Involvement of Angiogenic Factor in Cancer and Inflammation

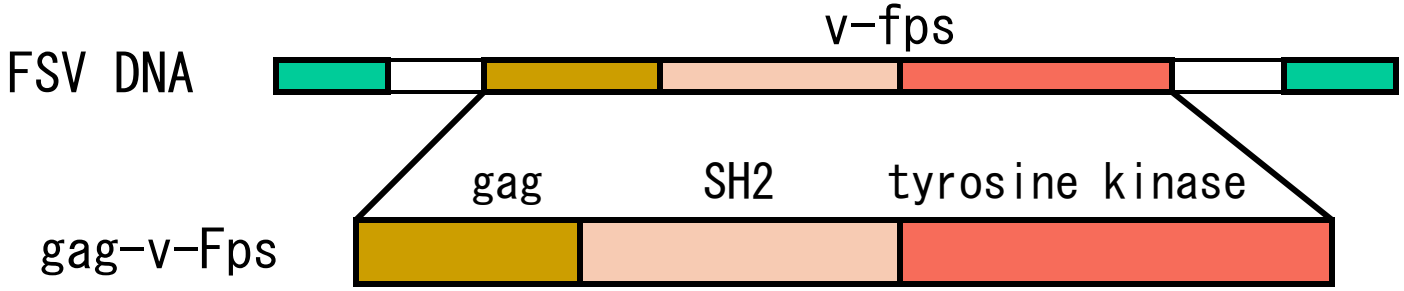
−−−−−−− Focusing on VEGF System −−−−−−−

2007.3.9

Masabumi Shibuya

1. Fujinami Sarcoma Virus ―――――――――――― v-fps cancer gene

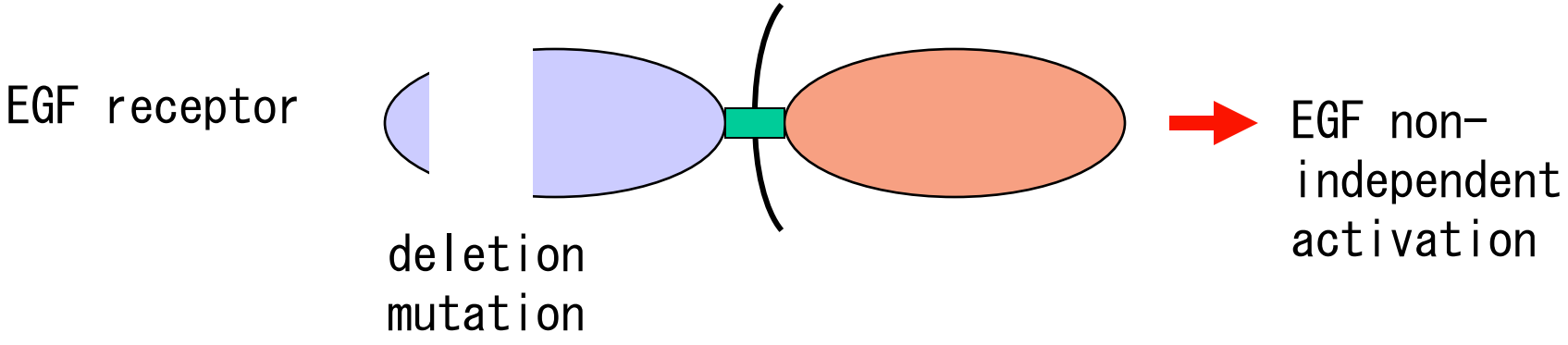
1913 Prof.Hanafusa 1980 Shibuya et al. Cell,1982



2. Discovery of structural anomaly in c-ErbB (EGFR)gene

in human brain tumor (glioblastoma)

Yamazaki et al. Mol Cell Biol,1988; Yamazaki et al Jpn.J.Cancer Res. 1990.



VEGF Independent/Non-Independent Vascular System Regulation

1. Characteristics of VEGF system and comparison with other angiogenic factors. Characteristics of the receptor VEGFR2.

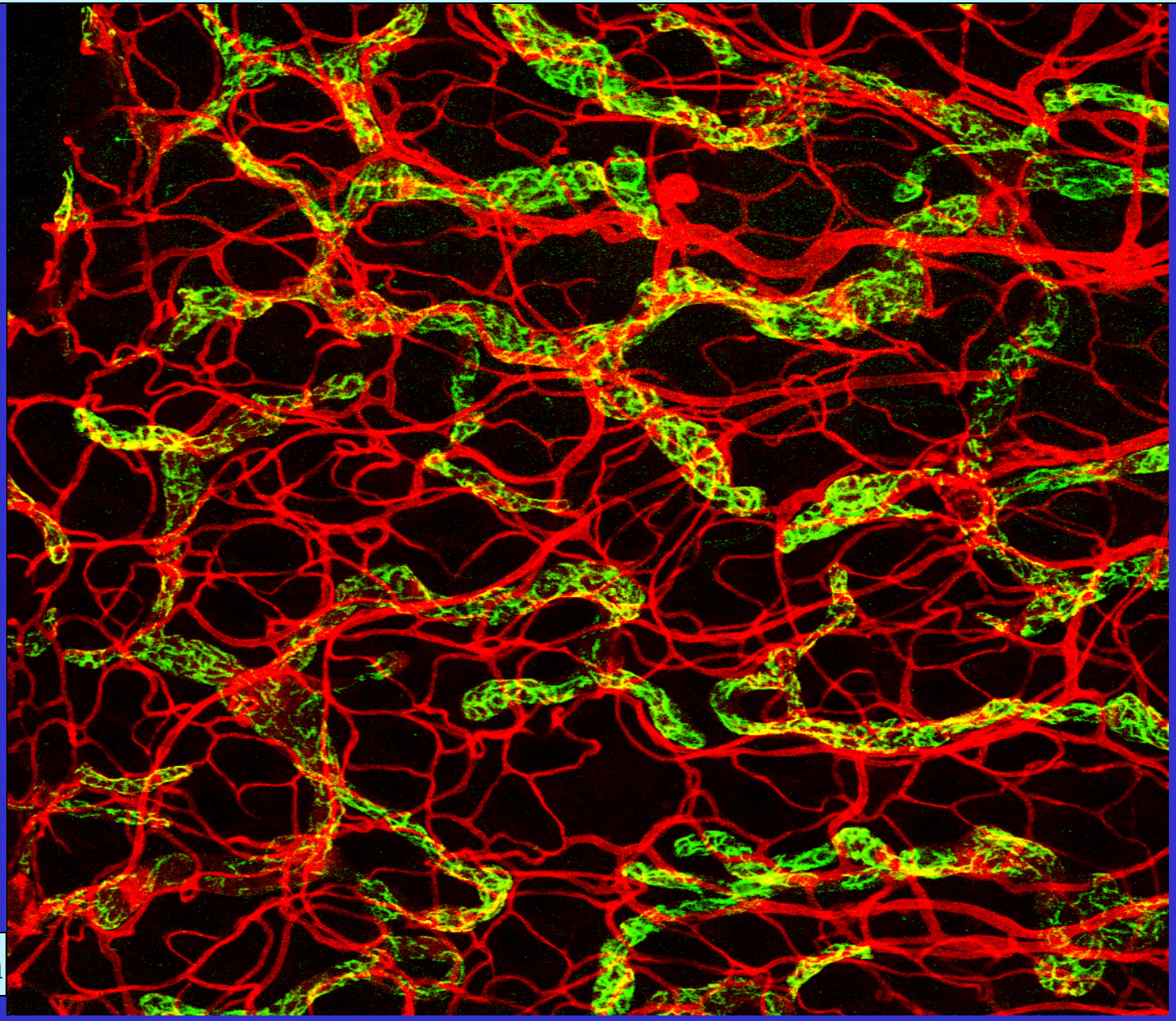
2. Responsibility of VEGFR1 to metastatic carcinoma, inflammatory diseases, and pregnant toxicosis

3. Toward regeneration of vessel, characteristics of VEGF-E, mechanism of vascular permeability

4. Regulation of VEGF non-independence

5. Summary of angiogenesis inhibiting treatment

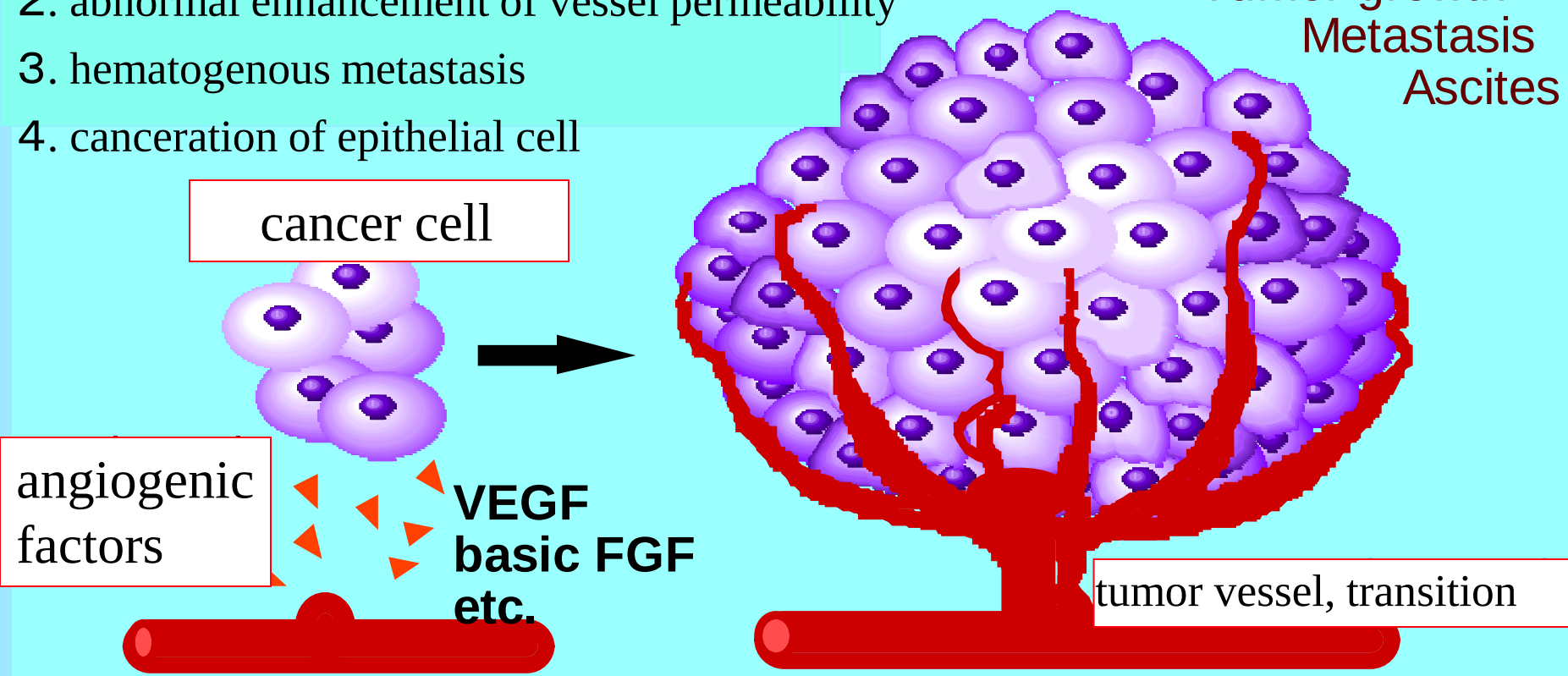
Blood Vessel and Lymph Vessel of Skin



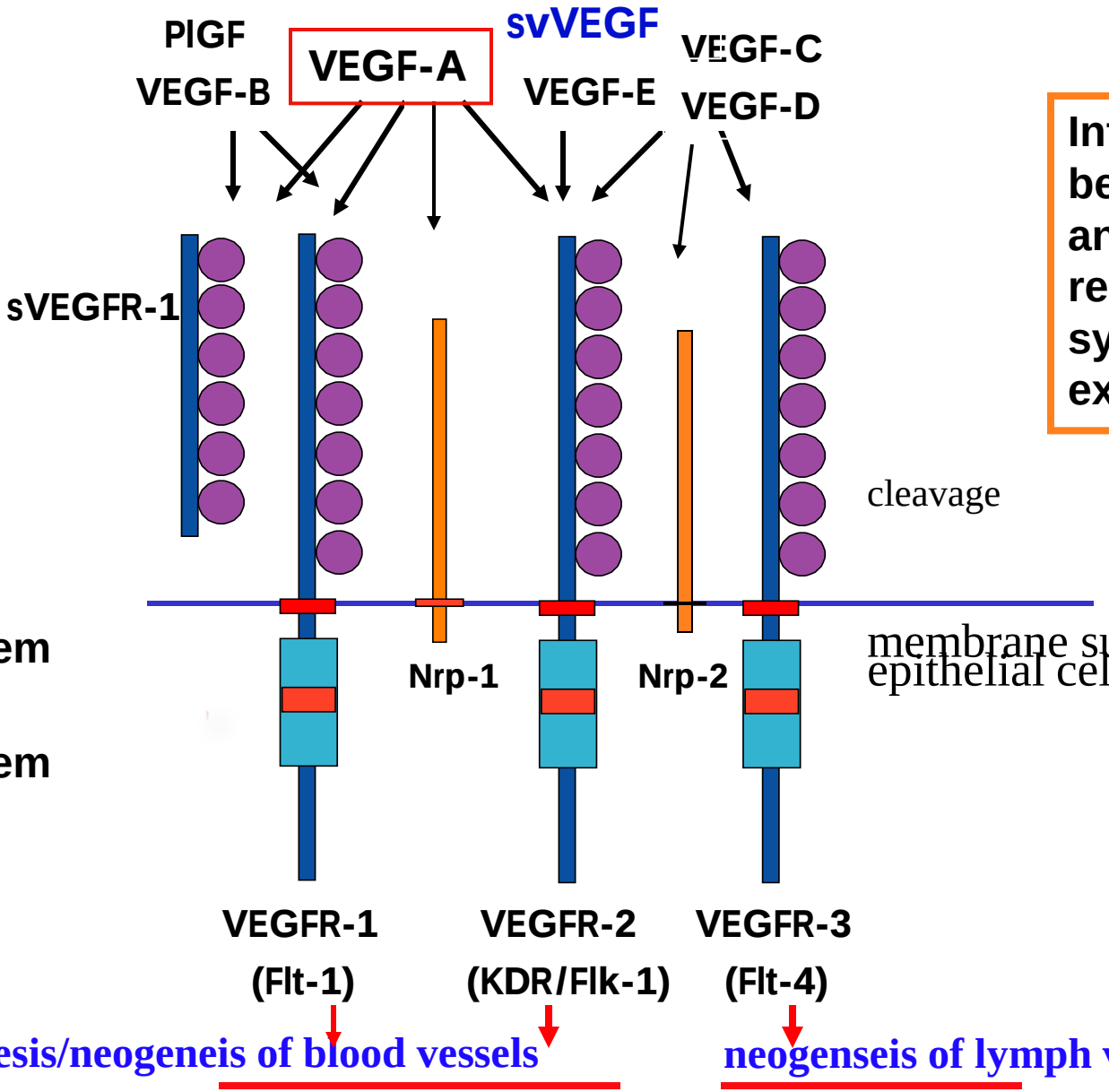
width 1 mm

Malignant Conversion of Cancer and Vessel : Propagation, Transition, Ascites

1. formation of tumor vessel
2. abnormal enhancement of vessel permeability
3. hematogenous metastasis
4. canceration of epithelial cell



VEGF-VEGF Receptor System

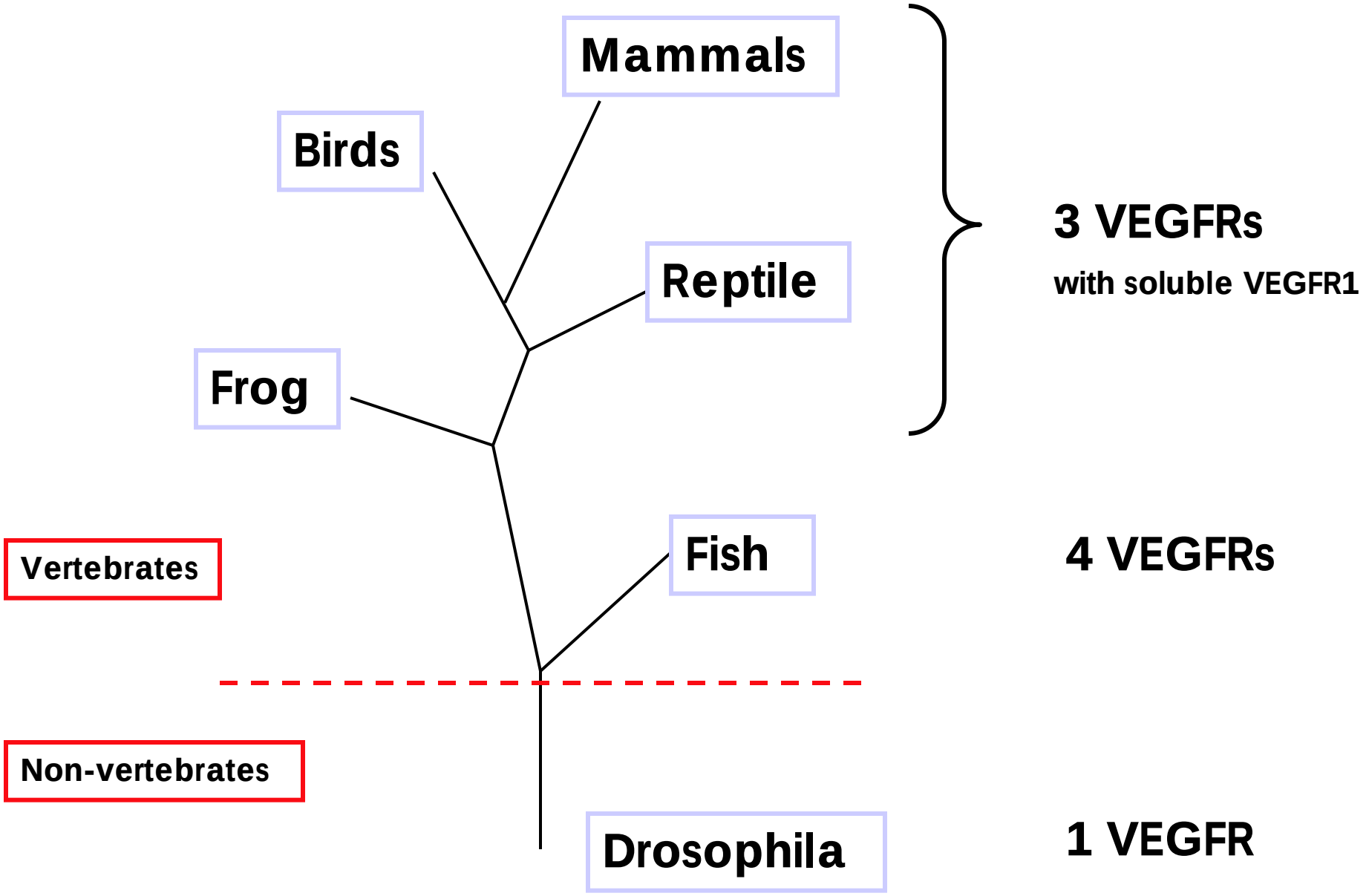


Interaction between angiogenic regulation system except VEGF

Birds:
3VEGFR system

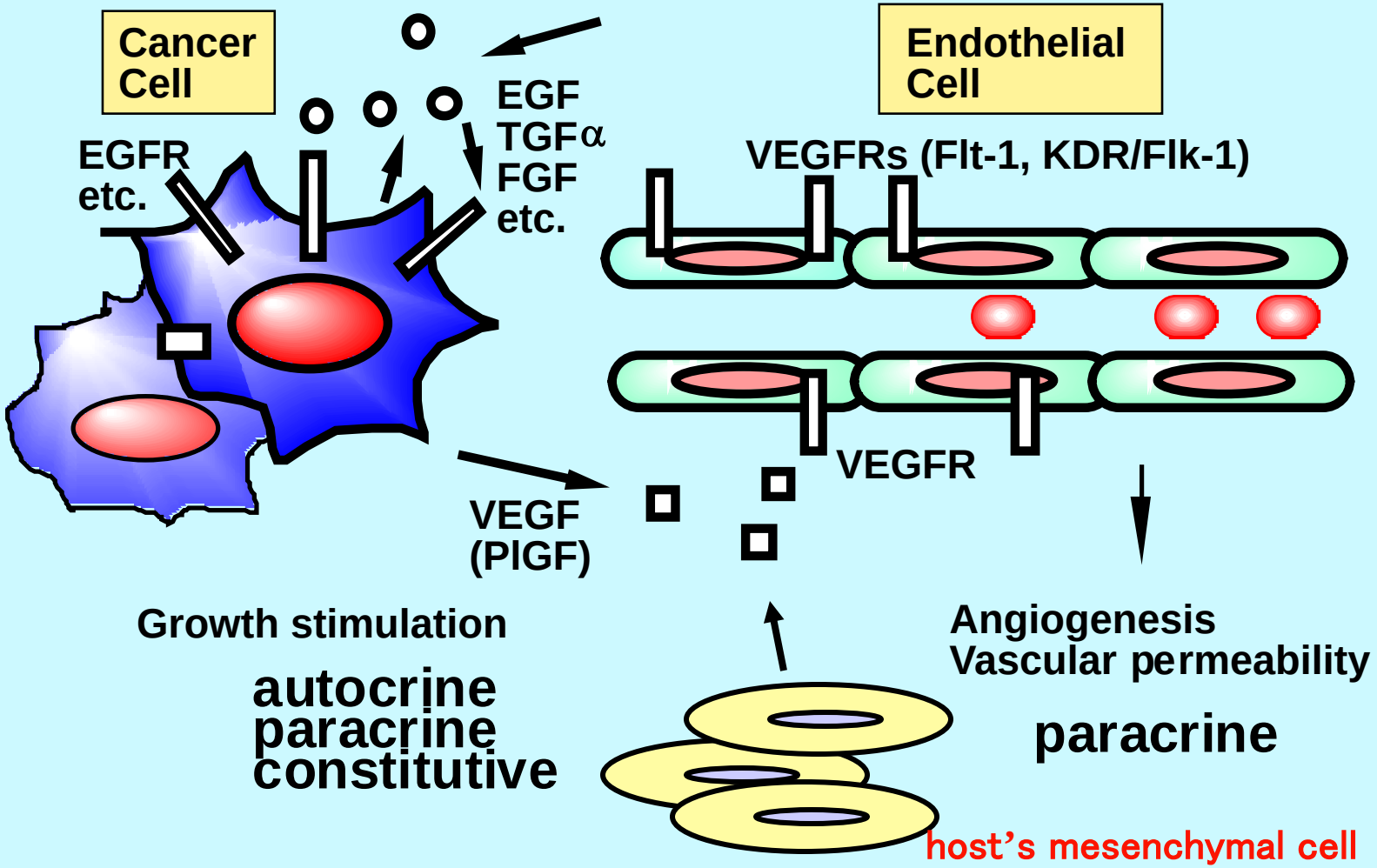
Drosophila:
1VEGFR system

Phylogeny of 7 Ig-tyrosine Kinase Receptor (VEGFR family)



Role of Tyrosine Kinase in Canceration

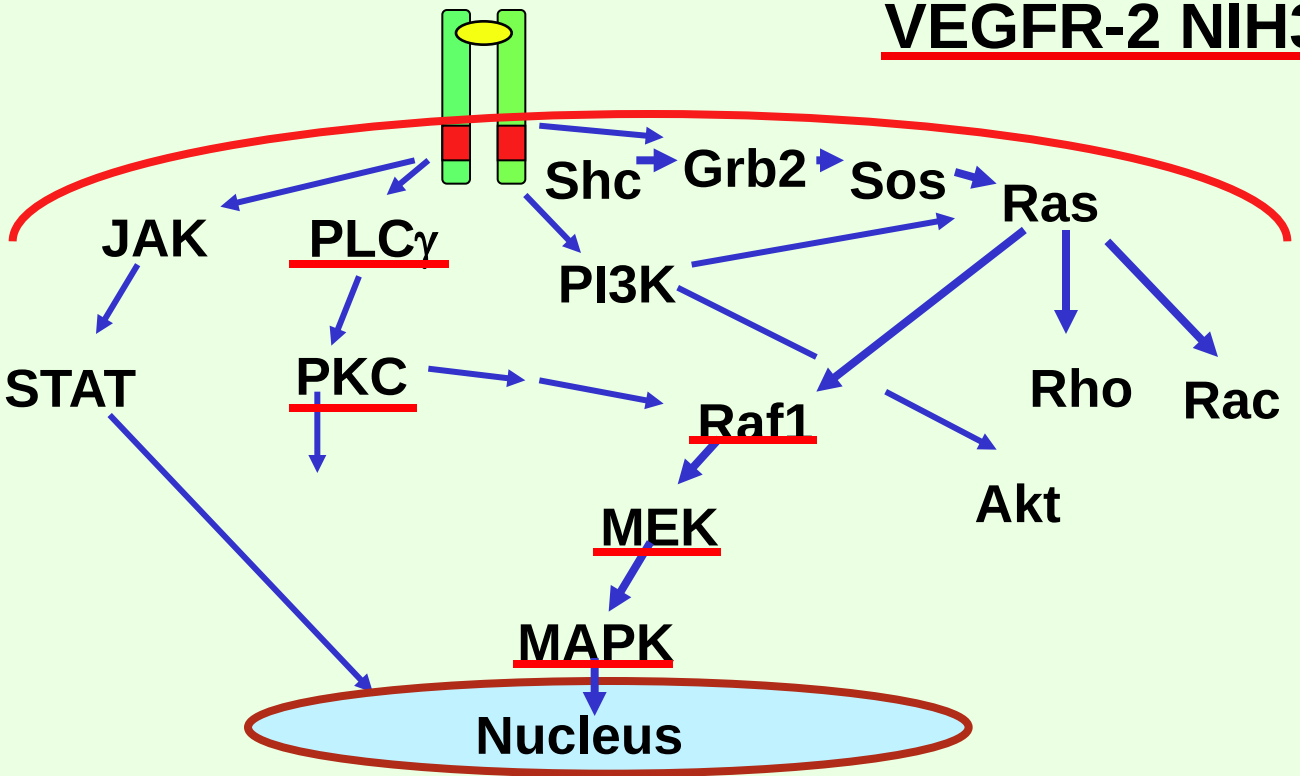
Paracrine Effect of VEGF and Its Receptor



Tyrosine Kinase Receptors and Intracellular Signaling

	Transform with ligand	DNA synth with ligand
EGFR NIH3T3:	+++	+++
VEGFR-1 NIH3T3:	-	-/(+)
VEGFR-2 NIH3T3:	-	+

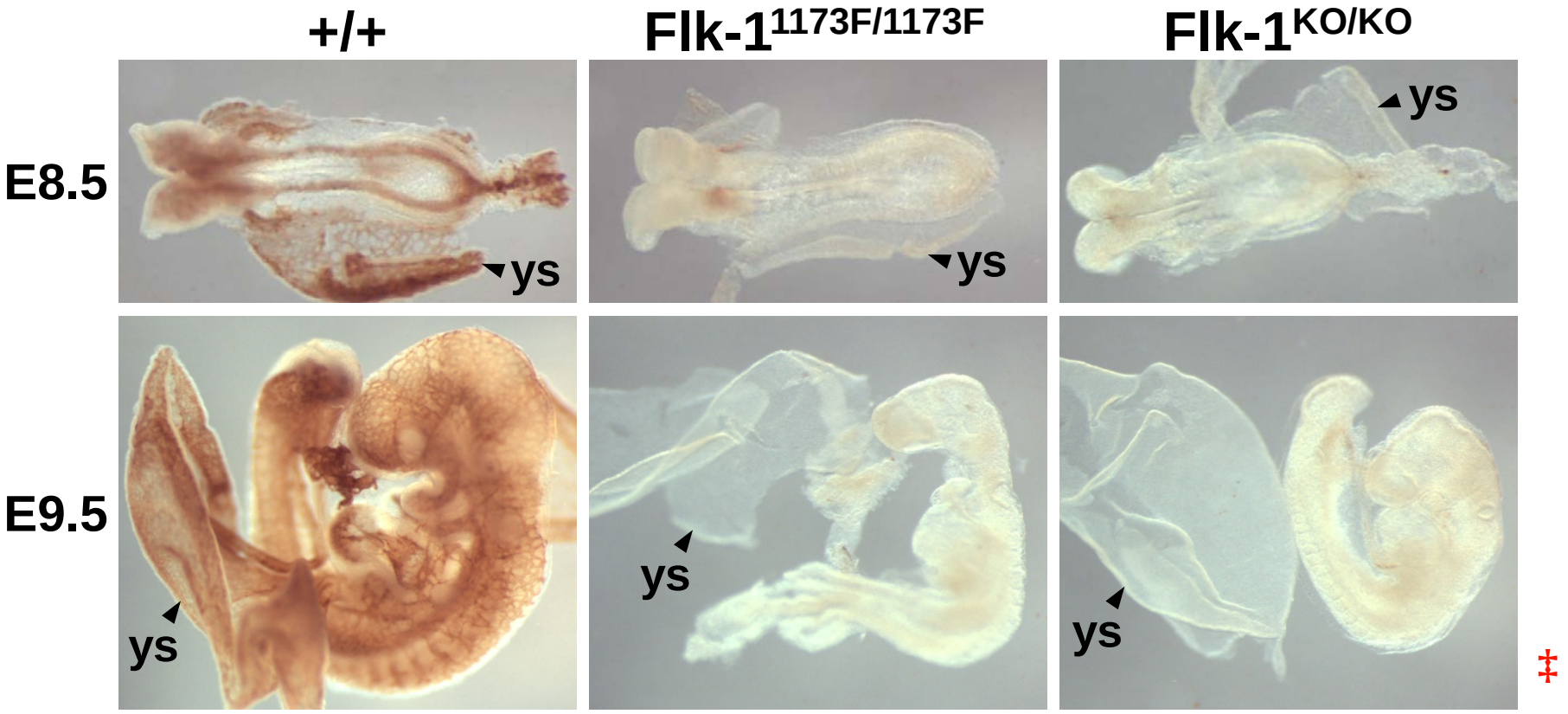
VEGFR-2 (KDR/Flk-1)



Characteristics of VEGFR-2 signal transduction:
mainly uses C-kinase system, not so dependent on Ras system

PECAM-1-Positive Blood Vessels Were Absent in *Flk-1*$^{1173F/1173F}$ and *Flk-1*$^{KO/KO}$ Embryos

PECAM-1 immunostaining

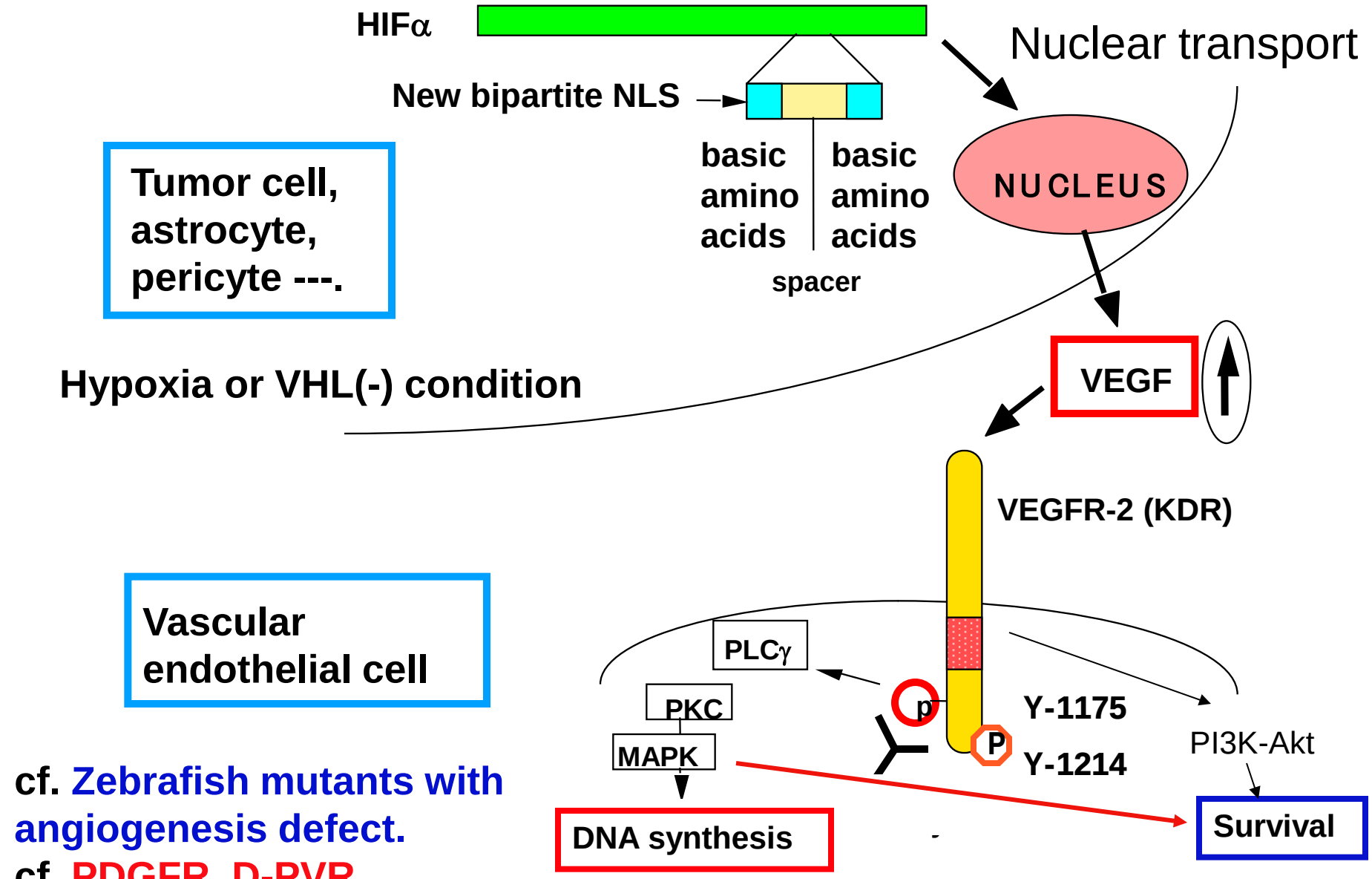


‡ ys, yolk sac

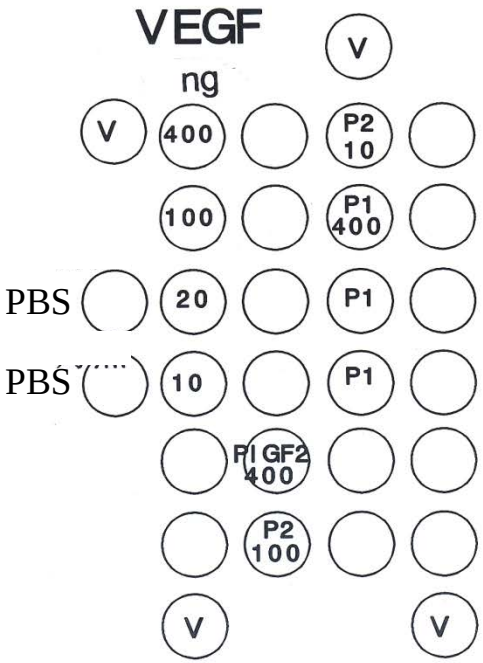
Sakurai et al. PNAS, 2005

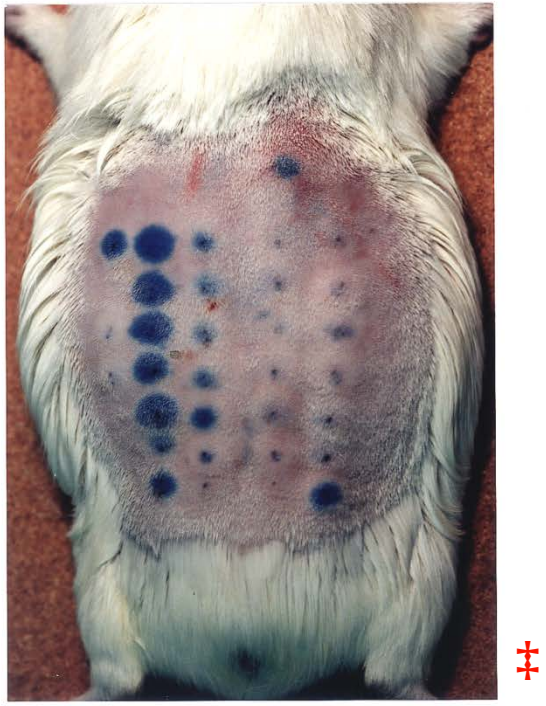
Induction of VEGF Expression and Cell Proliferative Signal from VEGF Receptor-2

use pY1175 - PLCγ - PKC - MAPK for DNA synthesis



Enhancing Activity of VEGF on Vessel Permeability --- Miles Assay





ascites

In every mouse ascites cancer, large amount of VEGF is accumulated in ascites.

Mouse ascites cancer cell MM2

(8 days)

right: control

left: anti-VEGF neutralizing antibody administrated

Mouse ascites cancer

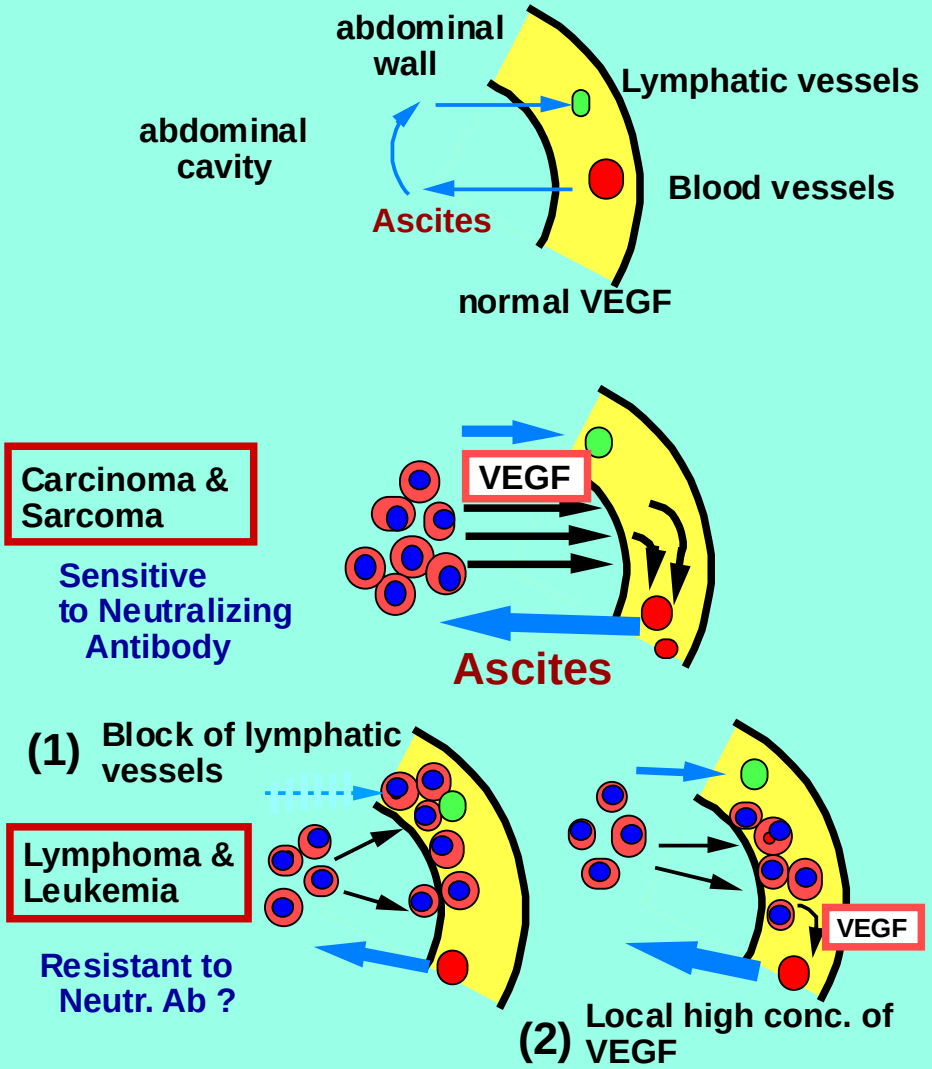
MM2 breast cancer-derived cell strain.

VEGF: 85 ng/ml ascites

anti-mouse VEGF antibody therapy (1w)

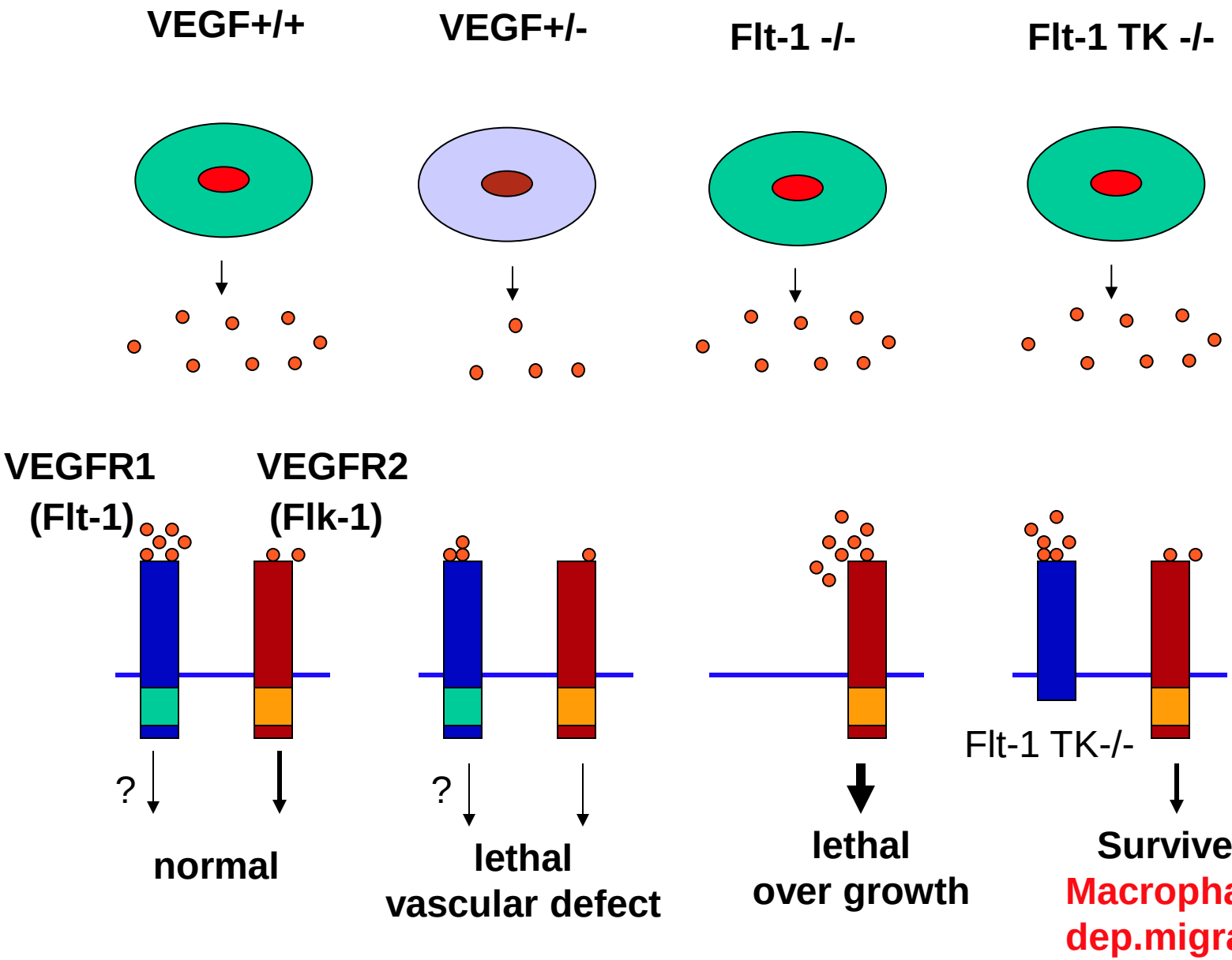
- **decrease in ascites (to 35%)**
- **decrease in tumor cells (to 35%)**
- **decrease in bleeding (to 10%)**

Models for Ascites Formation



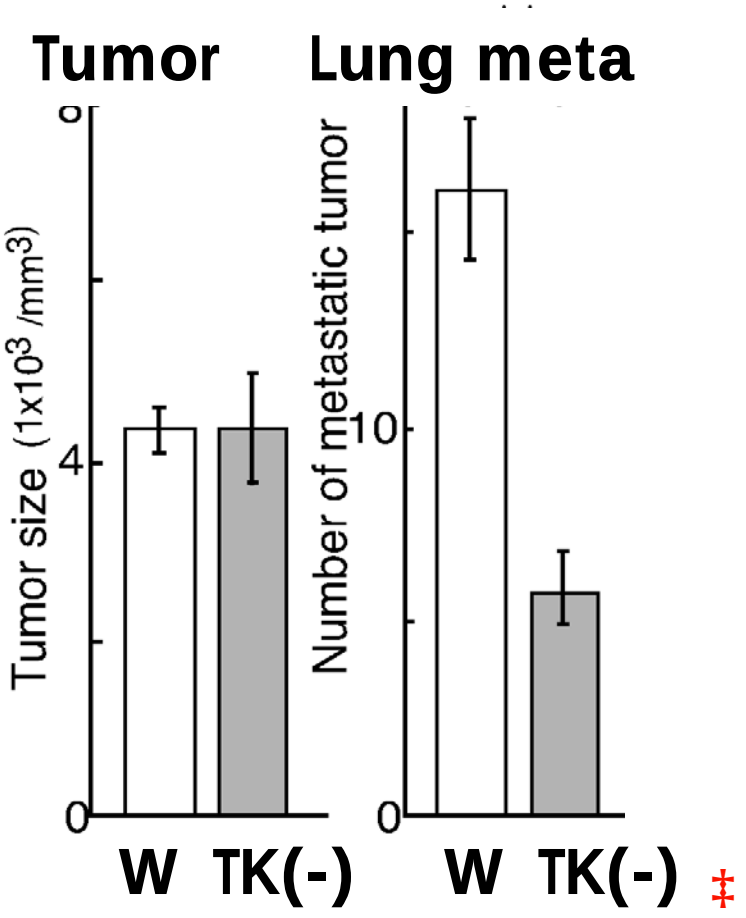
1. other VPFs (Bradykinin, Histamin etc.)
2. Suppression of absorption by cytokines
3. Abnormal coagulation

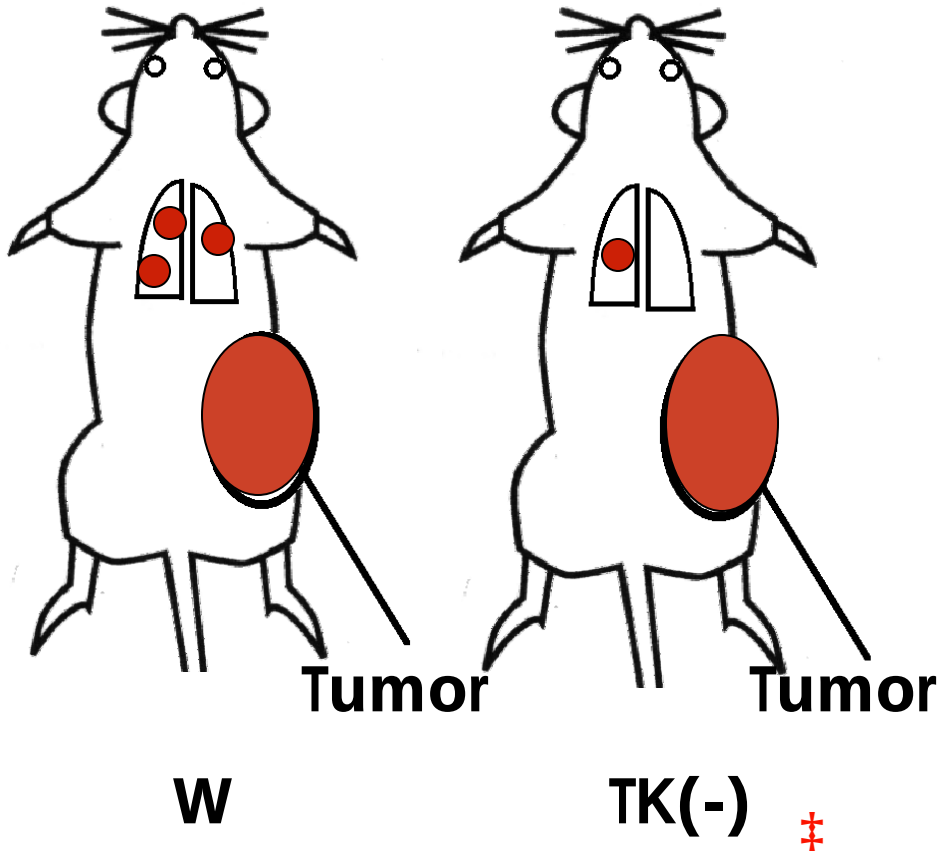
Knockout Mouse Analysis of VEGF, VEGF Receptor Gene



Mouse Model of Tumor Transition to Lung

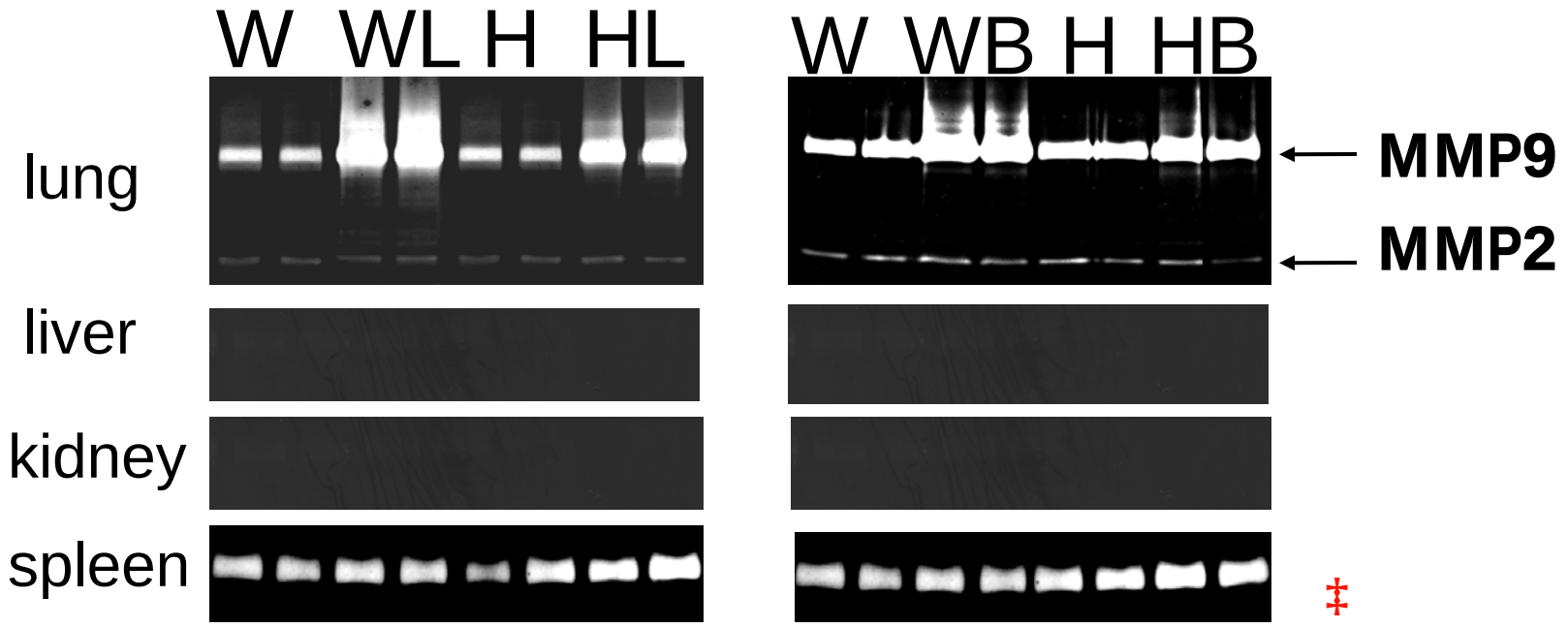
Transition to lung decreases in a mouse with VEGFR-1(Flt-1) signal defect.





W, wild type; TK(-), VEGFR-1 TK(-/-); Tumor: Lewis lung ca

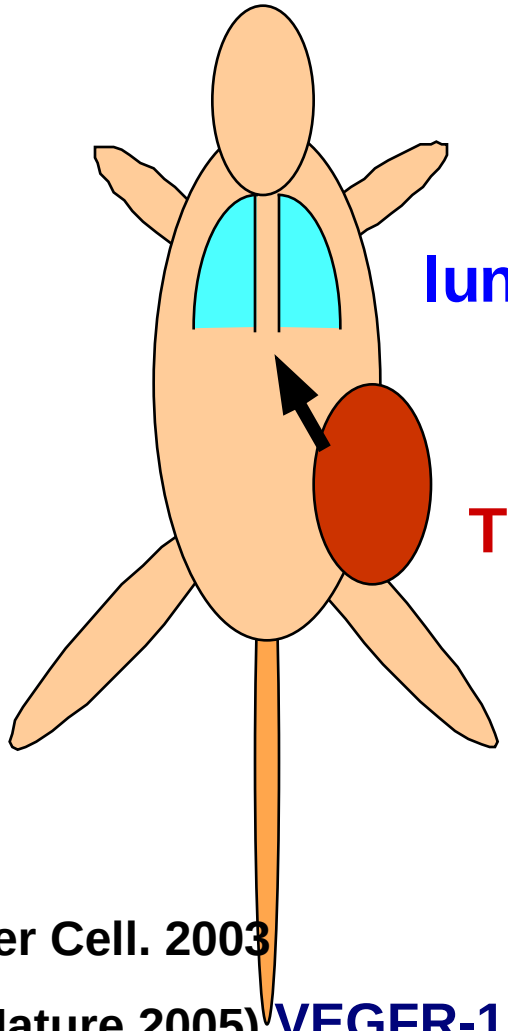
Pulmonary induction of MMP9 via primary tumor is significantly decreased in *VEGFR1 (flt-1) TK*(-/-) mice.



W: wild type; H: *VEGFR-1 (flt-1) TK-/-*

L: Lewis lung carcinoma; B: B16 melanoma

Mechanism of New Cancer Transition Dependent on VEGF Receptor-1 (Flt-1) Kinase



Tumor tissue

↓

(x)

↓

VEGFR-1(Flt-1) ⊢ TK(-/-)

↓

Lung endothelial cell
macrophage

↓

MMP9 induction ⊢ MMP9(-/-)

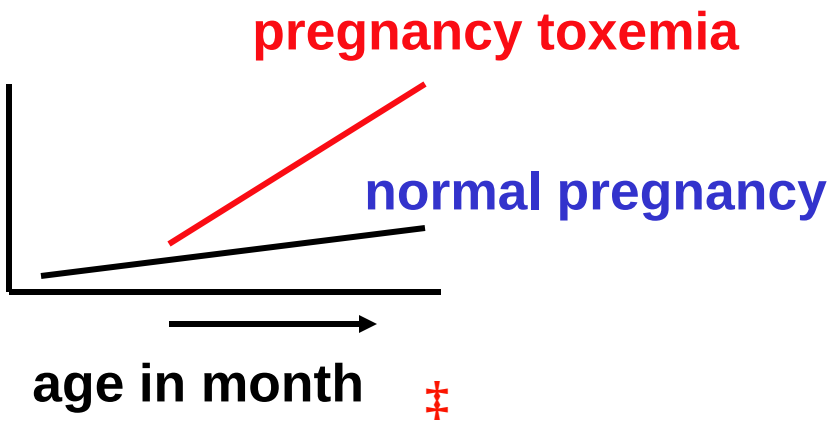
Metastasis

Cancer Cell. 2003

(c.f. Nature 2005) VEGFR-1 TK(-/-) and MMP9(-/-) block this phenotype.

Expression of Soluble VEGFR1 (flt-1) mRNA in Placenta

In pregnancy toxemia,
sol. Flt-1 in mother's blood increases abnormally.

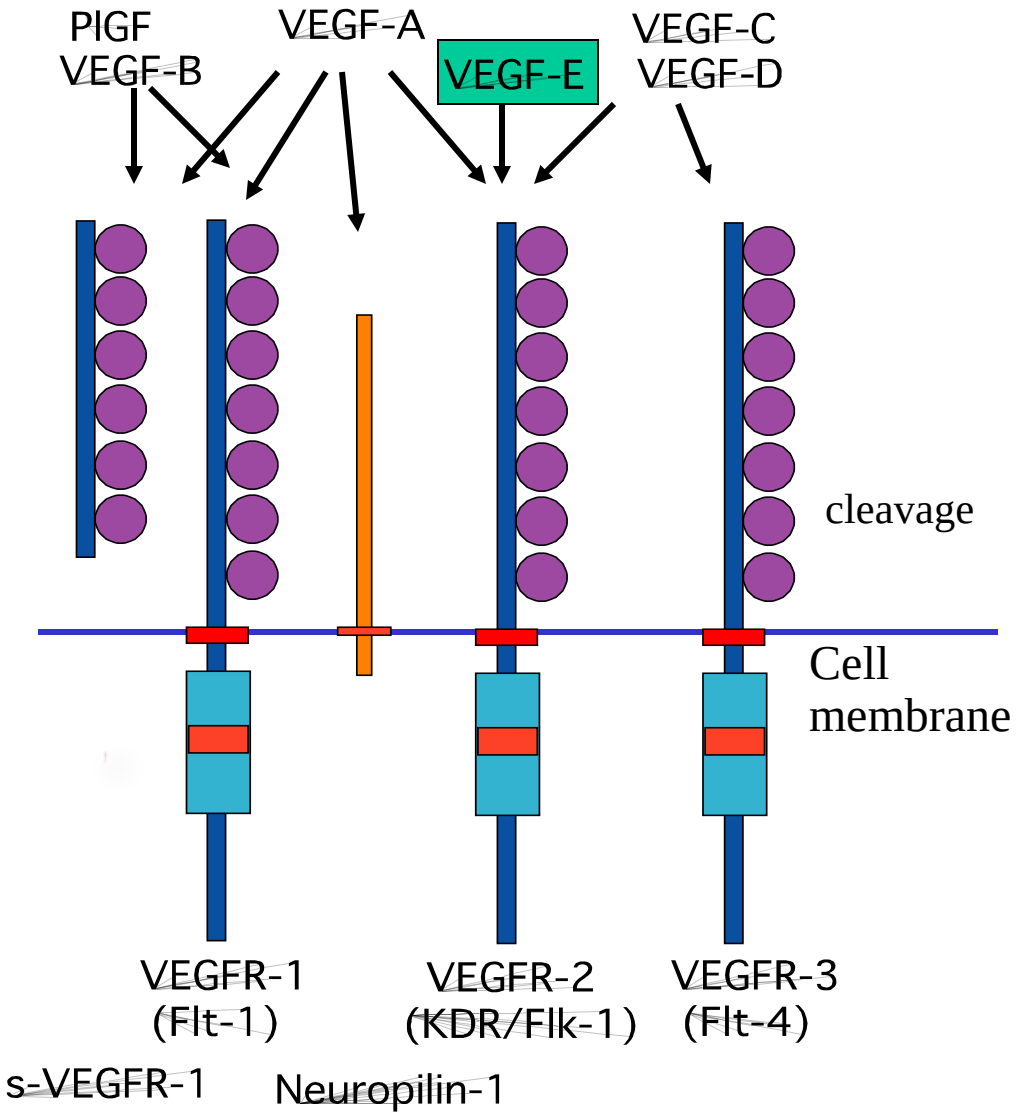


← Full length

← Soluble form

New Type Angiogenic Factor, **VEGF-E**

Binds only to VEGFR-2
Promotes angionenesis well in vivo.
Epithelial cells adhere well.
edema（—）
hemorrhagic lesion（—）



Phenotypes of Human VEGF-A_{165} Transgenic mouse

VEGF-A_{165} Tg **control**

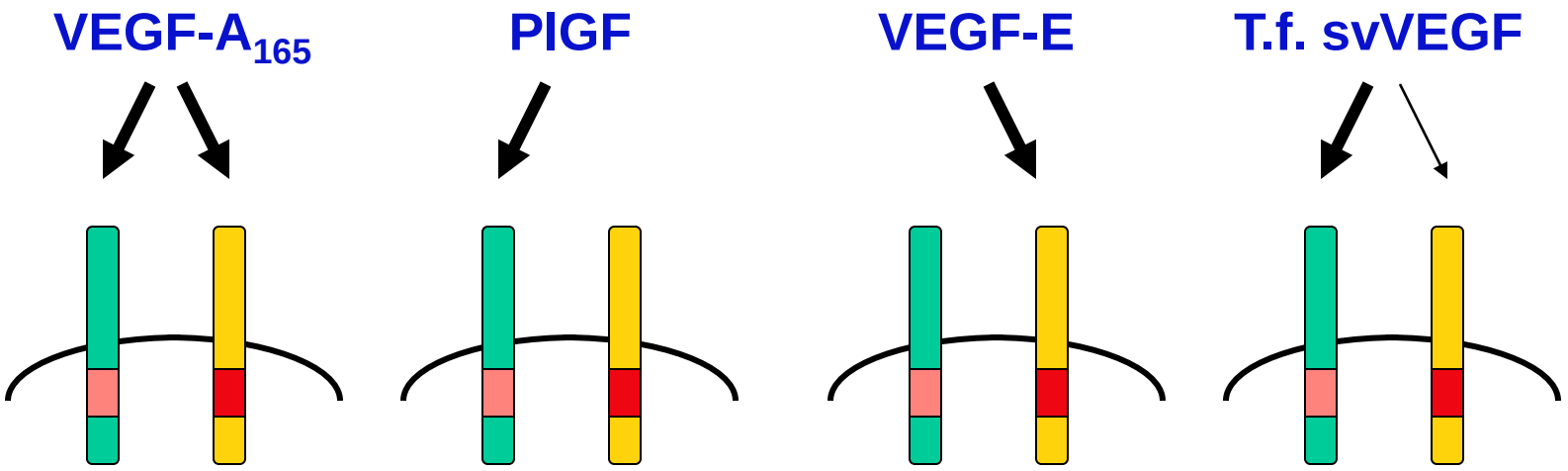
‡

VEGF-A_{165} Tg **control**

‡

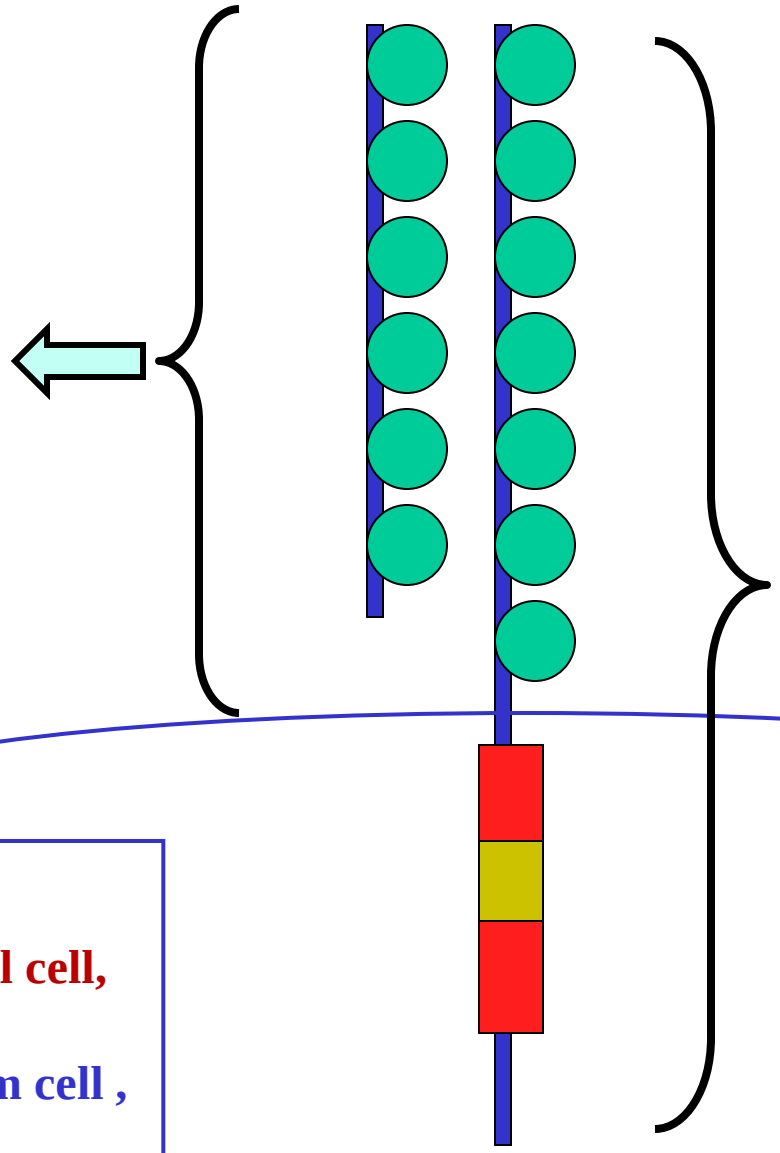
	blood vessels	leakiness	inflamm.	body w. loss
VEGF-A165	+++ (thin)	+++	+++	+++ (2nd, +)
VEGF-E (NZ7)	+++	+	-	-
PlGF	+	-	-	-

When VEGFR1 and VEGFR2 are activated at same time, promoting signal of vascular permeability increases.



	VEGF-A$_{165}$	PlGF	VEGF-E	T.f. svVEGF
VEGF activity	+++	+/-	+++	+
VPF activity	+++	+	+ (++ in acute phase)	+++

Biological Functions of VEGFR-1 (Flt-1) in Mammal



(1)
Inhibitory regulation of angiogenesis during fetal life

(2)
Barrier function in placenta? (pregnancy toxemia)

(3)
Ambulato of macrophage

(4)
Slight angiogenesis, high promoting of permeability

(5)
Reconstruction of bone marrow

(6)
MMP9 induction, promotion of transition to lung, promotion of inflammation

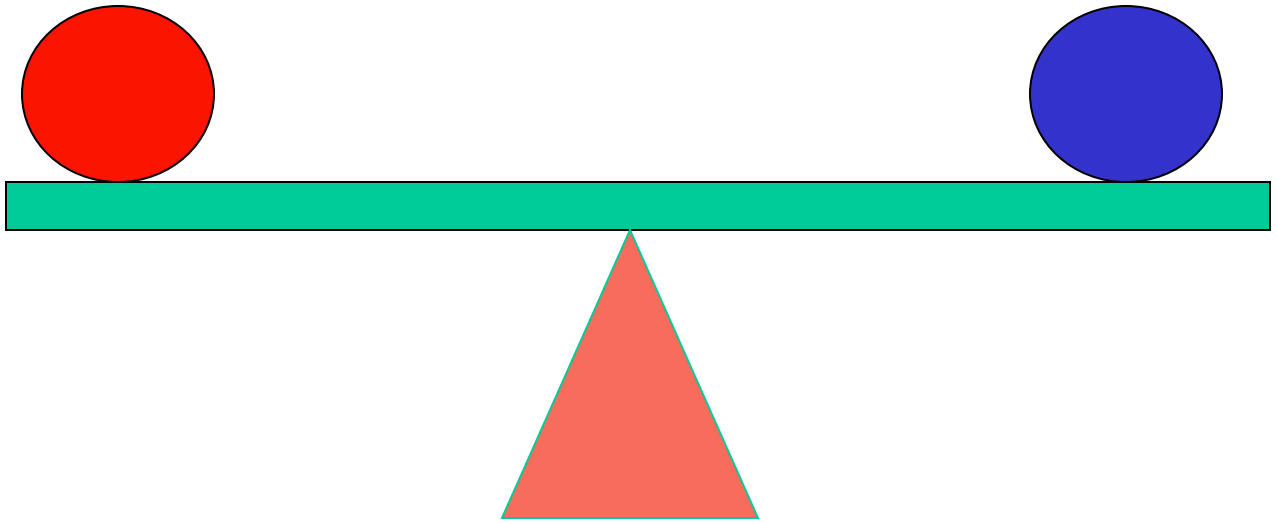
Expressing cells:

vascular endothelial cell,
macrophage,
haematopoietic stem cell ,
plain muscle cell

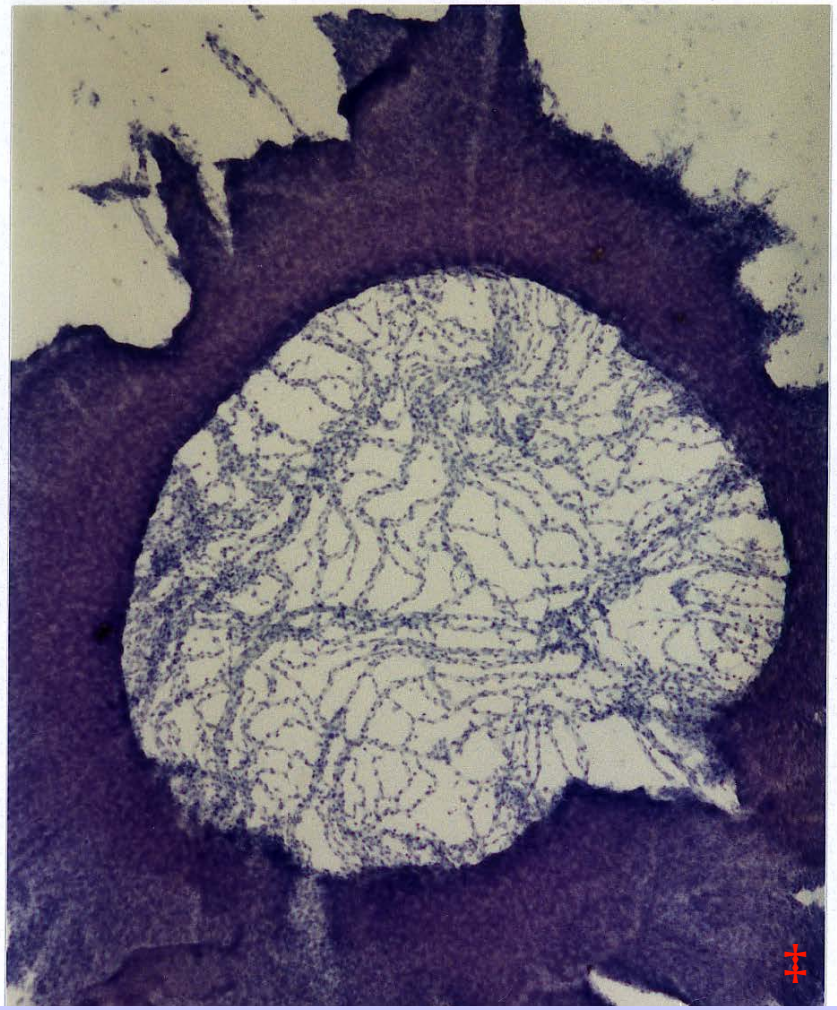
Summation of Angiogenesis

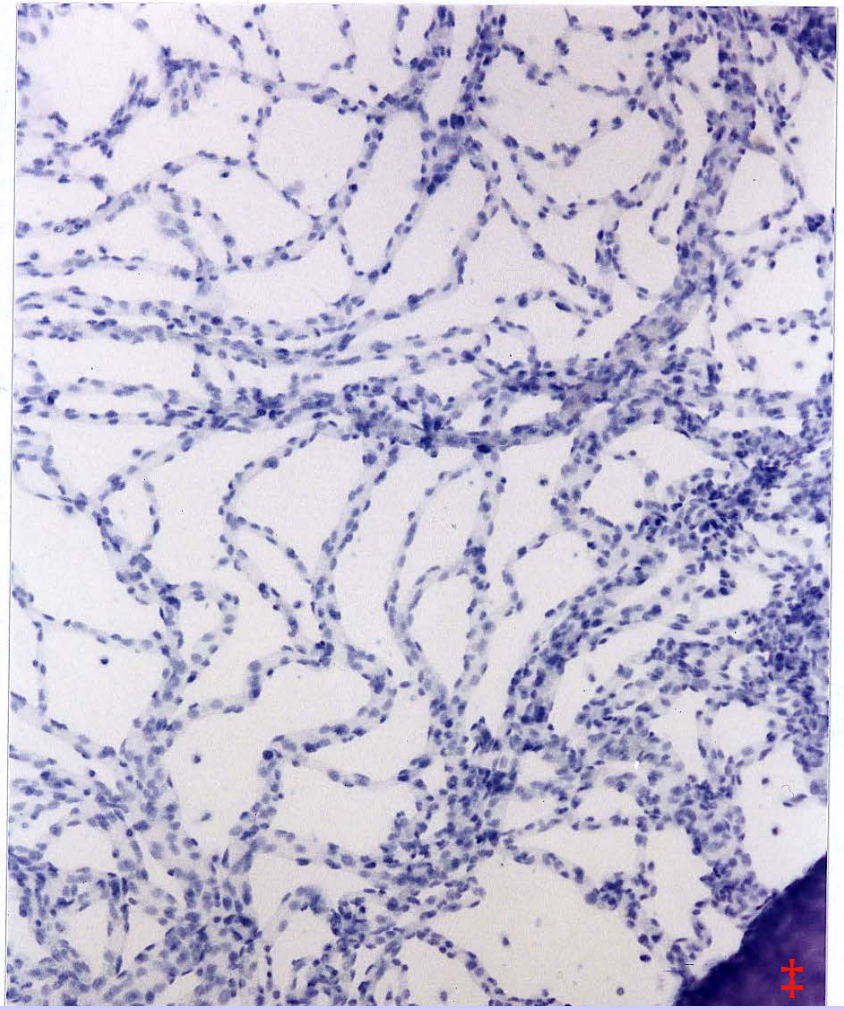
Pro-angiogenic

Anti-angiogenic



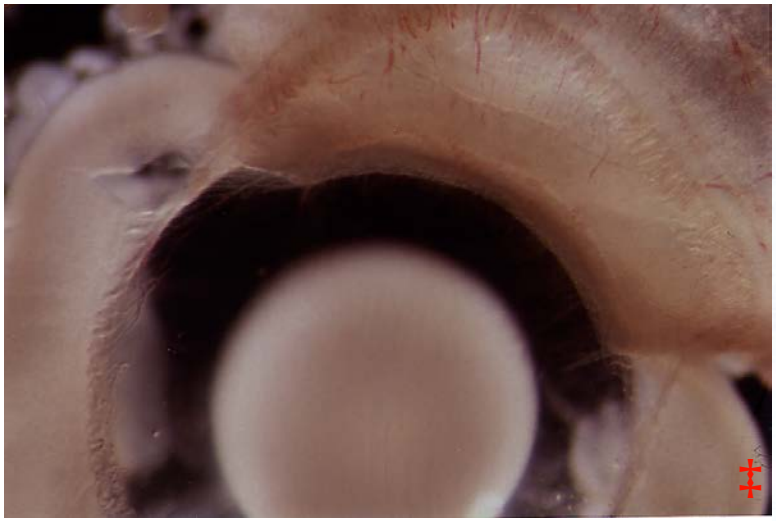
Capillary Network in Rat Pupillary Membrane at Day-8.

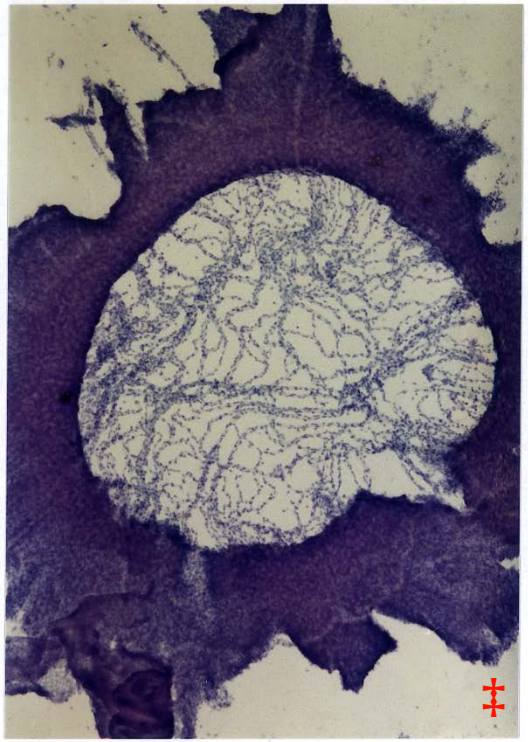


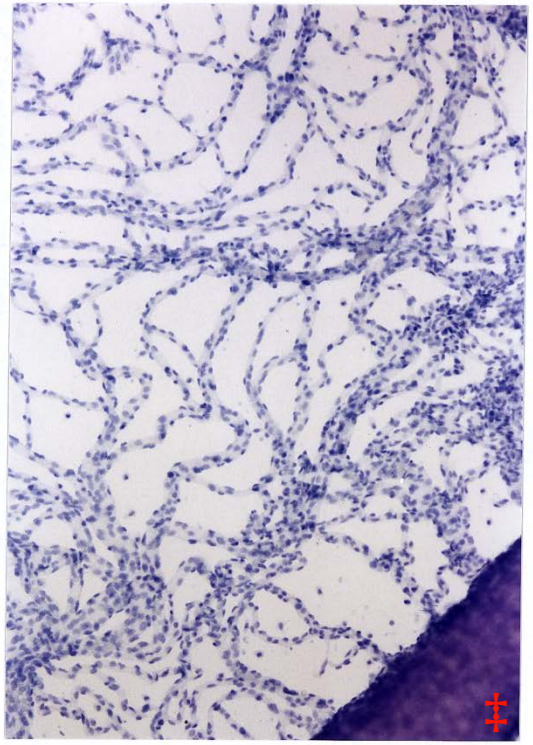


Lens epithelial cell produces BMP4, and induce apotosis of blood vessel.

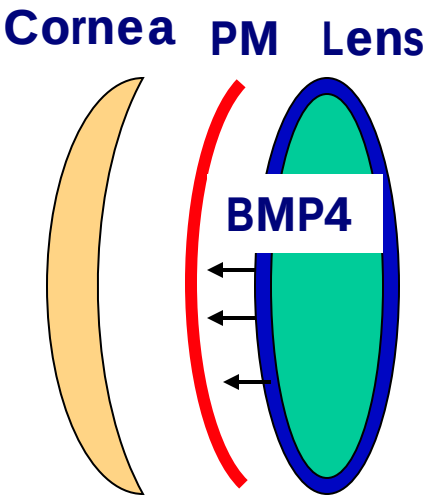
Rat Pupillary Membrane with Vascular Network

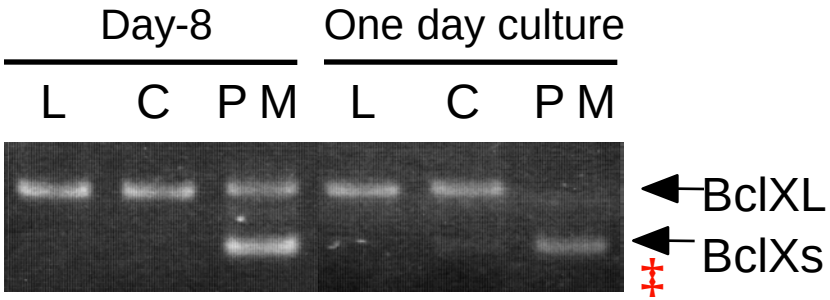






Day-8 rat

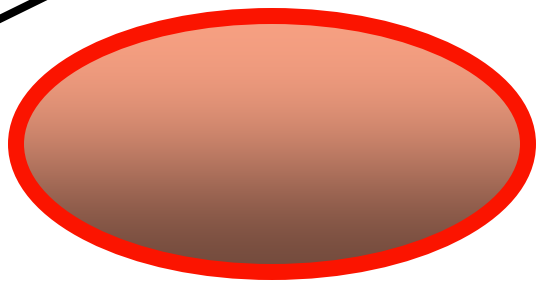




surgery

actinotherapy

chemotherapy
molecular targeting



immunotherapy

anti-angiogenic therapy

VEGF (VEGF-A) Neutralizing Antibody

the third phase clinical experiment

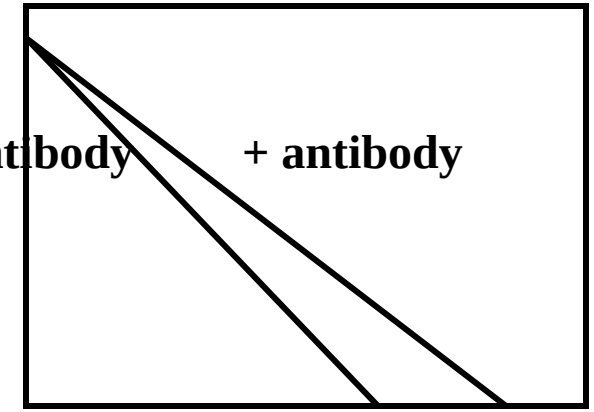
Stage-3,-4 colon cancer patients: randomized trial (400 x 2)

chemotherapy ± anti-VEGF antibody

survival time：－antibody, 15.6M; + antibody, 20.3M

by-effect：slight high blood pressure（no bleeding）

strain on kidney



cf. surgical benefit in breast cancer and lung cancer (NSCLC)

N. Ferrara et al. (Genentech Inc.), Gordon Conf. 2003, etc.

Laboratory to (Bench) Clinic (Bedside)

----- Cancer Therapy by Tumor Vessel Inhibition----（1）

Why Drug Discovery Was Efficient in VEGF-VEGFR System

1. VEGF-VEGFR system was almost the central system of vascular regulation in mammals.
2. Fundamental research in lab and application research in firm supported each other well.
3. Similar effect of sol-Flt-1(s-VEGFR1) was recognized without anti-VEGF antibody.
4. Cancer patients participated positively, which enabled steady clinical experiments.
5. By-effects not being so heavy could be predicted not only from animal experiments, but from the case of pregnancy toxemia.

Laboratory to (Bench) Clinic (Bedside)
----- Cancer Therapy by Tumor Vessel Inhibition----（2）

Challenge That Lies Ahead in Japan and the World

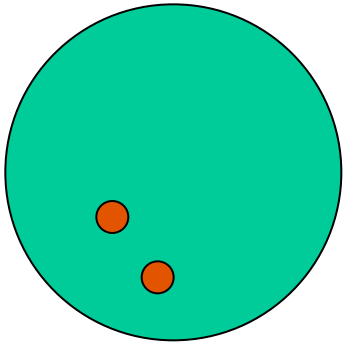
1. **Clinical experiments on VEGF~VEGFR inhibitor must be proceeded immediately in Japan and Asian countries.**

2. **Is there any difference in the effect between races? How about in stomach cancer or liver cancer? Any effect on scirrhous gastric cancer?**

3. **Are there any patients resistant to anti-VEGF antibody? Is tumor vascular inhibitor needed to be administrated individually? Are there individual variations in bi-effects?**

4. **What happens and what should be done when effects of anti-VEGF antibody lower? Any other inhibitor of VEGF-VEGFR system (TK-inhibitor etc.)？**
   Any dependence on systems other than VEGF?---- FGF, PDGF etc.
   Any non-vascular-dependent propagation of cancer? New evaluation system?

To Young Researchers ----From My Experience-------



1. Find a rule used individually.

------- cyclic GMP

2. Concentrate when needed.--- From cancer gene to blood vessel.

3. Basically, research is a personal duty, but it cannot be done without supports from many people.

Experience in TR and clinics in Japan, proactive participation of patients is essential.

4. Acquire a global sense------ Lesson from studying abroad（Hanabusa Lab）

Acknowledgements：Co-Researchers

VEGFR-1(Flt-1)

Shimehito Matsu
Sachiko Yamaguchi
Asako Sawano
Toshio Ikeda
Akira Yamane
Kenji Wakitani
Keiji Tanaka
Sachie Hiratuka
Yoshiro Maru
Shinobu Iwai
Kunio Kondo
Lata Seetharam
Satuki Kobayashi
Masato Murakami
Rei Koide
Makoto Watanabe
Tsuyoshi Nagase
Kumi Iwata
Akira Muramatsu

VEGFR-2(KDR/Flk-1)

Tomoko Takahashi
Yoshiko Sakurai
Naoyuki Yabana
Kaori Oogimoto
Ako Masuda
Junko Kami
Takahiro Kamiga
Saeed Samarghandian

VEGF/VEGF-E/svVEGF /BMP

Jin-Cai Luo
Sachiyo Ogawa
Atsushi Kiba
Mari Seino
Momomi Saito
Hiroyuki Takahashi
Yujuan Zheng
Mai Yamauchi
Seiji Yamamoto

External institutions, labs

Tetsuo Noda（Tohoku U.）
Nobuaki Yoshida（I. Med. Sci., U. Tokyo ）
Shunpei Niida（NCGG）
Tatsutoshi Nakahata（Kyoto U.）
Shinichi Nishikawa（Riken）
Kanji Sato（Tokyo Women's Medical U.）
Hitoshi Yoshizawa（Nara Medical U.）
Mayumi Ono（Kyushu U.）
Kensuke Egashira（Kyushu U.）
Kari Alitalo (Helsinki U.)
Peter Carmeliet (Belgium)
J. Waltenberger (Germany)

Kyowa Hakko Laboratory
（Kenya Shitara etc.）
Kirin Beer
（Kazuhide Nakamura etc.）
Daiichi Seiyaku（Noriko Tanaka etc.）

Acknowledgements：Co-researchers（２）

Cancer gene・tyrosine kinase

Noriko Goto
Lu-Hai Wang
Jun Yokota
Hitoshi Yamazaki
Kiyoshi Ariizumi
Toshihiko Seki
Yoshiyasu Kaneko
Shinsaku Hirosawa
林　雪芬
Misako Sato
Bertrand Pain

Arinobu Tojo
Yasuhisa Fukui
Terumasa Tsuchiya
Atsushi Kanno
Sachiko Misawa
Masayuki Hino
Katsuya Yamamoto
Michiko Kido
Masashi Toyoda
Taku Watanabe

External institutions, labs

Yoshihito Kamiyama（Tokai U.）
Kenichi Tamaki（Tokai U.）
Shigero Mori（I. Med. Sci., U. Tokyo ）

Prof. Yoshihito Kamishiro, Prof. Takashi Sugimura, Prof. Haruo Kanno
Prof. Hidesaburo Hanabusa, Prof. Kumao Toyoshima, Prof. Takeshi Odaka