Center for Vascular & Inflammatory Disease

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Center for Vascular and Inflammatory Diseases
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To integrate molecular and cell biology with applied and clinical sciences specifically in the areas of:

- biochemistry
- vascular biology
- immunology
- cancer biology
- hematopoiesis
- stem cell biology
- transplantation biology
## Faculty

<table>
<thead>
<tr>
<th>Tenure/Tenure track faculty</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Professors</td>
<td>8</td>
</tr>
<tr>
<td>Associate Professors</td>
<td>3</td>
</tr>
<tr>
<td>Assistant Professors</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>15</strong></td>
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<table>
<thead>
<tr>
<th>Non-tenure track faculty</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Assistant Professors</td>
<td>2</td>
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<tr>
<td>Research Associates</td>
<td>6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>8</strong></td>
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</tbody>
</table>
Departments

- Biochemistry
- Pathology
- Physiology
- Microbiology and Immunology
- Surgery
Funding

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Funding</th>
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</thead>
<tbody>
<tr>
<td>FY06</td>
<td>$2,000,000</td>
</tr>
<tr>
<td>FY07</td>
<td>$6,000,000</td>
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<tr>
<td>FY08</td>
<td>$4,000,000</td>
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<tr>
<td>FY09</td>
<td>$8,000,000</td>
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<td>FY10</td>
<td>$10,000,000</td>
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<tr>
<td>FY11</td>
<td>$8,000,000</td>
</tr>
<tr>
<td>FY12</td>
<td>$6,000,000</td>
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Comments on the value of basic research and relationship to pharmaceutical research
Value of NIH funding on economic growth. NIH funding has ...

- Boosted economic growth and contributes to growth of bioscience clusters
- NIH funding is complementary with private dollars
- For every NIH $1.00 in basic research, $8.38 is invested in private R&D
- For every $1.00 of NIH funding - $2.21 of output from biotech industry (companies respond to new information)

Source: Milken Institute
Real NIH award funding

Source: NIH Milken Institute
R&D spending by PhRMA member companies

US$ billions

* Estimated.

Source: Pharmaceutical Research and Manufacturers of America (PhRMA).

Source: Milken Institute
## Top funded states, 2011

<table>
<thead>
<tr>
<th>Rank</th>
<th>State</th>
<th>Real funding (US$ millions)</th>
<th>Real output in Bioscience industry (US$ millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>California</td>
<td>3,152</td>
<td>28,328</td>
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<tr>
<td>2</td>
<td>Massachusetts</td>
<td>2,223</td>
<td>11,550</td>
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<tr>
<td>3</td>
<td>New York</td>
<td>1,785</td>
<td>12,582</td>
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<tr>
<td>4</td>
<td>Maryland</td>
<td>1,486</td>
<td>4,149</td>
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<td>5</td>
<td>Pennsylvania</td>
<td>1,266</td>
<td>10,296</td>
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<td>6</td>
<td>Texas</td>
<td>947</td>
<td>6,844</td>
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<td>7</td>
<td>North Carolina</td>
<td>934</td>
<td>10,988</td>
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Source: Milken Institute
Role of lipoprotein receptors in protecting the vasculature
CVD – leading cause of death in USA, Europe and some of Asia

Deaths in USA (in thousands)

Source: American Heart Association
Mummies from 4 populations

- Ancient Egyptians
- Ancient Peruvians
- Ancestral Puebloans (Southwest America)
- Unangan hunters (Aleutian Islands – Alaska)

*Figure 2: Frequency of atherosclerosis by age group*
Deaths due to CVD (USA 1900-2008)

CVD (ICD-10 I00-I99) does not include congenital. Prior to 1933, data are for a death registration area and not the entire US. Source: NCHS
The problem

- 60,000 mile network of vessels in a closed environment

for comparison - 46,876 miles of Interstate highways in USA

- Adult: 4.7 L blood
- Child: 2 L of blood
Strickland DK, Gonias S, Argraves WS 2002 Trends in End & Metab
LDL receptor-related protein 1 (LRP1)

Required for development

Binds more than 30 structurally unrelated ligands

Mediates several intracellular signaling kinases by NPxY

Undergoes constitutive endocytosis

LRP1 is abundantly expressed in SMC and is atheroprotective

Hypothesis: smLRP1 plays a prominent role in vascular development and integrity
Anatomy of arterial vessels

Elastic fibrils

- major component
- elasticity
- resilience
- does not turnover
- produced by SMC

Collagen

- produced by SMC
- provides strength
- turnover
- Increased deposition with age – leads to hypertension
Genetic models to investigate smLRP1

LRP1 flox/flox  x  sm22Cre^tg

sm22Cre+ = smooth muscle cell LRP1 deficient mouse

‘smLRP1 –/–’
Effective genetic deletion of LRP1 in SMC

LRP1+/+  smLRP1-/-

LRP1 -  

Aortic extracts

tubulin -

α-SMA  LRP1  merged

Aortic SMC

Effective genetic deletion of LRP1 in SMC
What happens when the *Lrp1* gene is deleted in vascular smooth muscle cells?
Three distinct phenotypes are noted

- Loss of elastic fiber integrity
- Dilatation of aorta

*Marfan’s syndrome* – defects in EL fiber formation - aortic aneurysm
I - Loss of elastic fiber integrity

LRP1+/+  smLRP1-/

Elastic Van Gieson staining, 40x

marked fragmentation and disarray of elastic fibers
Echocardiography measurements

Aortic root diameter (mm)

Aortic cross sectional area (mm²)

LRP1+/+ smLRP1-/-

LRP1+/+ smLRP1-/-

*
How does LRP1 protect against this Marfans syndrome-like phenotype
Global proteomic analysis of ECM

remove adventitia

differential extraction
Didangelos et al. 2011

Quantitative differential proteomic analysis of WT and KO enriched ECM
Global proteomic analysis of ECM

~ 800 proteins identified

- Unchanged: 61%
- Upregulated: 19%
- Downregulated: 19%
Elastic laminae degradation and aortic dilatation: deregulation of proteases
HtrA1 is upregulated in smLRP1-/- vessels

![Graph showing upregulation of HtrA1 in aortic extracts of LRP1+/+ and smLRP1-/- mice.](image)
High temperature requirement factor A1 (HtrA1)

- Secreted trypsin family of serine protease
- Involved in degradation of ECM molecules – fibronectin, aggrecan, collagen II, tropoelastin
- Impairs elastogenesis by degrading fibulin 5
HtrA1 is an LRP1 ligand

\[ K_D = 70 \text{ nM} \]
Increased inflammation in smLRP1-/- vessel wall

- Proteolytic products of ECM are pro-inflammatory
- Macrophages source of MMPs
Increased MMPs in smLRP1-/- vessel wall

LRP1+/+  smLRP1-/-

MMP9 -
proMMP2 -
active MMP2 -
Increased MMPs in smLRP1-/- vessel wall

**MMP9 protein**

- LRP1+/+ vs. smLRP1-/-

**MMP9 mRNA**

- LRP1+/+ vs. smLRP1-/-

**MMP2 protein**

- LRP1+/+ vs. smLRP1-/-

**Active MMP2 protein**

- LRP1+/+ vs. smLRP1-/-

**MMP2 mRNA**

- LRP1+/+ vs. smLRP1-/-

* indicates statistical significance.
LRP1 protects the EL by regulates protease levels

- HtrA1
- MMP9
- MMP2
- MT1-MMP
Three distinct phenotypes are noted

- Loss of elastic fiber integrity
- Dilatation of aorta
- Age related thickening of the media due to increased collagen deposition
Increased collagen deposition in smLRP1-/- vessel wall

Masson’s Trichrome Staining, 40x
III – Age-dependent increase in collagen deposition in media

Age (months)
Aortic media thickness (μm)

0 5 10 15
0
20
40
60
80
100
*
* * smLRP1-/-
LRP1+/+

*
CTGF is upregulated in smLRP1-/- vessels

CTGF protein

CTGF mRNA

α-CTGF, 40x
Connective tissue growth factor

- LRP1 ligand
- CCN family of ECM associated, heparin-binding proteins
- Key mediator of fibrosis and matrix deposition
- cell adhesion, migration, proliferation, tissue wound repair,
Proteomic analysis reveals upregulation of ECM proteins.
In the vessel wall LRP1:

- Regulates protease levels - deregulation of proteases in smLRP1-/- lead to EL degradation of HtrA1, MMP2, MMP9,MT1-MMP

- Regulates matrix deposition by regulating levels of CTGF – excess matrix deposition occurs in smLRP1-/- vessels

- Protects against recruitment of macrophages into the vessel wall
Working model

LRP1+/+

CTGF
HtrA1

Lower levels of CTGF, HtrA1

Normal elastogenesis

LRP1+/+

smLRP1−/−

CTGF
HtrA1

Elevated levels of CTGF, HtrA1

Dysregulated elastogenesis

Collagen deposition

Matrix degradation

Elastic lamina disruption

Increased protease activity

Macrophage infiltration