Society of Cardiovascular Pathology
USCAP Companion Meeting Symposium
“Atherosclerosis: New Insights on an Old and Future Scourge”
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“Vascular Endothelium and the Pathobiology of Atherosclerosis:
New Insights & Future Directions”

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Homeostasis in the cardiovascular system is maintained by a hierarchy of interacting genetic programs, the dysregulation of which contributes to the pathogenesis of complex disease processes, such as atherosclerosis and hypertension, and their complications--myocardial infarction and stroke.

Vascular endothelium, the continuous lining of the cardiovascular system, is a locus of “critical regulatory nodes” in this homeostatic network.
“While working on the problem of arteriosclerosis, I have realized not only how little I knew about *endothelium*, but also how much I ought to know for the proper understanding of arteriosclerosis.”

Altschul, 1954
Human Coronary Artery Atherosclerosis
ORIGINAL “RESPONSE TO INJURY” HYPOTHESIS

Central role of endothelial denudation

Ross & Glomset, 1976
Pathophysiologicaal Stimuli of “Endothelial Dysfunction”

- ENVIRONMENTAL AGENTS
- CYTOKINES
- BACTERIAL PRODUCTS
- VIRAL INFECTION/TRANSFORMATION
- ADVANCED GLYCOSYLATION ENDPRODUCTS
- OXIDIZED LIPOPROTEINS
- HOMOCYSTEINE
- HEMODYNAMIC FORCES

GIMBRONE, 1980
Non-random Distribution of Early Lesions of Atherosclerosis in Rabbit and Mouse Models

WHHL Rabbit

LDLR-/- Mouse

Cybulsky & Gimbrone
Cybulsky et al.
HYPOTHESIS

Hemodynamic forces, in particular wall shear stresses, can act as both positive and negative pathophysiological stimuli in atherogenesis.

These biomechanically mediated effects can impact endothelial functional phenotype via gene regulation.
In Vitro Model System

\[ \tau = \mu \frac{du}{dy} \]

- Cone and plate flow apparatus
- Confluent endothelial monolayer
  - small coverslips (12 x 1 cm\(^2\))
  - large plate (250 cm\(^2\))
- Shear stress (0.1 - 35 dyne/cm\(^2\))

Flow Influences Endothelial Structure

Cultured HUVEC

Flow direction

STATIC

LAMINAR SHEAR STRESS
(10 dyn/cm², 24 h)
### Endothelial Genes Modulated by Fluid Shear Stress

<table>
<thead>
<tr>
<th>tPA</th>
<th>c-fos</th>
<th>TGF-β</th>
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<tr>
<td>ICAM-1</td>
<td>c-myc</td>
<td>PDGF-A</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Egr-1</td>
<td>PDGF-B</td>
</tr>
<tr>
<td>ACE</td>
<td>NF-κB</td>
<td>bFGF</td>
</tr>
<tr>
<td>Hsp70</td>
<td>Smad 6</td>
<td>eNOS</td>
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<tr>
<td>Thrombomodulin</td>
<td>Smad 7</td>
<td>COX-2</td>
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<td>SOD</td>
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EXPERIMENTAL STRATEGIES

- Probe the complexity of *endothelial phenotypic modulation* by hemodynamic forces utilizing the power of high-throughput genomic analyses.

- More realistically model *“disturbed flow”* as a hemodynamic *“risk factor”* in atherogenesis.
BIOINFORMATICS TOOLS

ARGUS

Comander J, Weber GM, Gimbrone MA Jr, Garcia-Cardena G.
Argus – A New Database System for Web-based Analysis of Multiple Microarray Data Sets. 

Z-POOL

Comander J, Natarajan P, Gimbrone MA Jr, Garcia-Cardena G.
Improving the Statistical Detection of Regulated Genes from Microarray Data using Intensity-based Variance Estimation. 

www.vessels.bwh.harvard.edu
Dynamic Flow System
Prototypical Arterial Waveforms derived from Regions of the Human Carotid Artery Bifurcation that are Relatively Resistant or Susceptible to Atherosclerosis

“Athero-protective” Waveform

“Athero-prone” Waveform
Hierarchical Clustering of Biomechanically Regulated EC Genes

cytochrome P450, family 1, subfamily B, polypeptide 1 (CYP1B1)
MAD, mothers against decapentaplegic homolog 7 (MADH7)
prostaglandin-endoperoxide synthase 2 (PTGS2)
MAD, mothers against decapentaplegic homolog 6 (MADH6)
serine (or cysteine) proteinase inhibitor, clade E member 2 (SERPINE2)
alddehyde dehydrogenase 1 family, member A3 (ALDH1A3)
jagged 1 (Alagille syndrome) (JAG1)
hairy/enhancer-of-split related with YRPW motif 1 (HEY1)
solute carrier family 38, member 2 (SLC38A2)
inhibin, beta A (INHBA)
inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
protease, serine, 3 (mesotrypsin) (PRSS3)
protease, serine, 2 (trypsin 2) (PRSS2)
chromosome 8 open reading frame 1 (C8orf1)
KIAA1128 protein (KIAA1128)
sperm associated antigen 9 (SPAG9)
ephrin-B2 (EFNB2)
CDK4-binding protein p34SEI1 (SEI1)
cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1)
translocase of inner mitochondrial membrane 8 homolog A (TIMM8A)

interleukin 8 (IL8)
angiopoitin 2 (ANGPT2)
nuclear transport factor 2 (NUTF2)
dickkopf homolog 1 (Xenopus laevis) (DKK1)
metallothionein 1G (MT1G)
m metallothionein 1X (MT1X)
chemokine (C-X-C motif) receptor 4 (CXCR4)
pentaxin-related gene, rapidly induced by IL-1 (PTX3)
placental growth factor (PGF)
cysteine-rich, angiogenic inducer, 61 (CYR61)
connective tissue growth factor (CTGF)
thrombospondin 1 (THBS1)

decidual protein induced by progesterone (DEPP)
kinesin family member 20A (KIF20A)
ubiquitin-conjugating enzyme E2C (UBE2C)
kinesin family member 11 (KIF11)
transmembrane 4 superfamily member 2 (TM4SF2)
MAD2 mitotic arrest deficient-like 1 (MAD2L1)
topoiso merase (DNA) II alpha 170kDa (TOP2A)
T-LAK cell-originated protein kinase (TOPK)
hyaluronan-mediated motility receptor (RHAMM) (HMMR)
cyclin B1 (CCNB1)
lamin B1 (LMNB1)
kinesin family member 23 (KIF23)
transmembrane 4 superfamily member 2 (TM4SF2)
tumor necrosis factor (ligand) superfamily, member 10 (TNFSF10)
CDC20 cell division cycle 20 homolog (CDC20)
antigen identified by monoclonal antibody Ki-67 (MKI67)
serine/threonine kinase 12 (STK12)
cell division cycle 2, G1 to 5 and G2 to M (CDC2)
centromere protein F, 350/400kDa (mitosis) (CENPF)
kinesin family member 23 (KIF23)

ATP-binding cassette, sub-family G, member 2 (ABCG2)
gap junction protein, alpha 4, 37kDa (connexin 37)
guanylate cyclase 1, soluble, alpha 3 (GUCY1A3)

scale

-3 1 3
VCAM-1 Cell Surface Expression in HUVEC Preconditioned by Exposure to Athero-protective or Athero-prone Waveforms

no IL-1

IL-1 (1U/ml, 6 hrs.)
Hemodynamic Forces and Atherogenesis

“Athero-protective” biomechanical stimulation can *offset* the induction of pro-atherogenic genes by *systemic risk factors*, such as inflammatory cytokines, in human endothelial cells.

What are the upstream regulators of this transcriptional program?
KLF2 Is Differentially Upregulated by Atheroprotective Flow

Athero-protective Waveform

Athero-prone Waveform

Shear Stress (dyn/cm²)

KLF2 expression

Biomechanical stimulus
Kruppel-like Factor 2 (KLF2)

- Zinc Finger Transcription Factor

  Embryonic lethality
  Spontaneously Activated T Cell Phenotype


- Upregulated 45X by Arterial Abdominal Waveform in cultured human EC (Kratz et al (Garcia-Cardena) *unpublished*).

- Expressed in the endothelium of human arteries *in vivo* (Dekker et al, 2003)
KLF2 Overexpression induces ~1/3 of the “Athero-protective Genes” Associated with Biomechanical Stimulation

![Venn Diagram](image)

- **Athero-protective waveform**: 255 genes
- **Static**: 76 genes
- **Adenovirus-KLF2**: 662 genes
- **Adenovirus-GFP**: 76 genes
KLF2 Overexpression in HUVEC Suppresses VCAM-1 Gene Induction by Pro-inflammatory Cytokines

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>IL-1β</th>
<th>TNF–α</th>
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<tbody>
<tr>
<td>Adeno-KLF2</td>
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<td>+</td>
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<td>VCAM1</td>
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<td>exo-KLF2</td>
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KLF2 is Critical for Inhibition of Leukocyte Adhesion by Athero-protective Flow

4 h IL-1 (1 U/ml) EC activation  →  ↑ Leukocyte adhesion
+/- 24 h preconditioning with athero-protective flow
+/- KLF2 mRNA silencing
KLF2 mediates the Endothelial Resistance to Oxidative Stress Conferring by Athero-protective Flow

<table>
<thead>
<tr>
<th>Static (No Flow)</th>
<th>(-\text{H}_2\text{O}_2)</th>
<th>(+\text{H}_2\text{O}_2)</th>
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<tbody>
<tr>
<td>Ad-GFP</td>
<td>Ad-GFP</td>
<td>Ad-KLF2</td>
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<tr>
<td>ctrl siRNA</td>
<td>ctrl siRNA</td>
<td>KLF2 siRNA</td>
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<th>Athero-protective Flow</th>
<th>(-\text{H}_2\text{O}_2)</th>
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<td>KLF2 siRNA</td>
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Using a combination of biochemical and molecular biological approaches, we have found that KLF2 is necessary for the Statin-mediated regulation of several “atheroprotective” endothelial genes.

These observations thus point to a novel mechanism for the well recognized non-lipid lowering beneficial cardiovascular effects of Statins (HMG-CoA reductase inhibitors).

These findings have been independently validated; Sen-Banerjee (Jain) et al. Circulation 112:270 (2005).
Dual Regulation of Transcription Factor KLF2 in Endothelium

**Biomechanical Stimulation**

**Pharmacological Stimulation**
Atheroprotective Flow

↑ KLF2

Endothelial Cell Vasoprotective Phenotype

Statins
(Other drugs?)

Inflammation
- VCAM-1
- E-Selectin
- Multiple chemokines/Chemokine receptors
- Elafin
- IL-11

Vasomotor Reactivity
- eNOS
- CNP
- Adrenomedullin
- Endothelin

Thrombosis
- Thrombomodulin
- Tissue Factor
- PAI-1

(Other drugs?)
Expression of KLF2 in the Mouse Aorta

Garcia-Cardena (Unpublished)
Endothelial KLF2 is a “Critical Regulatory Node” in Vascular Homeostasis

- *Atheroprotective flow* activates KLF2 in endothelial cells *in vitro*.

- Endothelial KLF2 expression is localized to *athero-resistant geometries in vivo*.

- KLF2 orchestrates a multi-functional *transcriptional program* in vascular endothelium that is *vasoprotective*.

- Endothelial KLF2 is directly induced by the *Statins*, mimicking the flow-induced *vasoprotective endothelial phenotype*.

- The identification of endothelial KLF2 as a “critical regulatory node” in vascular homeostasis points the way to new *diagnostic, therapeutic and preventive strategies* in cardiovascular disease.
Identify *additional “critical regulatory nodes”* important in the maintenance of the “athero-protective” phenotype in vascular endothelium.


Critically test the pathophysiologica significance of biomechanically regulated “athero-protective” genes, *in vivo*, in murine (loss/gain of function) transgenic models of atherosclerosis.

Explore the influence of mutations/polymorphisms in “athero-protective” genes as risk factors in human atherosclerotic disease.

Identify small molecules that function as activators of endothelial “master-switches” in athero-protection, and test their therapeutic potential in human subjects.
“The true mystery of the universe is its comprehensibility”

- Albert Einstein
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<th>Gimbrone Lab</th>
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<tr>
<td>Belinda Yap</td>
<td>Kush Parmar</td>
<td>Myron Cybulsky</td>
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<td>Guohao Dai</td>
<td>Benjamin Larman</td>
<td>Tucker Collins †</td>
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<td>Brett Blackman</td>
<td>Vinod Nambudiri</td>
<td>Ramzi Cotran †</td>
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<td>Jason Comander</td>
<td>Eric Wang</td>
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<td>Yvonne Ou</td>
<td>Yuzhi Zhang</td>
<td>Peter Libby</td>
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<td>Ivy Ku</td>
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Bioengineering Department
MIT

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