Upstream Therapy for Atrial Fibrillation

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CA Heart Rhythm Symposium
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Syllabus

• What does upstream mean?
• Evidence for role of fibrosis in AF
• Biology of fibrosis
• EP Effects of fibrosis
• Mechanism of fibrosis in AF
• Atrial selectivity of fibrosis
• Potential therapies

Disclosure

Grant
Zoll

Grant/Honorarium
Gilead

Common AF: An Acquired Disease

Genetic Influences
Environmental Influences
Aging
Other Diseases (HTN, CHF, OSA, obesity)

REMODELING

Electrical
Structural
Neuro-humoral

Trigger
Substrate

ATRIAL FIBRILLATION
AF: Current Treatment

Genetic Influences --> Environmental Influences --> Aging

Other Diseases (HTN, CHF, OSA, obesity) --> REMODELING

Electrical --> Structural --> Neuro-humoral

Ablation --> Trigger --> Substrate

AA Drugs

Atrial Fibrillation

What is Does Upstream Mean?

Genetic Influences --> Environmental Influences --> Aging

Other Diseases (HTN, CHF, OSA, obesity) --> UPSTREAM

Electrical --> Structural --> Neuro-humoral

Aging

Atrial Fibrillation

ACEI/ARB/ALDIO Use to Prevent AF

Khatib, et al. *Int J Cardiol* 2012

ACEI/ARB Use to Prevent AF

Khatib, et al. *Int J Cardiol* 2012
Statin Use to Prevent AF


Fish Oil: Afib Recurrence after CV

Kowey, et al. JAMA 2010
Kumar, et al. Heart Rhythm 2012

Up a Creek Without a Paddle: Anti-arrhythmic Drugs

Exploiting Gaps in the Market

Kumar, et al. Heart Rhythm 2012
Fibrosis as Final Common Pathway

- Senescence
- Mitral valve disease
- HTN
- Heart Failure
- Lone AF

Atrial Fibrillation and Fibrosis

- Sirius Red Collagen IH

Atrial Fibrillation and Fibrosis: LA

Collagen Content (collagen/GAPDH)

<table>
<thead>
<tr>
<th></th>
<th>Lone AF SR</th>
<th>Lone AF Parox</th>
<th>Lone AF Chronic AF</th>
<th>MVD SR</th>
<th>MVD Parox</th>
<th>MVD Chronic AF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>0.0 ± 0.1</td>
<td>0.0 ± 0.1</td>
<td>0.0 ± 0.1</td>
<td>0.0 ± 0.1</td>
<td>0.0 ± 0.1</td>
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</table>

Fibrosis in LA: Lone AF

Heart Failure Induced Atrial Remodeling

Li, et al. Circulation 1999

TGF-β1 Transgenic Mouse


AF Inducibility


TGFβ1 Mice: Cellular Electrophysiology

Cardiac Cell Subpopulations

- **Myocyte**
  - Lymphocyte
  - Endothelial Cell
  - Fibroblast

Fibroblast Transformation

- Fibroblast exist as small, round cells that have low level of collagen production
- When stimulated they begin to express large quantities of actin and become factories for collagen and cytokines
  - Stimulated by stretch, cytokines, injury, stress

Cardiac Fibroblasts

- Make up about 50-60% of cell types in the heart
  - Higher in the atria
- Responsible for maintaining the extracellular matrix in the heart
  - Collagen turnover—about 5% per day
  - Integrates contractile force across cardiomyocytes
  - ECM conserves 3-dimensional cyto-architecture for efficient conduction and contraction
- Can transform to collagen & cytokine producing “factories” called myofibroblasts
- Can actively and passively exert electrical effects

Active and Passive Effects of MFb

**PROPAGATION**
- Secrete Collagen—insulator
- Larger size and coupling
  - Couple to CMYocyte via Connexin
  - Passive conductor across MFb
  - Electrotonic interaction with CMYocyte

**IMPULSE GENERATION**
- Collagen improves source—sink for automaticity (like sinus node)
- MFb ion channel expression and coupling to CMYocyte
  - Spontaneous depolarization and automaticity

What does fibrosis do?
- Slows conduction (regional)
- Fibrillatory conduction/wavebreak
- Substrate for rotors
- Facilitates automaticity

Isotropic Conduction

Non-Uniform Anisotropy
Conduction Heterogeneity

Normal  AF Substrate

Verhuele, et al  *AJP* 2004

Effects of MFb

Propagation  Automaticity

Rohr, *Heart Rhythm* 2009

Mechanism of Atrial Fibrosis

- What is the mechanism of atrial fibrosis?
- Does preventing fibrosis prevent atrial fibrillation?

adapted from Burstein & Nattel, *JACC* 2008
Canine Heart Failure: TGFβ

Atrial TGFβ1 Expression


Human Atria: TGFβ1 Levels

Human atria: TGFβ1 levels were measured in human atria. The graph shows the relative protein levels of TGFβ1 in different groups.

AF C C AF C AF C AF C C C C C

Relative Protein Level

Effect of Pirfenidone

Conduction AF Vulnerability

Why is the atria uniquely susceptible to fibrosis?

Effect of Pirf on AF Substrate

TGFβ1 Mice: Selective Atrial Fibrosis

Recovery from Heart Failure
Differential Fibrosis: Canine HF


TGFβ1 Tx Mice: Differential Gene Expression

Log2 (Tx/A vs WtA)

Significant difference TxA vs WtA

Significant difference TxV vs WtV

Log2 (TxA/WtA)

Atria (Wt)

Atria (Tx)

Ventricle (Wt)

Ventricle (Tx)

MEEBO Array

TGFβ Signaling

[Modified from Derynk]

Atrial Specific

[Modified from Derynk]
Rescue of TGFβ Overexpression

- Is this relevant to human atrial fibrillation?

TGFβ1 Content: Human Hearts

Chamber Differences in Fb

- Failing or Non-Failing Human Hearts
- Fb Culture
- TGFβ
- Cell isolation
- qRT-PCR
- IHC
- Collagen
- LV
- Atria
- Morphology
Isolated Human Fibroblasts: Response to TGFβ1

Collagen I mRNA

Non-Failing Hearts

Failing Hearts

Conclusion

- Atrial fibrosis is an important substrate for AF
- TGFβ1 plays a central role in animal models and human AF
- The atria are uniquely susceptible to TGFβ-induced AF, especially in failing hearts
- Targeting fibrosis may be important target for prevention and treatment of AF—a new anti-arrhythmic approach

Swimming Upstream
Thank You

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Croft Thomas
Jacob Vogan

CTDN

Effect of Increasing Fibrosis
Simulated Tissue Propagation

Jacquemel & Henriquez  Heart Rhythm  2009

Rotor Driving AF
**ACE Inhibition in AF: CHF**

Li, et al. *Circulation* 2001

**Isolated Human Fibroblasts: Response to TGFβ1**

LOX mRNA

<table>
<thead>
<tr>
<th>Non-Failing Hearts</th>
<th>Failing Hearts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrial Fb</td>
<td>ctrl</td>
</tr>
<tr>
<td>Ventricle Fb</td>
<td>ctrl</td>
</tr>
</tbody>
</table>

Fold Change

| ctrl | tgfβ1 |
| ctrl | tgfβ1 |

**Human Atria: Activated TGFβ1**

PAI1-Luc Activity in MLEC Assay

**Up-a-creek**

Even with a paddle, being up shit creek is kind of a drag.
ACEI for AF Prevention: Meta Analysis

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment n/N</th>
<th>Control n/N</th>
<th>RR (95% CI random)</th>
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<tbody>
<tr>
<td>1. ACEI inhibitor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Re🌟</td>
<td>2/7</td>
<td>7/11</td>
<td></td>
</tr>
<tr>
<td>SOLVD</td>
<td>101/196</td>
<td>49/105</td>
<td></td>
</tr>
<tr>
<td>TRACE</td>
<td>22/700</td>
<td>42/767</td>
<td></td>
</tr>
<tr>
<td>Lis off</td>
<td>15/00</td>
<td>32/75</td>
<td></td>
</tr>
<tr>
<td>CAPRA</td>
<td>117/1492</td>
<td>135/1463</td>
<td></td>
</tr>
<tr>
<td>STOP-H2</td>
<td>200/2056</td>
<td>977/4409</td>
<td></td>
</tr>
<tr>
<td>GISSI</td>
<td>695/886</td>
<td>721/846</td>
<td></td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>1034/12415</td>
<td>1338/15009</td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity chi-square = 22.26 df = 6 P = 0.00001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect z = -2.53 P = 0.01</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

| 2. ARB | | | |
| Medtrix | 9/79 | 29/75 | |
| CHARM | 170/2780 | 105/1490 | |
| LIFE | 179/4117 | 250/1387 | |
| Subtotal (95% CI) | 402/474 | 663/411 | |
| Test for heterogeneity chi-square = 5.26 df = 3 P = 0.15 | | | |
| Test for overall effect z = -4.12 P = 0.00004 | | | |

Total (95% CI) | 1517/27909 | 2002/29020 | |
| Test for heterogeneity chi-square = 45.30 df = 10 P < 0.00001 | | | |
| Test for overall effect z = -3.74 P = 0.00002 | | | |

TGFβ Tx Mice: TGFβ Signaling

- TGFβRI
- TGFβRII
- Smad-2/3
- pSmad-2
- β-actin

TGFβ Tx Mice: Effect of TGFR Kinase Inhibition

- IL-6
- ET-1
- PAI-1

AP-1 Regulated Genes

- CRP
- RANTES
- ICAM-1
**TGFβ Tx Mice:**
Effect of TGFR Kinase Inhibition

- Collagen mRNA levels (% WtA)
- WtA, TxA, TxA+Ki26894, WtV, TxV, TxV+Ki26894

**Sinus Node: Trichrome Stain**

- Fibrosis required to
  - Minimize electrotonic interaction for impulse generation
  - Overcome source-sink mismatches to allow SN to drive atria

**Sanchez-Quintana, et al. Heart 2004**

**Ovine HTN Model**
Atrial Fibrosis

- Genetic Influences
- Environmental Influences
- Aging
- Other Diseases (HTN, CHF, OSA, obesity)

- Atrial Collagen Content
- AF Inducibility

- Kistler et al. Eur Heart J. 2006
Effect of TGFβ1 on Fibroblasts

TGFβ1 ➔ Collagen
Fibroblasts ➔ Myofibroblasts

Effect of TGFβ1 on Fibroblasts

Pirfenidone
TGFβ1 ➔ Collagen
Fibroblasts ➔ Myofibroblasts

Effects of TGFβ1
- Major activator of fibroblasts to produce collagen
- Organ and vascular development
- Bone turnover
- Wound healing
- Cancer?

Cardiac Effects of TGFβ1
- Hypertrophy
- Fibrosis
- Apoptosis
Varied TGFβ1 Effects

- Different receptor subtype combinations
- Extracellular modifiers of TGFβ activity and receptor binding
- “Co-factors”
- Different intracellular signaling
- Coordination with other cytokines and transcription factors

![TGFβ1 Activation Diagram](image)

**TGFβ1 Activation**

Latency Associated Peptide → TGF-β

- Small Latent Complex
- LAP-dimer
- TGF dimer
- Large Latent Complex
- Extracellular Matrix
- LTBP-1

![Activation Isochronal Maps](image)

**Activation Isochronal Maps**

- LA
- LV
- Wt
- Tx
- Effective Refractory Period
- AF Inducibility (%)

**Statistical Graphs**

- Graph A
  - AF Inducibility (%)
  - P < 0.001
- Graph B
  - Effective Refractory Period
- Graph C
  - Representative Optically-Derived Action Potentials
- Graph D
  - Activation Isochronal Maps
  - Activation
  - MMP Integron αβ
  - LTBP-1
**Tx Mice: cys\textsuperscript{33}ser TGFβ1**

- Latency Associated Peptide
- TGF-β
- LAP-dimer
- LTBP-1
- Extracellular Matrix
- Constitutively Active TGFβ1

**CHF and Atrial Fibrosis**

- Conduction Velocity (m/s)
- HF Contr
- Non-uniform Anisotropy