Therapeutic Angiogenesis for Peripheral Arterial Occlusive Disease

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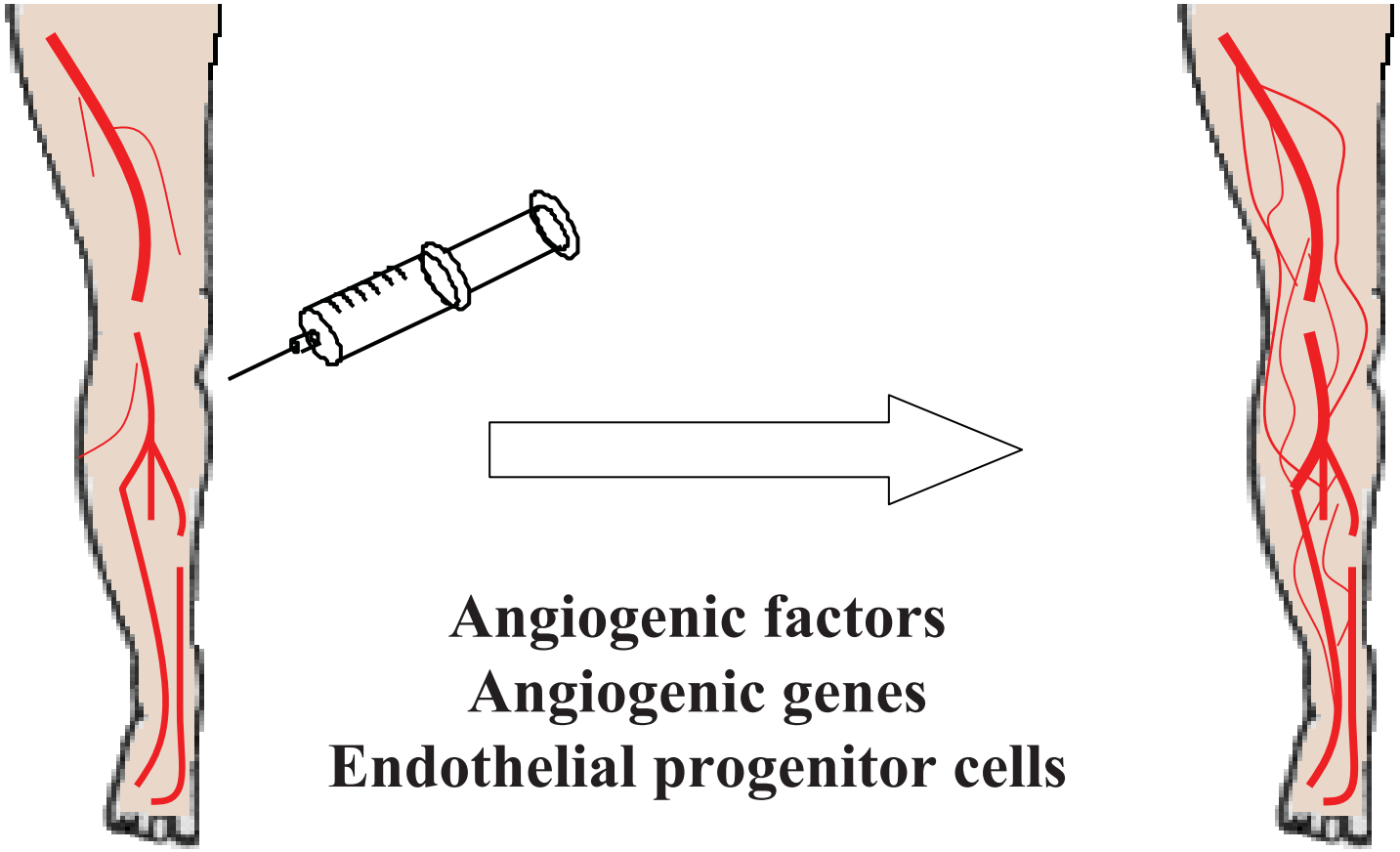
Therapeutic Angiogenesis



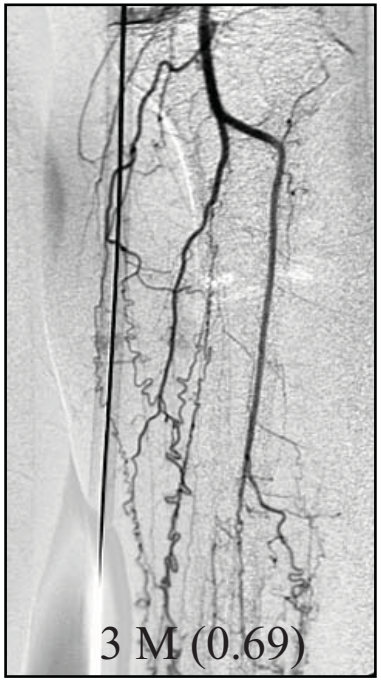
Angiogenic factors
Angiogenic genes
Endothelial progenitor cells

Angiogenic Gene Therapy

- hVEGF165 naked DNA
- No option patients with chronic severe PAOD
- Phase I trial: 9 patients (dose-escalating 2 mg, 4 mg, 8 mg)
- Phase II trial: currently undergoing
  - 8 mg: 125 microgram/site x 16 injections x 4 times q 1 month

First KFDA-approved gene therapy trial

M/46



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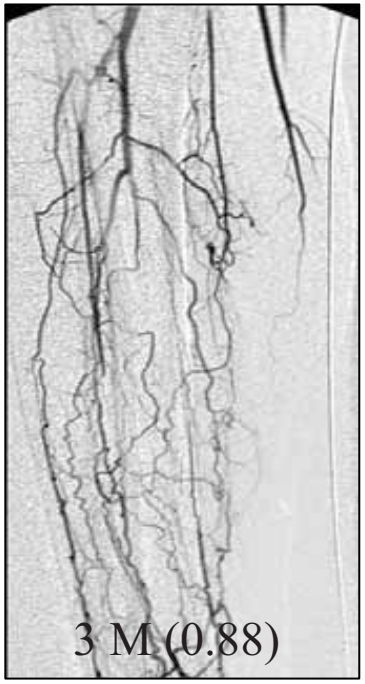

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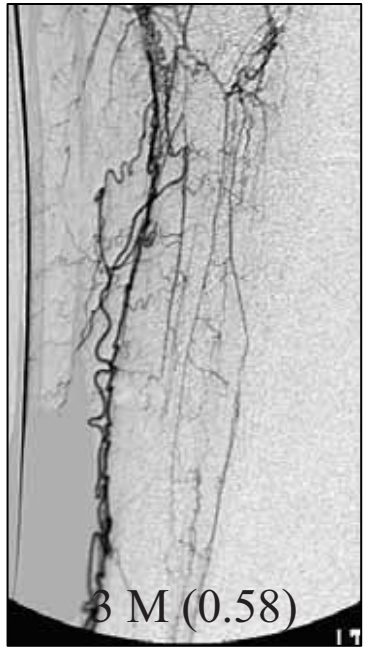
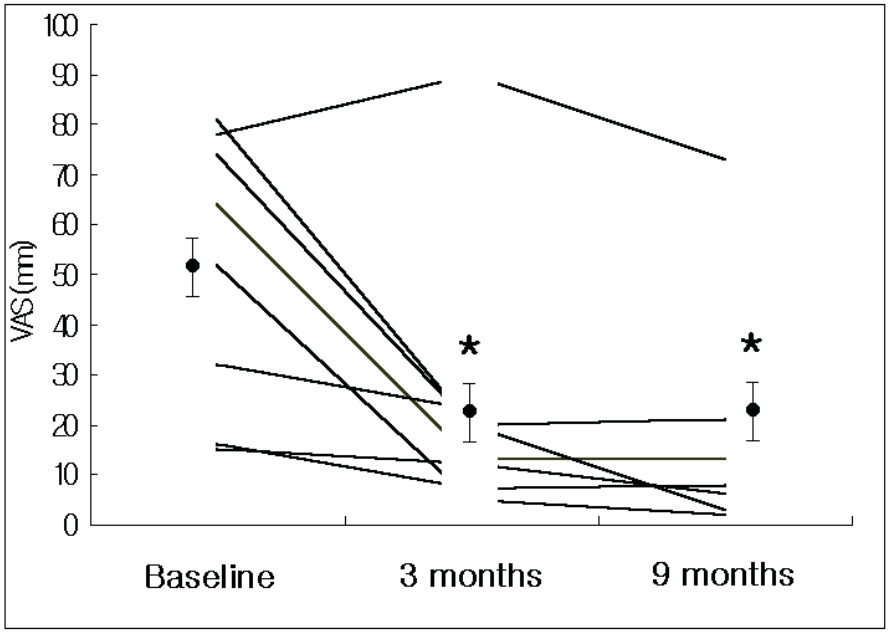


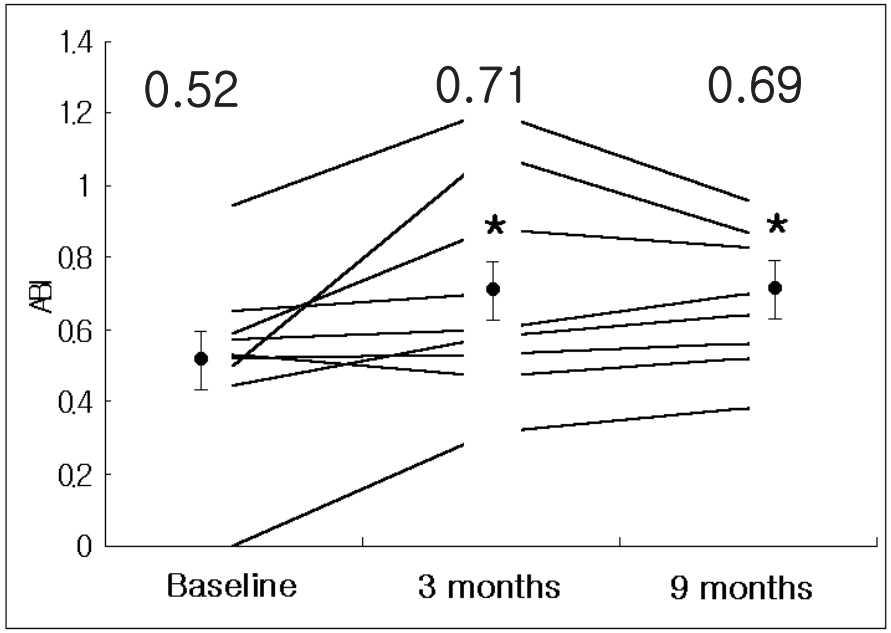
Case #2; Foot Ulcer

Secondary endpoints : Pain (VAS) & ABI

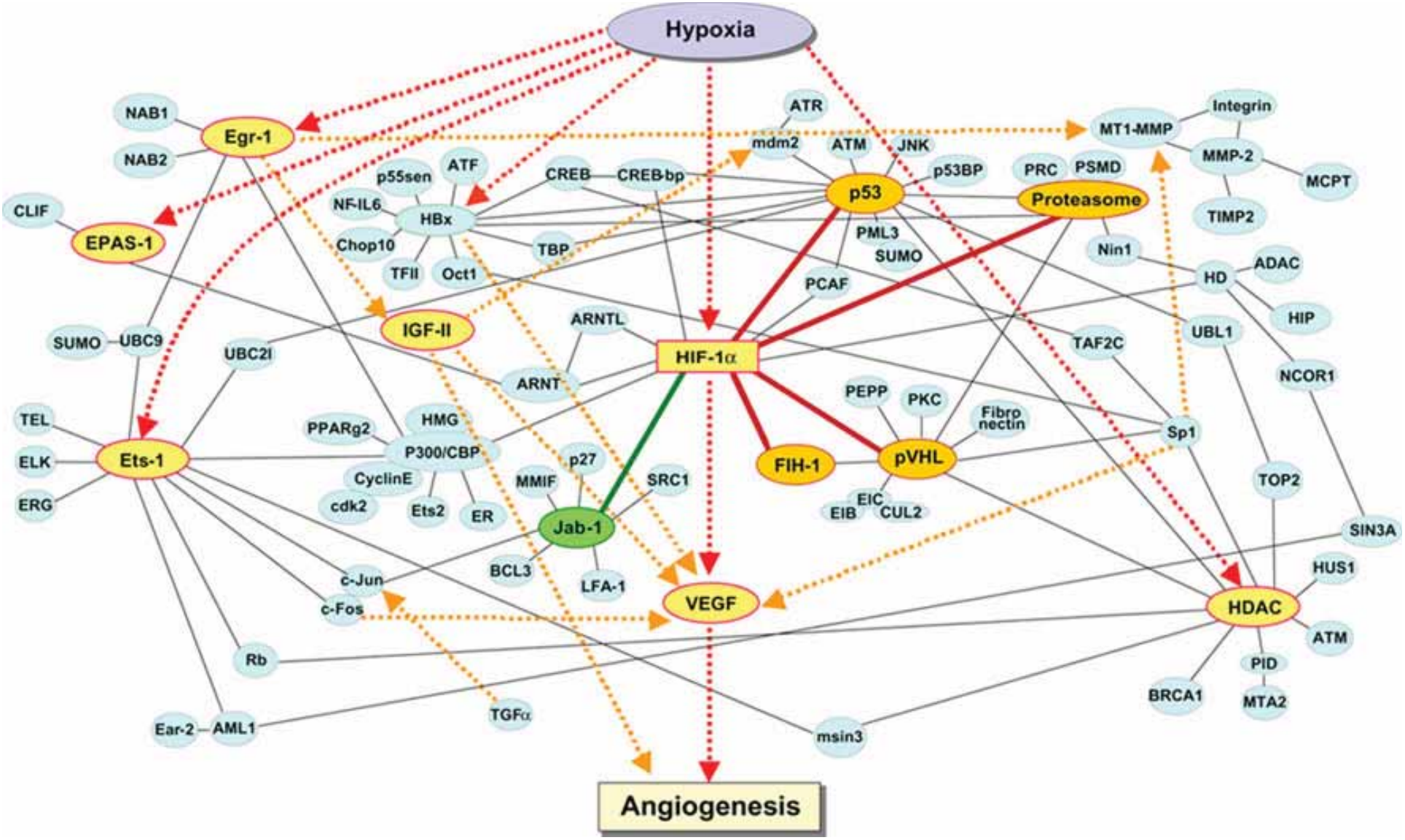
A



B



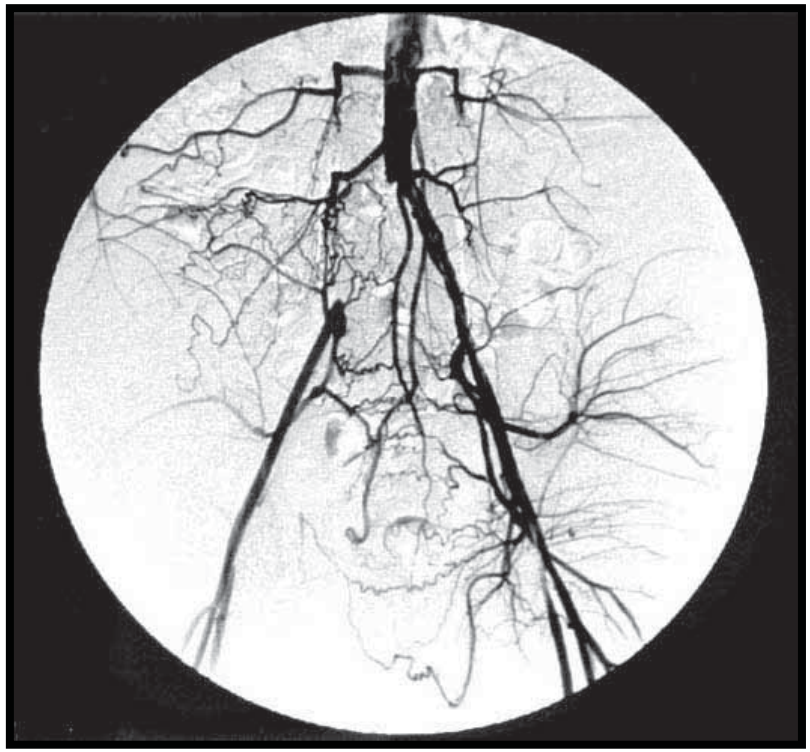
Orchestrated biology



Kim KW 2002

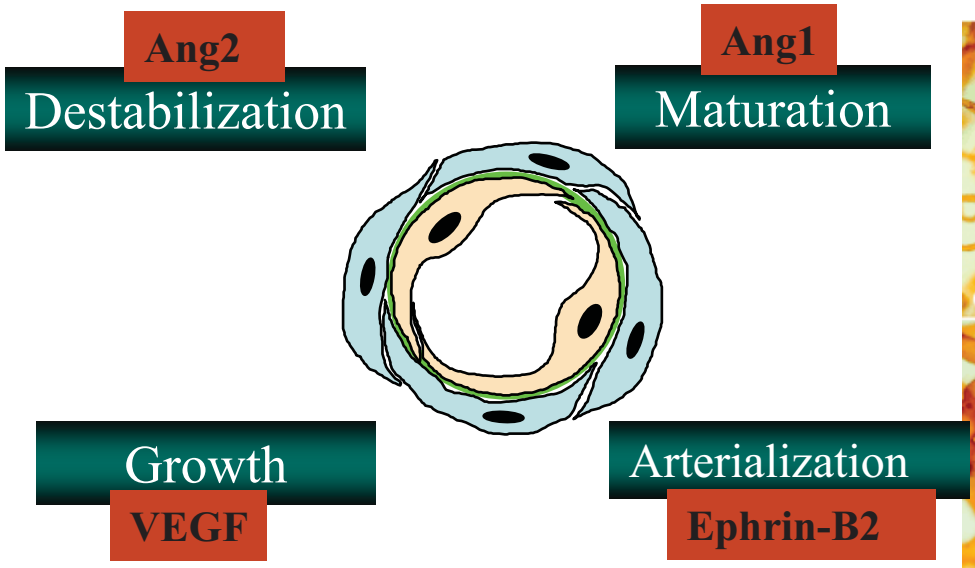
Therapeutic arteriogenesis/collateralogenesis

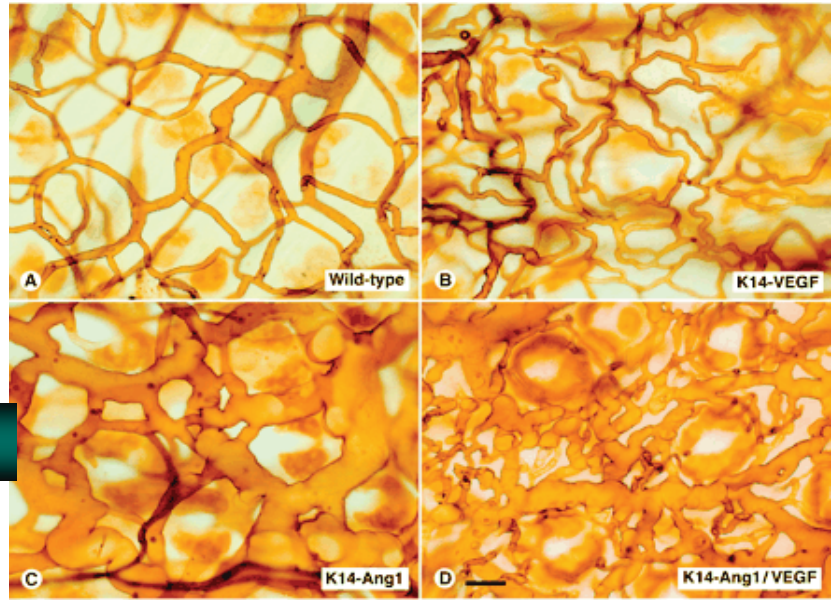
- Combination of angiogenic factors
  - Development of angiogenic assay to test multiple angiogenic genes
  - Test transcriptional factor to turn-on multiple angiogenic genes



67/M, mild claudication, ABI: 0.9/11

Arteriogenic/Collateralogenic Therapy





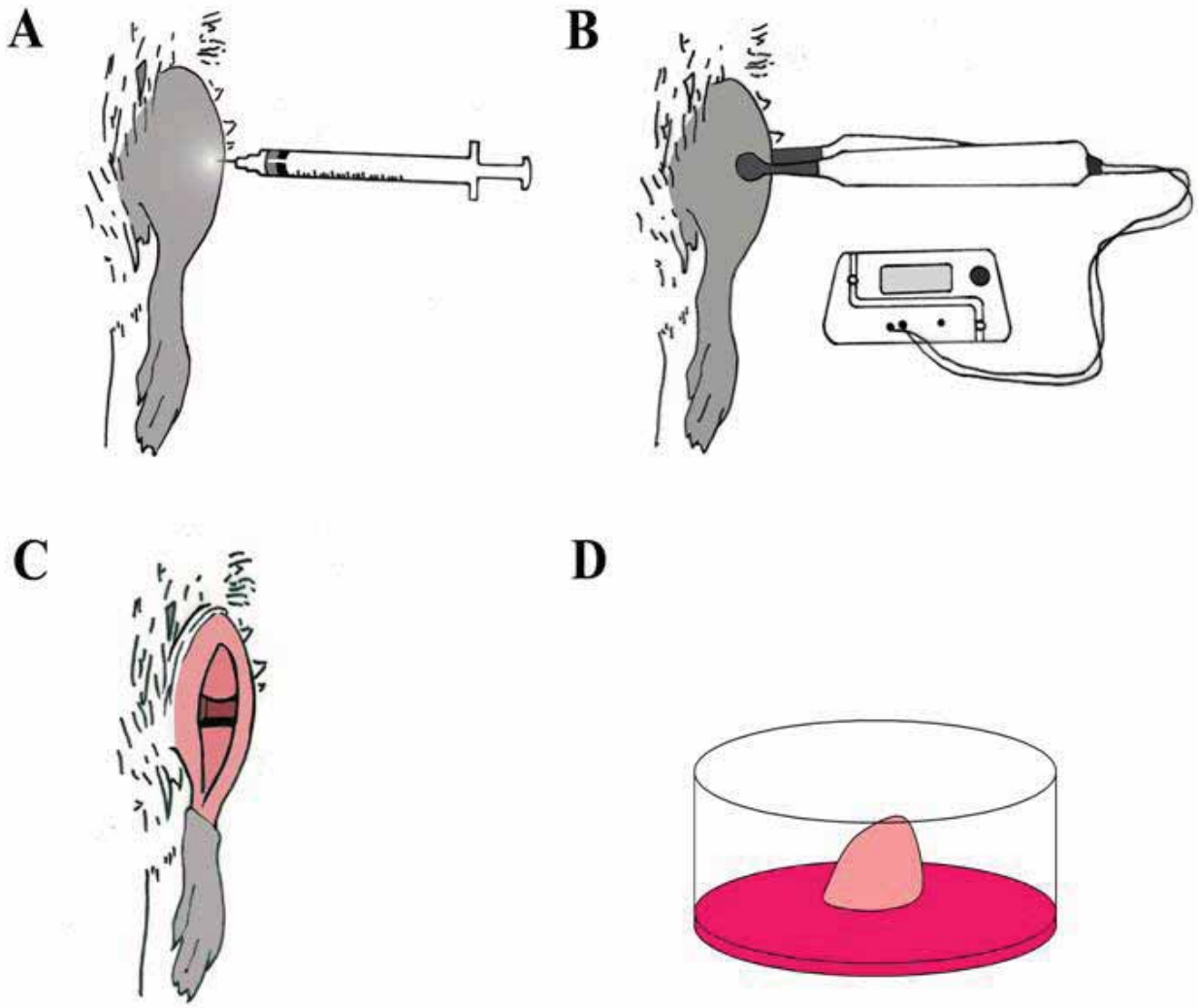
Combination therapy ?

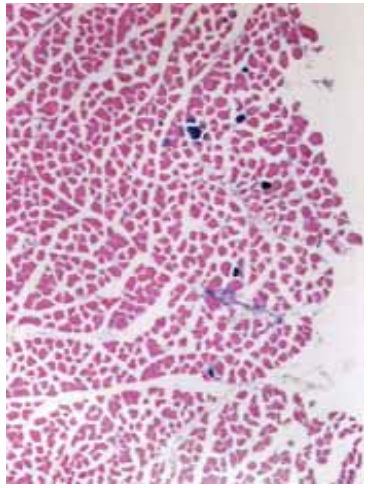
Thurston et al, Science 2000

A novel angiogenesis assay

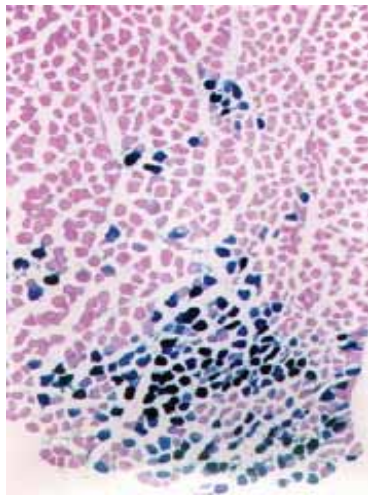
- A simple, reproducible, and quantitative assay to test angiogenic genes would be useful for the development of gene therapy for therapeutic angiogenesis.
- Most of conventional angiogenesis assays were designed to test protein factors.
- Naked DNA
  - Simple
  - Skeletal muscle
    - highly vascularized
    - endocrine factory for the secretion of therapeutic proteins
    - target organ of angiogenic gene therapy for patients with PAOD
  - Electroporation increases transfer efficiency

A novel *ex vivo* angiogenesis assay



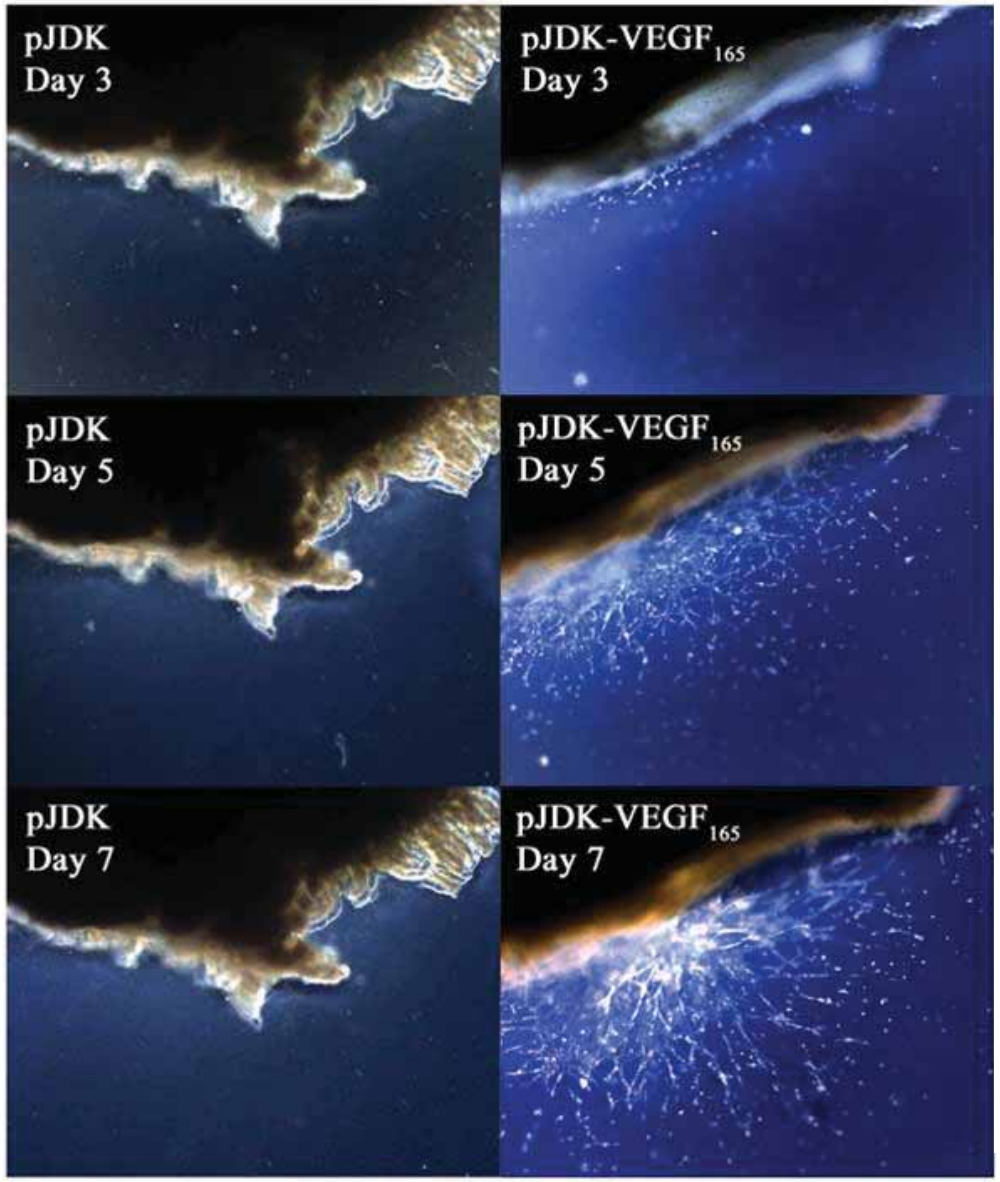


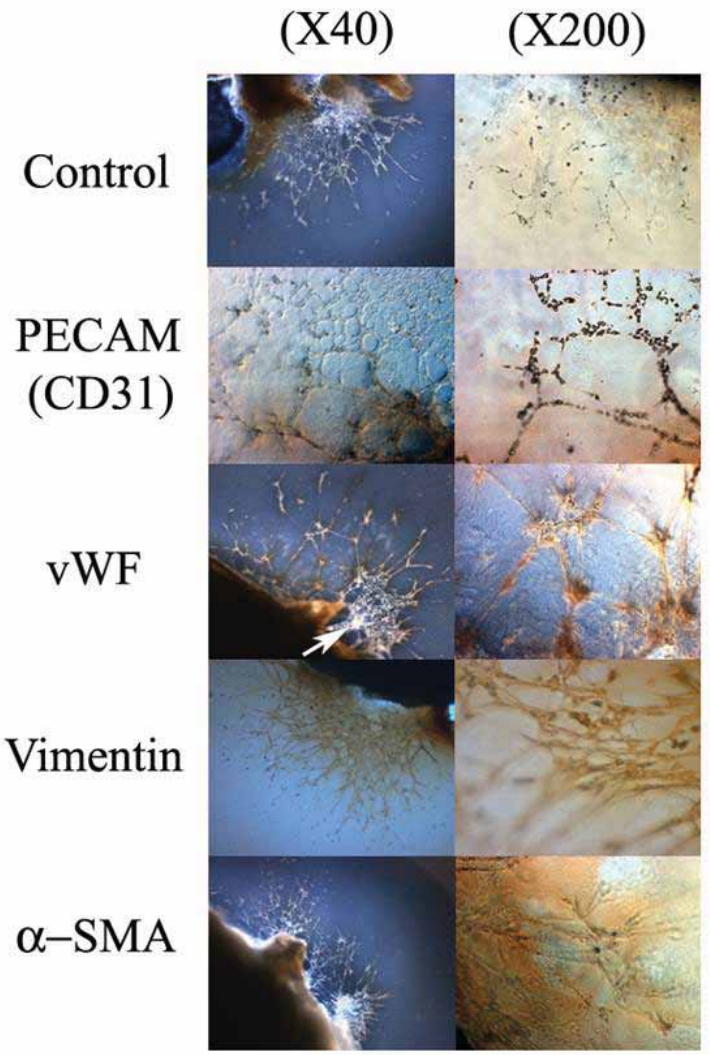
ES (−)



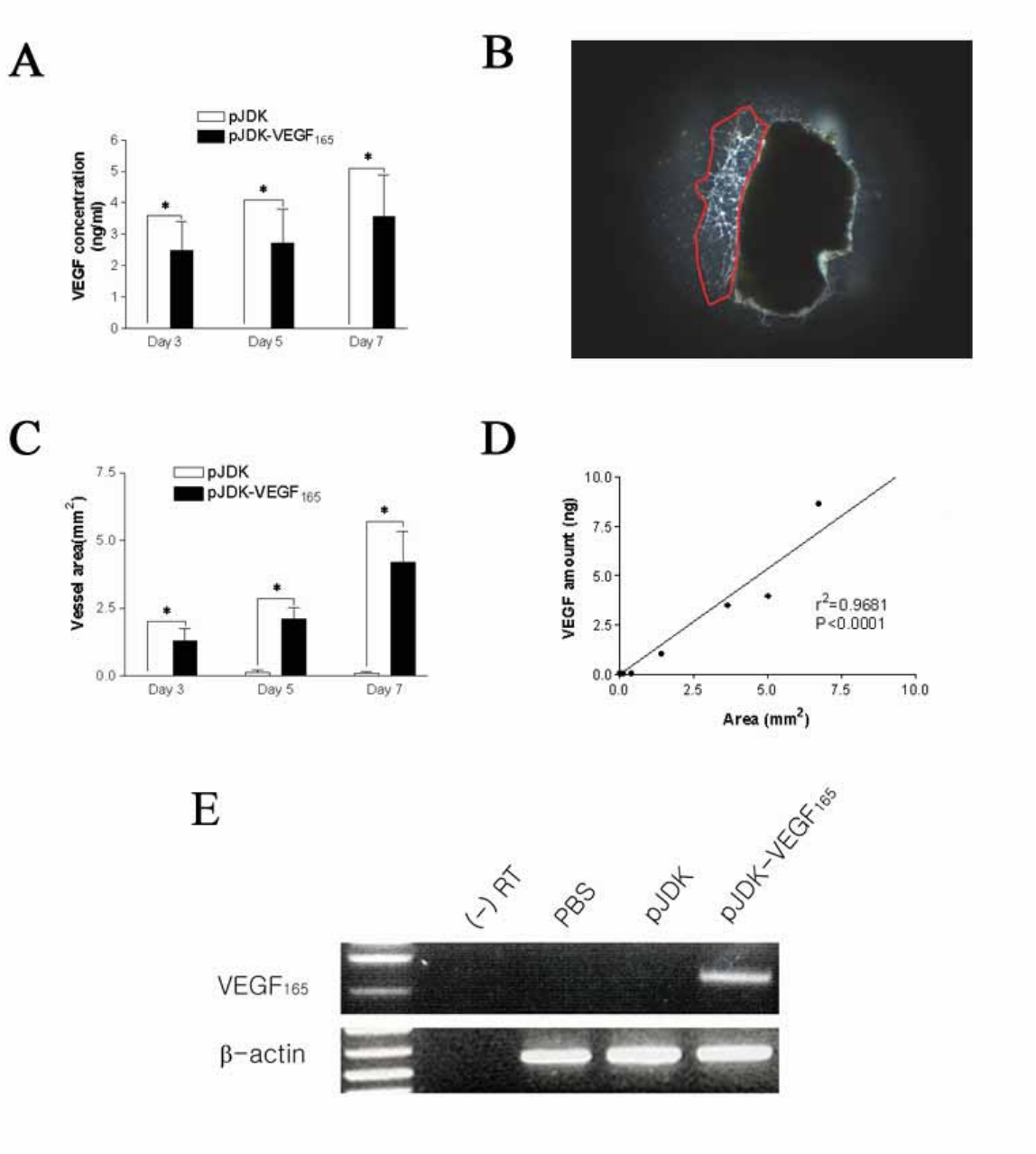
ES (+)

A novel *ex vivo* angiogenesis assay

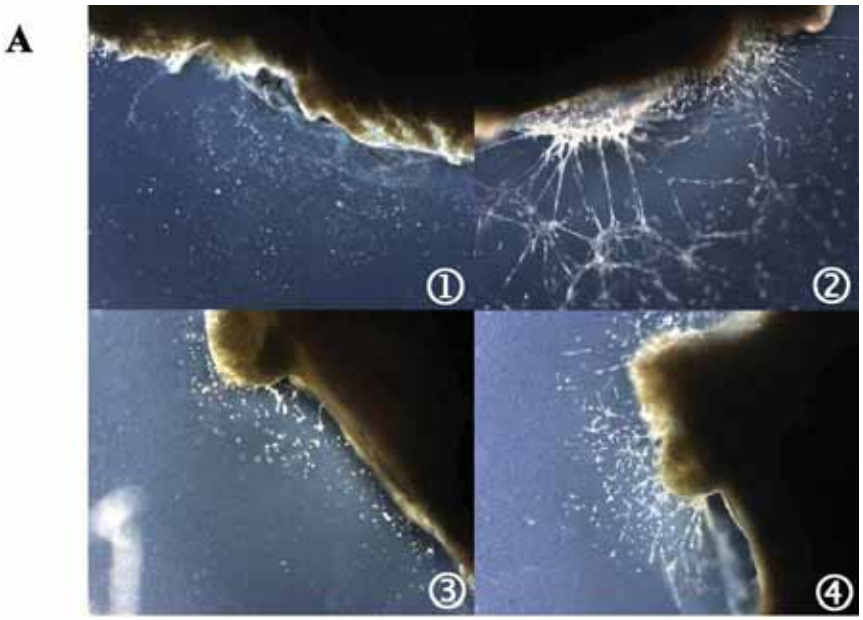


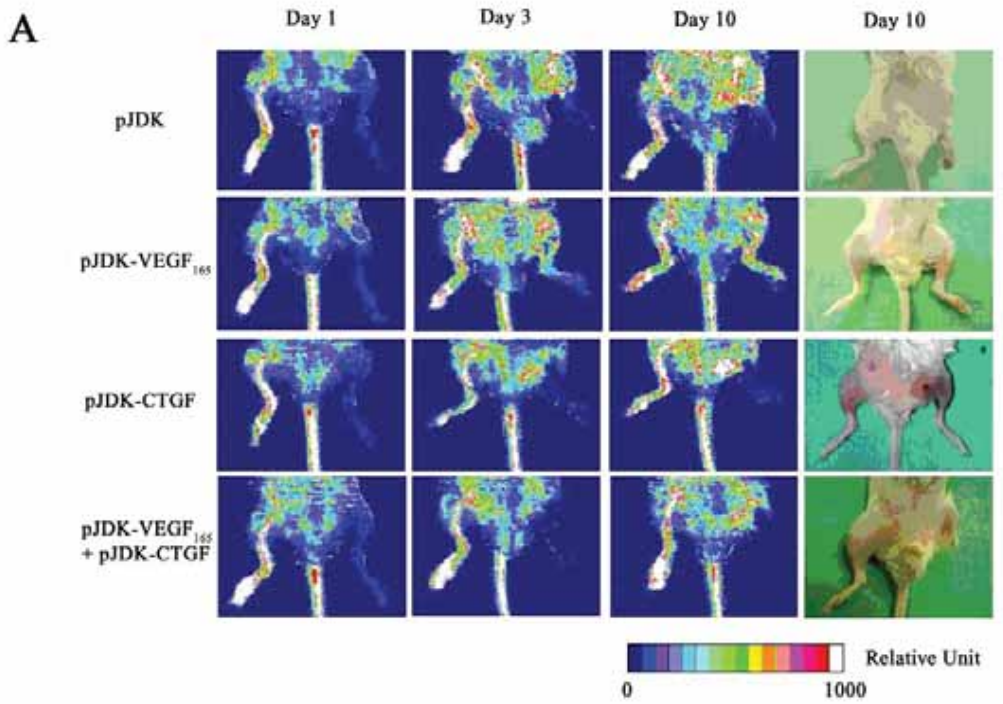


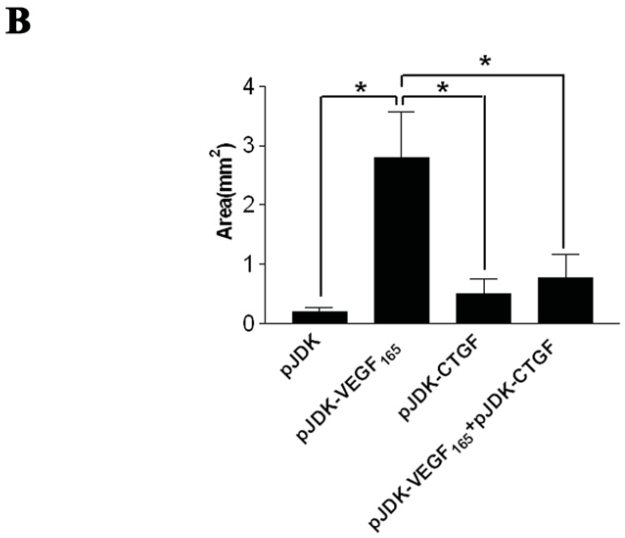
A novel *ex vivo* angiogenesis assay

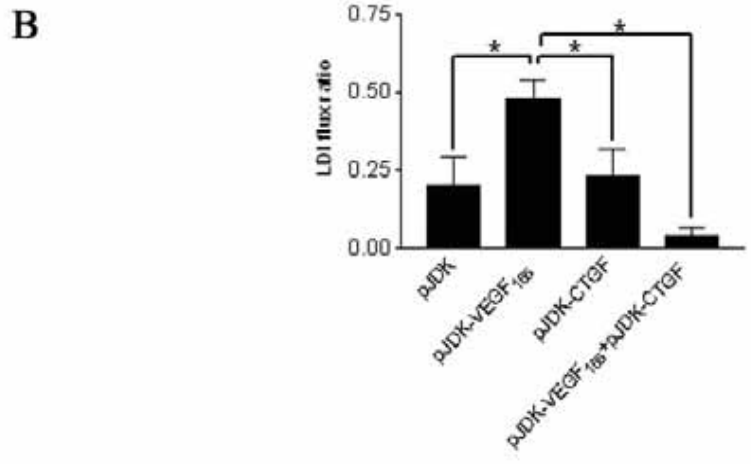


A novel *ex vivo* angiogenesis assay









Factors with potential for therapeutic vascular growth

Growth Factors	Chemokines	Transcription factors	Others
VEGF,-B,-C,-D,-E	MCP-1	HIF-1α, ***Egr-1***, Prox-1	Del-1, Cyr61, PR39
FGF-1, -2, -4, -5			Tissue kallikrein
Ang 1, Ang 2			Secreted frizzled-related protein
HGF, PDGF-BB			eNOS, iNOS
GM-CSF, neurotrophin			
IGF-1, IGF-2			

Seppo Yla-Herttuala & Kari Alitalo, 2003

Egr-1 (Early Growth Response Factor -1)

- 3 tandemly repeated Cys_2His_2 zinc-finger motifs
- rapidly induced to a variety of stimuli: hypoxia, injury, physical forces, cytokines
- short-lived protein located in nucleus
- phosphorylated on serine
- consensus binding sequence : GCG(T/G)GGGCG.
- co-activator : CREB-binding protein (CBP), p300
- co-repressor : NAB1, NAB2
- M.W.: 80- to 82-kDa

Egr-1 in angiogenesis

- Egr-1 upregulates the expression of a diverse array of proangiogenic genes: bFGF, PDGF, TGF-β, IGF I, II, ICAM-1 (*Gene 2003*)
- Egr-1 supports FGF-dependent angiogenesis (*Nat Med 2003*)
- Overexpression of NAB2 inhibits the angiogenic responses of endothelial cells (*J Biol Chem 2003*)
- NAB2 blocks Egr-1-mediated growth factor activation and angiogenesis (*Biochem Biophys Res Commun 2001*)

Hypothesis

Egr-1 gene delivery

↓

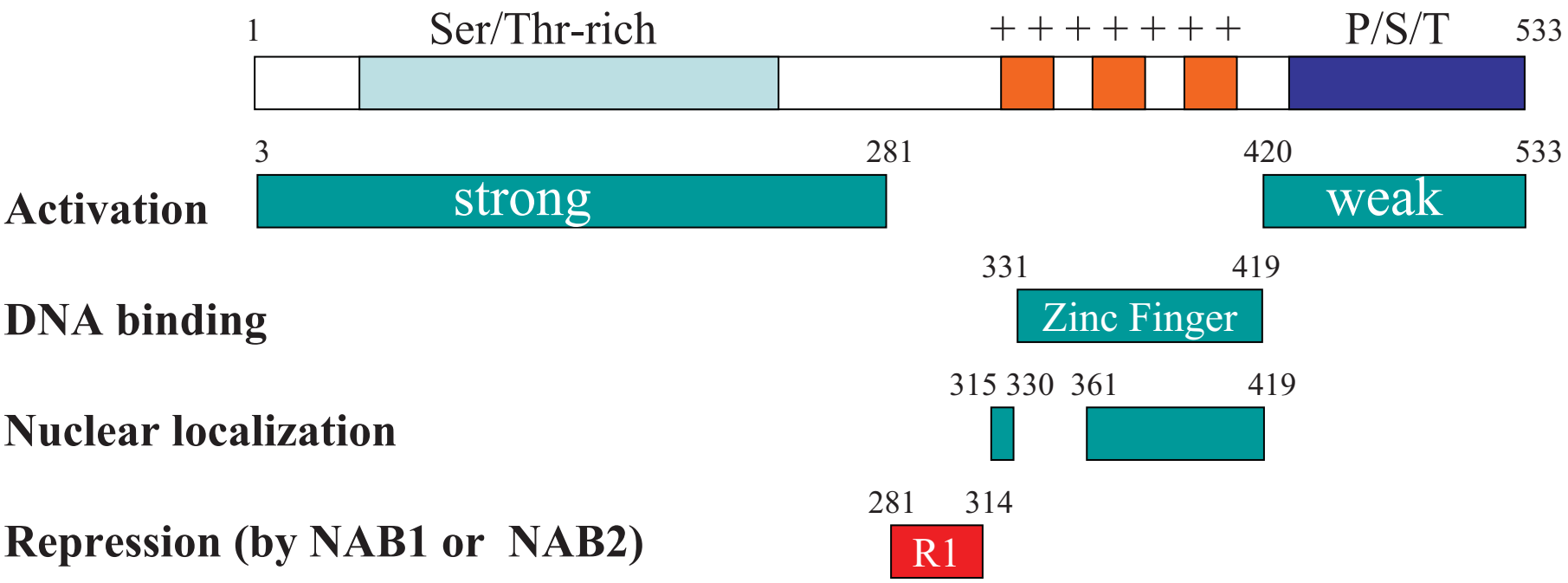
Upregulation of angiogenesis/arteriogenesis-related genes:
bFGF, PDGF, TGF-β, IGF II...

Multifactorial, Combined & Orchestrated Effects

↓

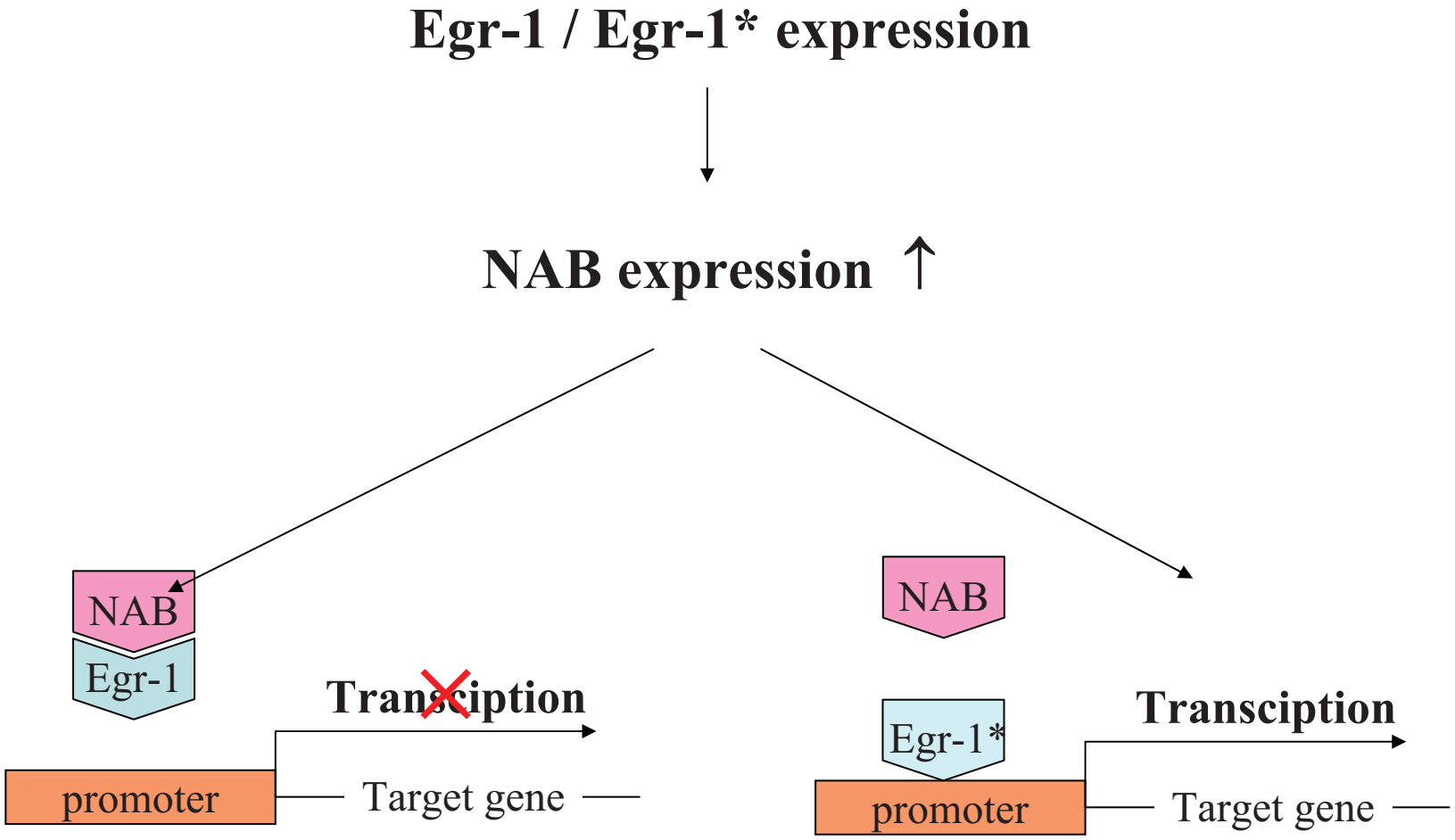
Improvement of perfusion

Modular structure of Egr-1 and Egr-1*

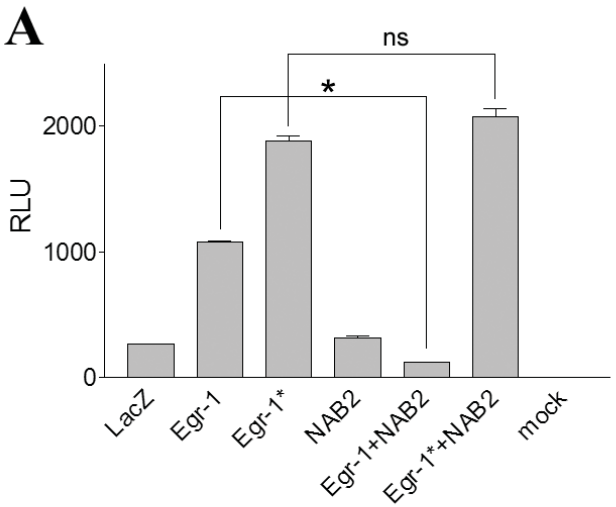


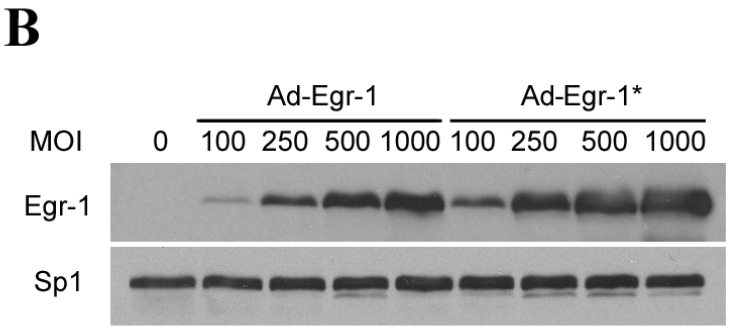
Egr-1*

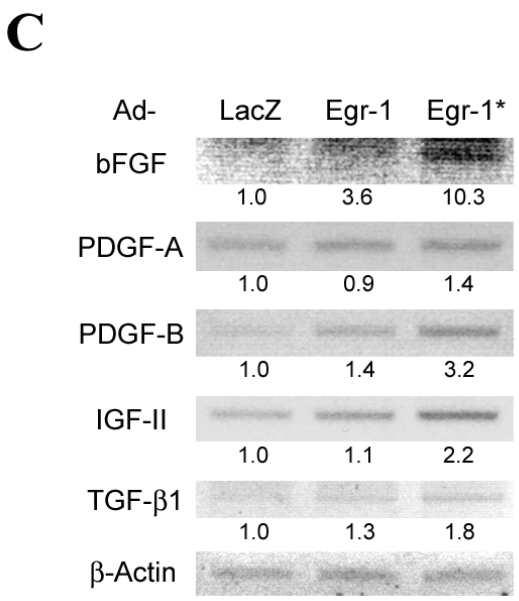
- Mutation of Ilu_{293} in R1 domain → Phe
- NAB-insensitive Egr-1 (Constitutively active Egr-1)

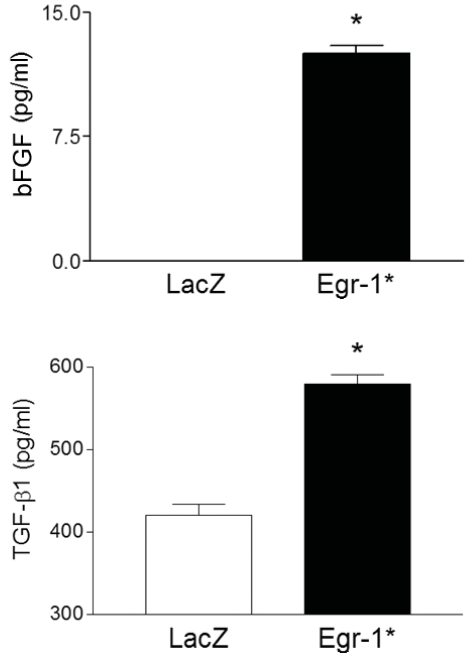

Egr-1 / Egr-1* expression
NAB expression ↑
NAB
Egr-1
Transcription
promoter
Target gene
NAB
Egr-1*
Transcription
promoter
Target gene

Upregulation of multiple angiogenic genes

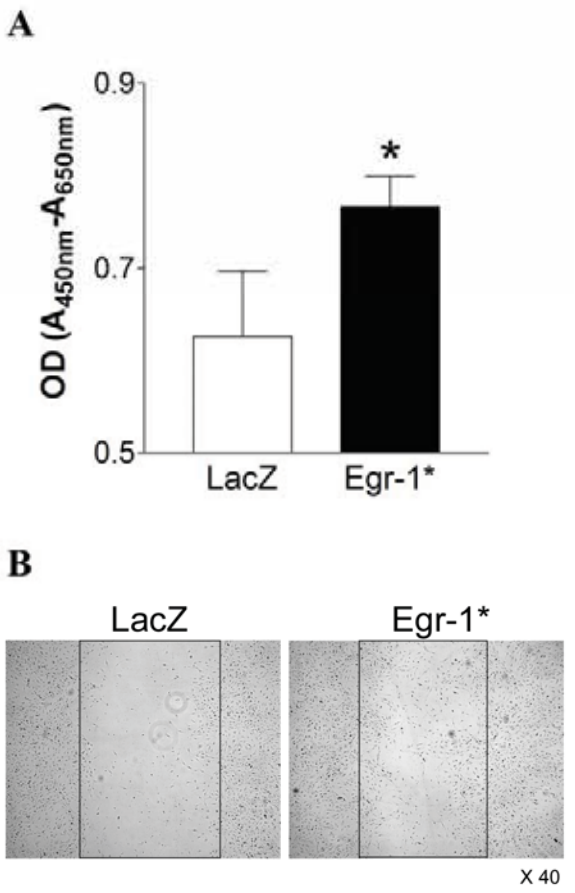


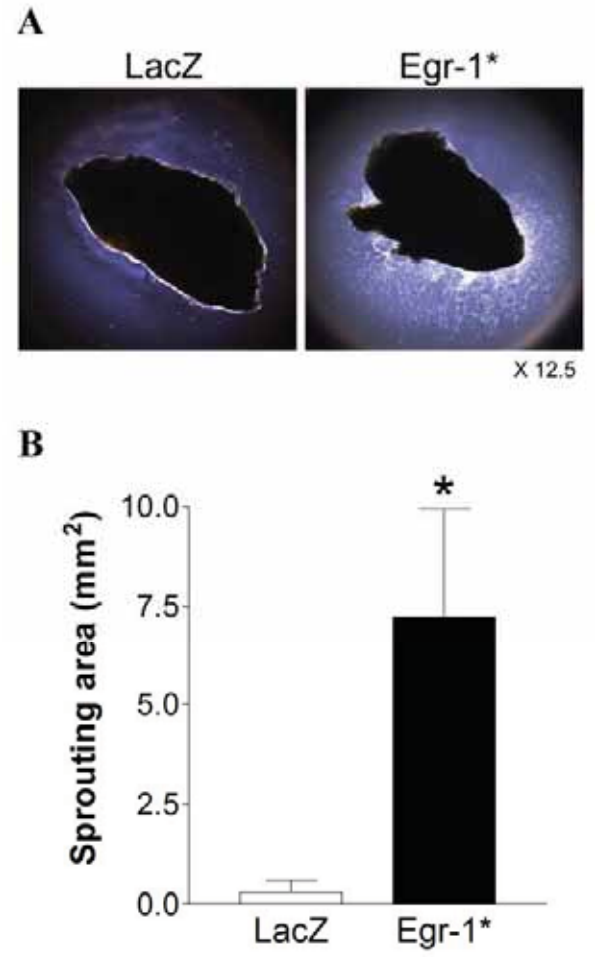


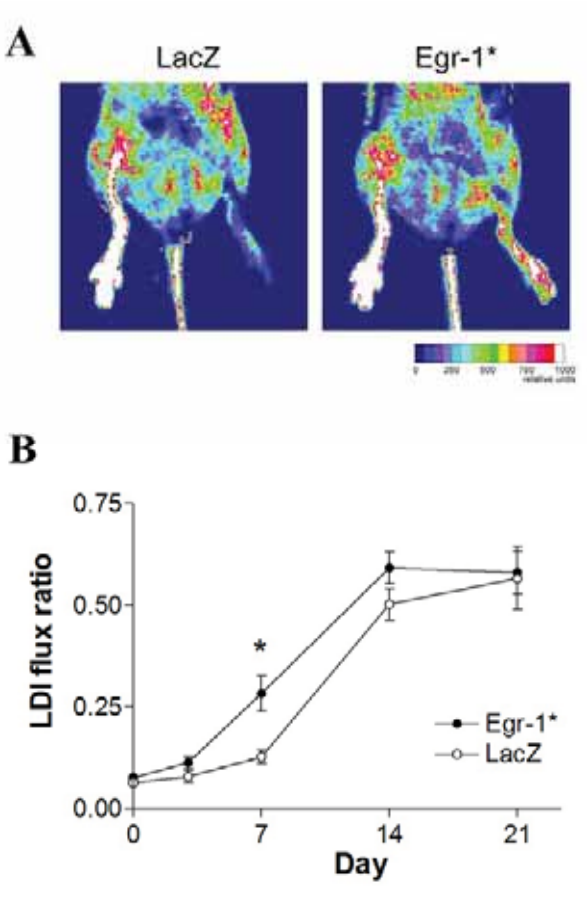




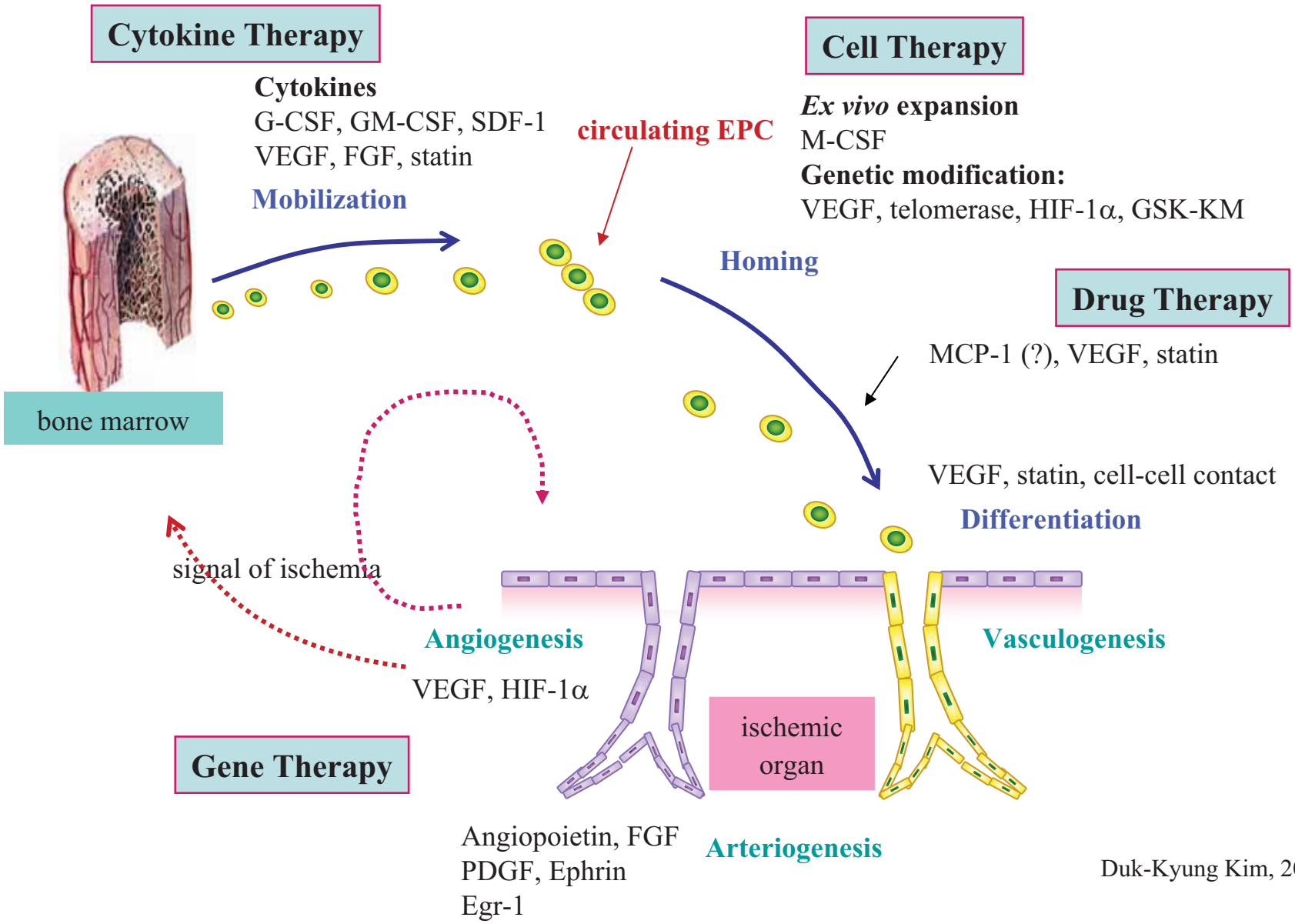
In vitro, ex vivo and in vivo evaluation of Ad-Egr-1* for angiogenic activity







Therapeutic Angiogenesis

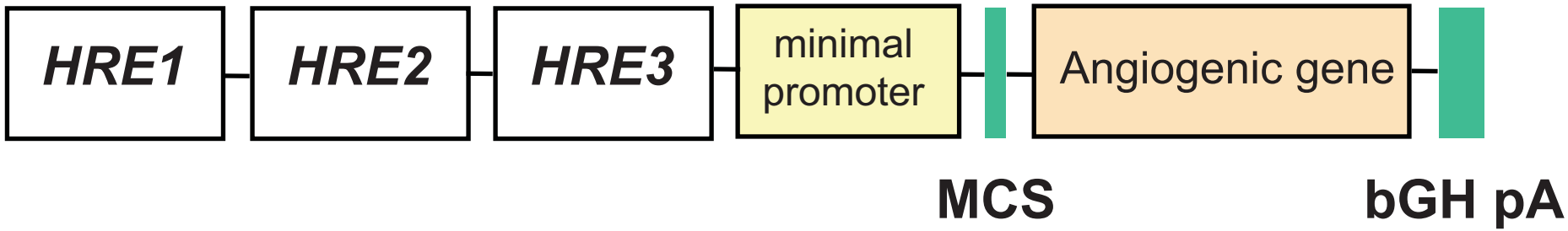


Duk-Kyung Kim, 2003

Contributors and collaborators

Dong-A Pharm Co.
ViroMed.
SNU Sunyoung Kim

Hypoxia-inducible vector



Hypoxia-responsive mechanisms

- HIF1α-dependent: HRE
- HIF1α-independent:
  - MTF-1: MRE
  - Egr-1: EBS

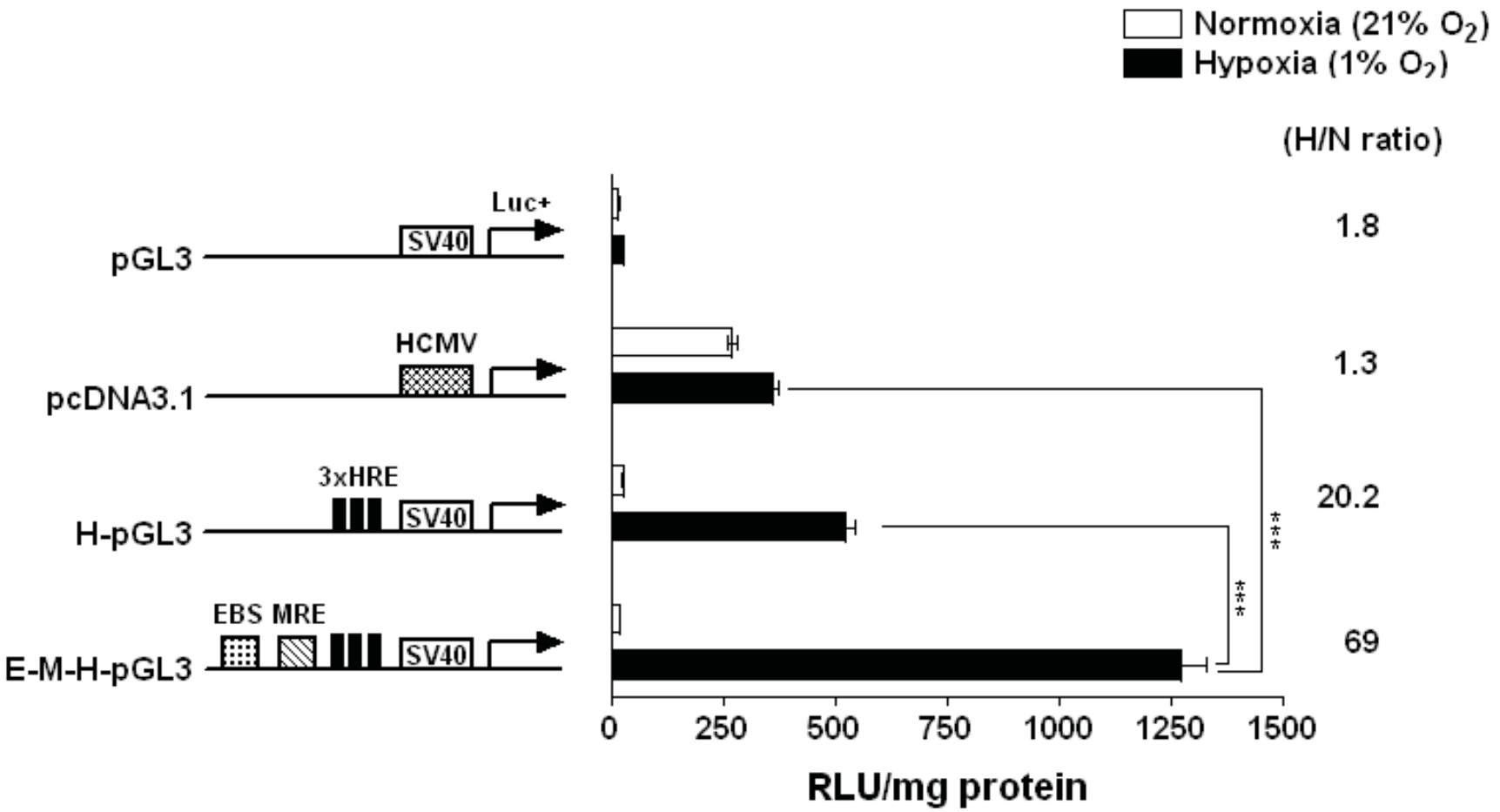
DNA motifs responsive to hypoxia

Table 1. Oligonucleotides used for generation of 3xHRE, MRE, and EBS.

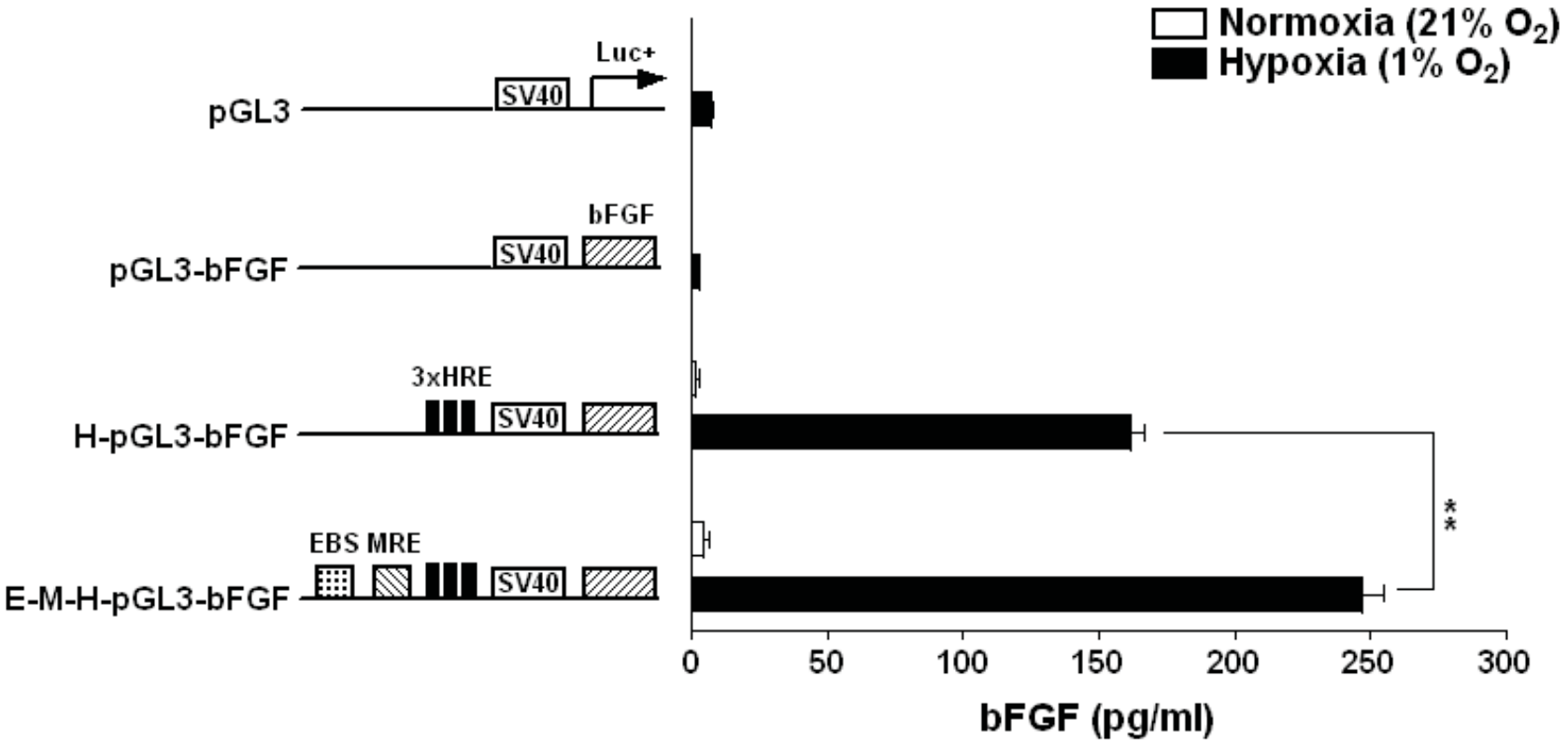
cis-acting element	Sequence of oligonucleotides
EBS	**5'- CTAGCCGCCCTCGCT –3'**
MRE	**5'- CTAGCAGGGAGCTCTGCACTCCGCCCGAAAAGT -3'**
3xHRE	**5'- CTAGCGTCGTGCAGGACGTGACATCTAGTGTCGTGCAGGACGTGACAT CTAGTGTCGTGCAGGACGTGACAT -3'**

The consensus binding sites for Egr-1, MTF-1, and HIF-1 are boxed. The functionally essential sequences of HRE are underlined. All oligonucleotides were designed with 5' *Nhe* I/ 3' *Xba* I sites on the ends such that each enhancer could be cloned into *Nhe* I sites to generate chimeric combinations. EBS; Egr-1 binding site from murine Egr-1 promoter, MRE; metal response element from mouse metallothionein-I promoter, 3xHRE; three tandem copies of hypoxia response element from murine phosphoglycerate kinase-1.

Effect of three-enhancer combination



Validation with angiogenic gene



Induction by hypoxia-mimetics