Therapeutic Angiogenesis for Peripheral Arterial Occlusive Disease

Duk-Kyung Kim, MD, PhD
Sungkyunkwan University School of Medicine
Samsung Medical Center, Cardiac & Vascular Center
Laboratory of Cardiovascular Molecular Therapy
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
Case #2; Foot Ulcer
Secondary endpoints: Pain (VAS) & ABI
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

Ang2
Destabilization

Ang1
Maturation

Growth
VEGF

Arterialization
Ephrin-B2

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 \textit{ex vivo} angiogenesis assay
A novel *ex vivo* angiogenesis assay
A novel *ex vivo* angiogenesis assay

![Graph A](image1.png)

![Graph B](image2.png)

![Graph C](image3.png)

![Graph D](image4.png)

![Graph E](image5.png)
A novel *ex vivo* angiogenesis assay
## Factors with potential for therapeutic vascular growth

<table>
<thead>
<tr>
<th>Growth Factors</th>
<th>Chemokines</th>
<th>Transcription factors</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF,-B,-C,-D,-E</td>
<td>MCP-1</td>
<td>HIF-1α, <strong>Egr-1</strong>, Prox-1</td>
<td>Del-1, Cyr61, PR39</td>
</tr>
<tr>
<td>FGF-1, -2, -4, -5</td>
<td></td>
<td></td>
<td>Tissue kallikrein</td>
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<tr>
<td>Ang 1, Ang 2</td>
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<td>Secreted frizzled-related protein</td>
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<tr>
<td>HGF, PDGF-BB</td>
<td></td>
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<td>eNOS, iNOS</td>
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<td>GM-CSF, neurotrophin</td>
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<tr>
<td>IGF-1, IGF-2</td>
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</tbody>
</table>

Seppo Yla-Herttuala & Kari Alitalo, 2003
Egr-1 (Early Growth Response Factor -1)

- 3 tandemly repeated Cys$_2$His$_2$ 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

- 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*

1. Ser/Thr-rich
2. P/S/T 533
3. Activation: strong
4. DNA binding: strong
5. Nuclear localization
6. Repression (by NAB1 or NAB2): weak

Egr-1*
- Mutation of Ile$_{293}$ in R1 domain → Phe
- NAB-insensitive Egr-1 (Constitutively active Egr-1)
Egr-1 / Egr-1* expression

\[ \downarrow \]

NAB expression \[ \uparrow \]

promoter

Target gene

Transcription

Egr-1

NAB

Egr-1*

NAB

promoter

Target gene

Transcription
Upregulation of multiple angiogenic genes
In vitro, ex vivo and in vivo evaluation of Ad-Egr-1* for angiogenic activity
Therapeutic Angiogenesis

Cytokine Therapy
- Cytokines: G-CSF, GM-CSF, SDF-1, VEGF, FGF, statin
- Mobilization
- Circulating EPC

Cell Therapy
- Ex vivo expansion: M-CSF
- Genetic modification: VEGF, telomerase, HIF-1α, GSK-KM

Drug Therapy
- MCP-1 (?), VEGF, statin
- VEGF, statin, cell-cell contact

Gene Therapy
- Angiogenesis: VEGF, HIF-1α
- Vasculogenesis
- Arteriogenesis: Angiopoietin, FGF, PDGF, Ephrin Egr-1

Mobilization
- Homing
- Signal of ischemia

Bone marrow

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.

<table>
<thead>
<tr>
<th>cis-acting element</th>
<th>Sequence of oligonucleotides</th>
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<tbody>
<tr>
<td>EBS</td>
<td>5'- CTAGCGCCTCGCT -3'</td>
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<tr>
<td>MRE</td>
<td>5'- CTAGCAGGGAGCTTGCACTCCGCCCGAAAAGT -3'</td>
</tr>
<tr>
<td>3xHRE</td>
<td>5'- CTAGCGTTCGTCAGGACGTGACATCTAGGTTCGTCAGGACGTGACAT -3'</td>
</tr>
</tbody>
</table>

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

- pGL3
- pGL3-bFGF
- H-pGL3-bFGF
- E-M-H-pGL3-bFGF

Legend:
- Normoxia (21% O₂)
- Hypoxia (1% O₂)

Graph shows bFGF (pg/ml) levels under different conditions.
Induction by hypoxia-mimetics