# TNF-related Apoptosis-inducing Ligand is a Novel Regulator of Angiogenesis


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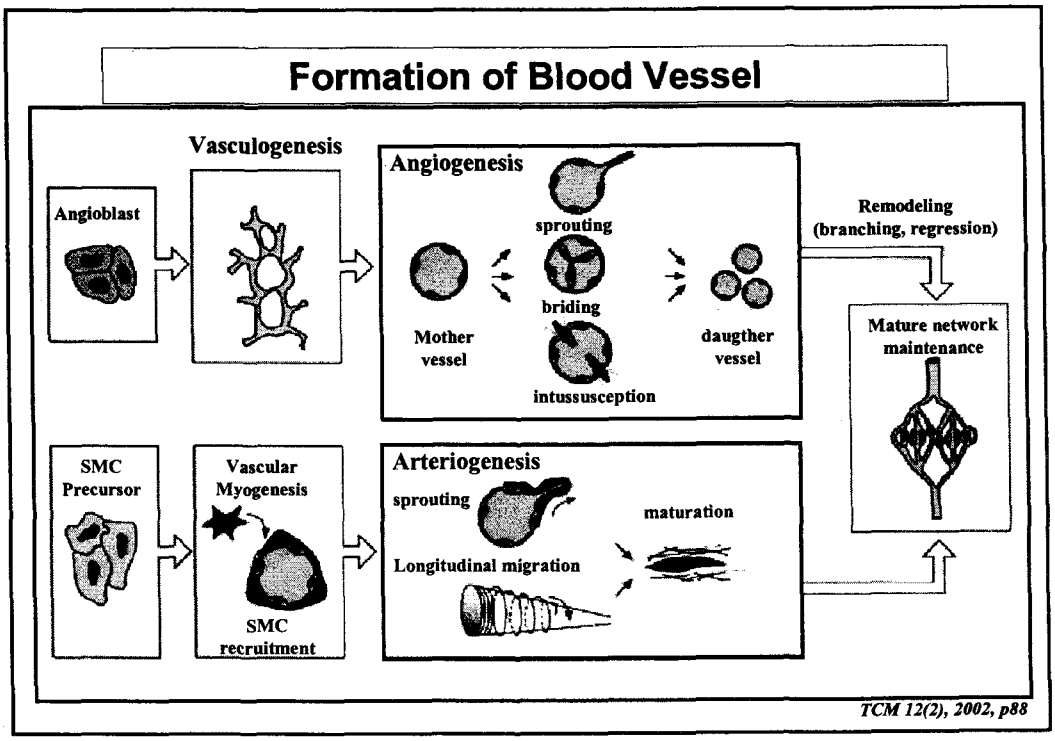
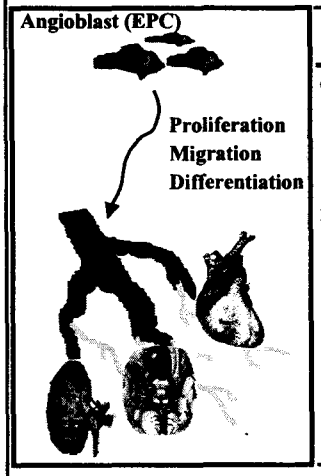


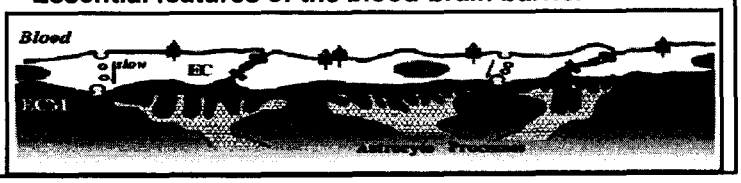
## Heterogeneity of endothelial cells in different adult tissues & organs



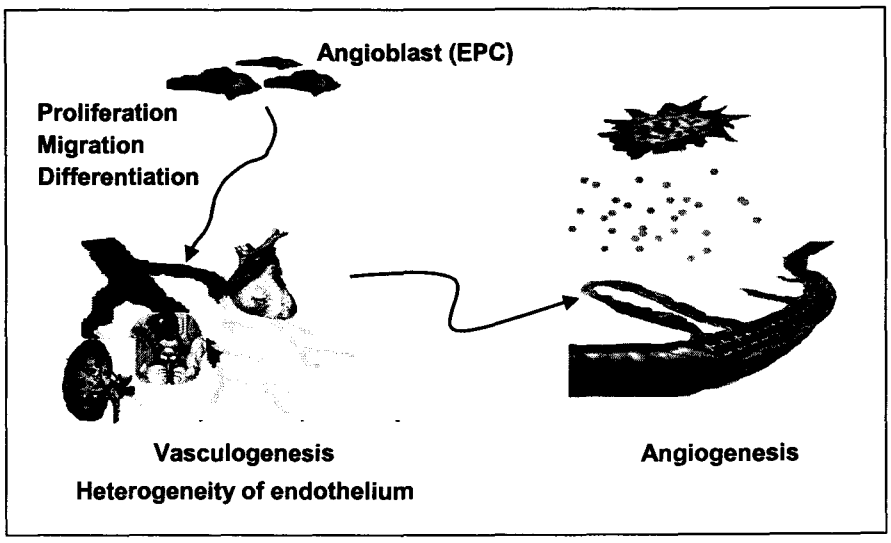

| | Tissue or Organ | Properties | Function |
|---|---|---|---|
| Continuous | | | |
| | CNS | Low number of vesicles, complex tight junctions | Blood-brain barrier |
| | Lymph nodes | High endothelial venules HEV | Lymphocyte homing |
| | Muscle | High number of vesicles | Exchange/transport |
| Discontinuous | | | |
| | Endocrine glands | Fenestrae | Secretion |
| | Gastrointestinal tract | Fenestrae | Absorption |
| | Choroid plexus | Fenestrae | Secretion |
| | Kidney glomeruli | pores | Fitration |
| | Liver | Large gaps | Exchange of particles |
| | Bone marrow | Marrow sinus | Hemopoiesis, delivery of blood cells |
| | Spleen | Splenic sinus of red pulp | Blood cell processing |

### Essential features of the blood-brain barrier

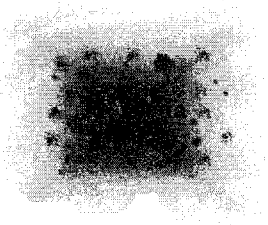
Tight junction: ●
Adherence junction: ■
P-glycoprotein: ▲




## Vasculogenesis and Angiogenesis



**Tumor** Benign tumor
Malignant tumor-cancer



Hypoxia inducible factor (HIF)

HIF-1α

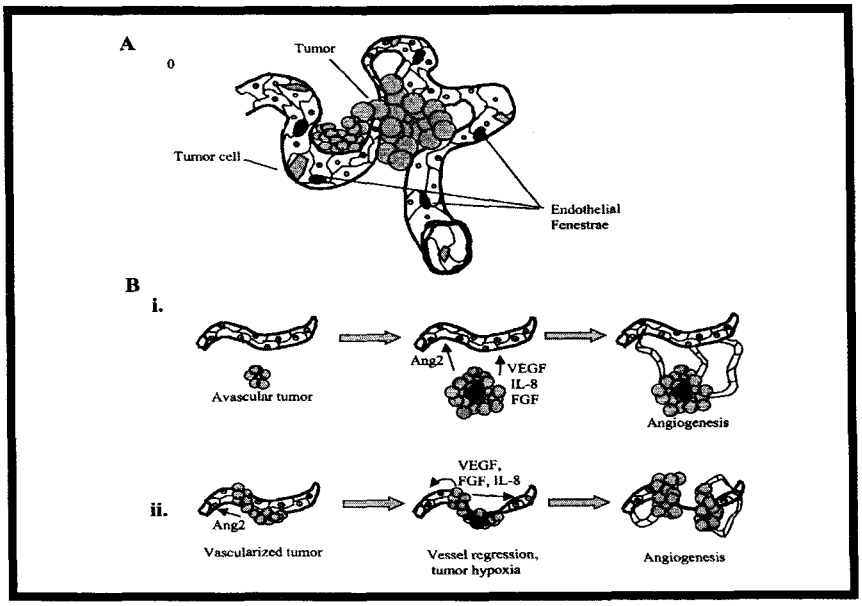
VEGF

HIF-1α HIF-1 β

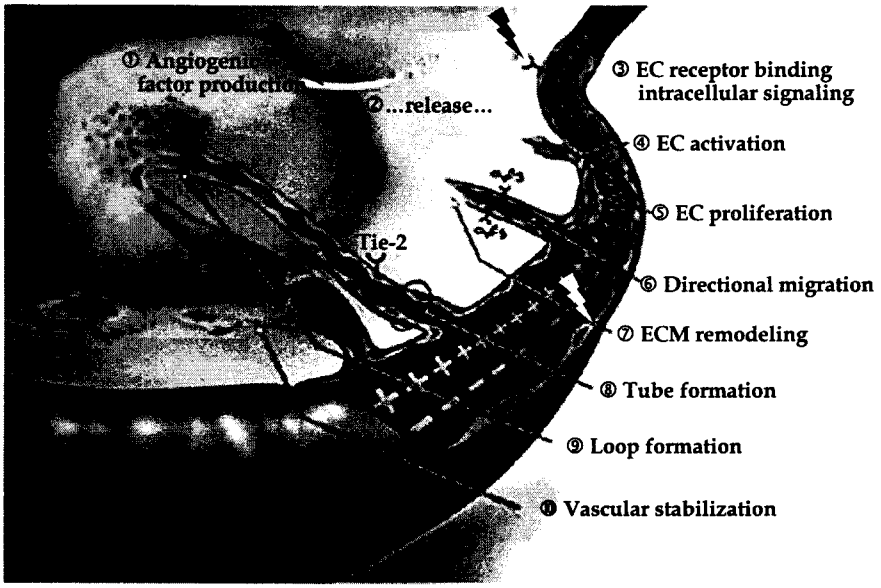
Target gene

Nucleus

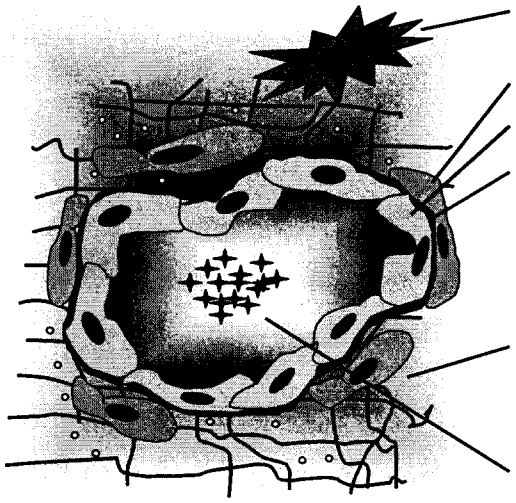
## Mechanism of tumor angiogenesis



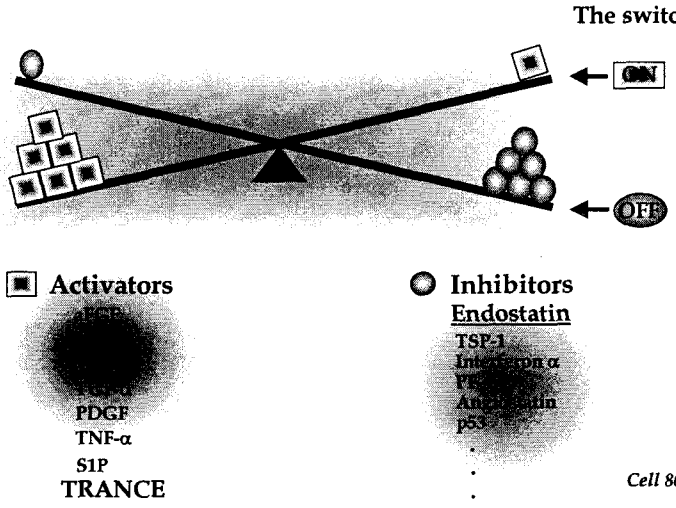
## The Angiogenesis Process: How Do New Blood Vessels Grow?



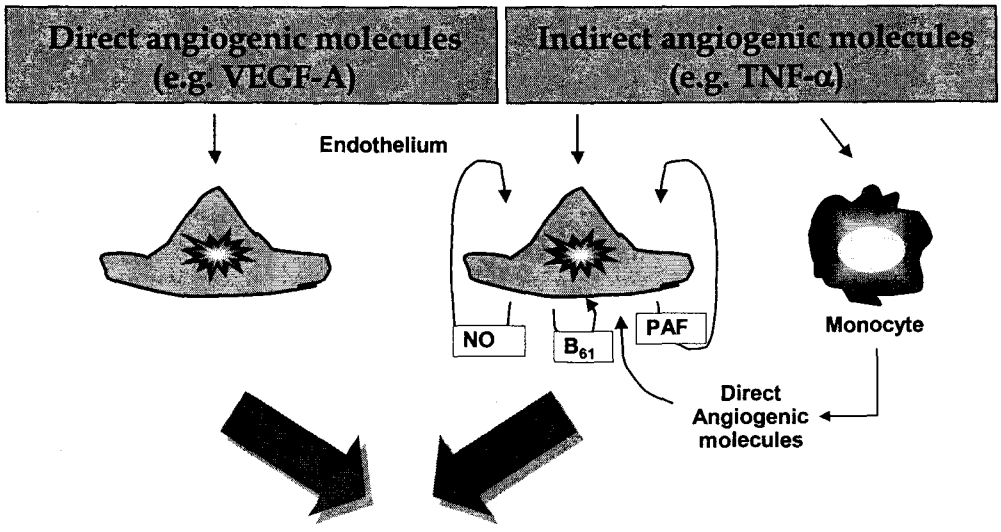

## Blood Vessel



## Balance hypothesis for the angiogenic switch

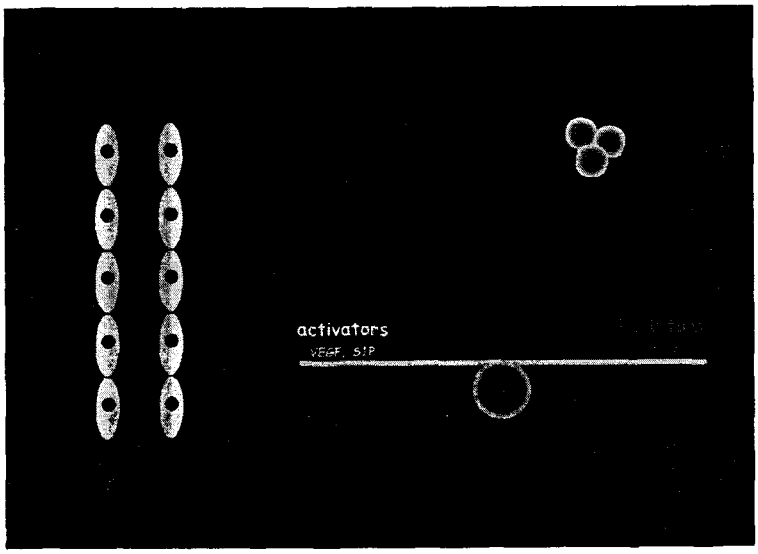



*Cell 86, 353 (1996)*




*TIBS, 22, 251 (1997)*

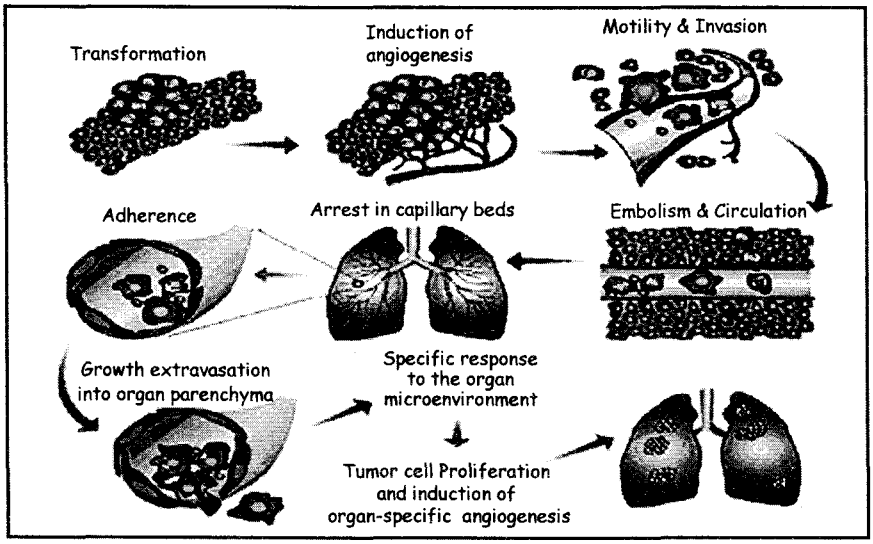
## ANGIOGENESIS




## Angiogenesis Dependent Diseases

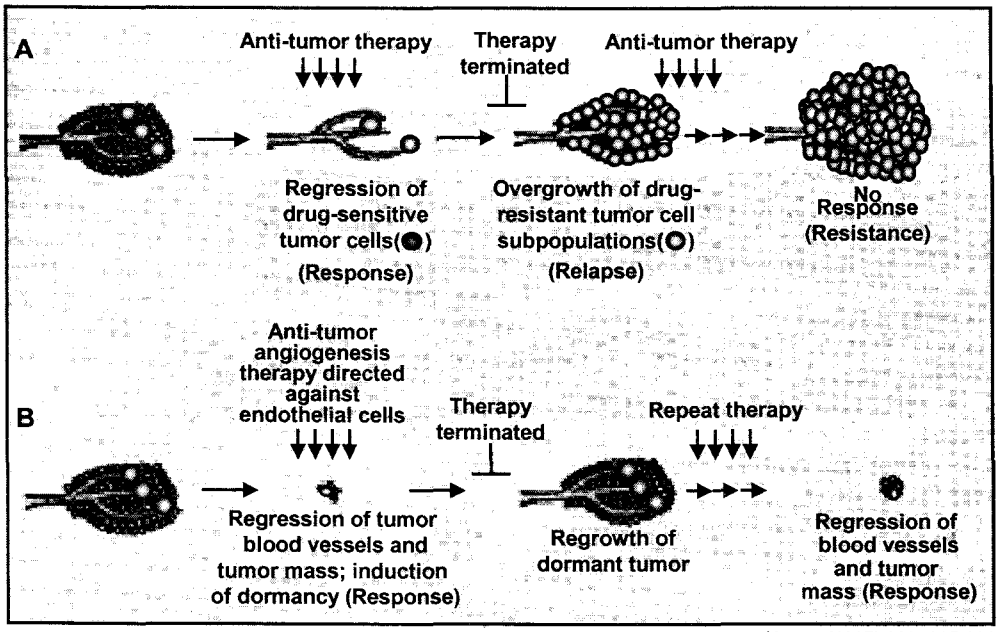
| Hypo-Vessel | Hyper-Vessel |
|---|---|
| Ischemia | Solid tumors |
| Myocardial infarction | Diabetic retinopathy |
| Atherosclerosis | Rheumatoid arthritis |
| Lated wound healing | Psoriasis |
| Ulcer | Hemangiomas |

## Tumor growth and Metastasis



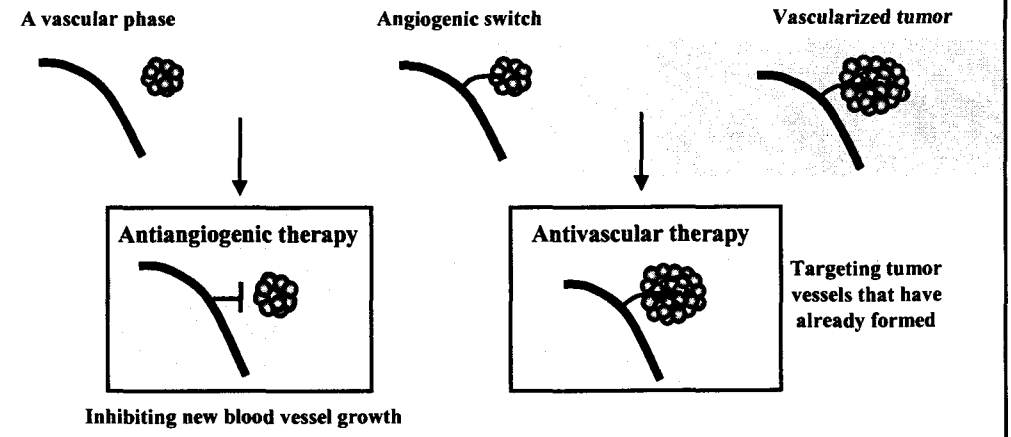

*Nature Med* (2000) 6:500

## A Cancer therapy resistant to resistance




*Nature* (1997) 390: 335-336

## Antiangiogenic and Antivascular Therapy

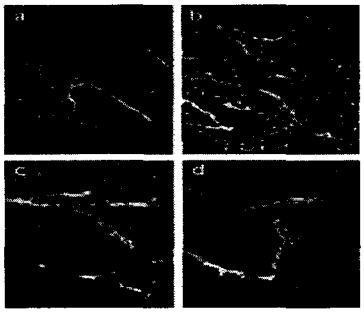



**Target molecules**

**Adhesion molecules-** Integrins, E-selectin, VCAM-1, PECAM-1
**Matrix components-** Oncofetal fibronectin, Tenascin
**Receptors-**VEGF-R, TGFβ-R, endoglin
**Microtubules**

*Curr Opin Pharmacol, 1, 2001, p378*

## Tumor blood vessels and their change in response to antiangiogenic therapy



A. Normal vessels are well organized and have even diameters

B. Vessels from a colon cancer are dilated, tortuous, and leaky

C. Angiogenesis inhibitors prune excess, inefficient vessels which initially "normalizes" the vasculature and helps chemotherapeutic drugs to reach tumors

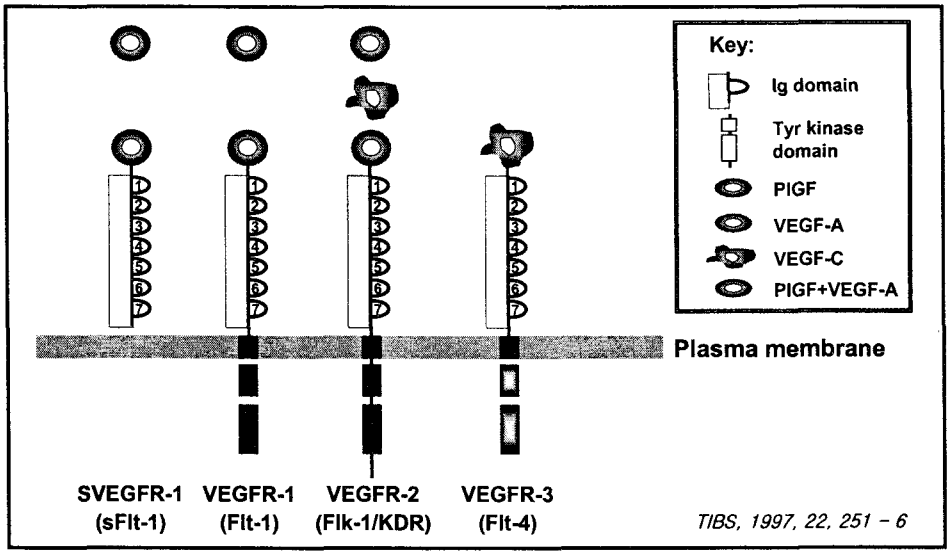
D. Eventually, though, increasing numbers of vessels begin to die

## Combine and Conquer

Antiangiogenic drugs could well be combined with any of the other approaches (such as surgery, chemotherapy and radiation) to improve the success rate.

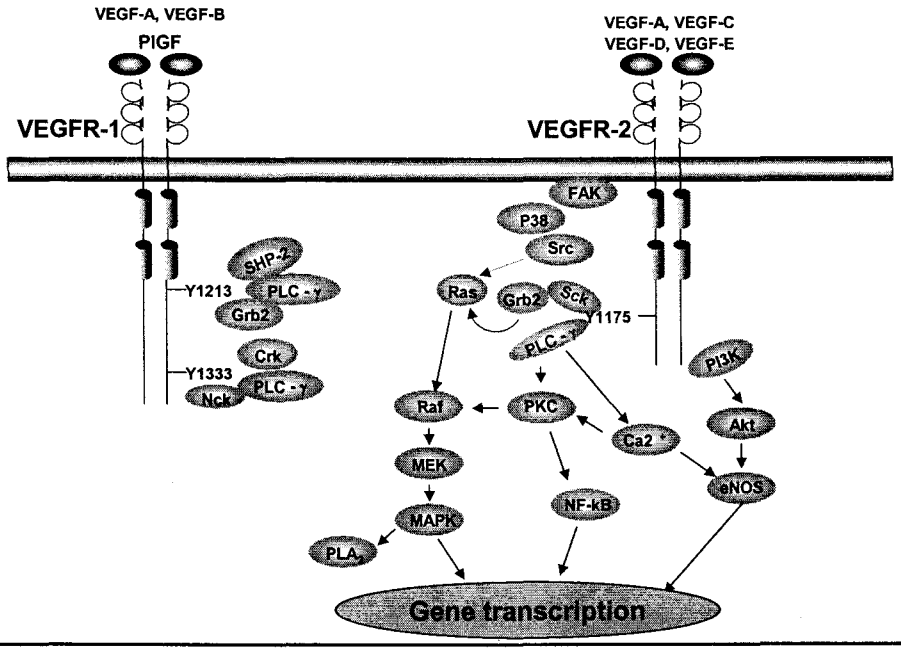
Optimal antiangiogenic therapy might consist of a cocktail of several angiogenesis inhibitors (increases efficacy and reduces acquired or induced resistance)

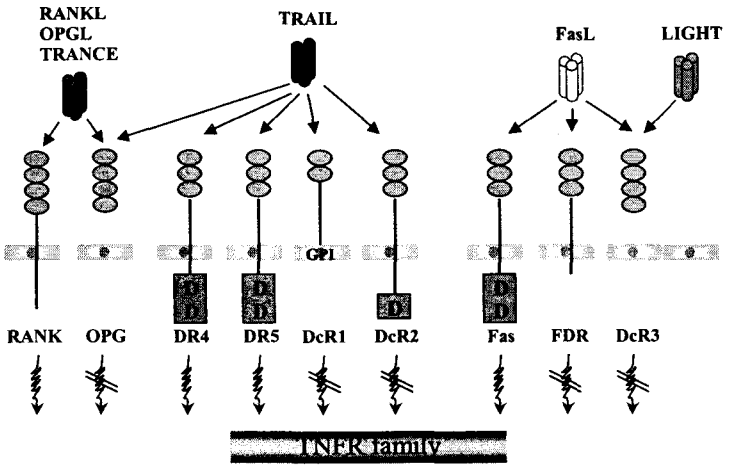
## Schematic Illustration of Vascular Endothelial Cell Receptors (VEGFR) and Their Ligands



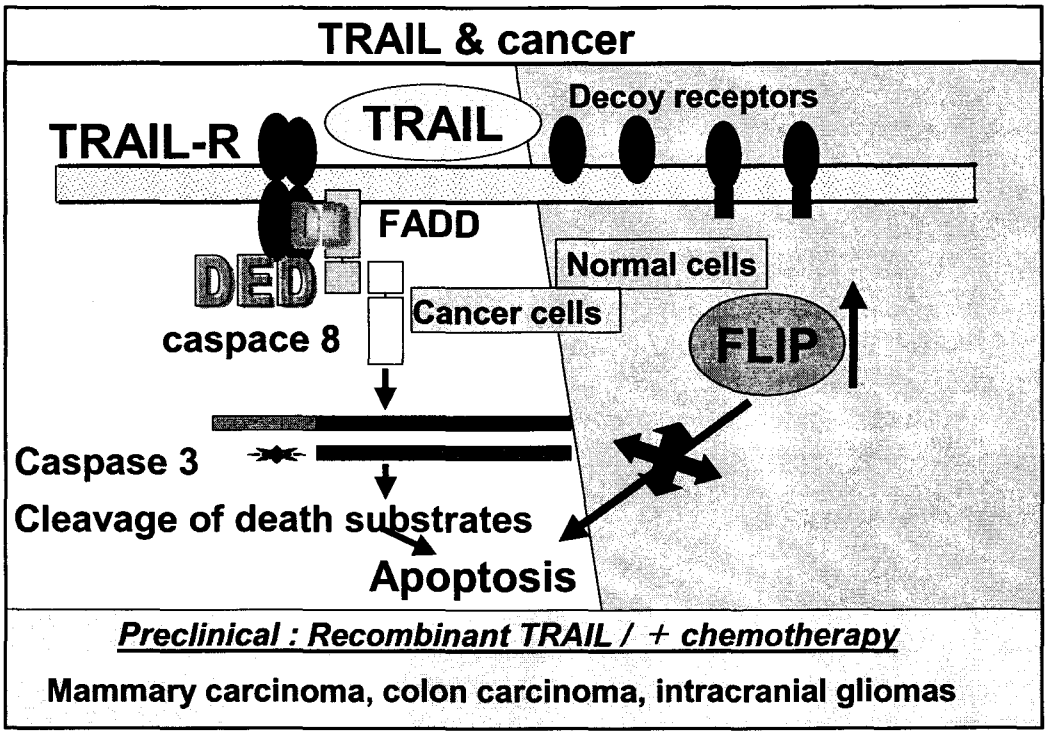

*TIBS, 1997, 22, 251 - 6*

## Overview of VEGFR-1 and VEGFR-2 signaling





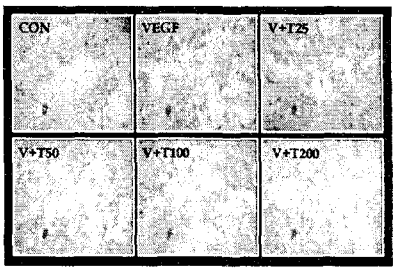

- **TRAIL Receptor 1(DR4), 2(DR5/TRICK2/Killer)** : Cysteine rich extracellular ligand binding domain, death domain(DD) – inducing apoptosis
- **TRAIL Receptor 3(DcR1/TRID/LIT)** : glycosyl-phosphatidylinositol(GPI) linked to the plasma membrane
- **TRAIL Receptor 4(DcR2/TRUNDD)** : lacks 52 of the 76 amino acids that encode the predicted DR4 and DR5 death domain, induced NF-kB activation
- **TRAIL Receptor 5(OPG)** : inhibits TRAIL-induced apoptosis of Jurkat cells

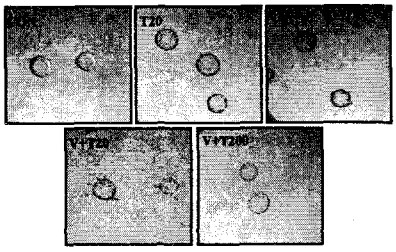


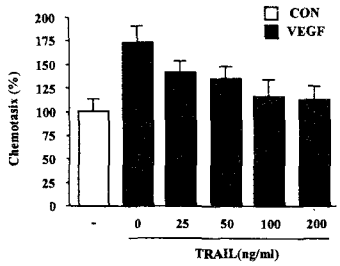
# TNF-related apoptosis-inducing ligand (TRAIL)

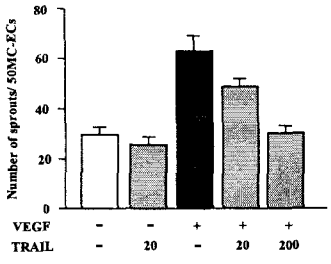
- 281 aa, located chromosome 3q26, TNF superfamily, apoptosis inducing ligand,
- Apo-2L : induced apoptosis of tumor cells (no cytotoxicity to normal cells)(1995,1996)
- TRAIL Receptor 1(DR4),2(DR5),3(DcR1),4(DcR2)(1997)
- TRAIL Receptor 5(OPG)(1998)
- Crystal structure of Human TRAIL(1999)
- On the TRAIL to a new cancer therapy(1999)
- Caspase-8 is required for TRAIL inducing apoptosis(2000)
- Decoy receptors regulate inflammatory cytokines and chemokines(2001)

# The effect of TRAIL on VEGF-induced HUVEC migration and sprouting

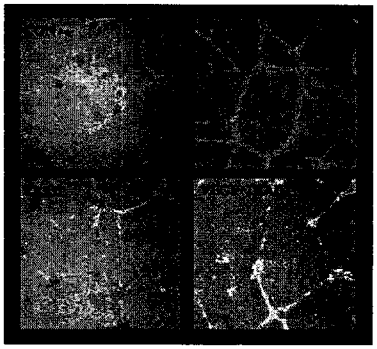


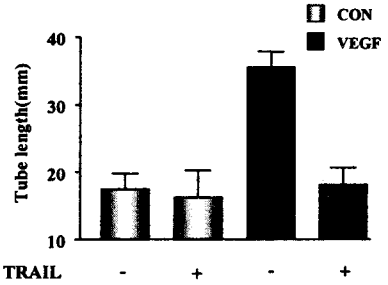



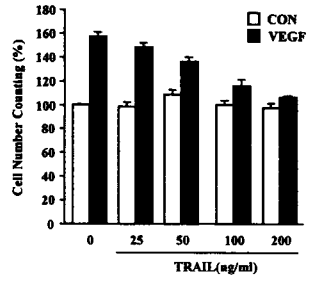



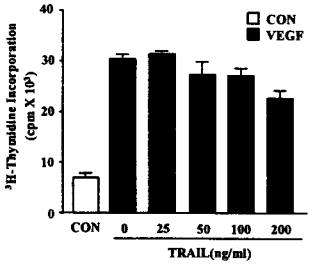
## The effect of TRAIL on VEGF-induced HUVEC tube formation

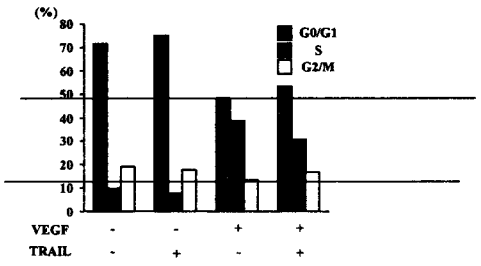




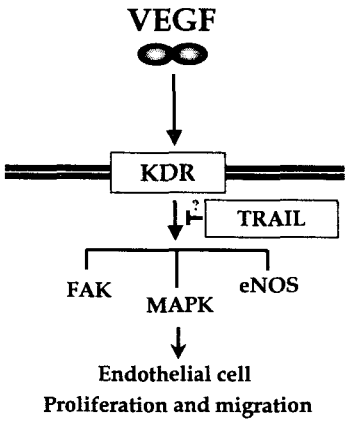

## The effect of TRAIL on VEGF-induced HUVEC proliferation

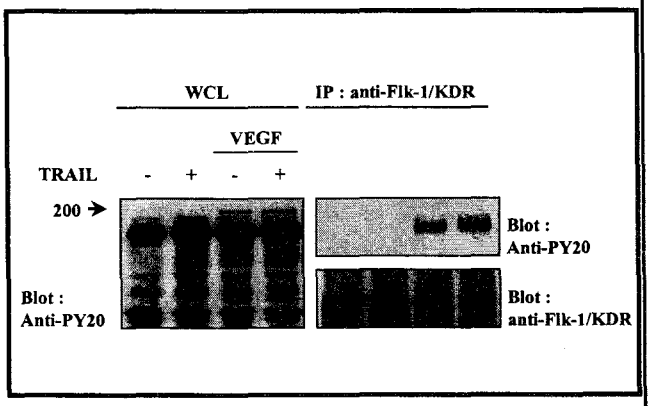


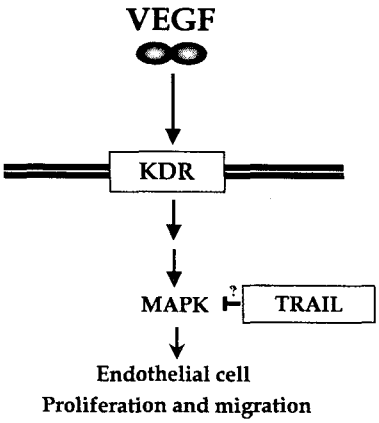



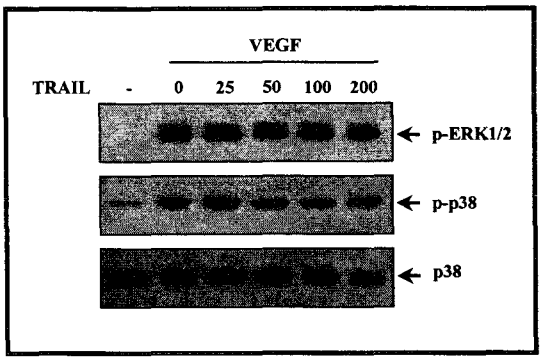
## The effect of TRAIL on VEGF-stimulated KDR phosphorylation in HUVEC




## Effects of TRAIL on VEGF-stimulated ERK1/2 and p38 activation

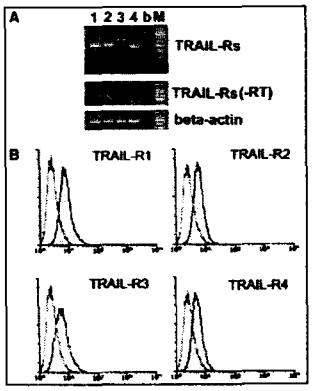


# TRAIL receptor in HUVEC

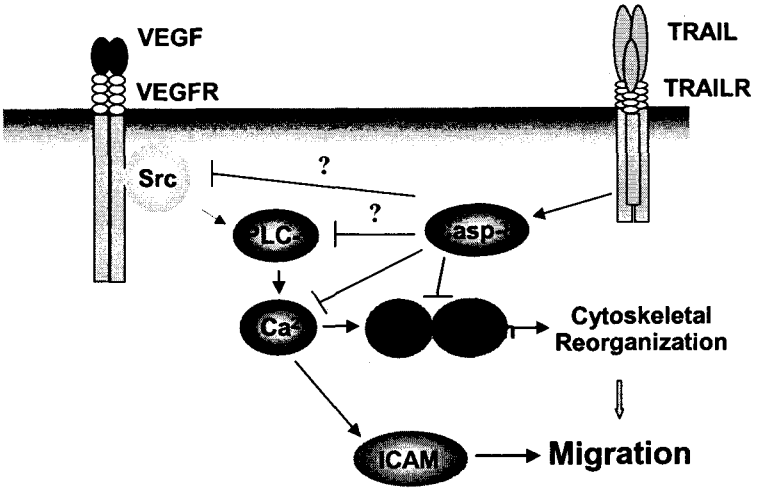
**Tumor Necrosis Factor–Related Apoptosis-Inducing Ligand (TRAIL) Sequentially Upregulates Nitric Oxide and Prostanoid Production in Primary Human Endothelial Cells**

Giorgio Zauli, Assunta Pandolfi, Arianna Gonelli, Roberta Di Pietro, Simone Guarnieri, Giovanni Ciabattoni, Rosalba Rana, Marco Vitale, Paola Secchiero

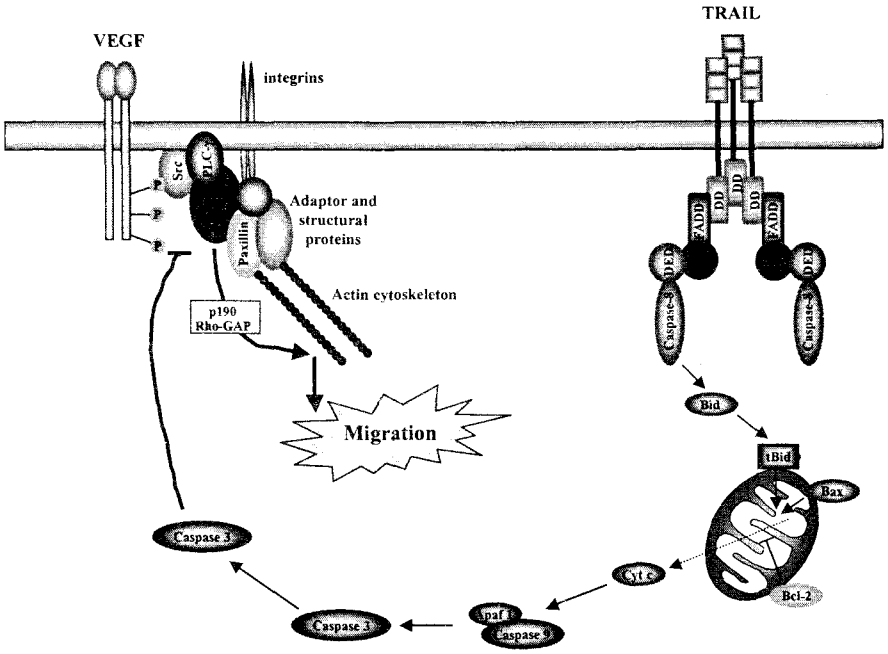



*Circulation Research* is available at http://www.circresaha.org

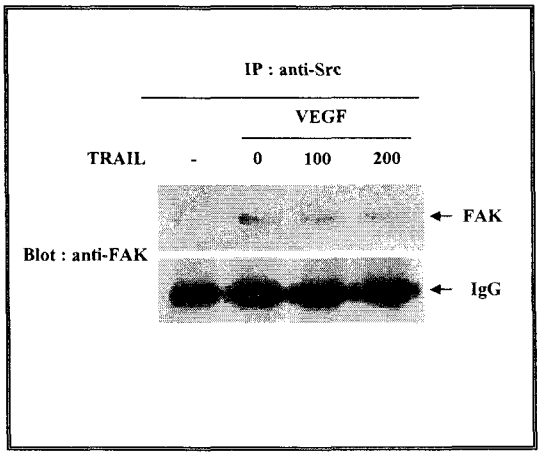
# Roles of TRAIL in VEGF-induced HUVEC Migration signaling pathways



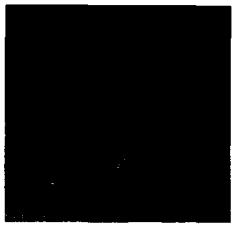
## Hypothesis I

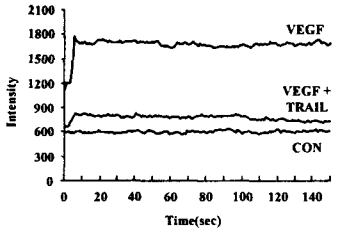


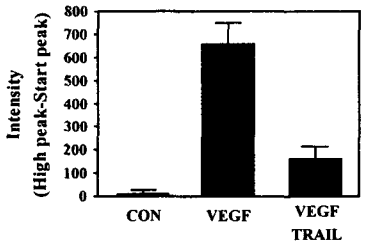

## Inhibition of VEGF-induced Src-FAK complex formation by TRAIL



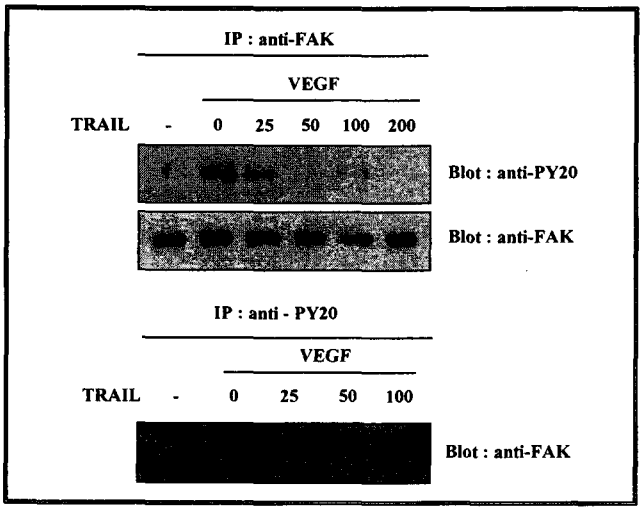
## Inhibition of VEGF-induced $Ca^{2+}$ release by TRAIL



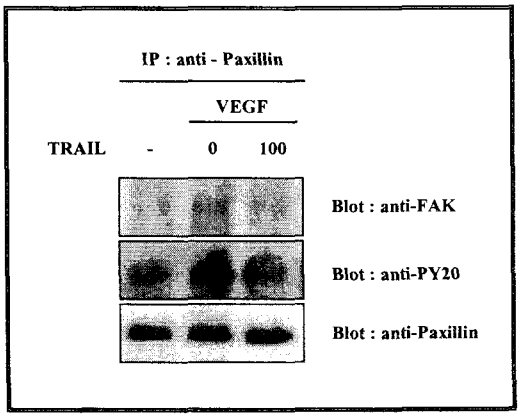


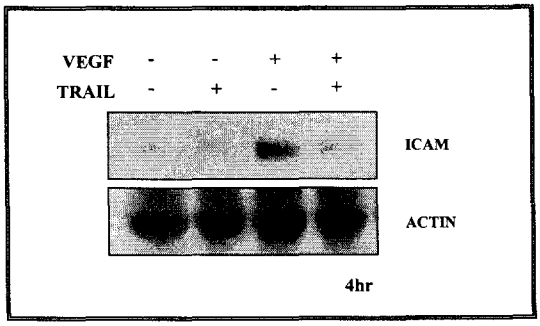

## Inhibition of VEGF-stimulated $p125^{FAK}$ Tyrosine phosphorylation by TRAIL



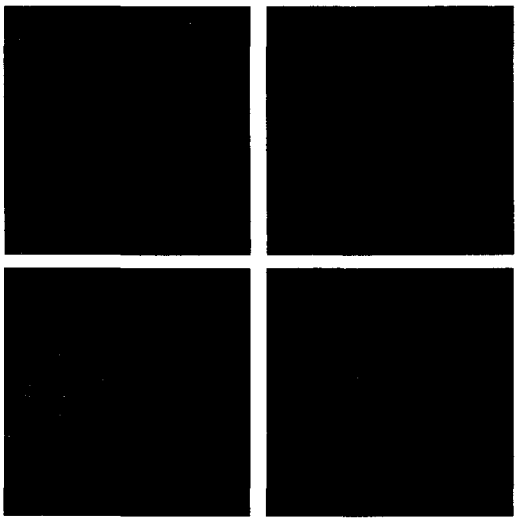
## Inhibition of VEGF-induced FAK-Paxillin complex formation and Paxillin Tyrosine phosphorylation by TRAIL




## Inhibition of VEGF-induced ICAM expression by TRAIL



## Inhibition of VEGF-induced actin remodeling by TRAIL



Confocal Microscope