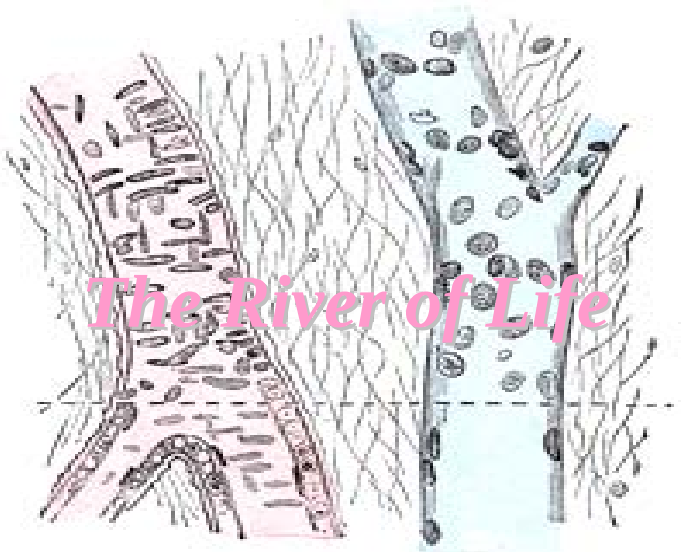
Positive & negative inflammatory signals in the vascular wall



Young-Guen Kwon

Vascular genomics Lab., Dept. of Biochemistry
Yonsei University

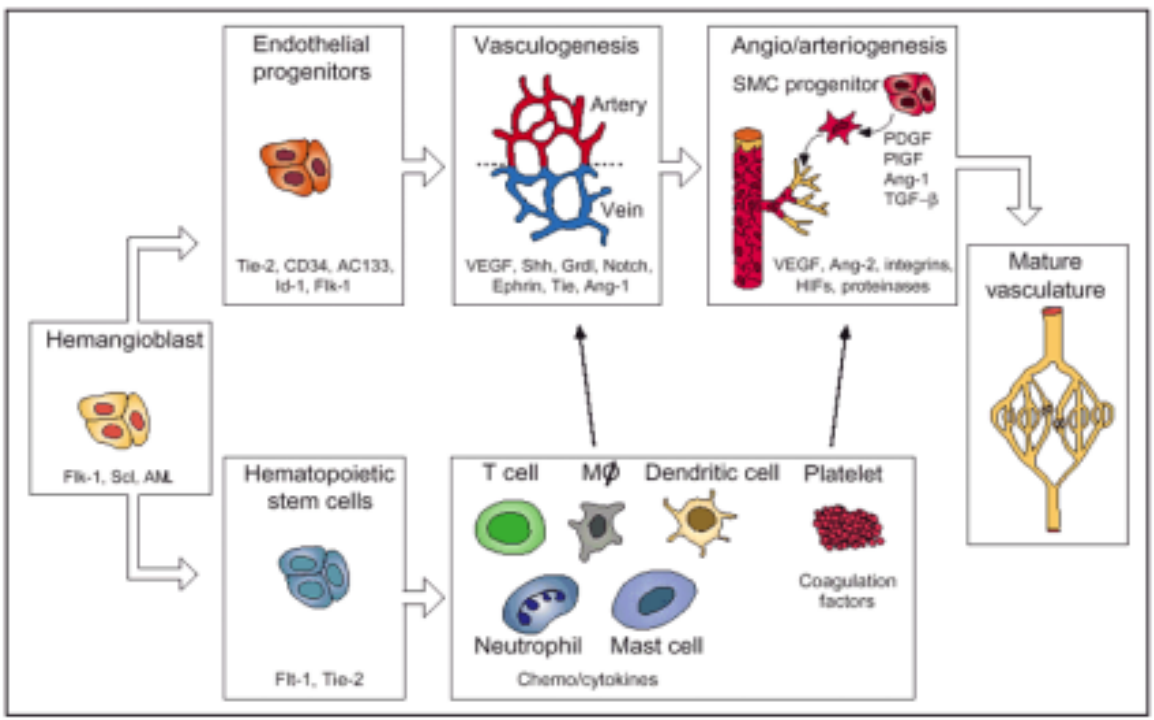
Formation of a vascular network

Two processes are responsible for the formation of new blood vessels:

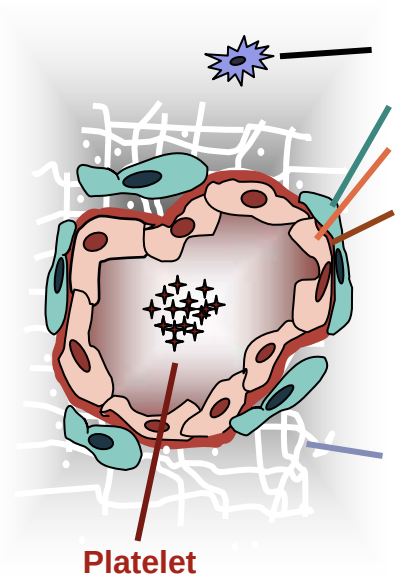
I. Vasculogenesis and Angiogenesis

Vasculogenesis: *in situ* formation of blood vessels from progenitor endothelial cells or angioblasts

Angiogenesis: formation of new blood vessels from preexisting blood vessles



Structure of Blood Vessel



Immune cell, **Pericytes**

Smooth muscle cell

Endothelial cell

Basement membrane

Collagen typeIV

Heparan sulphate proteoglycan

Entactin

Von Willbrand factor

Vitronectin

Interstitial matrix

Collagen types I, III, V and VI

Fibronectin

Other ECM components

Platelet

Therapeutic angiogenesis

Ways for treating myocardial ischemia and peripheral vascular disease

Cell therapy for ischemia

Gene or protein therapy

EPC

Endothelial progenitor cell

VEGF

bFGF

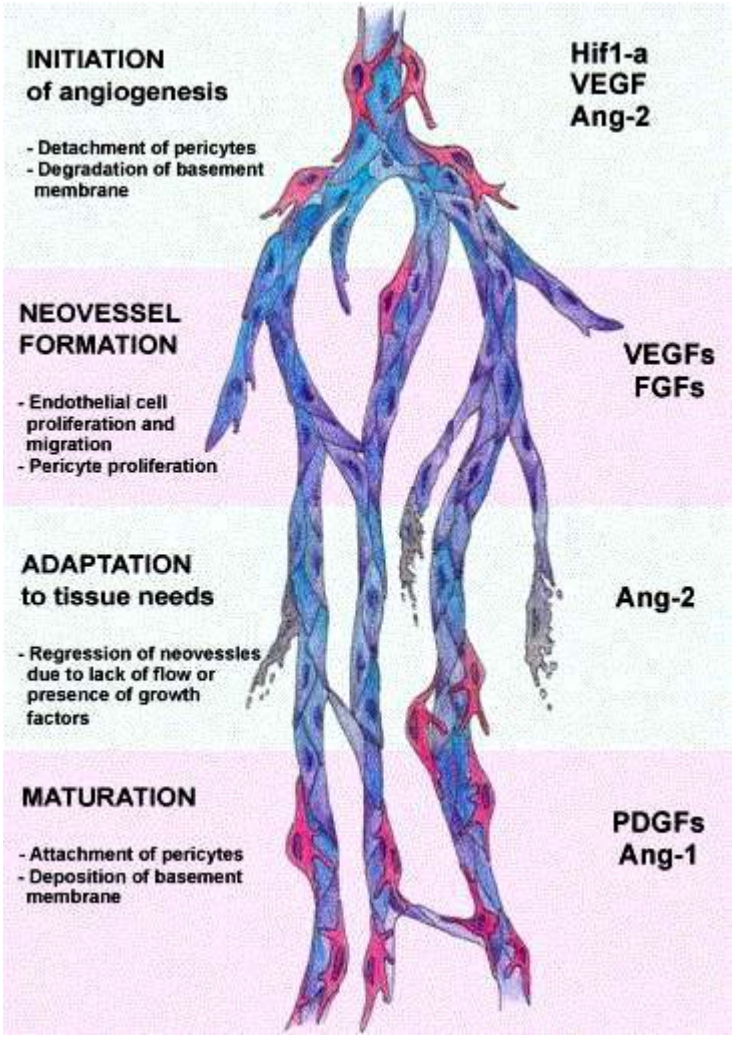
Will any single growth factor or stem cell therapy

be the "magic bullet" to cure vascular disease?

>>> Combination therapy?

Therapeutic Angiogenesis (Gene or protein therapy)

What to deliver?



Cardiovascular Research 2005

Clinical trials of angiogenic therapy

Protein	Trial type	N	Delivery
FGF1	Phase I, OL	20	Safe 41 Capillary blush at injection site
FGF2	Phase I, OL	30	Hypotension at high dosages Dilatation of epicardial coronaries
FGF2	Phase II, DBR	337	Safe/ No effect on ETT or SPECT Short-term improvement in symptoms compared to placebo
VEGF- A_{165}	Phase I, OL	15	Hypotension at low dosages Reduced SPECT defect size
VEGF- A_{165}	Phase II, DBR	165	No improvement in ETT, symptoms, or SPECT compared to controls
GM-CSF	Phase I/II, DBR	21	Improved collateral flow index in the GM-CSF group

What are the risks?

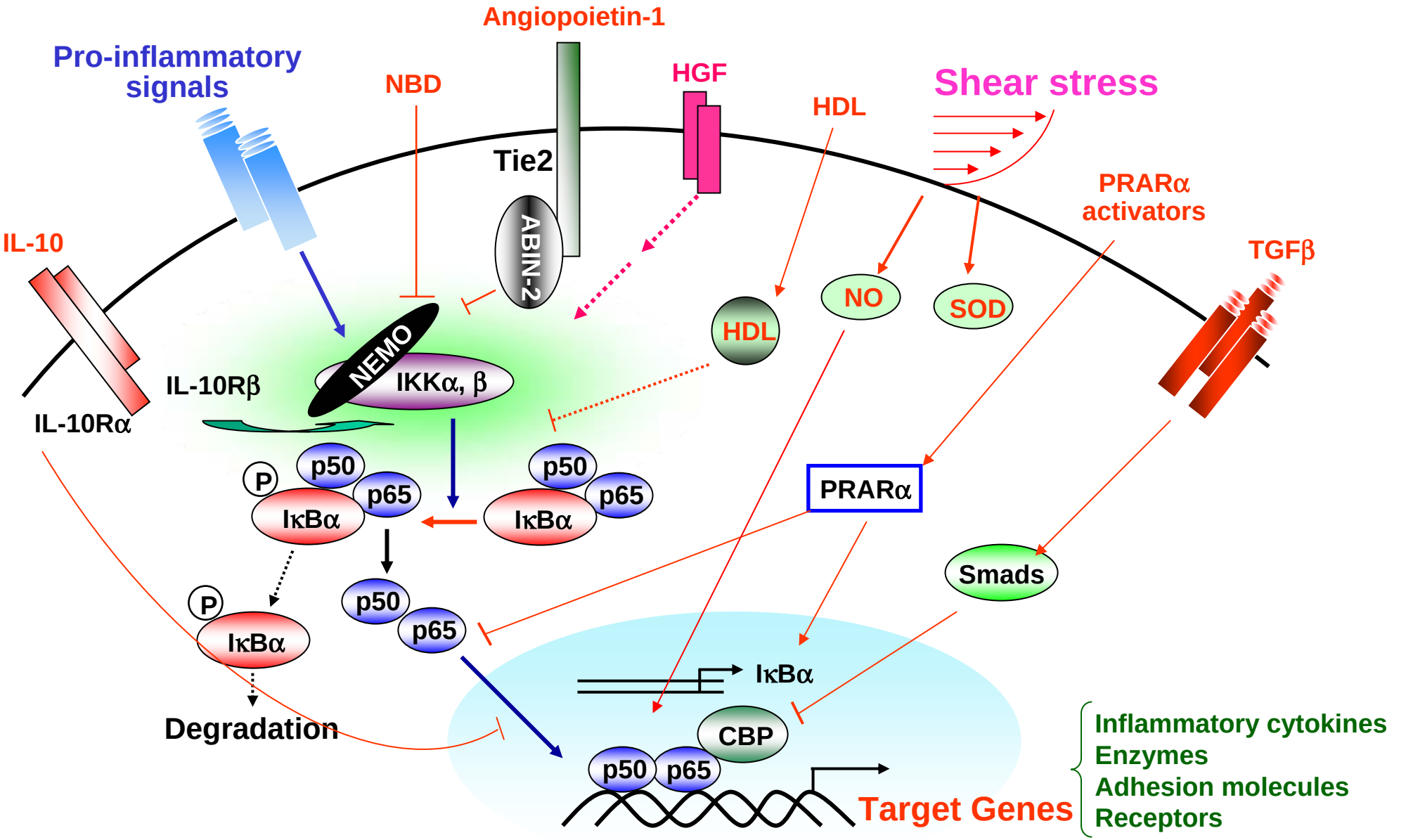
- Aberrant Vascular Proliferation in Nontargeted Tissues
- Increased Vascular Permeability and Inflammation
- Induction of the Development of Functionally Abnormal Blood Vessels
- Triggering Growth of Neoplasms
- Increase in Atherosclerotic Plaque Mass and Instability
- Vasodilatation and Hypotension During Short-Term Administration
- Hazards Associated With Viral Vectors
- Hazards Associated With Direct Myocardial Delivery

Molecular link between angiogenesis and inflammation ?

>> Pro- and Anti-Inflammatory Signals Acting on Vascular Cells <<

Mediators	Proinflammatory Signals	Anti-Inflammatory Signals
Cytokines	TNF, IL-1, IL-8, IFN-γ,	TGF-β, IL-10, IL-1ra,
	Oncostatin M, IL-4, IL-13	IL-13
Angiogenic factors	VEGF, TRANCE	Angiopoietin-1
Growth factors	PDGF	FGF, HGF
Vasoactive agents	Angiotensin II , endothelin	NO
Neuropeptides	Substance P	
Nuclear receptors		PPARs
Mechanial forces	Stretch	Shear stress
Other	LPS, phobol esters, thrombin	HDL, n3-fatty acids

NF-κB; a key molecule of vacular inflammation



Vascular Inflammation

- a basic pathological mechanism that underlies **atherosclerosis**, ischemia/reperfusion, rheumatoid arthritis, psoriasis, restenosis, and bronchial asthma.

> Inflammatory process

The inflammatory process requires extravasation of leukocytes from the microvasculature at sites of inflammation or injury

- **Increased endothelial permeability**
- **Up-regulation of leukocyte adhesion molecules**
- **Up-regulation of endothelial adhesion molecules (ICAM & VCAM-1)**
- **Migration of leukocytes into the artery wall**

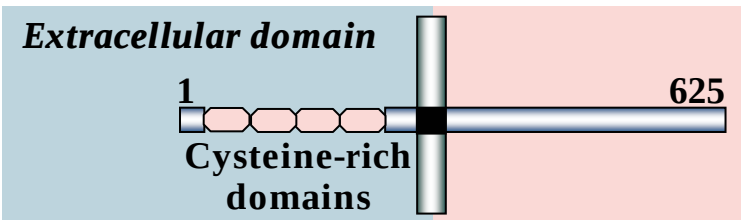
> Potential makers of Vascular Inflammation

Cellular adhesion molecules
Intracellular adhesion molecule-1
Vascular cellular adhesion molecule-1
Selectins
Chemokines
Monocyte chemoattractant protein-1
Cytokines
Interleukins 1, 6, 10, 18
Tumor necrosis factor-α
Proteases
Matrix metalloproteinases
Accessory signaling markers
CD40/CD40L
Acute phase proteins
C-reactive protein

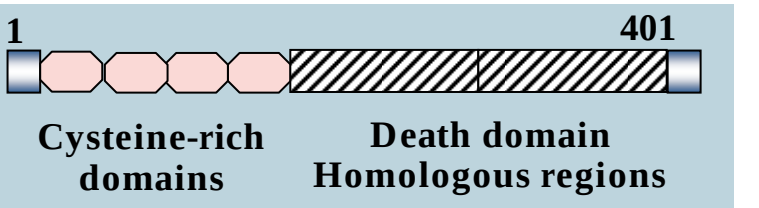
TRANCE, TNF-related activation-induced cytokine

- also called ODF, OPGL, and RANKL
- exists either in a cell-bound form or a truncated ectodomain variant cleaved by TACE
- The biological function from the phenotype of RANKL-deficient mice
  - *> osteoclast functions and bone remodeling*
  - *> immune cell cross-talks, dendritic cell survival, and lymph node organogenesis.*
  - *> Mammary gland development*

RANK, Receptor activator of NF-κB



OPG, Osteoprotegerin

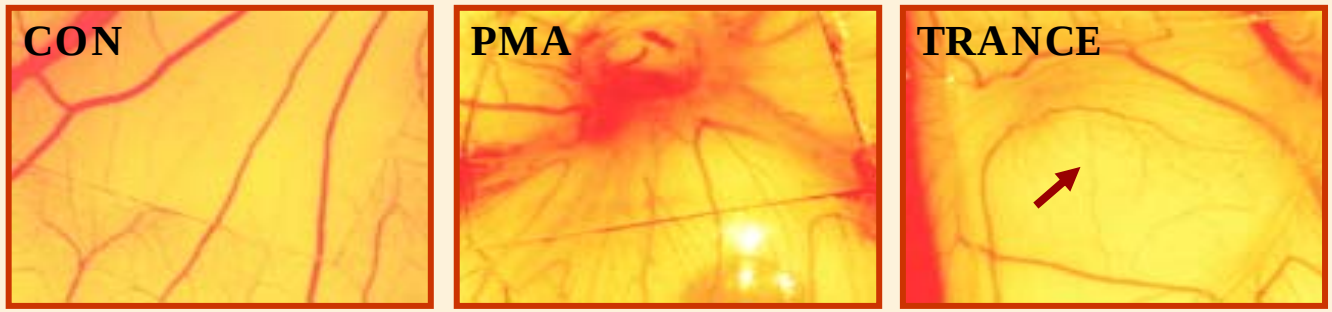


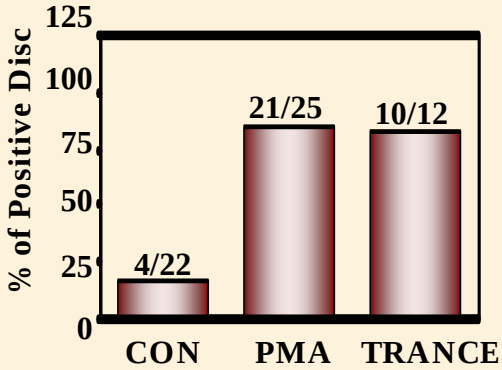
TRANCE in the vascular system

- **Prominent expression of TRANCE in the vascular cells *in vitro* and *in vivo***
- **Expression of RANK in endothelial cells**
  - >> up-regulation of RANK by VEGF in endothelial cells (JBC 2003)
- **A potential risk factor for progressive atherosclerosis and cardiovascular disease**
  - >> mice deficient in OPG exhibit arterial calcification in addition to early onset osteoporosis
- **TRANCE in angiogenesis and vascular inflammation**
  - >> induced angiogenesis in vitro and in vivo (JBC 2002)

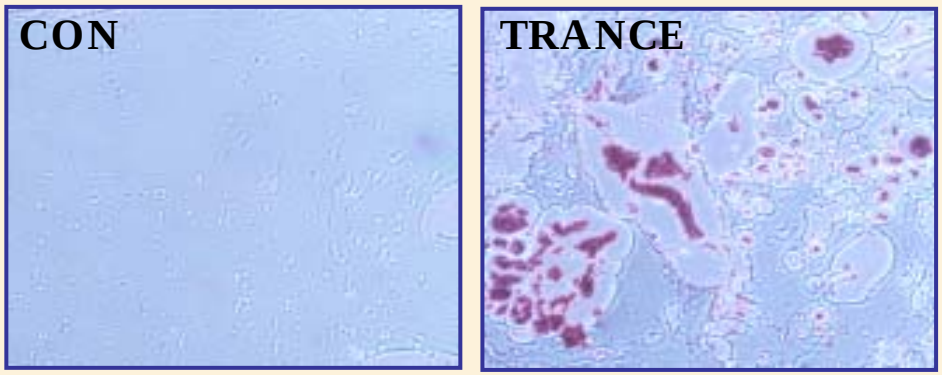
Soluble TRANCE Induces Angiogensis *in Vivo*

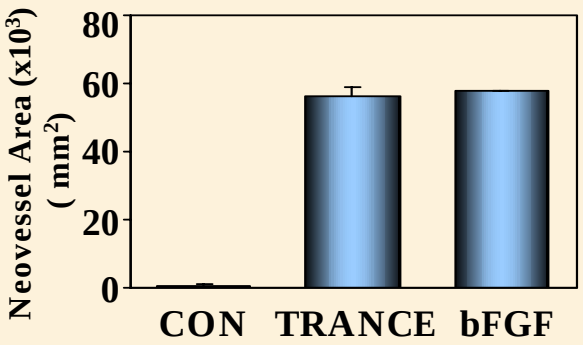
Chicken Chorioallantoic Membrane Assay





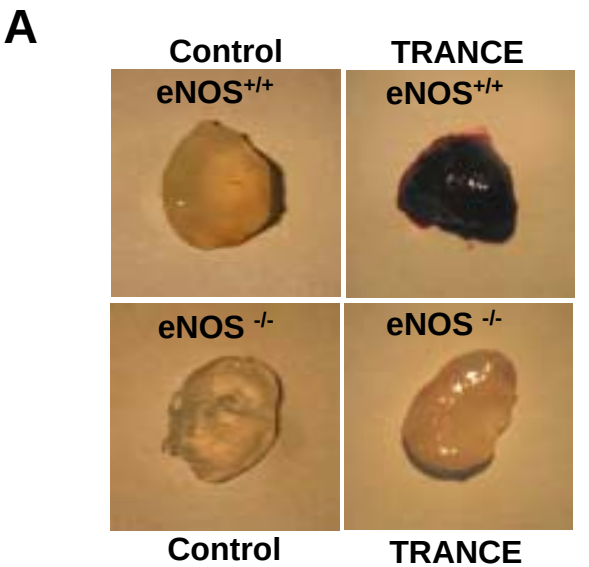
Mouse Matrigel plug assay

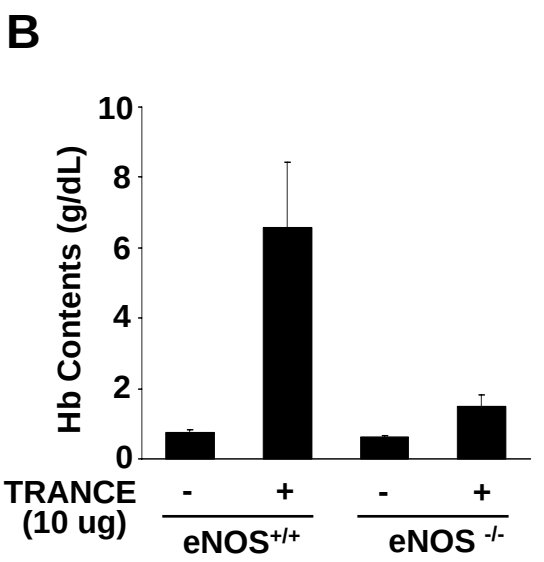


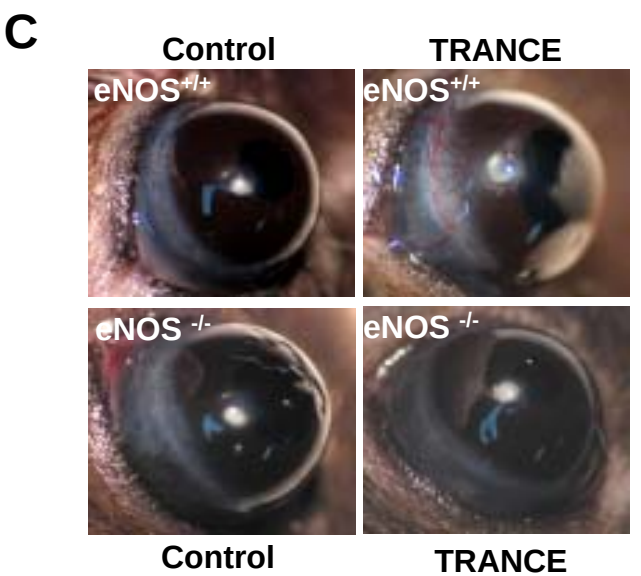


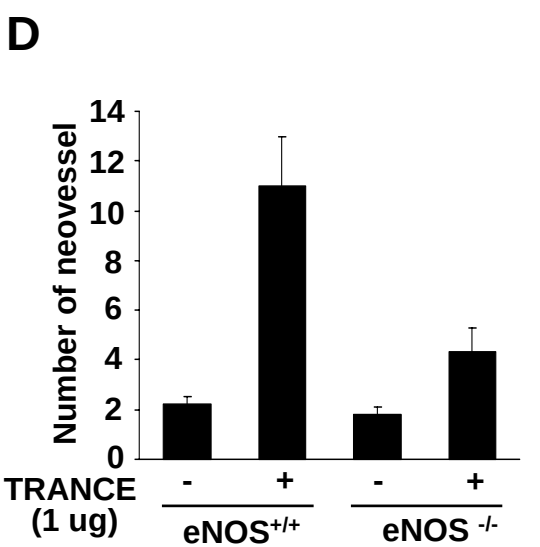
JBC 2002

TRANCE fails to promote angiogenesis in eNOS-knock out mice in vivo

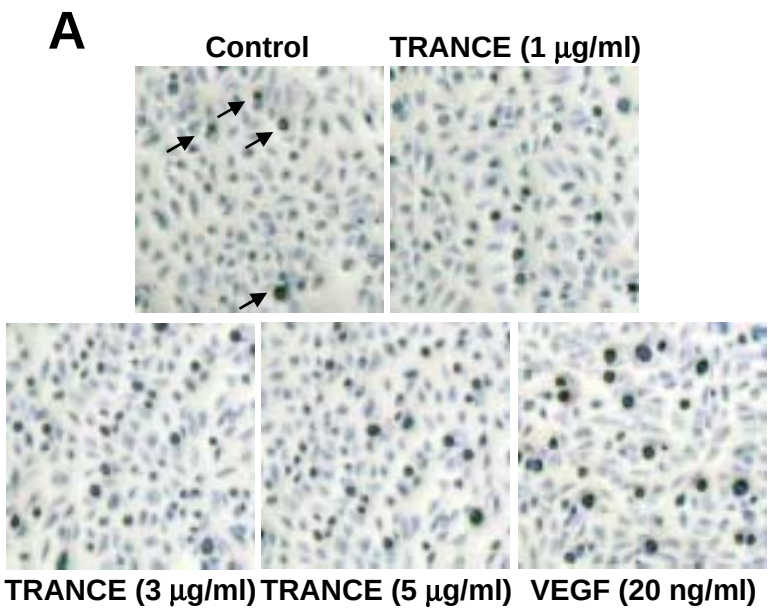


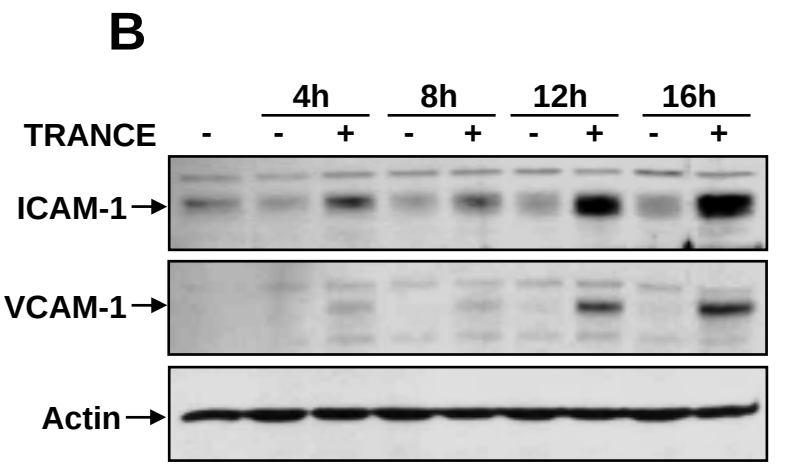


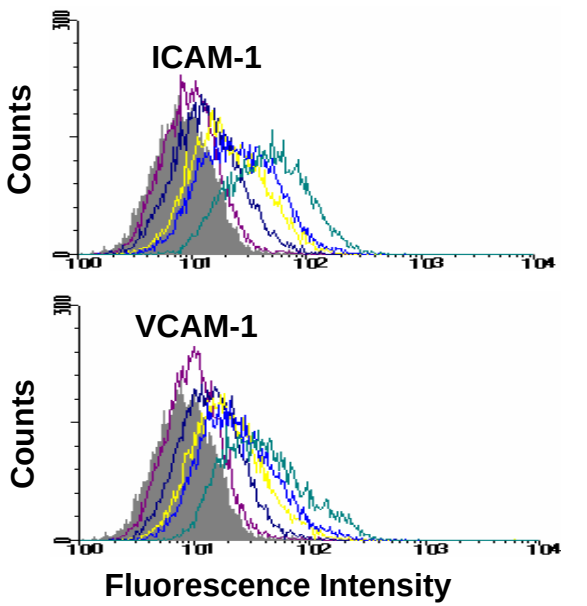


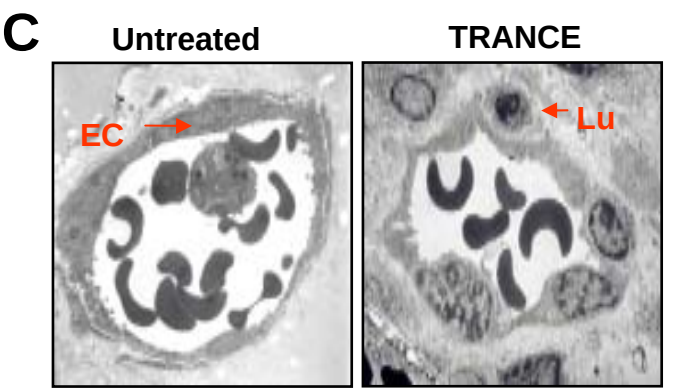


TRANCE increases monocyte adhesiveness and transendothelial migration of leukocyte *in vivo*



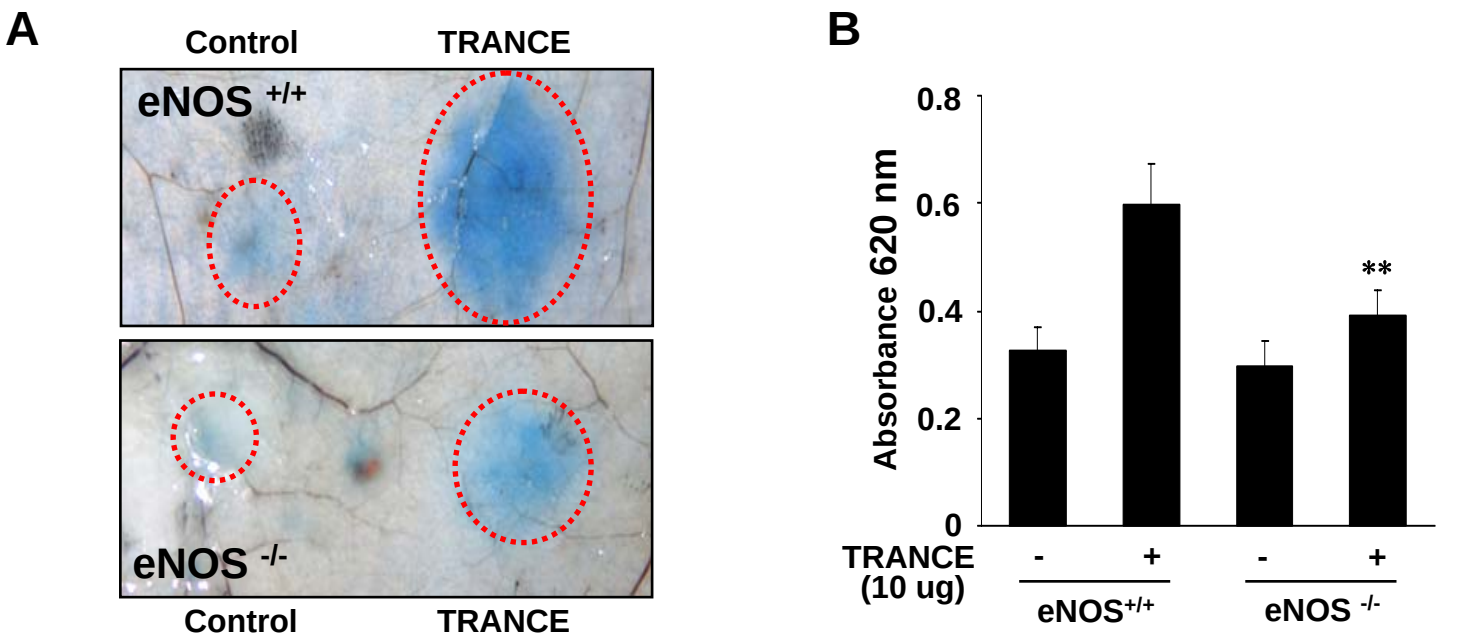




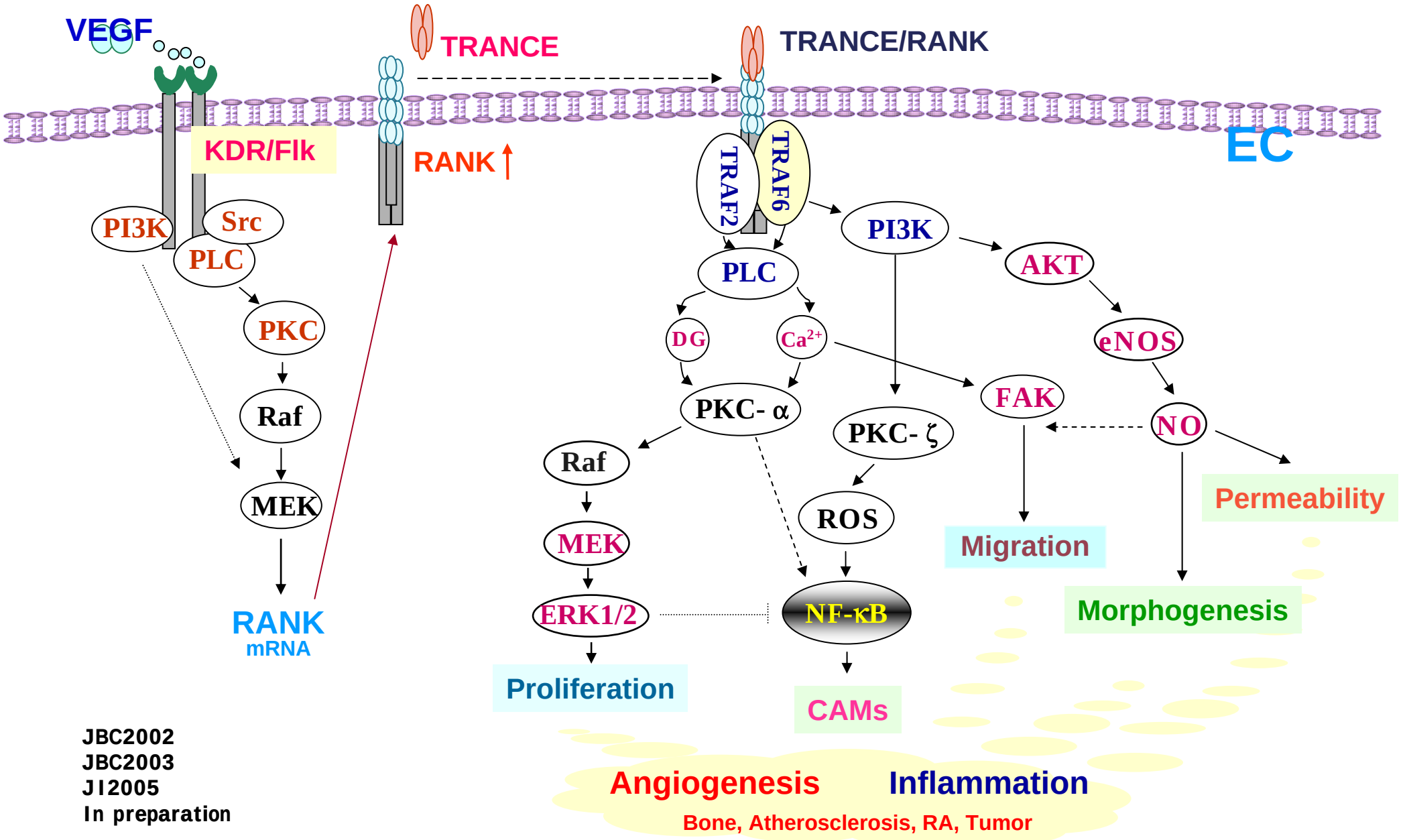


TRANCE induces Vascular permeability *in vivo*

; impairment in eNOS-deficient mice



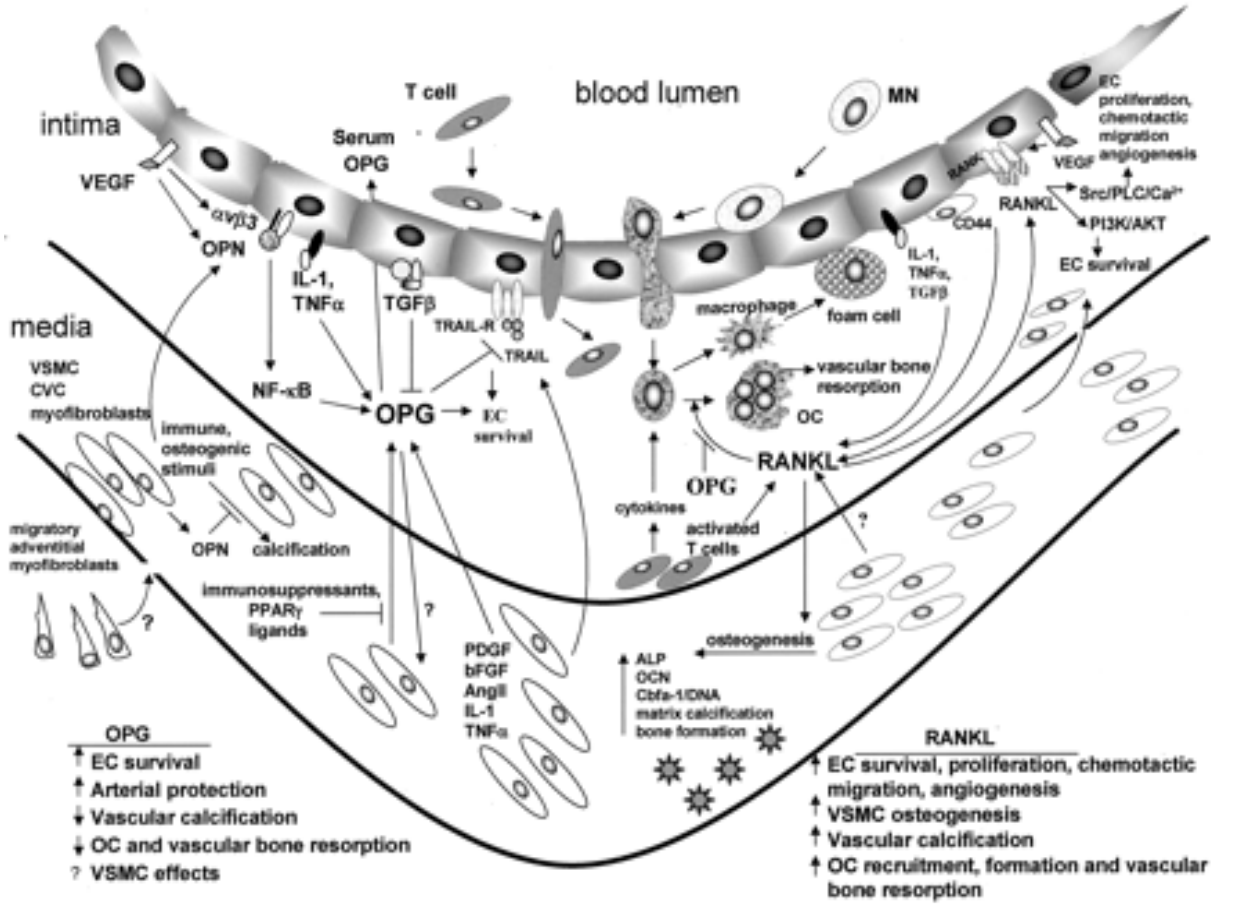
Schematic illustration of TRANCE-induced Angiogenesis & Inflammation



JBC2002
JBC2003
JI2005
In preparation

Circulation Research. **2004;95:1046**

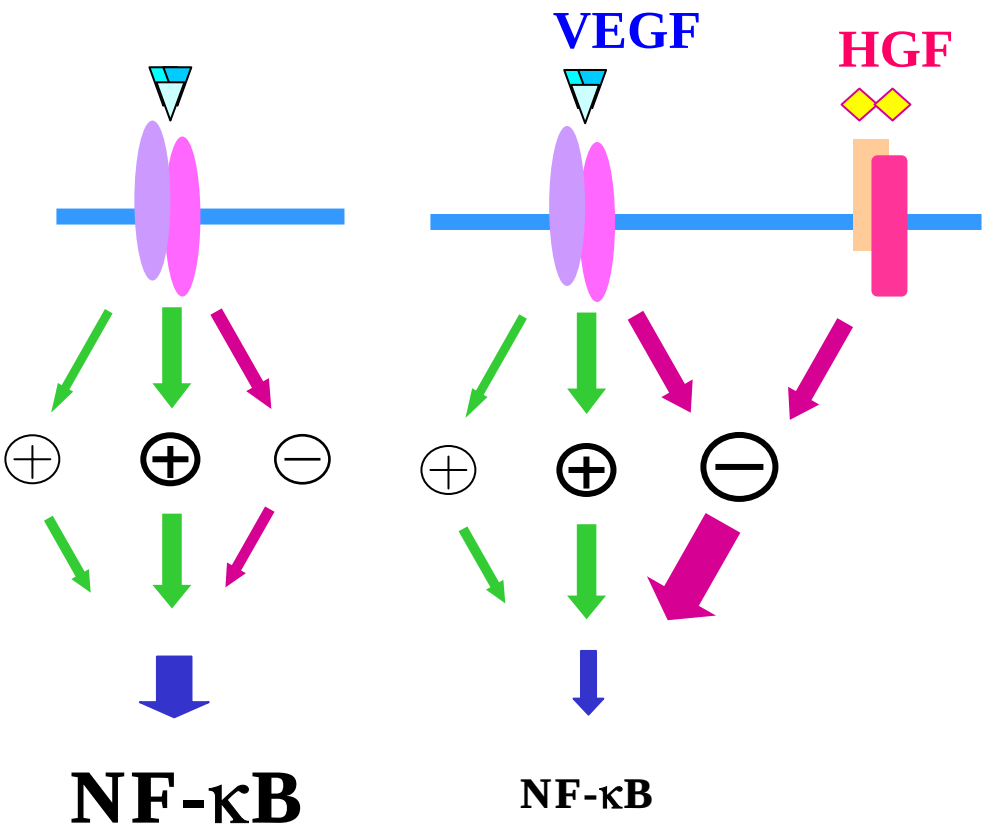
Schematic diagram of potential expression, regulation, and function of RANKL, RANK, and OPG in atherosclerotic vascular calcification.

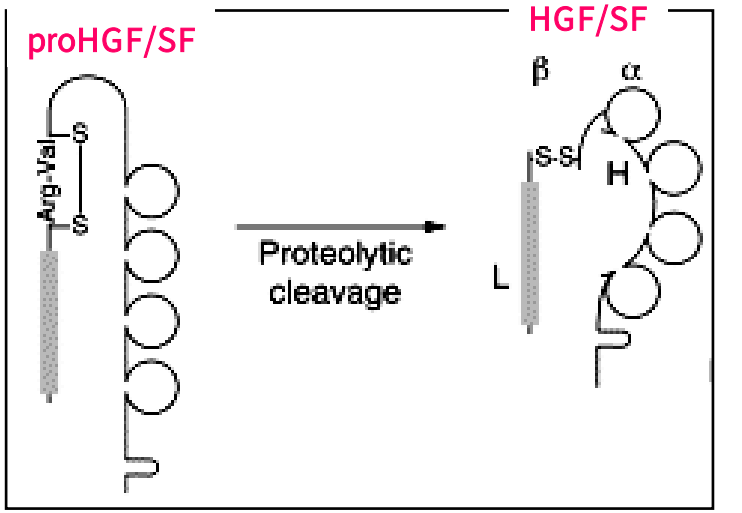


Elevated level of TRANCE in the vasculature may be importantly involved in the pathogenesis of atherosclerosis.

External regulation of inflammatory signals

HGF suppresses inflammatory responses in EC





Tubulogenesis by epithelial cells

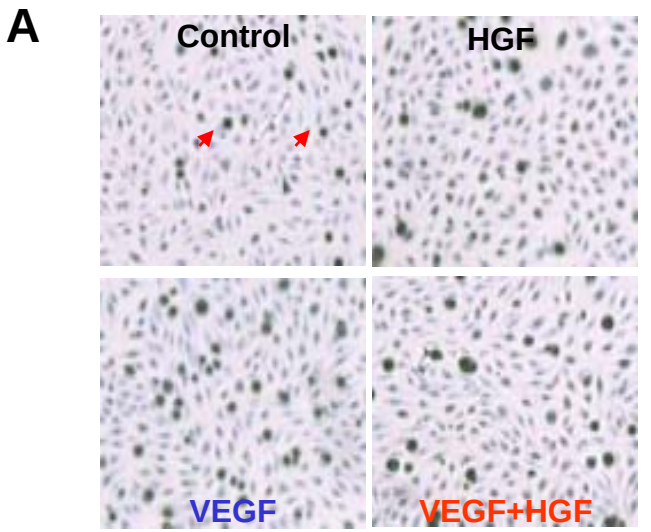
Angiogenesis by endothelial cells

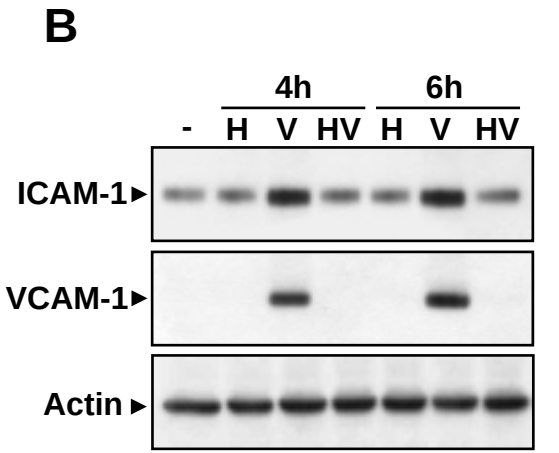
Scattering

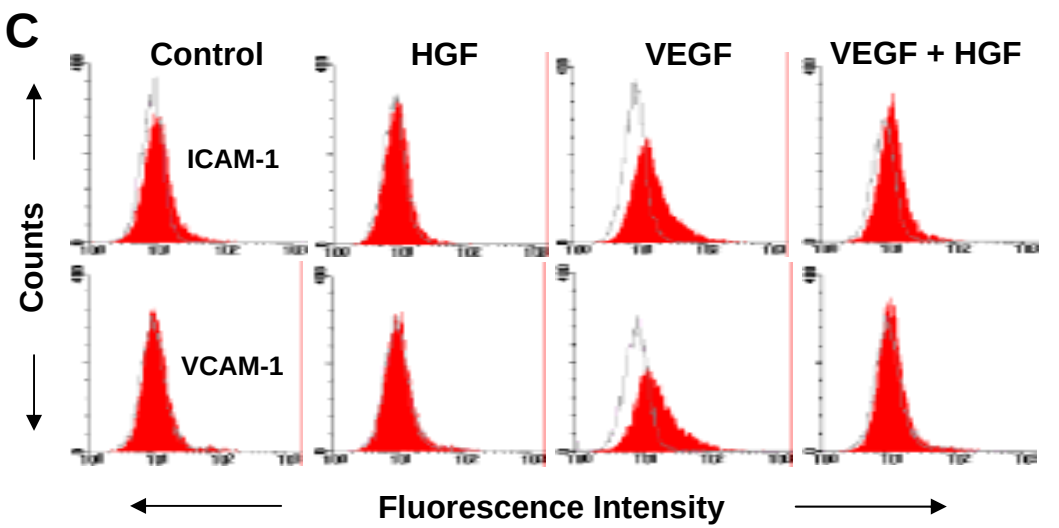
Invasion and metastasis

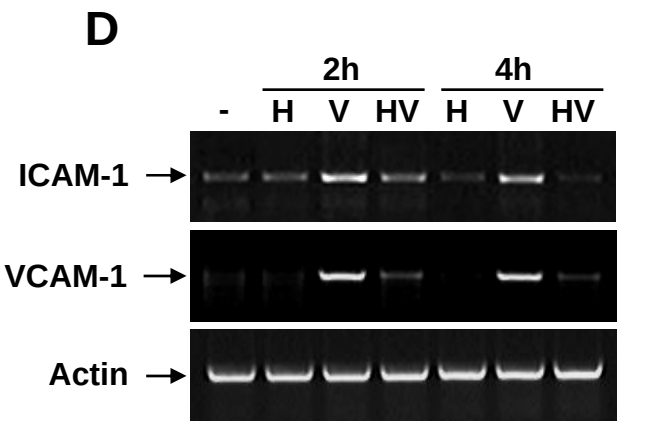
HGF; hepatocyte growth factor

HGF inhibits VEGF-stimulated leukocyte adhesion & CAM expression in HUVECs

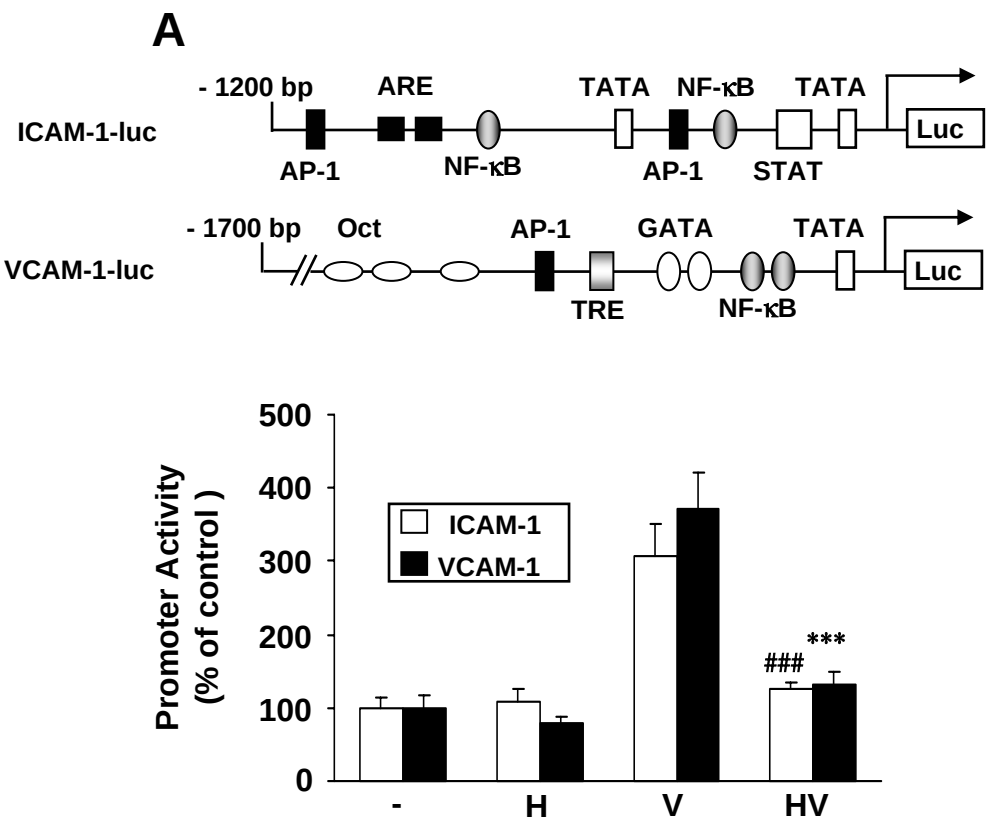


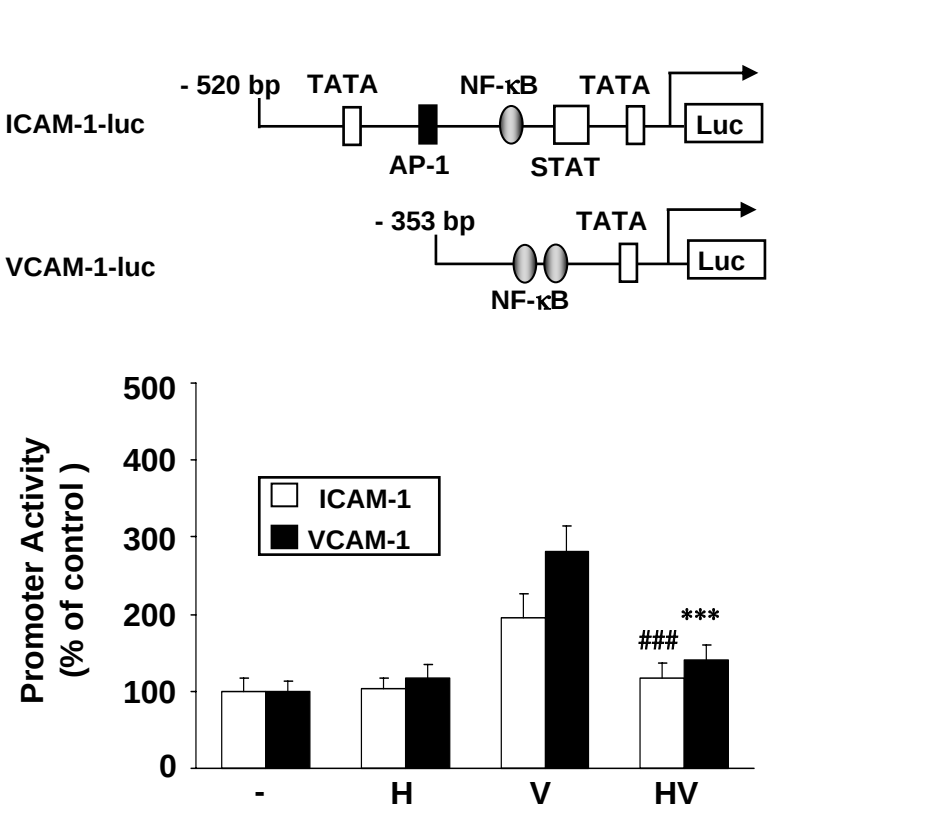




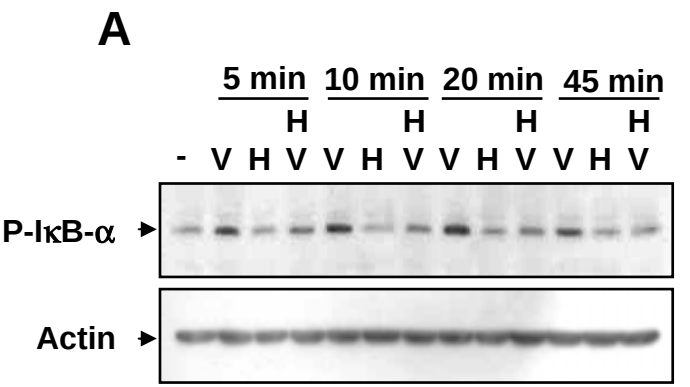


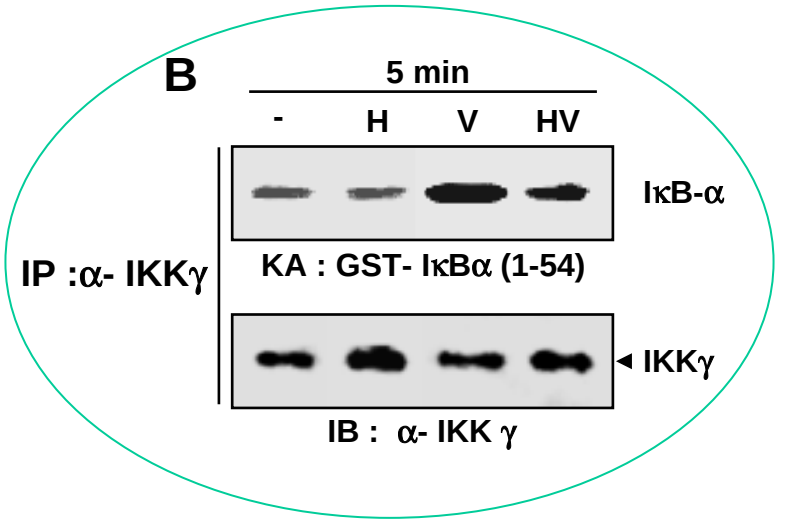
HGF suppresses VEGF-induced transcriptional activity through inhibition of NF-κB activation

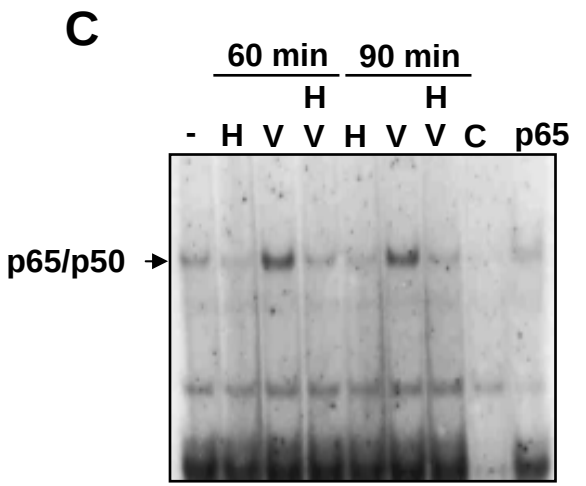


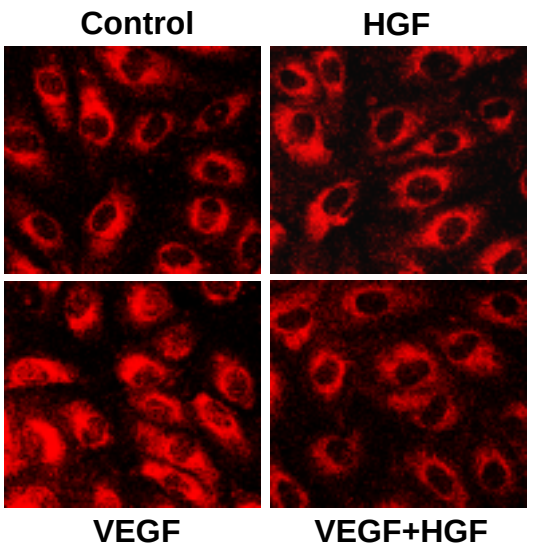


HGF inhibits VEGF-induced NF-κB activation through inhibition of IκB-α phosphorylation and degradation

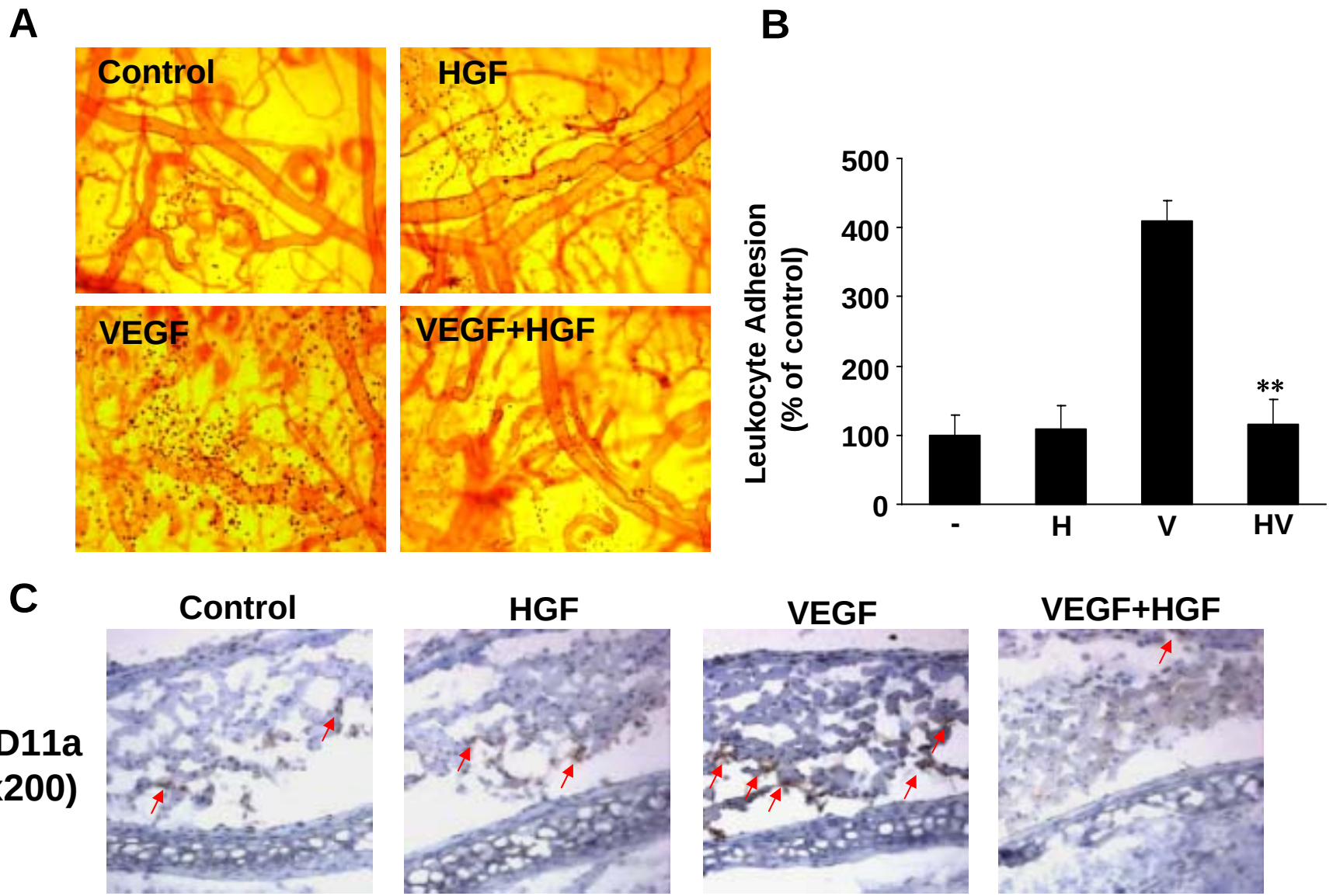




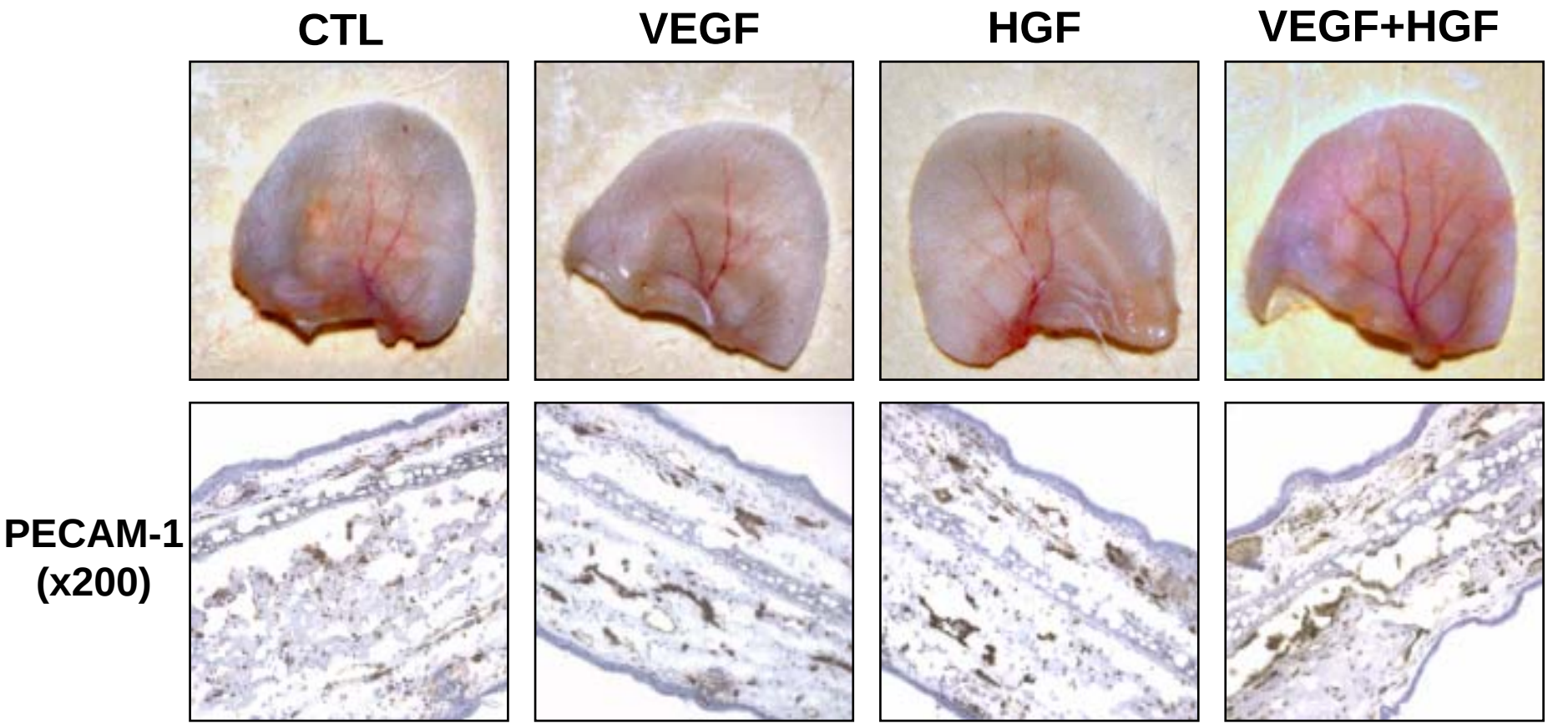




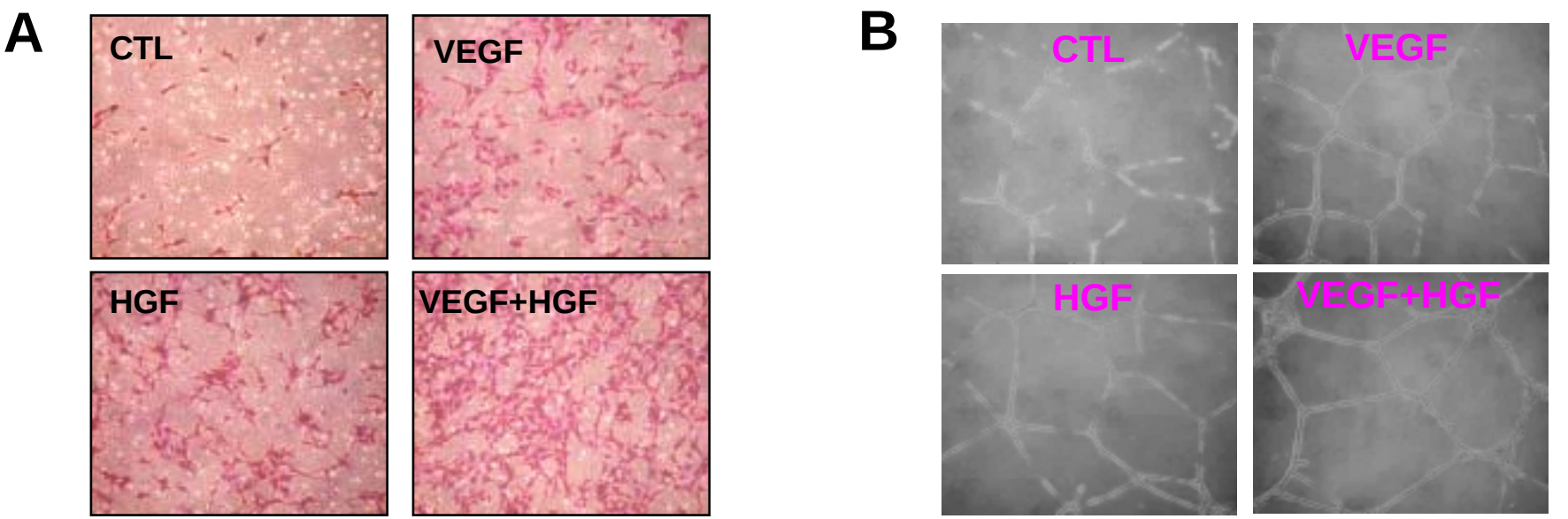
HGF inhibits VEGF-induced leukocyte infiltration *in vivo*



Co-treatment of HGF with VEGF shows the synergistic effect on neovessel formation in the mouse skin



VEGF and HGF synergistically stimulates angiogenesis



1.Xin X, Yang S, Ingle G, Zlot C, Rangell L, Kowalski J, Schwall R, Ferrara N, Gerritsen ME. Hepatocyte growth factor enhances vascular endothelial growth factor-induced angiogenesis in vitro and in vivo. *Am J Pathol.* 2001; 158: 1111–1120.

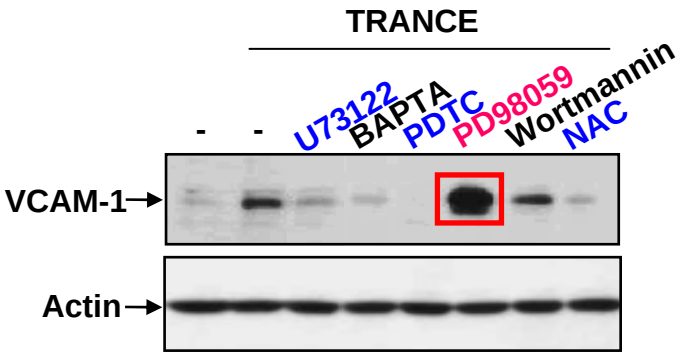
2. Mary E. Gerritsen, **HGF and VEGF: A Dynamic Duo,** Circ. Res. 2005 96: 272-273.

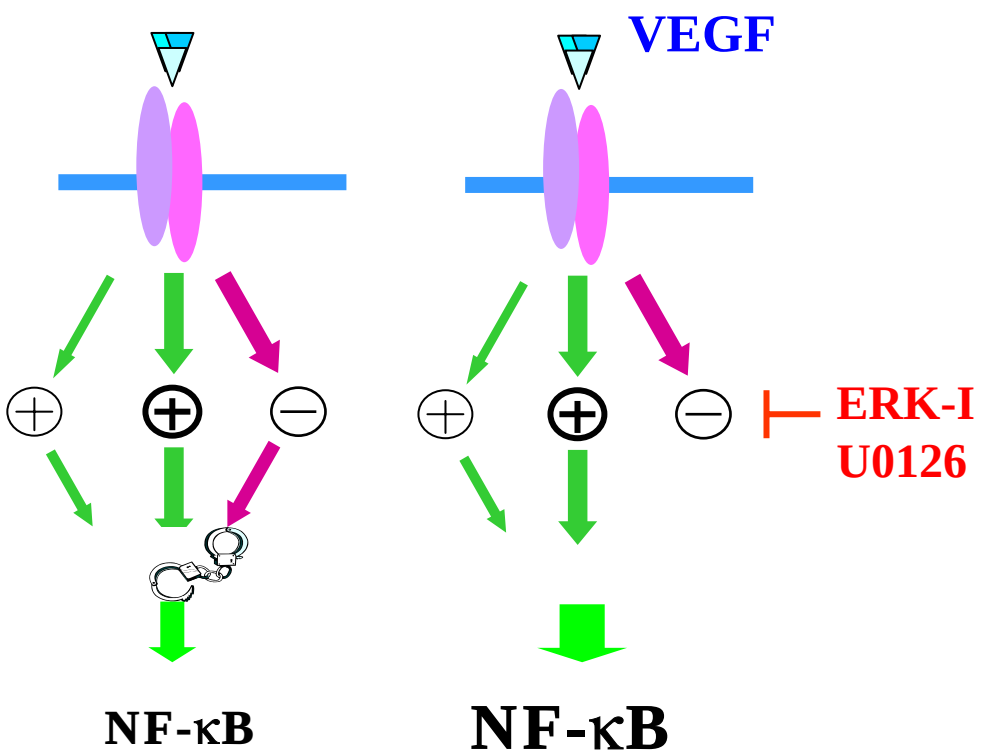
Therapeutic angiogenesis using VEGF & HGF co-administration may be more clinically applicable
Reduce inflammation & potentiate angiogenesis

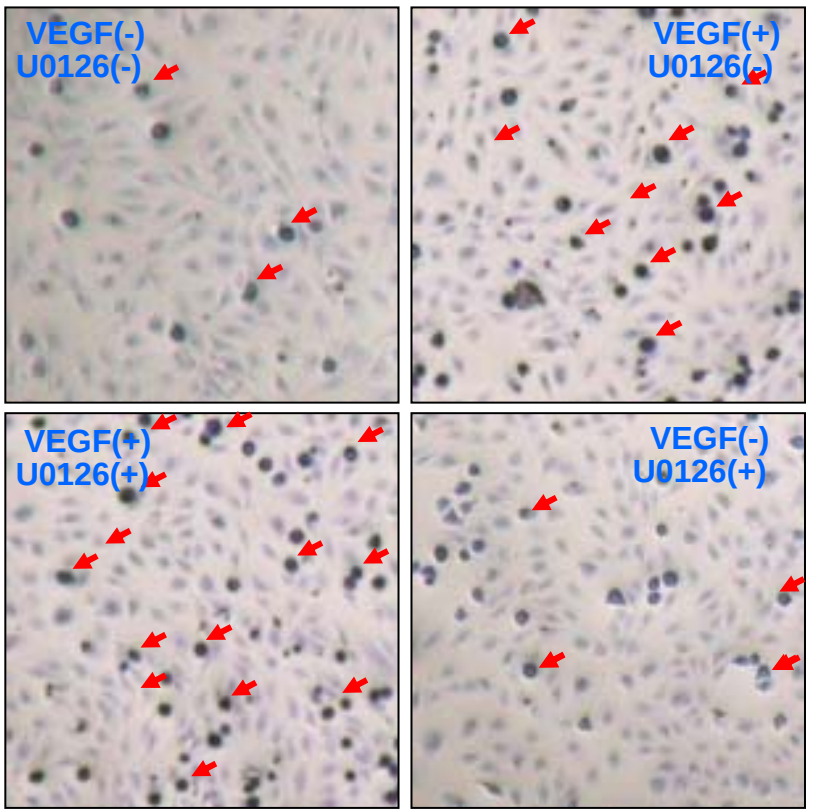
Min et al Circ. Res. 2005

Internal regulation of inflammatory signals

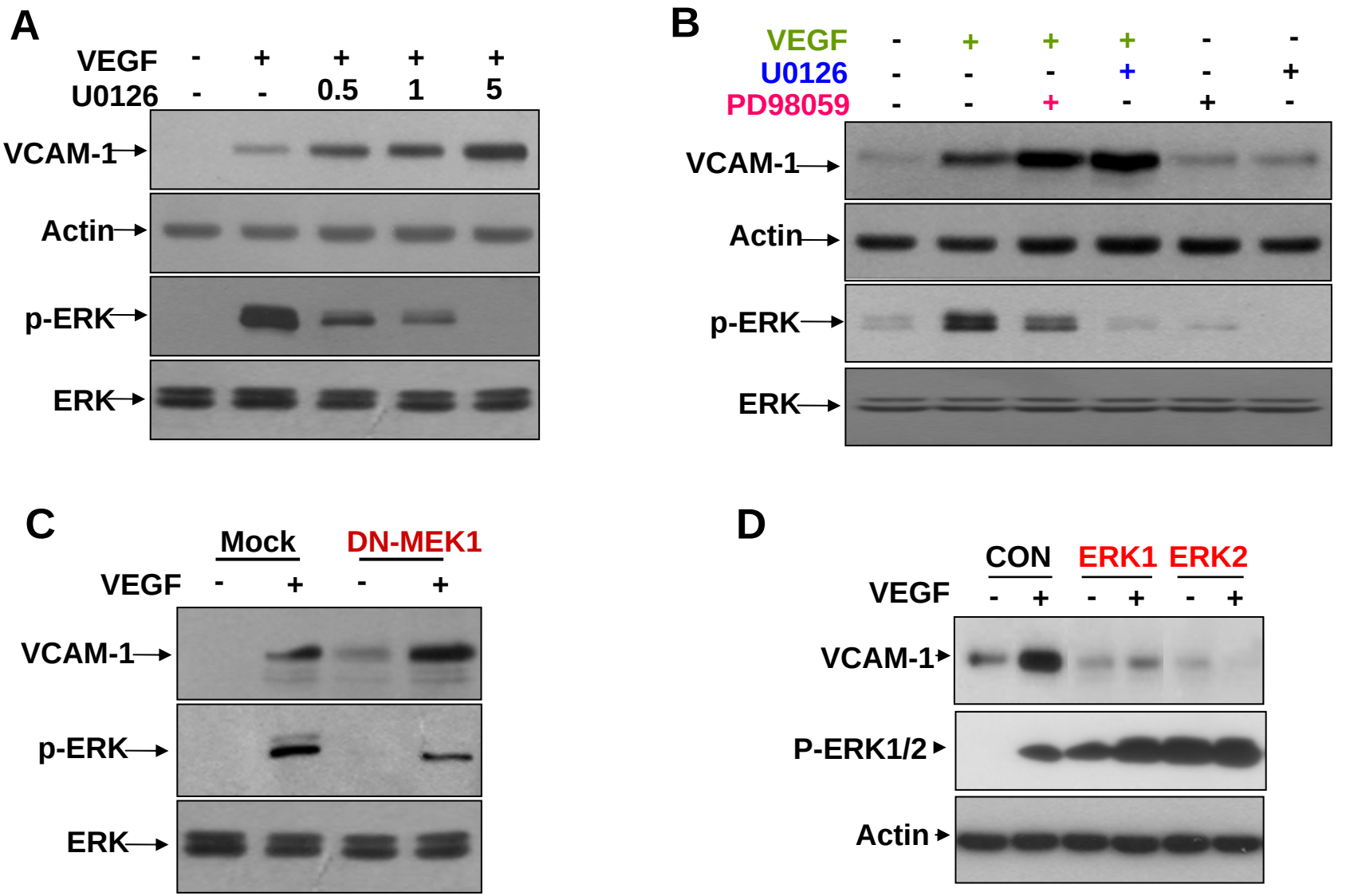
ERK suppresses inflammatory responses in EC



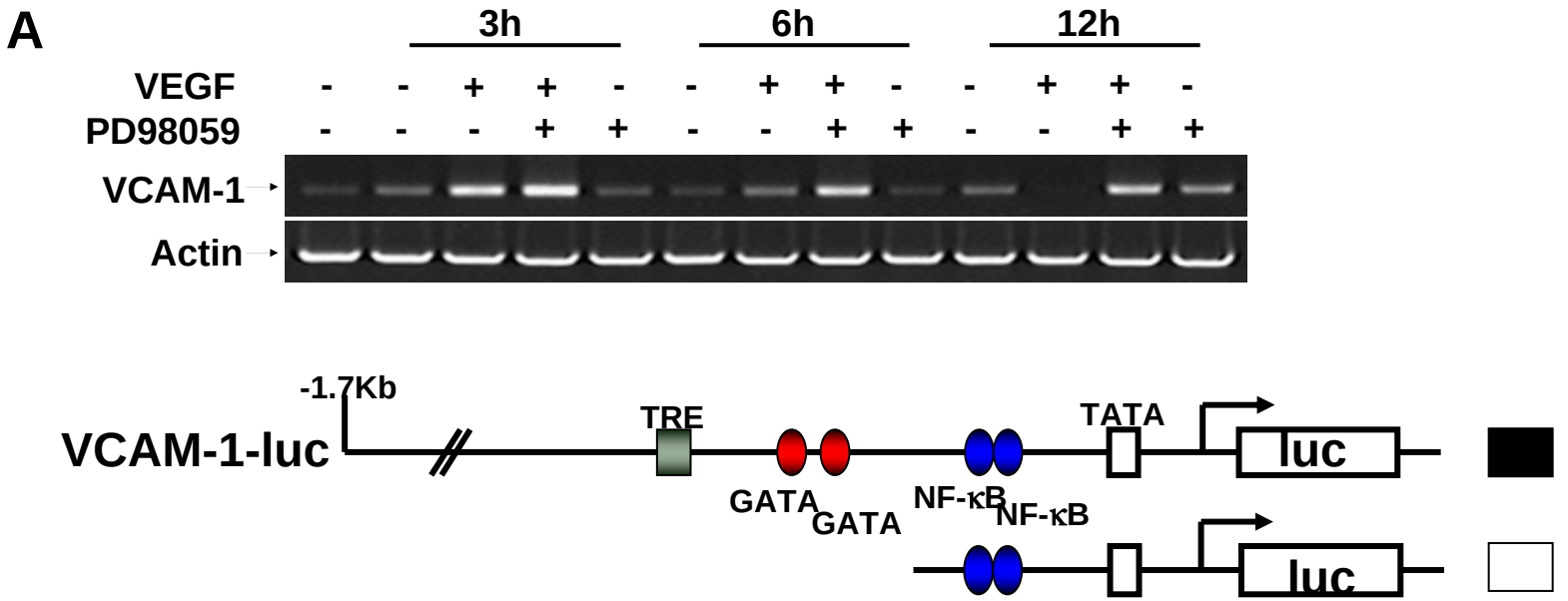




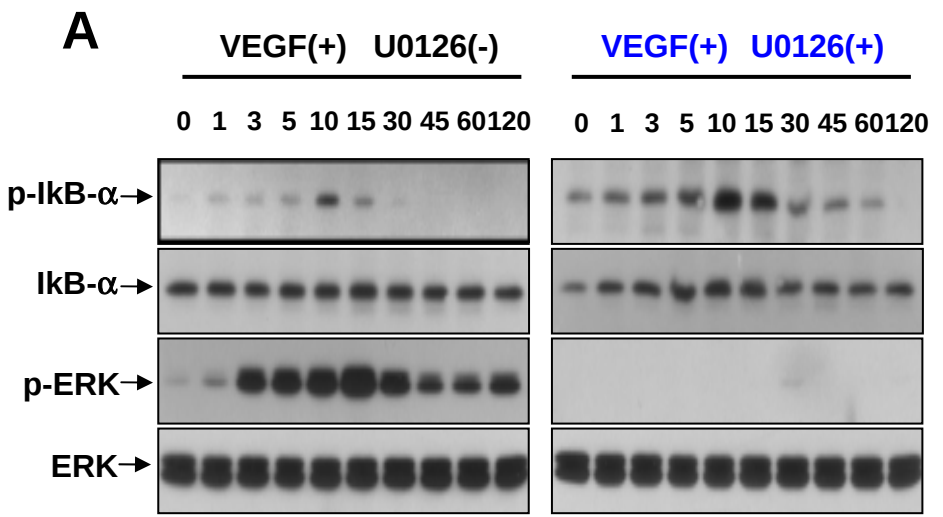
ERK negatively regulates VEGF-induced VCAM-1 expression in ECs

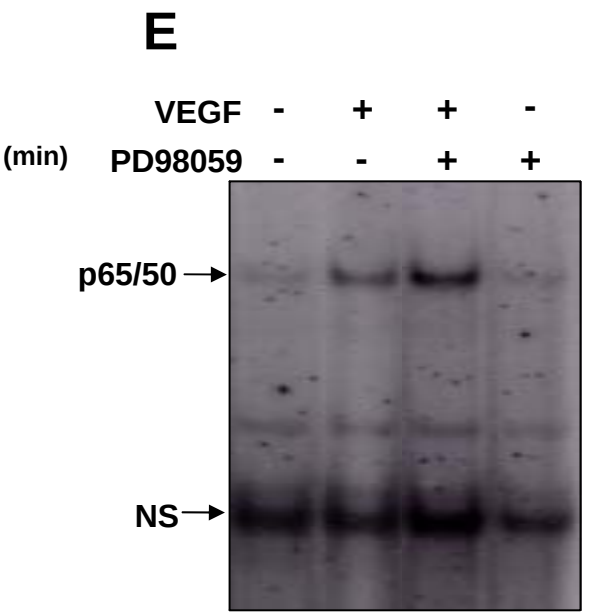


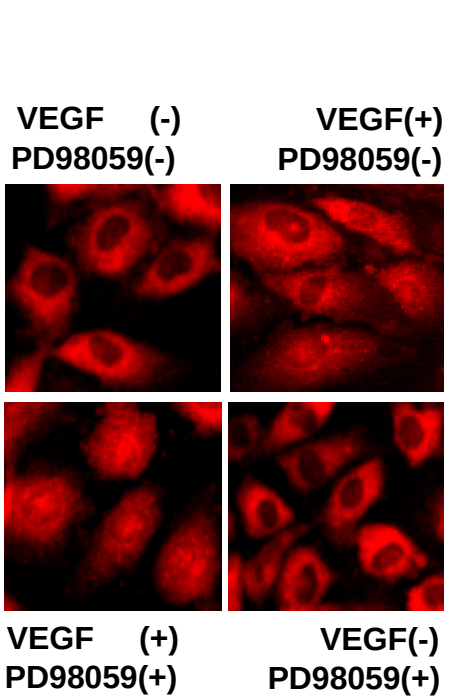
ERK negatively regulates VEGF-Induced transcriptional activity of VCAM-1

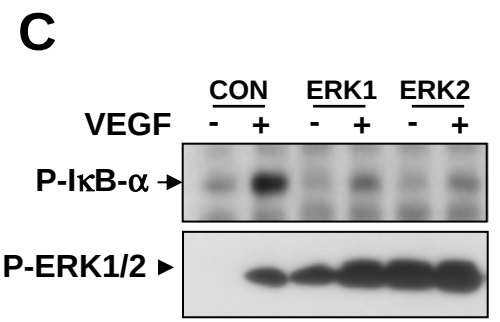


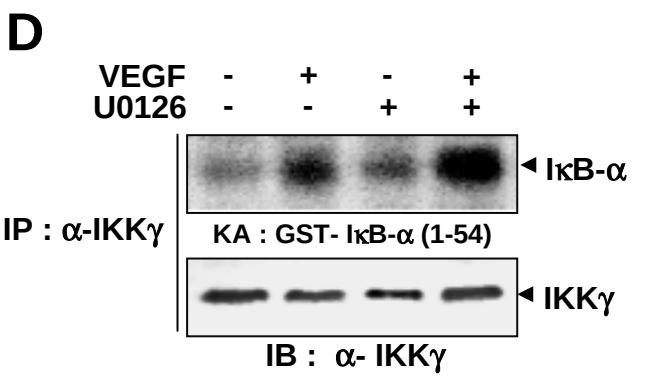
Inhibition of ERK increases VEGF-induced IκB-α phosphorylation and NF- κB activation



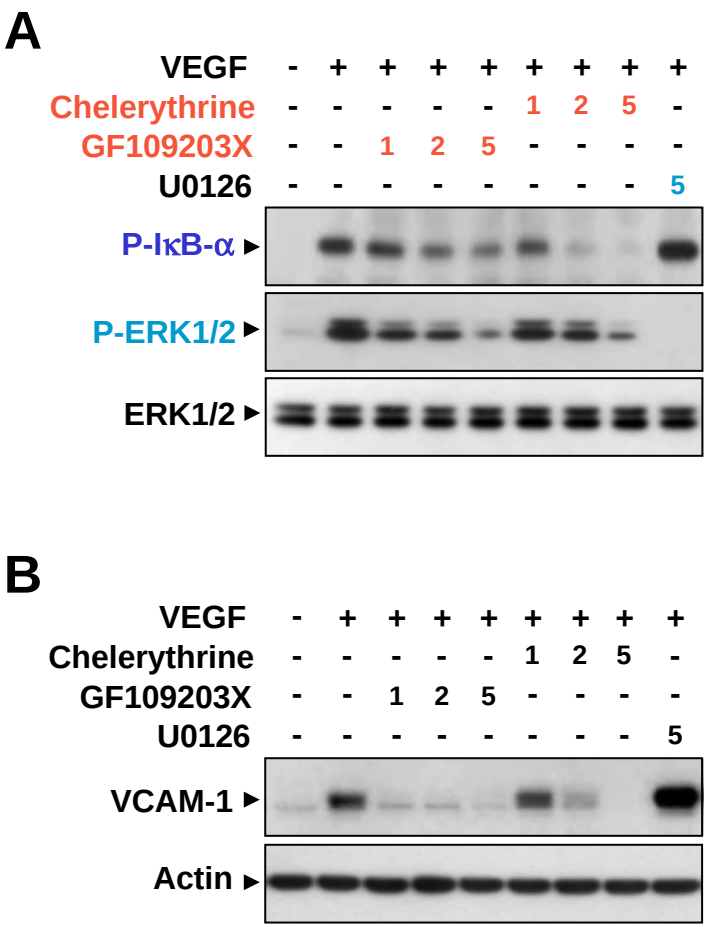


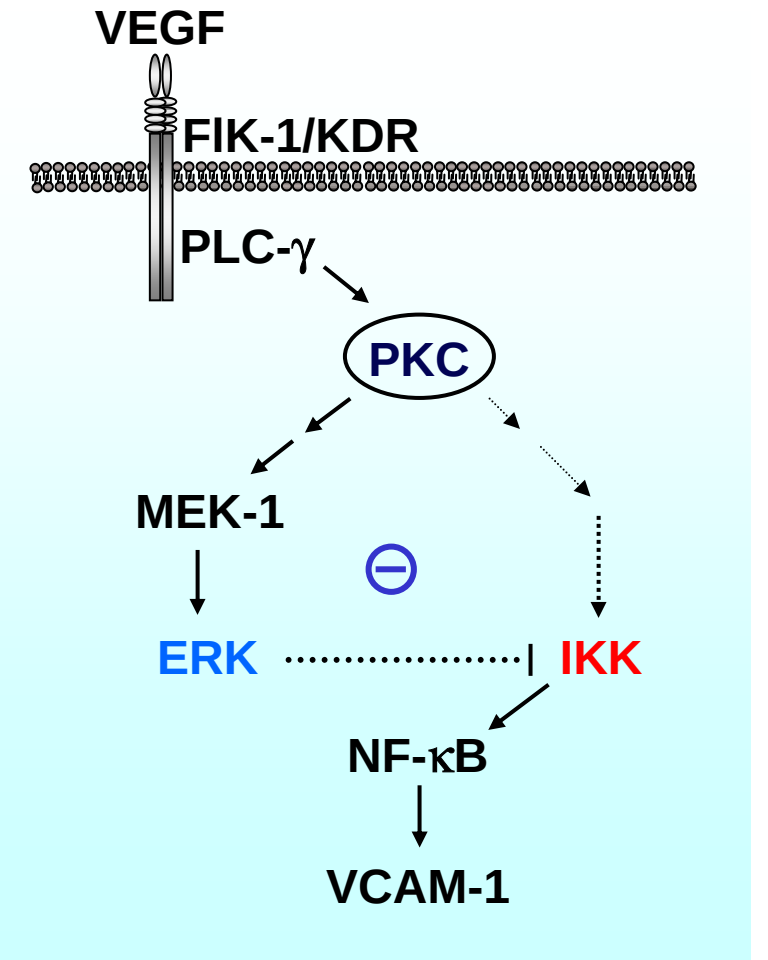




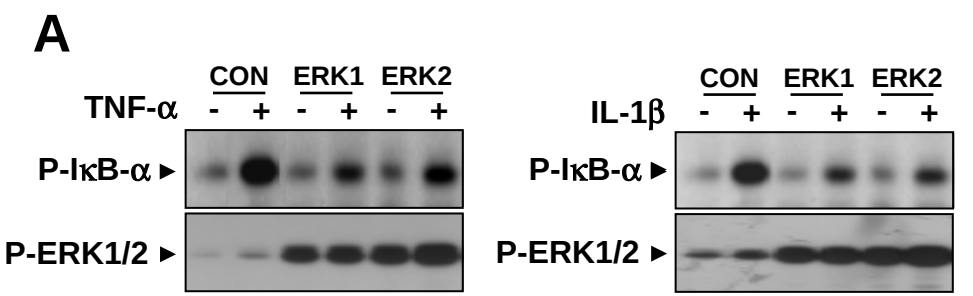


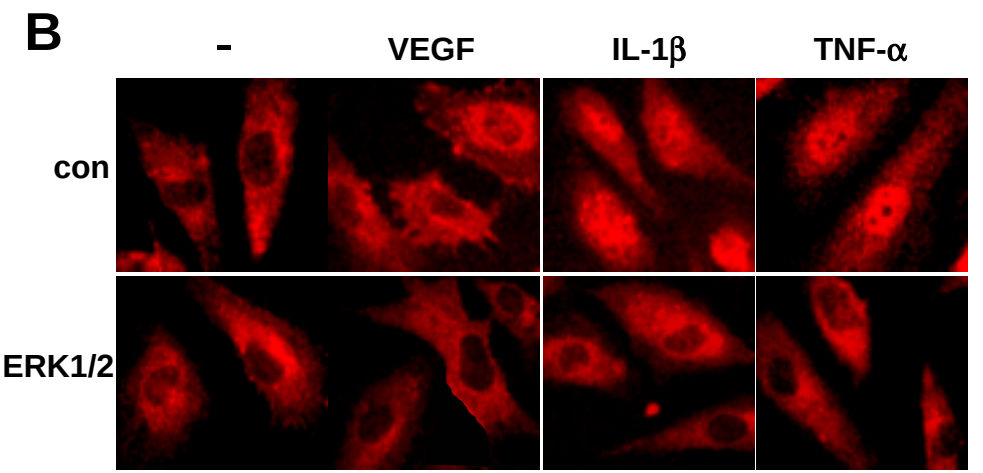
PKC mediates both ERK and NF- κB activation in response to VEGF

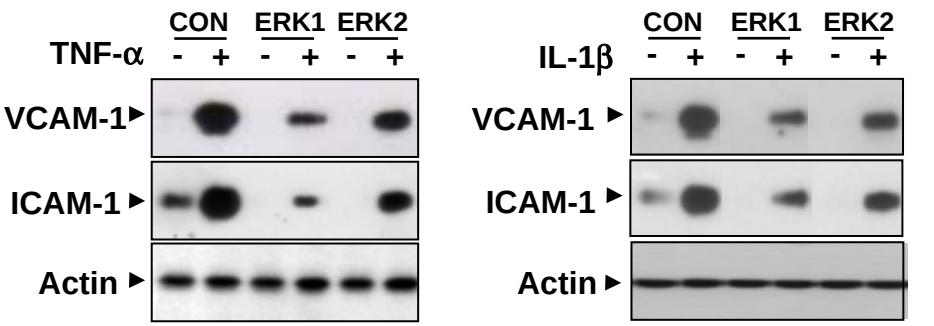


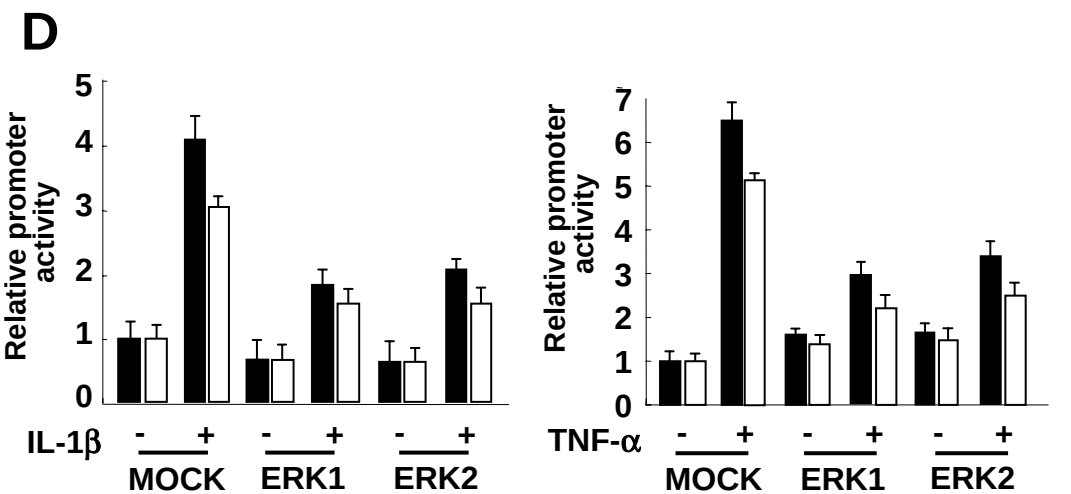


ERKs inhibit IL-1β- and TNF-a-induced CAM expression via inhibition of the NF-kB pathway in EC

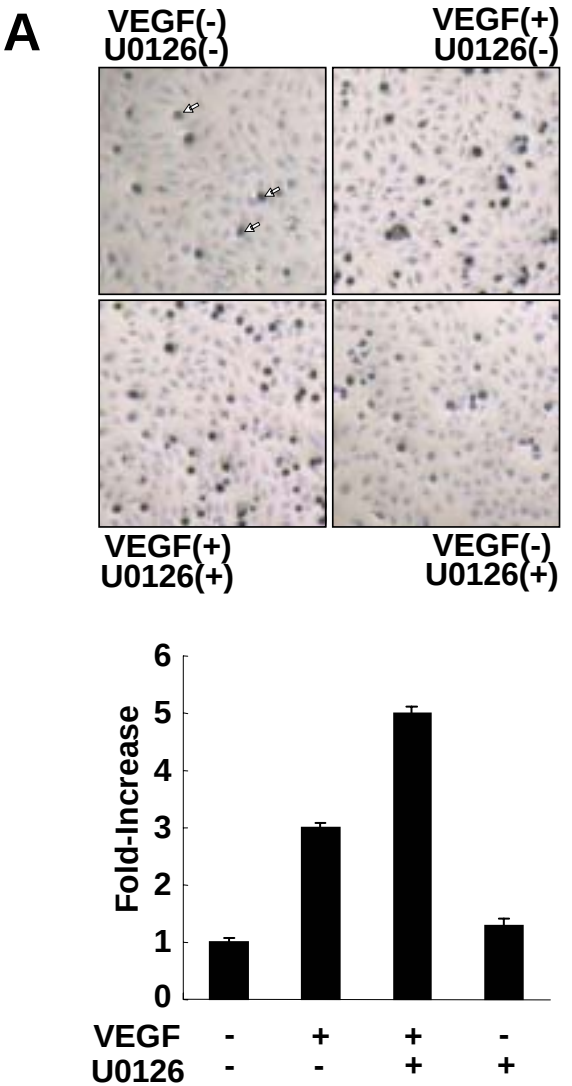


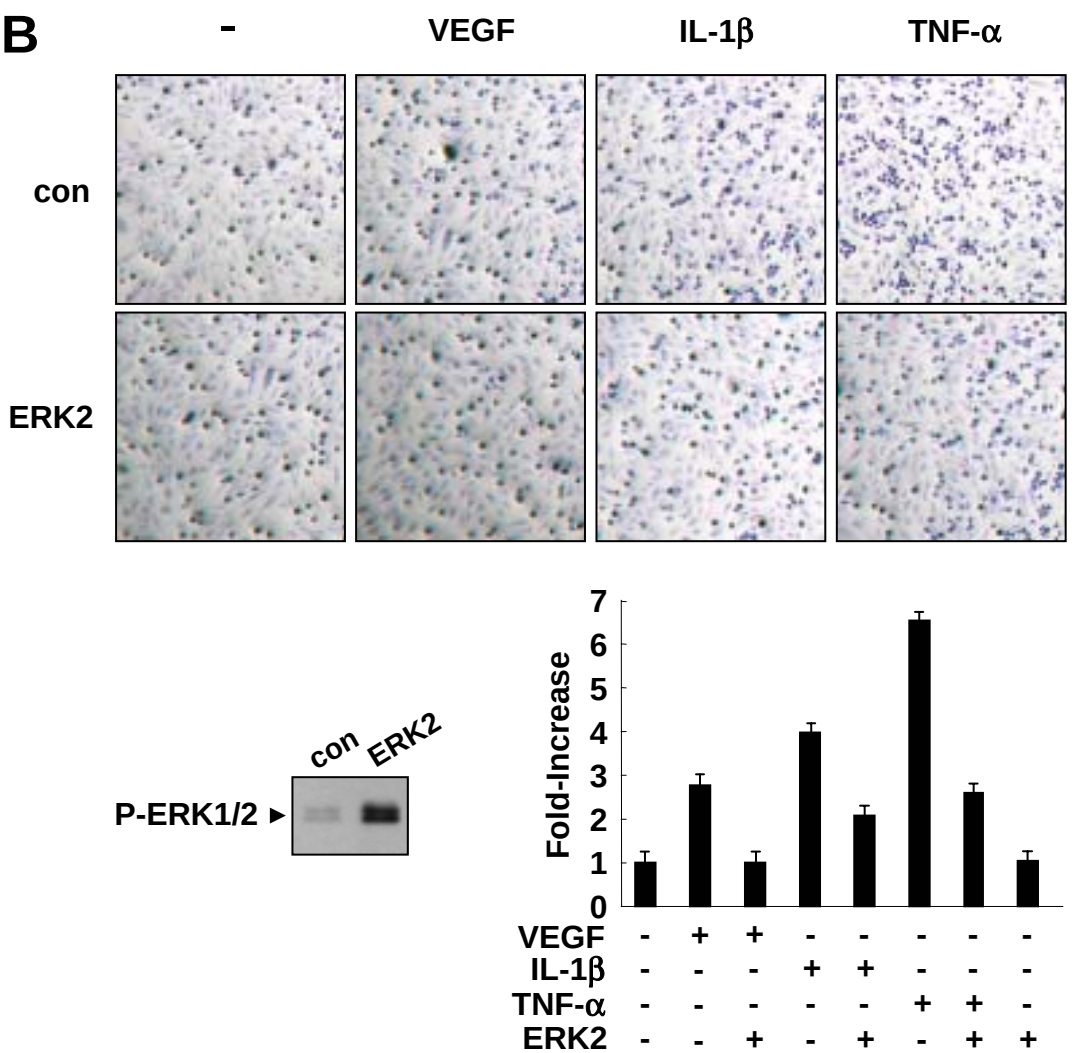




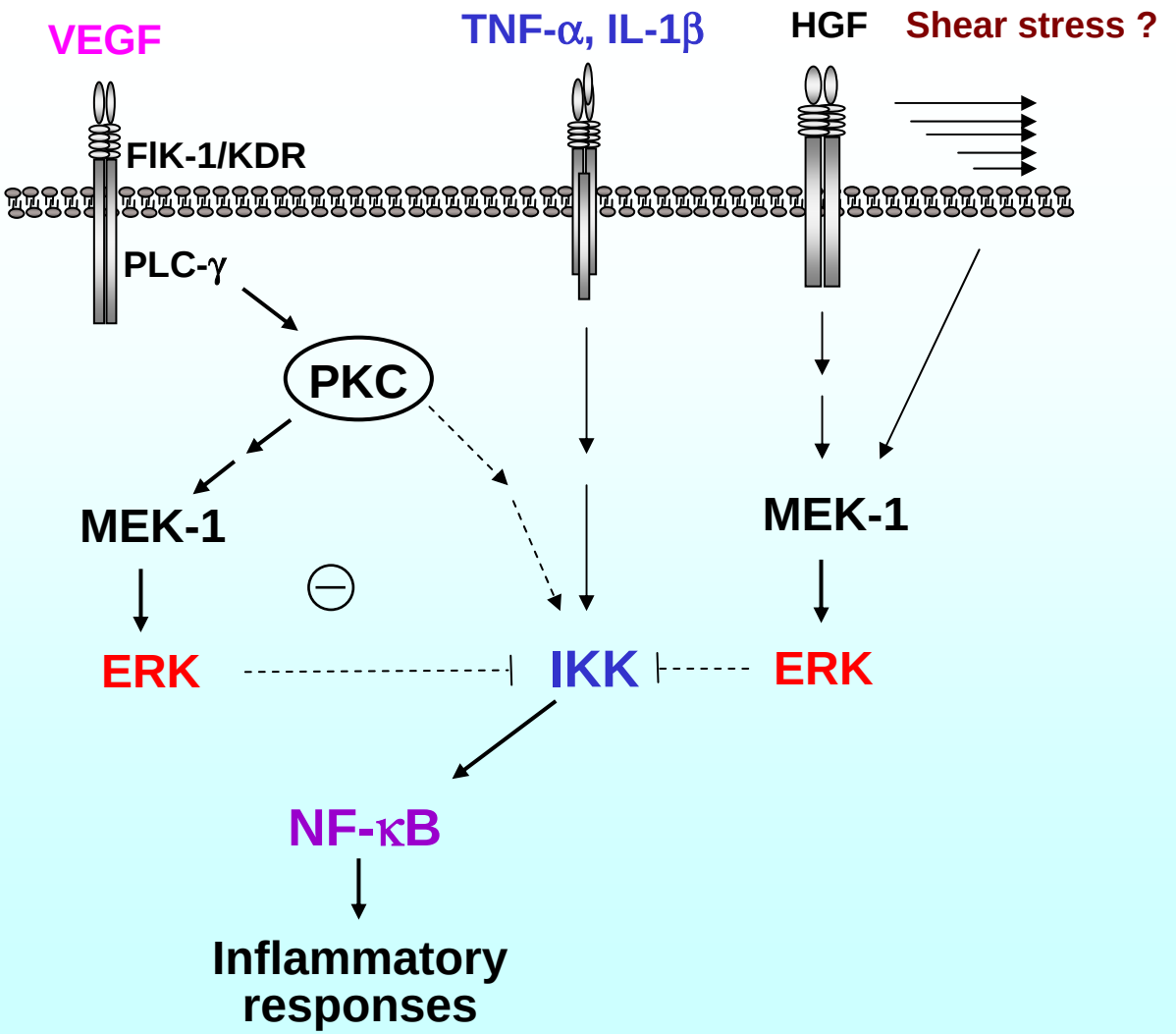


ERK inhibits inflammatory cytokine-induced leukocyte adhesion to ECs

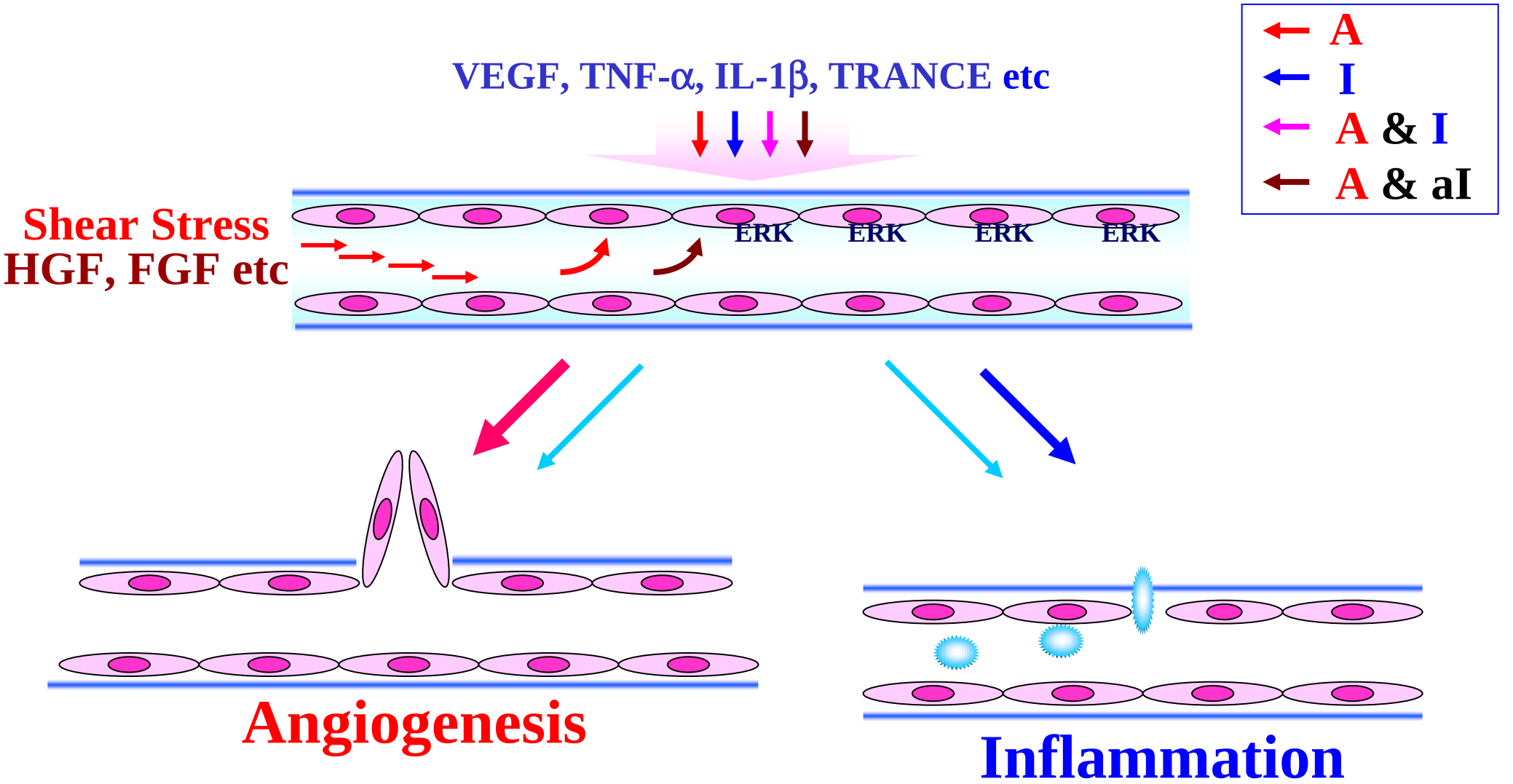




Schematic pathway for the negative regulation of VEGF-induced VCAM-1 expression by ERK



Balanced Regulation of Angiogenesis & Inflammation in the vascular wall



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