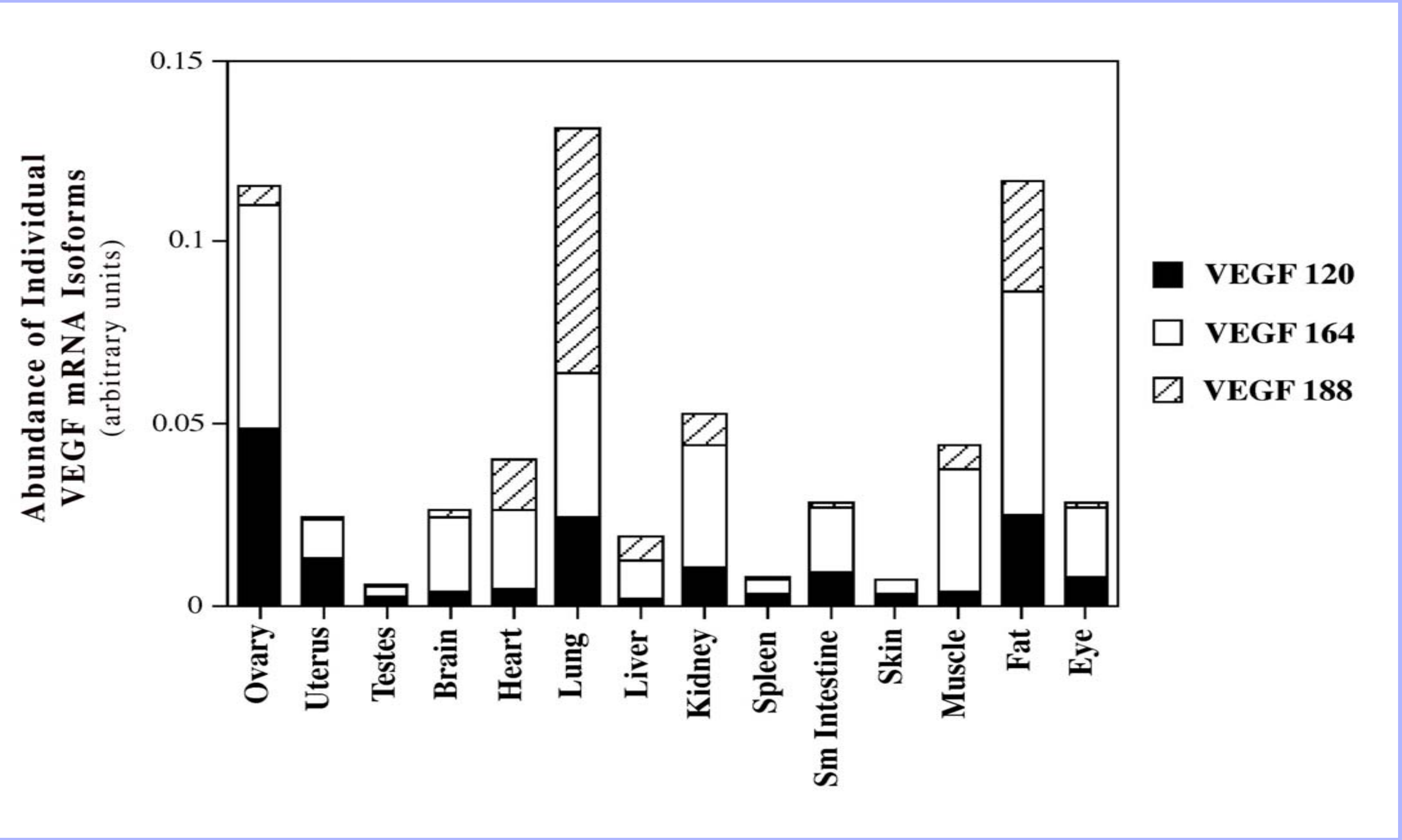
VEGF isoform gene structure



Differential Localization of Mouse VEGF Isoforms



VEGF Isoform Distribution in Adult Mouse Tissues



VEGF mRNA Expression in P0.5 Mouse Lung

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Lung Structure of P0.5 wt and VEGF120 Mice

Images removed for copyright reasons.

wt

VEGF120/120

VEGF expression in the kidney

β-gal, PECAM

Images removed for copyright reasons.

VEGF expression in the choroid plexus of the brain

β-gal

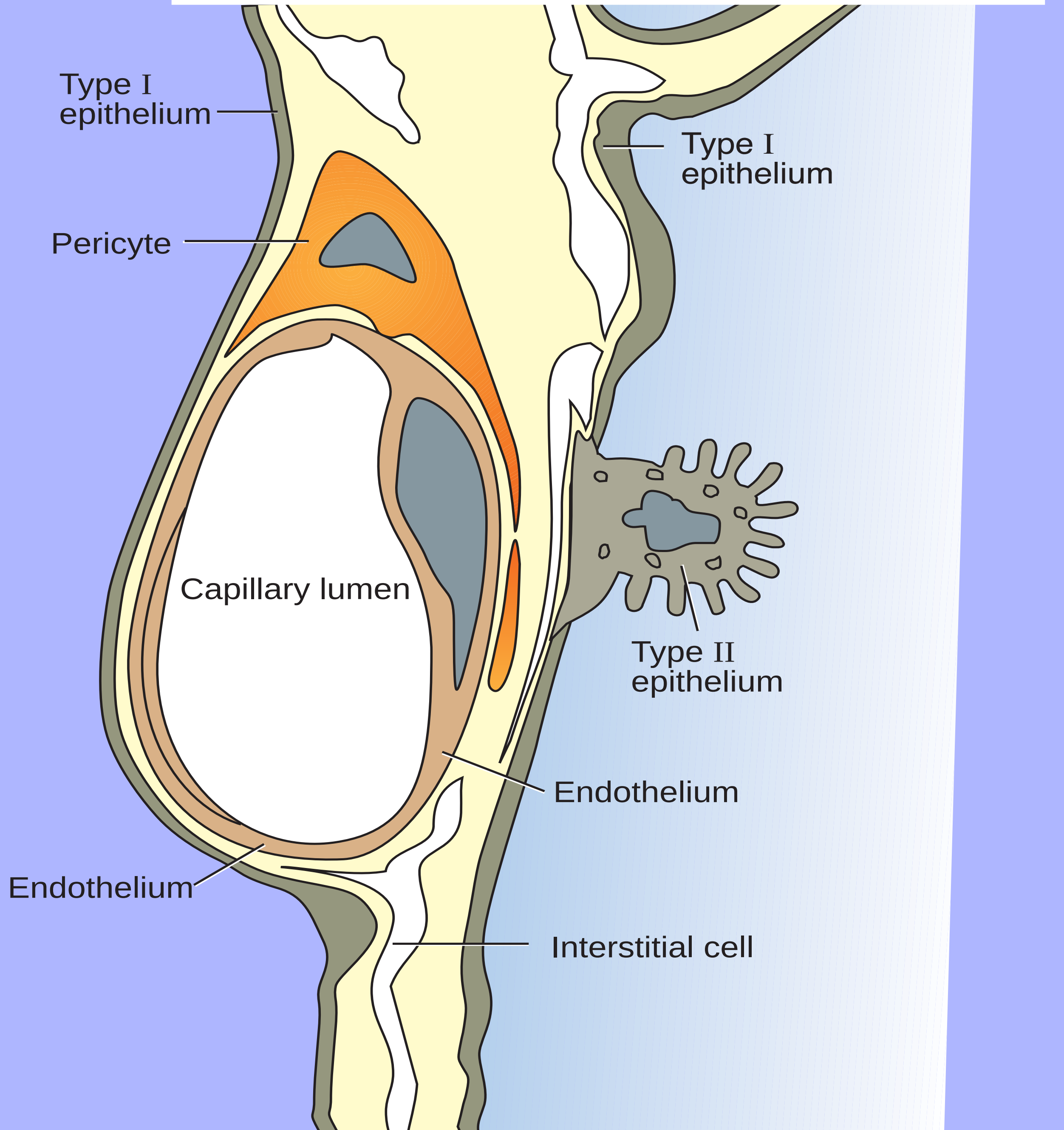
β-gal, PECAM

Images removed for copyright reasons.

VEGF expression in the choroid

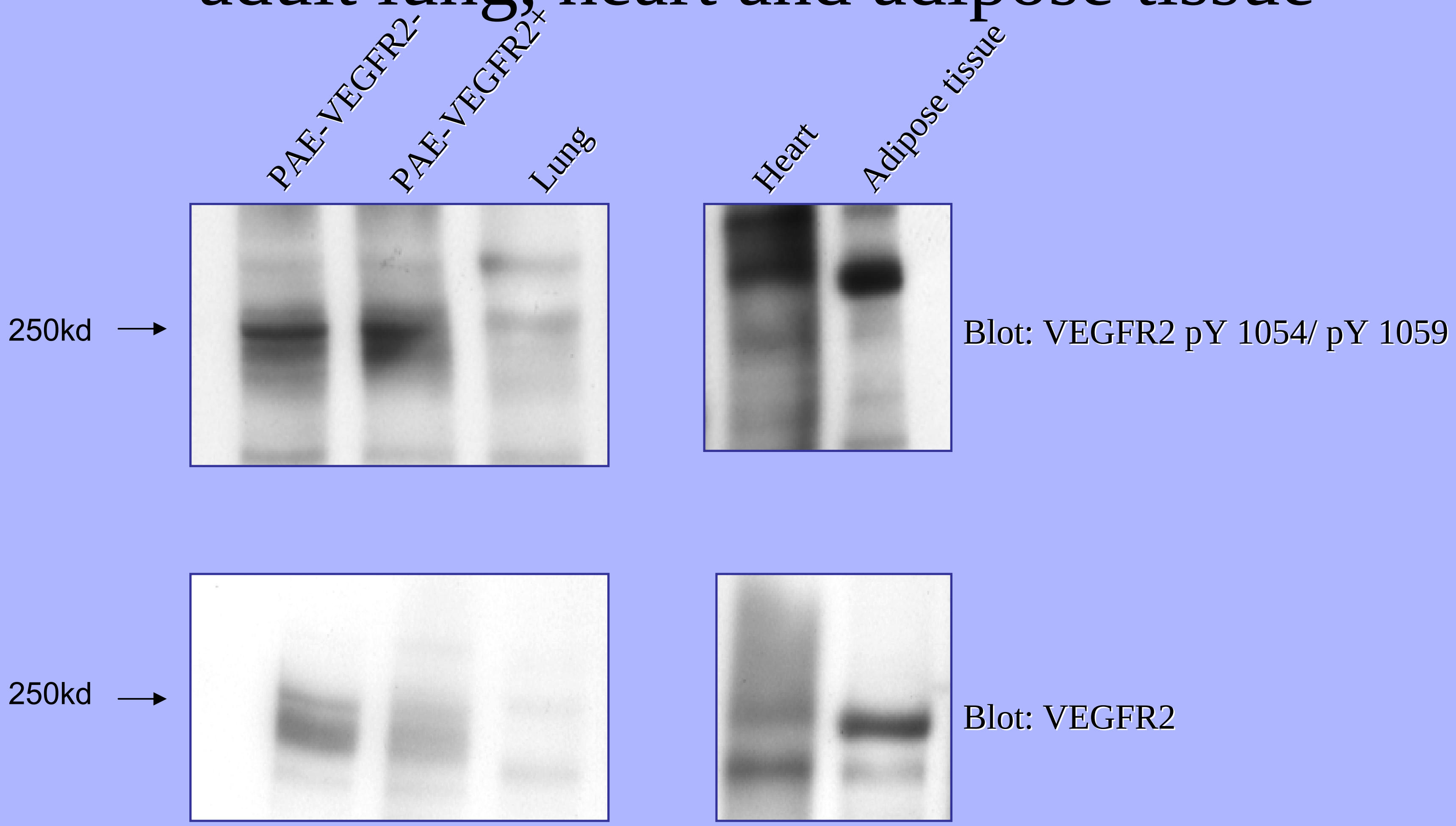
Images removed for copyright reasons.

50μm



Type I epithelium
Type I epithelium
Pericyte
Capillary lumen
Type II epithelium
Endothelium
Endothelium
Interstitial cell

VEGFR2 is constitutively activated in the adult lung, heart and adipose tissue



Effect of VEGF inhibition in the lung

Images removed for copyright reasons.
See: Fig. 1 (a, b) and 4 (a, b) in Kasahara Y, Tuder RM, Taraseviciene-Stewart L, Le Cras TD, Abman S, Hirth PK, Waltenberger J, Voelkel NF. "Inhibition of VEGF receptors causes lung cell apoptosis and emphysema." *J Clin Invest* **106** (2000): 1311-1319.

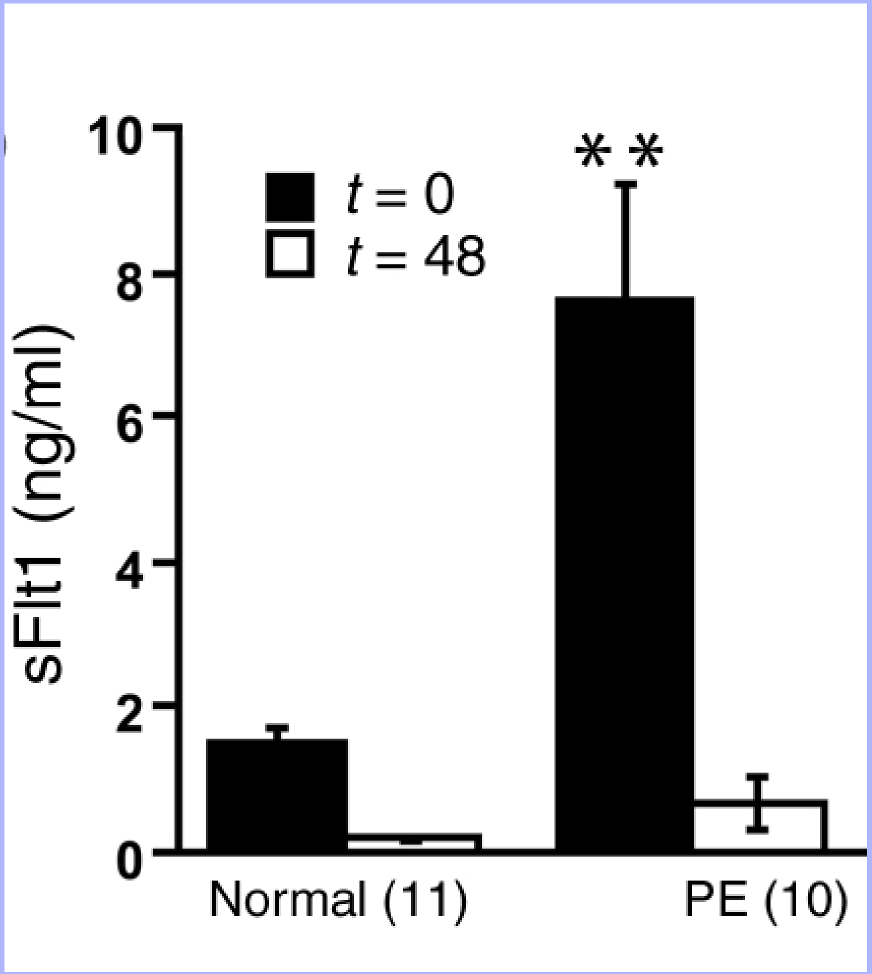
Caspase3

Effect of VEGF inhibition on tracheal vessels

Images removed for copyright reasons.
See: Fig. 1 in Inai T, Mancuso M, Hashizume H, Baffert F, Haskell A, Baluk P, Hu-Lowe DD, Shalinsky DR, Thurston G, Yancopoulos GD, McDonald DM. "Inhibition of vascular endothelial growth factor (VEGF) signaling in cancer causes loss of endothelial fenestrations, regression of tumor vessels, and appearance of basement membrane ghosts." *Am J Pathol* **165** (2004): 35-52.

Control AG013736 : 2 days 10days

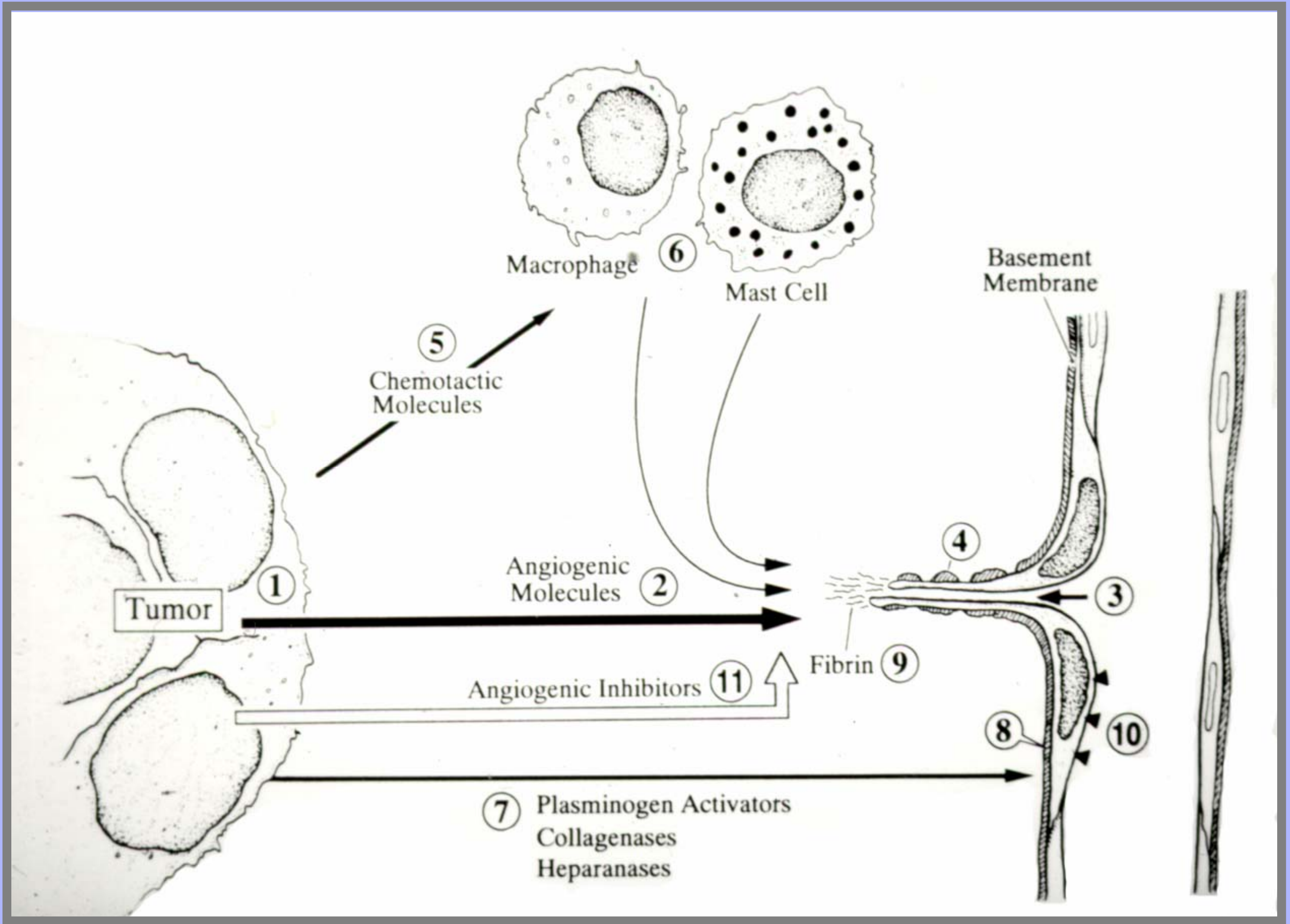
Serum levels of VEGF-A and sFlt-1 in preeclamptic patients



Effect of VEGF inhibition in the kidney

Images removed for copyright reasons.
See: Fig. 6 (a) in Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA. "Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia." *J Clin Invest* **111** (2003): 649-658.

Antiangiogenic Therapy: Points of Attack



Anti-Angiogenic Therapies in Clinical Trial

- Metalloproteinase inhibitors (Merimastat)
- Anti-VEGF
- Anti-VEGFR2
- Interferon alpha
- Interleukin 12
- Squalamine (cartilage-derived)
- Thalidomide
- TNP-470
- Anti-integrins
- Various cryptic fragments (e.g.angiostatin and endostatin)

TUMOR- STARVING DRUG CLEARS TRIALS

Author(s): Raja Mishra, Globe Staff Date: June 2, 2003 Page: A1 Section: National/ Foreign CHICAGO –

For the first time, a cancer treatment based on the groundbreaking work of Harvard's Dr. Judah Folkman has cleared clinical trials and is expected to be widely available soon to US patients.

The drug, called Avastin, extended the life spans of test colon cancer patients, shrank tumors in some cases, and overall delayed relapses, without the onerous side effects characteristic of many cancer treatments, according to a study of the drug's efficacy, presented here yesterday at the American Society of Clinical Oncology conference. While it could take months for the Food and Drug Administration to approve Avastin, pending review of the data, specialists predicted colon cancer patients around the country would have access to the drug sometime next year. ....