How to Apply OMICs Discoveries: A Novel Phenotypic Approach for Fibrotic Diseases

Prof Selim Cellek, MD, PhD, FBPhS
Faculty of Health, Education, Medicine and Social Care
Medical Technology Research Centre
Anglia Ruskin University, UK
selim.cellek@anglia.ac.uk

SMSNA, Nashville, USA
24th October 2019
Disclosure

I have no actual or potential conflict of interest in relation to this program/presentation
Fibrosis: an unmet need

- Fibrosis is the formation of excess fibrous connective tissue in an organ
- Underlying pathology in several chronic and progressive diseases
- Idiopathic pulmonary fibrosis, hepatic fibrosis (cirrhosis), kidney fibrosis, heart fibrosis, scleroderma, Dupuytren’s contracture, Peyronie’s disease
- **Accounts for 42% of all deaths in the world.**
- Pathophysiology is complex and multi-factorial
- **No medical treatment** with significant efficacy and safety is available
- Only approved drugs are for pulmonary fibrosis (*pirfenidone* and *nintedanib*) with limited efficacy and safety.
Targets in pulmonary fibrosis

Datta et al., Br J Pharmacol, 163 (1), 141-172, 2011
Single target approach

In vitro expression of the target

Phenotypic screening

In vivo

In vitro

Measure changes in function or biomarker

Lead identification

Lead optimisation

Candidate selection

O’Connor & Roth (2005) Nature Reviews Drug Discovery
Phenotypic vs single-target based drug discovery

Swinney & Anthony, 2011
Our approach: Inhibition of myofibroblast transformation

1. Patient recruitment and consent
2. Surgical excision of disease relevant tissue
3. Human tissue
4. Human primary fibroblasts
5. Cell growth and differentiation to myofibroblast in high throughput format
6. High throughput phenotypic assay

- Graph showing normalized fluorescence intensity with Z' = 0.89
Fibroblasts isolated from human tunica albuginea

**In Cell Western method**

Control + TGF-β1

**Immunofluorescence**

Control + TGF-β1

α-SMA staining

Medium/High throughput screening

<table>
<thead>
<tr>
<th>Compound</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sildenafil</td>
<td>Sigma-Aldrich, UK</td>
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<tr>
<td>Quercetin</td>
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<td>Apigenin</td>
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<td>Suramin sodium salt</td>
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<td>Tranilast</td>
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<td>Pirfenidone</td>
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<tr>
<td>5’ COOH Pirfenidone</td>
<td>Santa Cruz Biotechnology, USA</td>
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<td>Interferon α 2B human</td>
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<tr>
<td>4-Aminobenzoic acid potassium salt (PABA)</td>
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<td>L-Carnitine hydrochloride</td>
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<td>Pentoxifylline</td>
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<td>Tamoxifen citrate salt</td>
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<tr>
<td>α-Tocopherol</td>
<td>Sigma-Aldrich, UK</td>
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<tr>
<td>Thrombin</td>
<td>Sigma-Aldrich, UK</td>
</tr>
<tr>
<td>Vardenafil hydrochloride</td>
<td>Sigma-Aldrich, UK</td>
</tr>
</tbody>
</table>

Ilg et al, Eur Urol 2019
Synergy between PDE5i and SERM

Ilg et al, Eur Urol 2019
Vardenafil and tamoxifen synergise in inhibiting myofibroblast contraction

Ilg et al, Eur Urol 2019
Vardenafil and tamoxifen inhibit collagen and fibronectin production

Ilg et al, Eur Urol 2019
Peyronie’s disease animal model

- 40 male Sprague-Dawley rats (10-12 weeks old) randomly divided into 5 groups:
  - Intratunical TGF-β1 injection
  - Vehicle injection
  - TGF-β1 injection + vardenafil treatment (drinking water 1.5mg/kg/day)
  - TGF-β1 injection + tamoxifen treatment (intraperitoneal injection 5mg/kg/day)
  - TGF-β1 injection + vardenafil and tamoxifen treatment
- Drug treatment started the day after TGF-β1 injection and rats were sacrificed after 5 weeks after a 48 hours wash off period

Ilg et al, Eur Urol 2019
Vardenafil and tamoxifen prevent erectile dysfunction in Peyronie’s disease animal model

Ilg et al., Eur Urol 2019
Vardenafil and tamoxifen prevent upregulation of fibrotic marker mRNAs

Ilg et al, Eur Urol 2019
Vardenafil and tamoxifen prevent upregulation of fibrotic marker proteins

- WB showed a 50% reduction in ASMA content in the TGI group
- Not in the treatment groups
- Synergy in restoration observed in the combined group
- ASMA/Col I ratio significantly increased in the vardenafil and combined groups
- ASMA/Col III, and Col I/Col III ratios only significantly improved in the combined group

Ilg et al, Eur Urol 2019
Vardenafil and tamoxifen prevent fibrosis

Ilg et al, Eur Urol 2019
Screening of FDA-approved drugs

- 1,954 FDA approved drugs (Selleck Drug library) were screened at 10 μM concentration
- Drugs inhibiting myofibroblast transformation (>80%) and not affecting cell viability (<20%) were selected

_Cellek group, unpublished observations_
Screening of FDA-approved drugs

Total=41

Cellek group, unpublished observations
Conclusions

• Discovery of a synergistic action between PDE5i and SERMs in models of Peyronie’s disease
• Discovery of other approved drugs which may be repurposed for Peyronie’s disease
Future work

• Validating the 41 hits in vitro and in vivo
• Clinical trial: men with early stage PD PDE5i+SERM combination
• Same approach on burn scar tissue and lung tissue
Acknowledgements

• Anglia Ruskin University
  • William Stebbeds
  • Marta Mateus
  • Marcus Ilg

• University College London Hospitals
  • David Ralph
  • Asif Muneer
  • Nim Christopher
  • … all andrology research fellows
  • …and all patients

• KU Leuven
  • Maarten Albersen
  • Uros Milenkovic

• Funding
  • ESSM (RG14-01 and RG16-03)
Thank you
Back up slides
Wound healing: a product of evolution

- The pristine ability to heal by regeneration was lost during evolution.
- Repairs by inflammation and subsequent deposition of matrix protein (scar) evolved as the method of mammalian healing.
Wound healing vs fibrosis

Stone et al, Cell & Tissue Research, 2016
Fibrosis can affect any organ

Liver

Normal
Cirrhosis

Kidney

H&E
PAS
MT

Lung

NORMAL LUNG
IPF LUNG

Heart

A
B
How Is Pulmonary Fibrosis Treated?
There is no cure for pulmonary fibrosis. Current treatments are aimed at slowing the course of the disease, relieving symptoms and helping you stay active and healthy.

For more information, visit: Lung.org/pf
Why is phenotypic screening more successful than single target approach?

*Reason #1: It can predict unknown pathways better*

Marc K. Hellerstein; Exploiting Complexity and the Robustness of Network Architecture for Drug Discovery (2008; Journal of Pharmacology and Experimental Therapeutics)
Why is phenotypic screening more successful than single target approach?

**Reason #2: It is more translatable to human physiology**

Lars Olbe, Enar Carlsson & Per Lindberg
Nature Reviews Drug Discovery 2, 132-139 (February 2003)
Vimentin

Desmin

No TGF

Plus TGF

No TGF

Plus TGF

42kD

37kD

ASMA

Immunocytochemistry

PCR

42kD

37kD

Z' = 0.89

Well number

Ratios/Cells

Negative Control

Cells exposed to 10 ng/ml TGF-β1

Ratios/Cells/fields

Negative Control

Cells exposed to 10 ng/ml TGF-β1

Cells

Cells
Drug development strategy

- FDA-approved drugs library (n=900)
- Commercial/proprietary compound libraries

Primary screen using in-house ICW

- In vitro and in vivo validation
- Lead optimisation
- Preclinical development
- Clinical development

Indication switch

_Cellek lab, UNPUBLISHED_
Tamoxifen inhibits myofibroblast contraction
Vardenafil inhibits myofibroblast contraction