Renal Fibrosis: Mechanisms and novel therapeutic strategies

Jill Norman
Department of Renal Medicine
University College London

E-mail: j.norman@ucl.ac.uk

Chronic Kidney Disease

- Renal fibrosis hallmark of Chronic Kidney Disease (CKD)
- Diverse causes: Diabetes, hypertension, hyperlipidemia, obesity, chronic inflammation, chronic infection, kidney stones, kidney cysts, immune disorders, genetic disorders (ADPKD), age; recurrent Acute Kidney Injury
- Progressive damage and reduced function over time

Stages of Chronic Kidney Disease

Stage	Description	GFR (mL/min/1.73 m^2)	
1	Kidney damage with normal or ↑ GFR	≥90	
2	Kidney damage with mild ↓ GFR	60–89	
3	Moderate ↓ GFR	30–59	3a: 45-59 3b: 30-44
4	Severe ↓ GFR	15–29	
5	Kidney failure	<15	*RRT: Dialysis/Transplantation*

End-stage Renal Disease

Chronic kidney disease is defined as either kidney damage or GFR <60 mL/min/1.73 m^2 for ≥3 months. Kidney damage is defined as pathologic abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies.

Chronic Kidney Disease

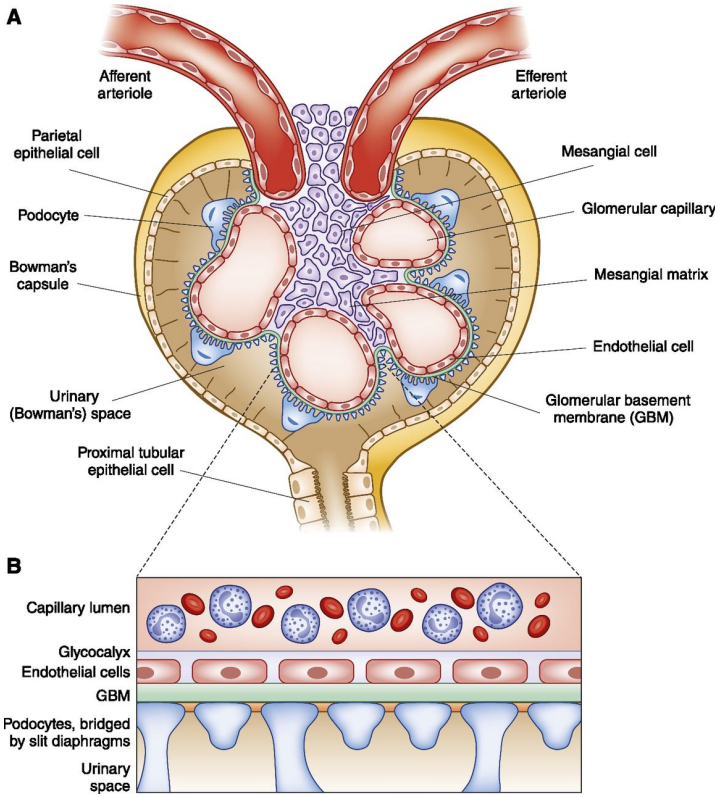
- WHO classified CKD as a major non-communicable disease, “silent epidemic”
- Affects >850 million people worldwide, one of the most common causes of death worldwide
- Globally >3.4 million people are kept alive by RRT; only half those who need it receive treatment
- Number of patients on RRT predicted to continue to increase (aging population, hypertension and diabetes)
- In the UK estimated 3 million people are at risk of moderate/severe CKD
- Economic cost: £1.45 billion a year in England
- Patient cost: reduced QoL, premature death (patients on RRT have 2.4-19x higher mortality than age-matched population)
- Those with CKD are up to 20 times more likely to die of other causes (largely cardiovascular diseases) before reaching ESRD

Challenges:

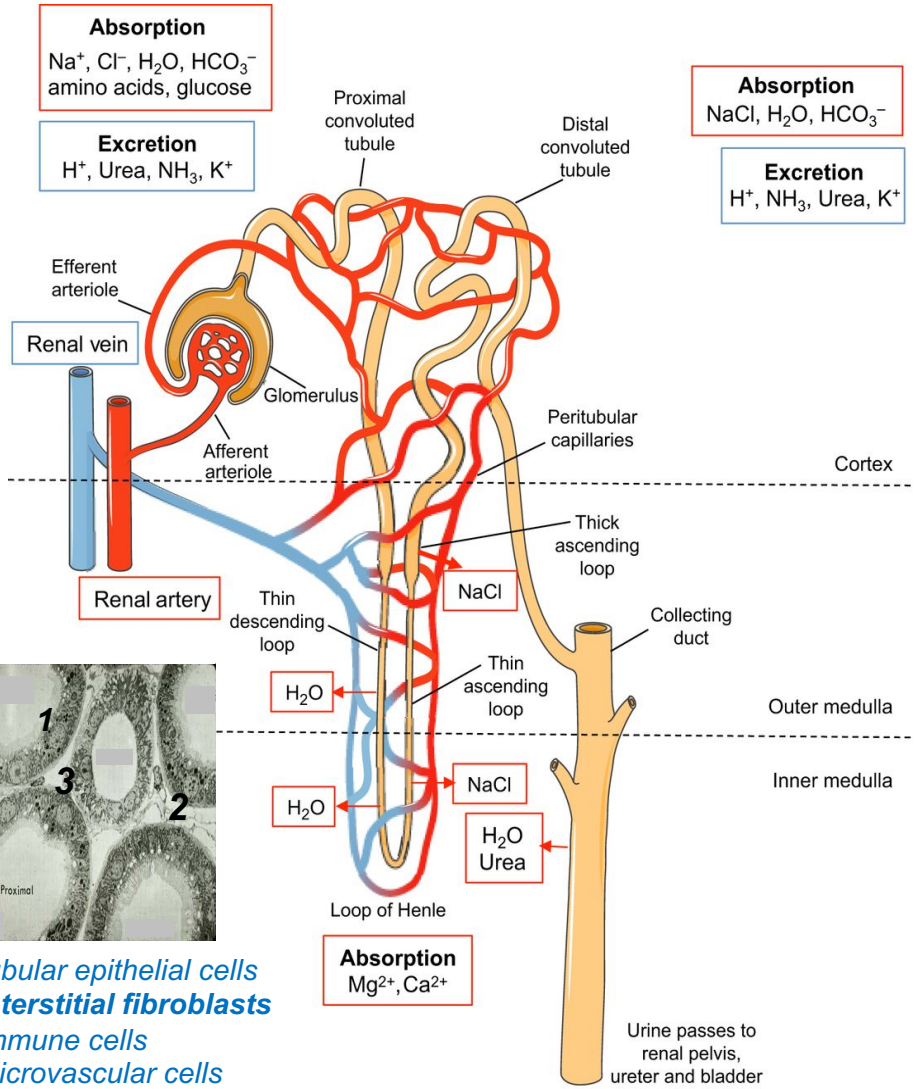
- Currently limited treatment options – novel therapies
- Predict who will develop CKD
- Predict rate of progression

Functional unit of the kidney: nephron

Glomerulus



Tubule



1. *Tubular epithelial cells*
2. ***Interstitial fibroblasts***
3. *Immune cells*
4. *Microvascular cells*

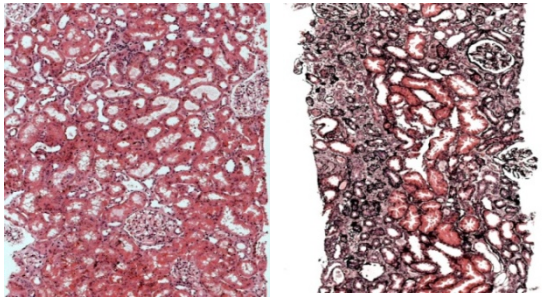
Tubulointerstititum

- Complex tissue ~26 different cell types
- Additional heterogeneity?

Chronic Kidney Disease and fibrosis

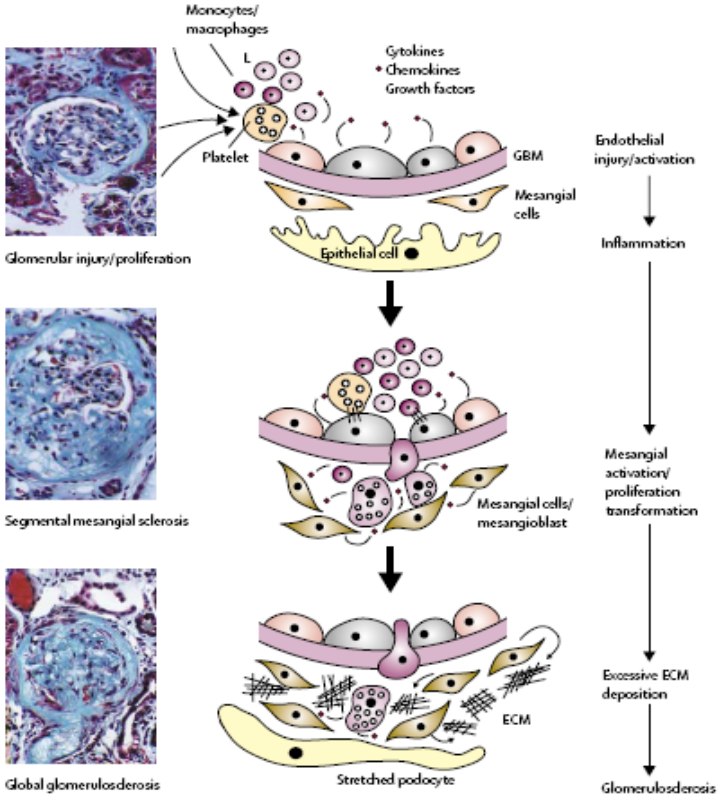
- **Multiple aetiologies**
- **Common pathology**

Normal *Chronic damage*

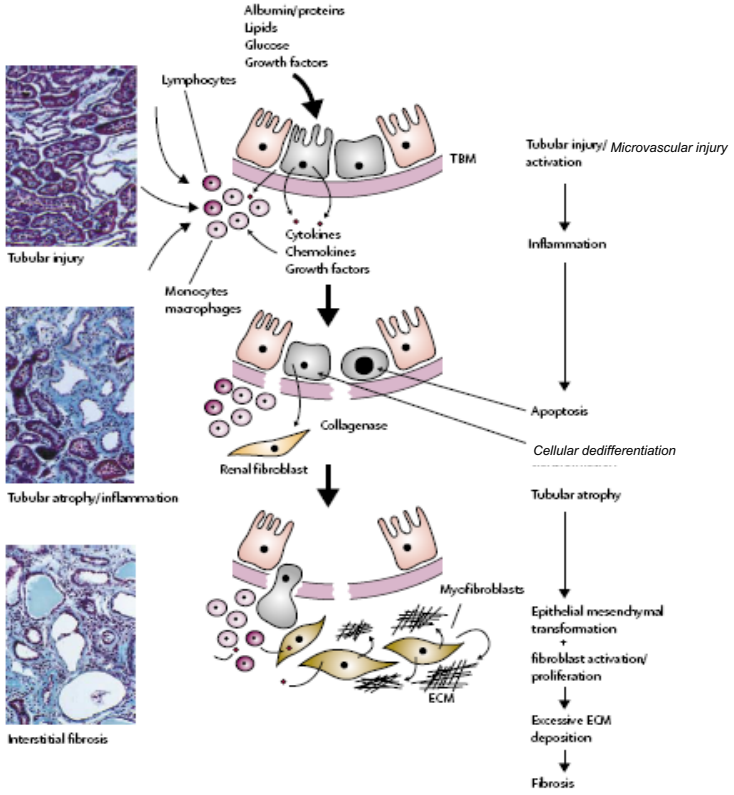


Courtesy of Prof. A Howie.
Dept Cell Path, Royal Free Hospital

Glomerulosclerosis



Tubulointerstitial fibrosis

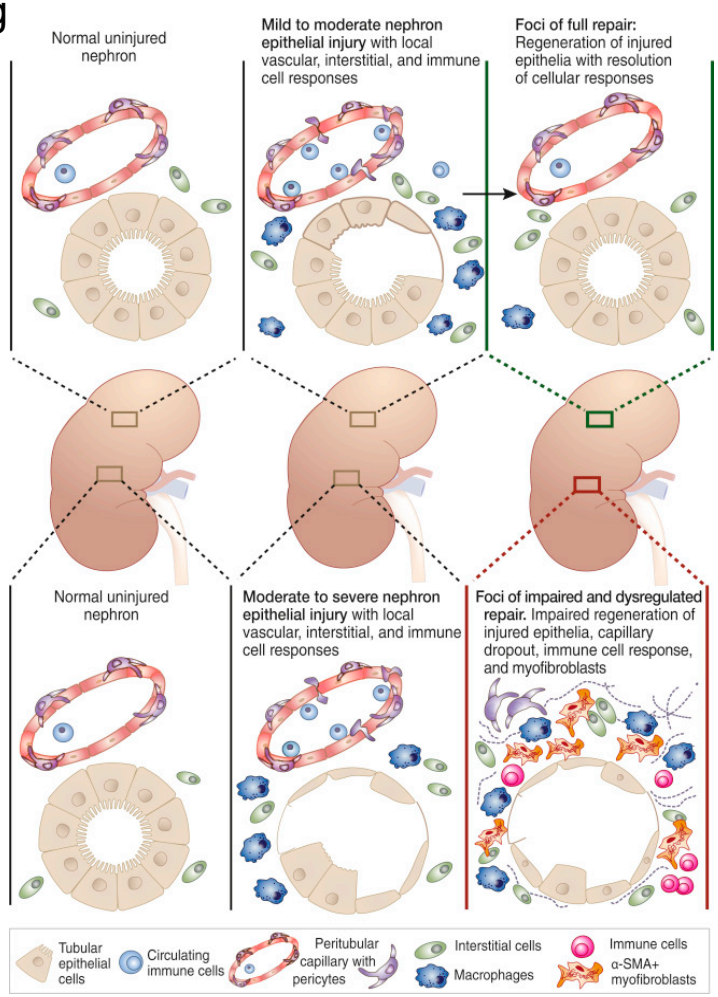


- **Tubulointerstitial fibrosis best predicts progression to ESRD.**

El Nahas AM, Bello AK.
Lancet 365:331-345, 2005.

Chronic Kidney Disease and fibrosis

- Fibrosis = pathological extension of normal wound healing
- Fibrosis characterised by:
  - Cellular injury/damage
  - Inflammation - persistent, non-resolving
  - Altered expression of growth factors and cytokines
    - * Increased pro-fibrotic cytokines (TGFβ)
    - * Reduced anti-fibrotic factors
  - Increased interstitial cell number
  - Appearance of myofibroblasts (αSMA^+)
  - Tubular atrophy and loss
  - Microvascular injury and loss
    - * Decreased O_2 and nutrients; hypoxia -> fibrosis
  - Accumulation of ECM
    - * Increased production
    - * Reduced turnover (MMPs/TIMPs; PAs/PAIs)
    - * Altered composition (EDA fibronectin, foetal proteins); altered cell/matrix interactions
    - * Post-translational modification (cross-linking collagen by TG-2)

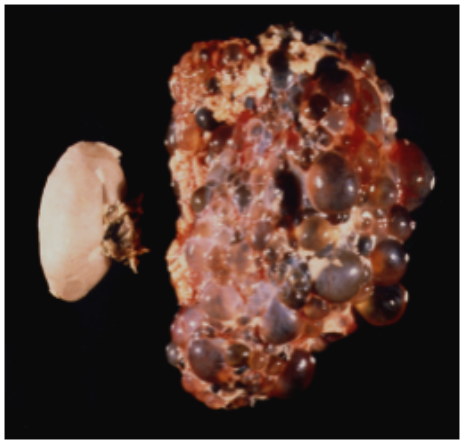


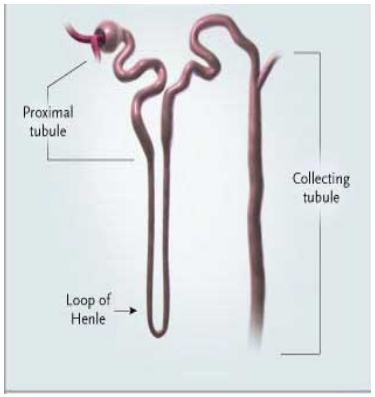
Kumar S. Kidney Int 93:27-40,2018

Autosomal Dominant Polycystic Kidney Disease

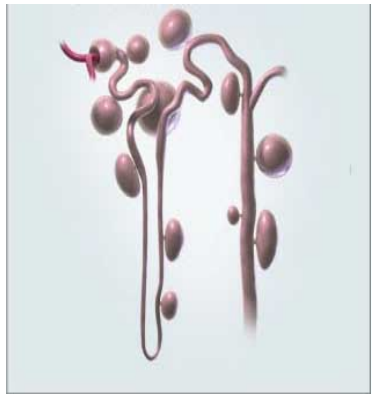
- Most common monogenic kidney disease; ~1:800 live births
- Mutations in PKD1 (PC-1) (85%) or PKD2 (PC-2) (15%)
- Affects ~12 million individuals worldwide
- Affects both genders, all racial, geographic and ethnic groups
- 50% of patients develop ESRD and require RRT (7-10% of dialysis population)
  - Wide variation in age of onset of ESRD (1-8th decade)
- Characterised by extreme bilateral kidney enlargement
  Cysts arise from all segments of the nephron, cyst expansion accompanied by interstitial fibrosis

Normal ***ADPKD***

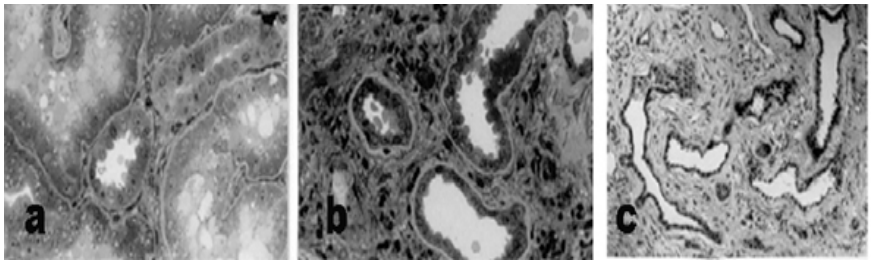




Normal



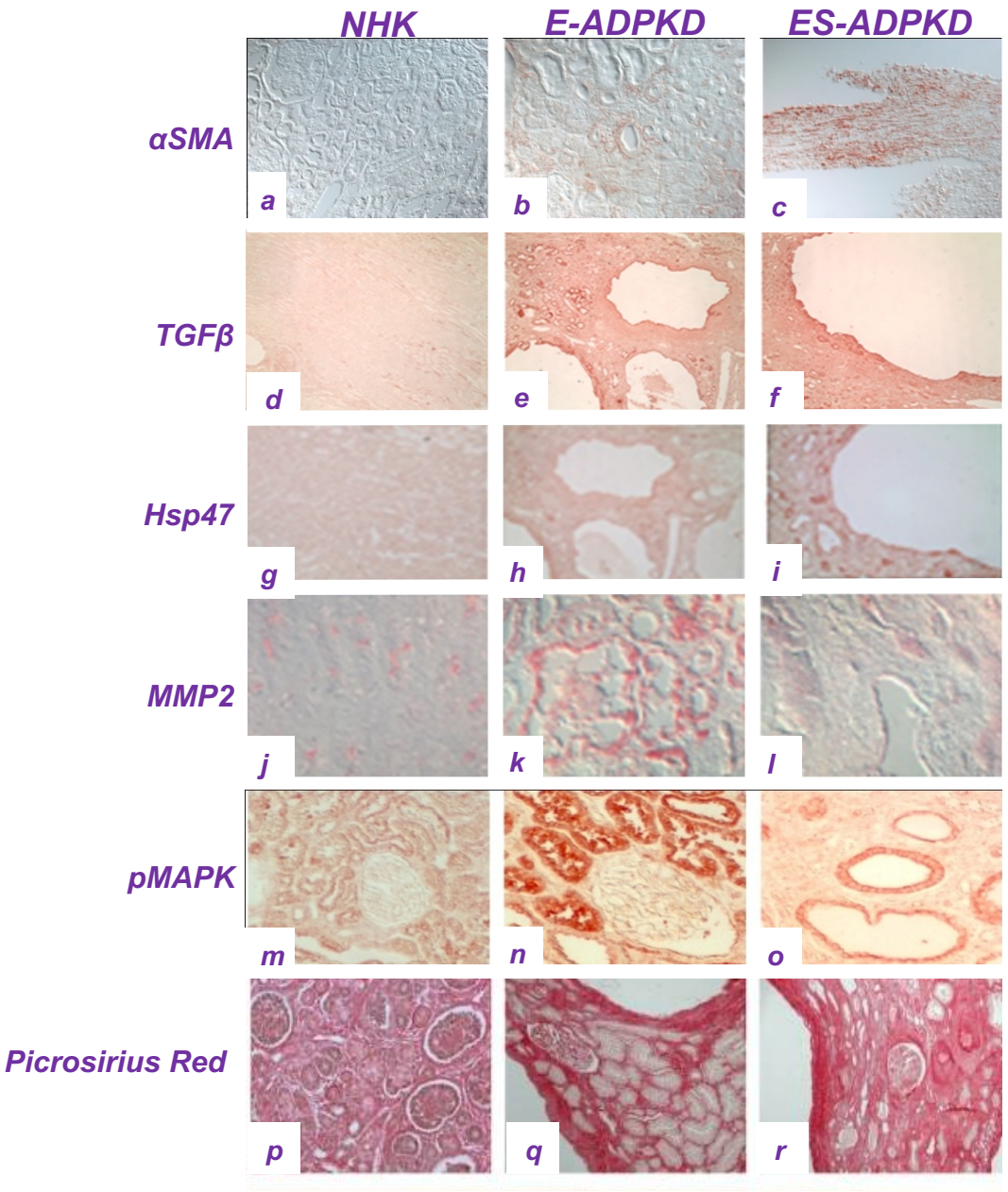
ADPKD



Normal ***Early ADPKD*** ***End-Stage ADPKD***

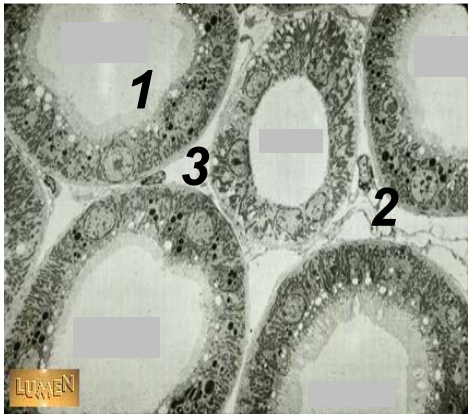
Wilson P NEJM 2004

Pro-fibrotic markers in human ADPKD



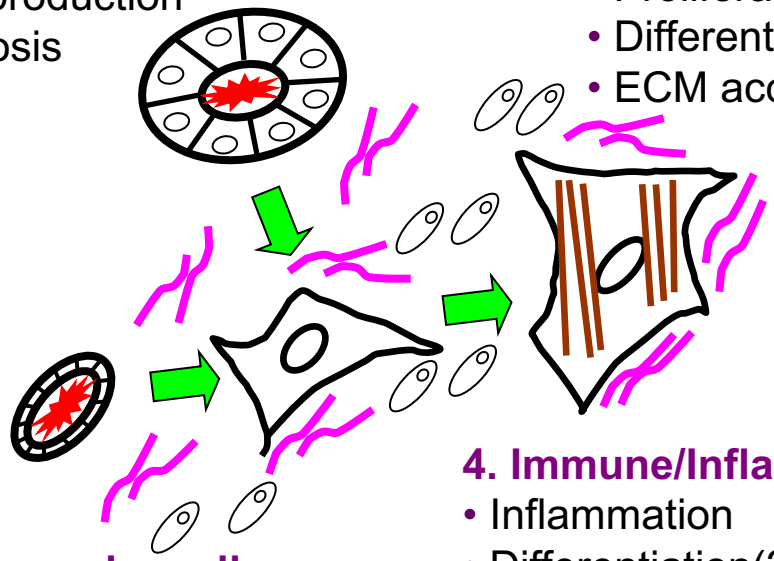
E-ADPKD: Early, pre-dialysis
ES-ADPKD: End-stage

Multiple cell types activated in fibrosis



1. Tubular cells
- Dedifferentiation
- Proliferation
- ECM production
- Apoptosis
- EMT?

2. Interstitial fibroblasts
- Proliferation
- Differentiation (αSMA)
- ECM accumulation



3. Microvascular cells

Endothelial cells
- Migration
- ECM production
- Apoptosis
- EndoMT?

Pericytes
- Differentiation
- ECM accumulation

4. Immune/Inflammatory cells
- Inflammation
- Differentiation(?)

5. Progenitor cells

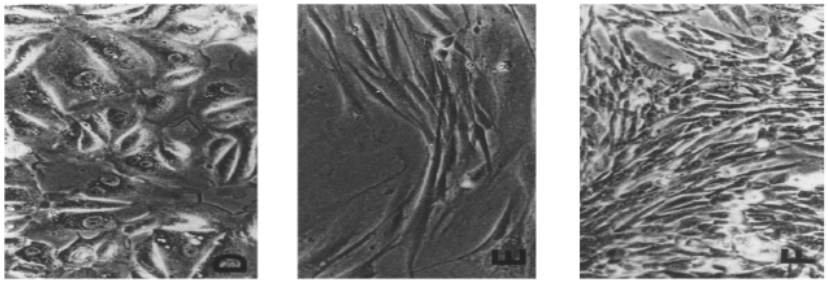
Resident

Circulating

ADPKD vs normal fibroblasts *in vitro*

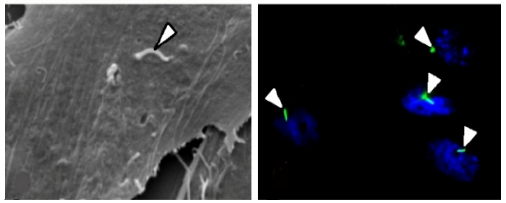
Compared normal fibroblasts and ADPKD (PKD1 mutant) fibroblasts:

- *Altered phenotype*



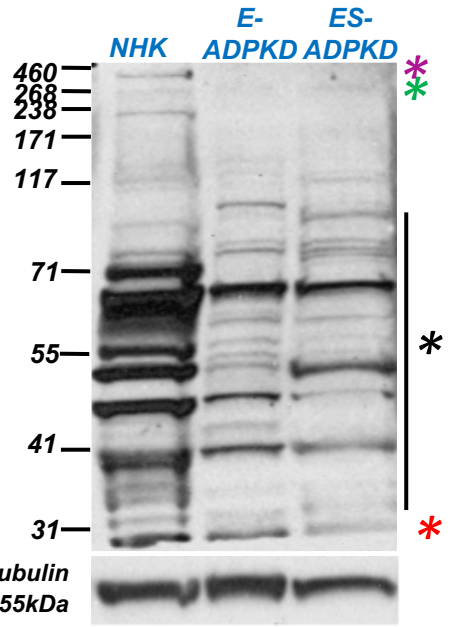
NHK E-ADPKD ES-ADPKD

- *Shortened cilia*



Group	Cilia length
NHK	5.8±1.5
E-ADPKD	4.3±0.6
ES-ADPKD	3.8±0.3*

- *Altered PC-1*



PC-1 protein in ADPKD fibroblasts:

* *Full-length PC-1 (~460kD) undetectable*

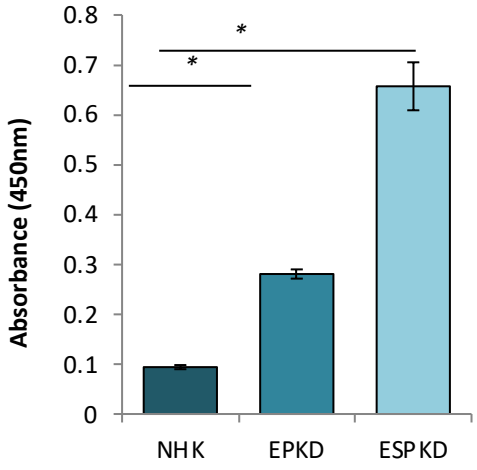
* *Reduced ~250kD fragment*

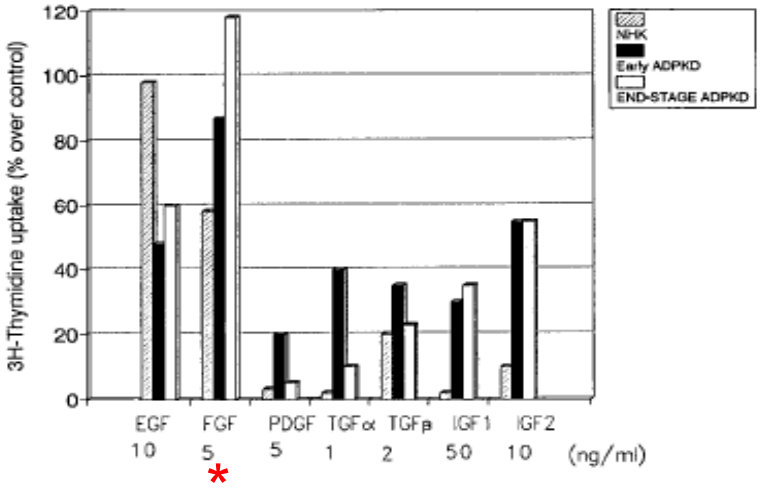
* *Decreased expression of C-terminal ~30kD fragment*

* *Other fragments (~30-100kD) generally decreased with disease stage*

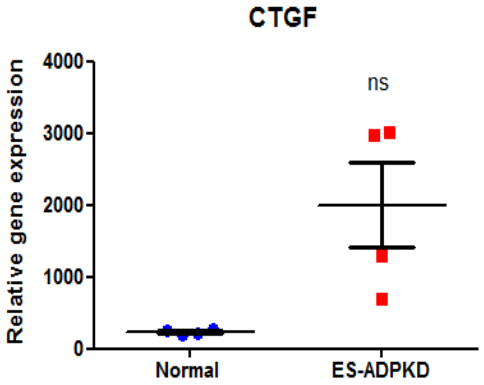
ADPKD vs normal fibroblasts *in vitro*

- Increased proliferation (basal) and differential response to growth factors





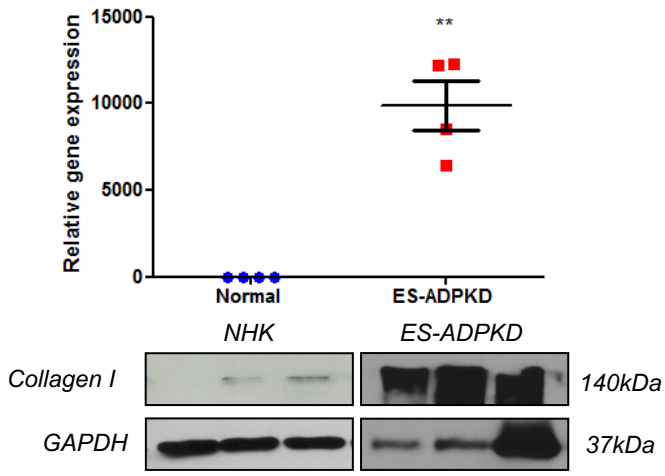
- Increased production of growth factors



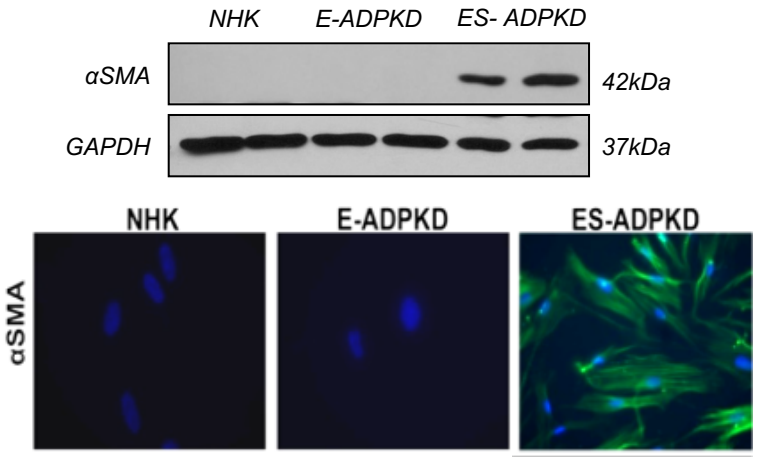
- Increased GF production: CTGF, FGF, TGFβ

ADPKD vs normal fibroblasts *in vitro*

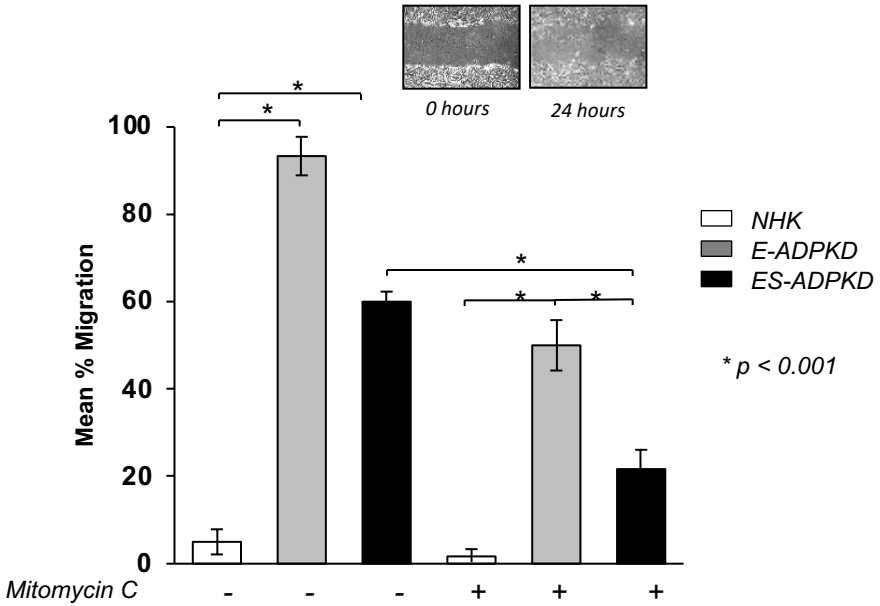
- Increased collagen expression



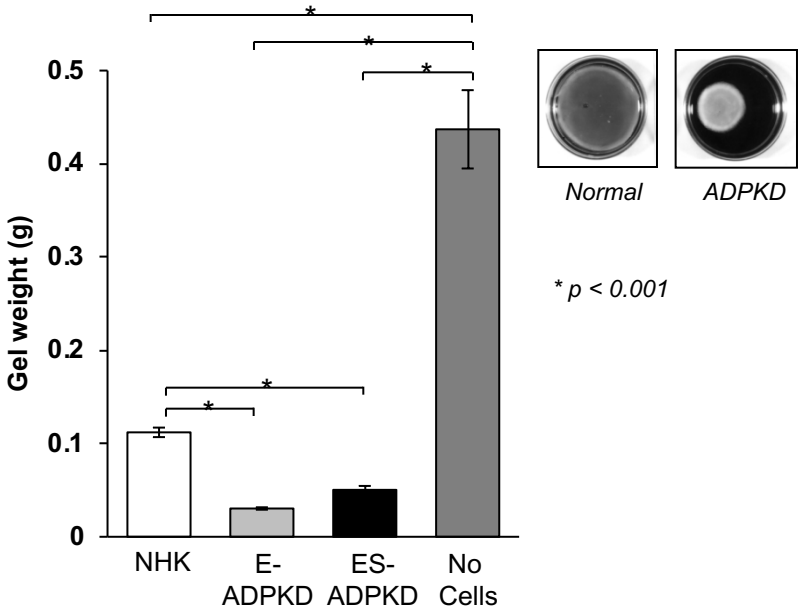
- Enhanced myofibroblast differentiation



- Increased migration

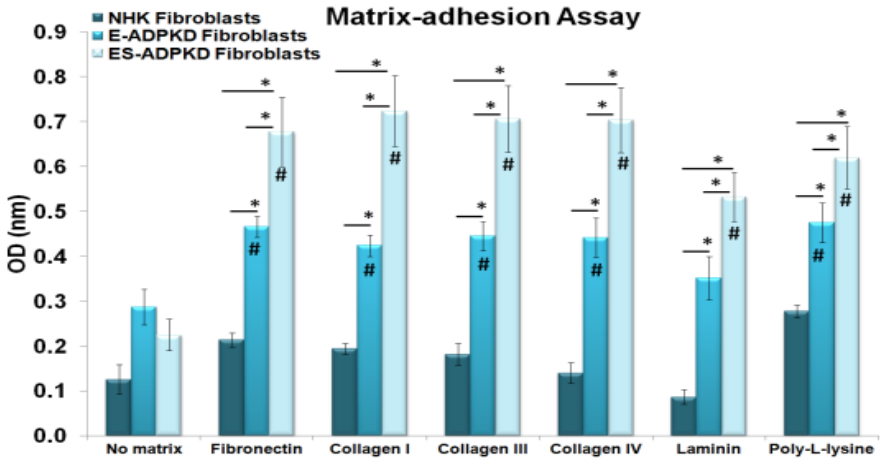


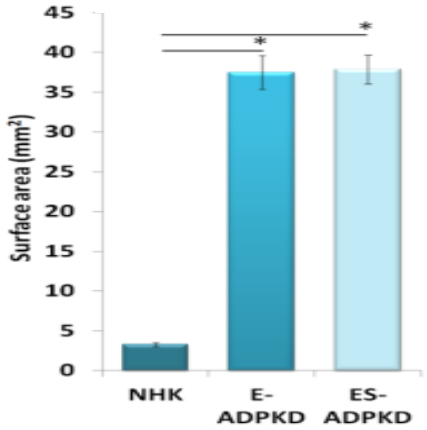
- Increased collagen gel contraction



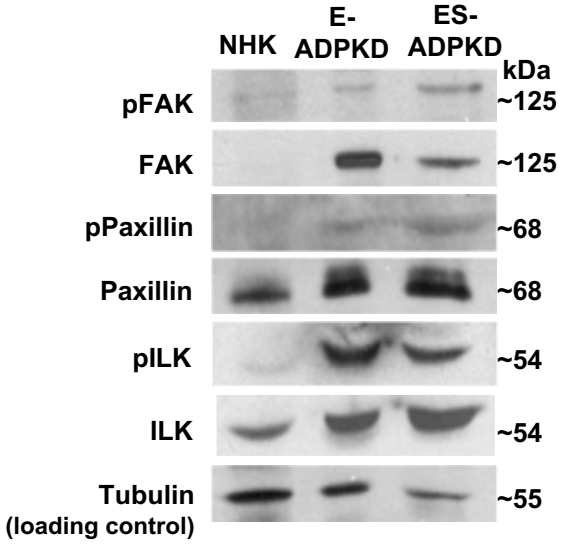
ADPKD vs normal fibroblasts *in vitro*

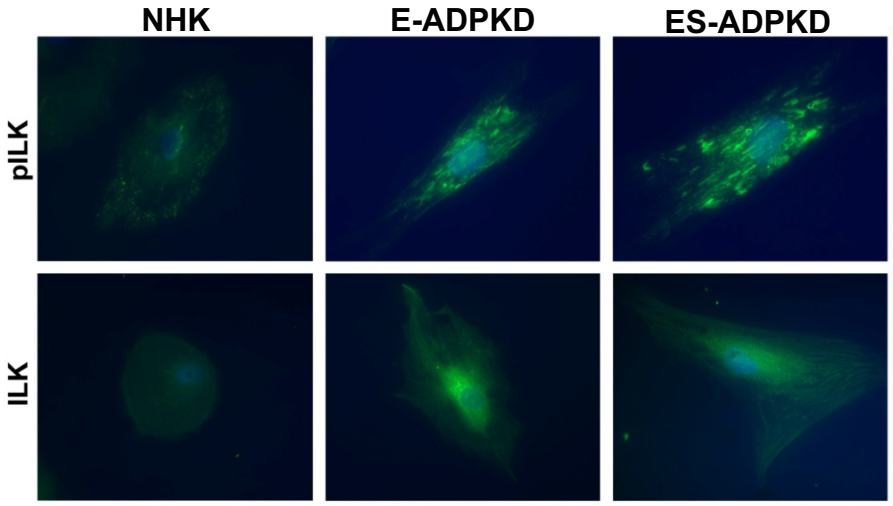
- Increased adhesion and spreading





- Changes in focal adhesions



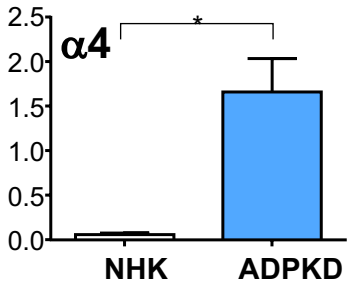


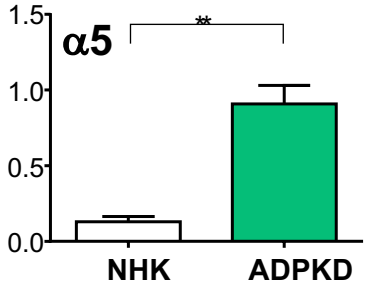
ADPKD vs normal fibroblasts *in vitro*

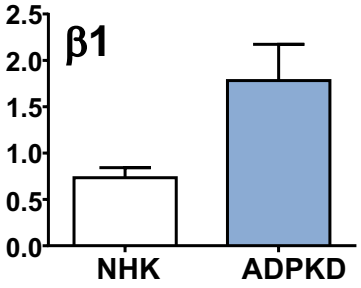
Dysregulated expression of ECM receptors

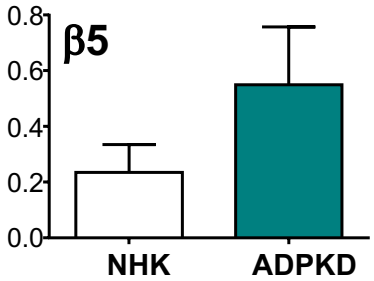
Integrins

Up-regulated

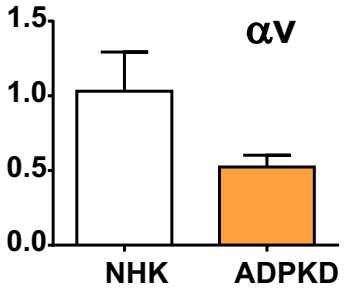


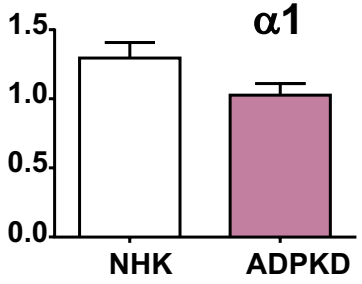


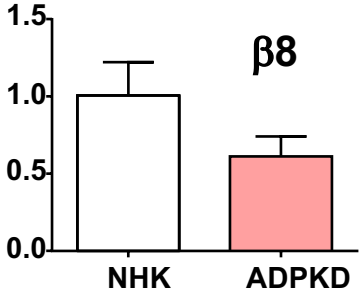




Down-regulated

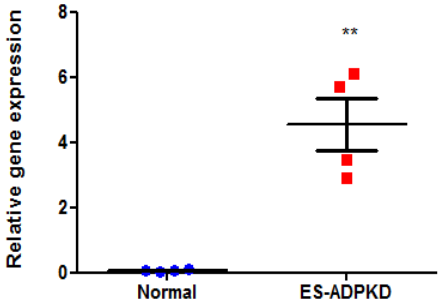




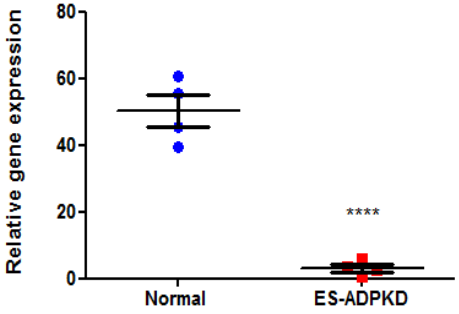


Heterodimers:
αvβ3, αvβ8

Discoidin domain receptor 2



Tetraspanins



Down-regulated:

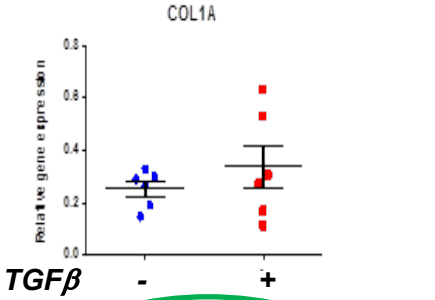
- TSPAN1
- TSPAN14
- TSPAN15
- TSPAN18

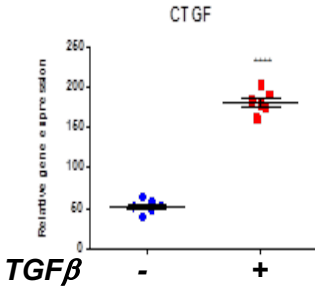
In vitro characterisation of ADPKD fibroblasts

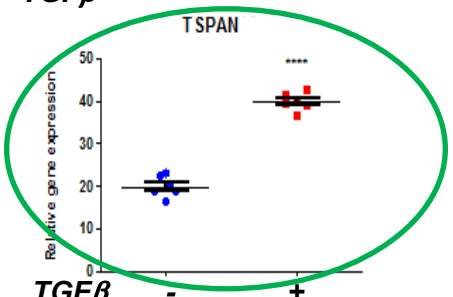
- **Compared to normal kidney fibroblasts ADPKD fibroblasts show:**
  - Decreased PC-1
  - Cilia defects (cilia known to integrate growth factor signalling, factors relevant to fibrosis PDGF, TGFβ)
  - Stage-dependent increase in proliferation and altered response to growth factors
  - Increased myofibroblast differentiation; up-regulation of αSMA incorporated into stress fibres
  - Increased contractility
  - Increased collagen production
  - Increased matrix adhesion and spreading
  - Up-regulation of FA-associated proteins and larger FA; dysregulated ECM receptor profile
- **Abnormalities reflect many of those seen in fibrotic fibroblasts from other organs**

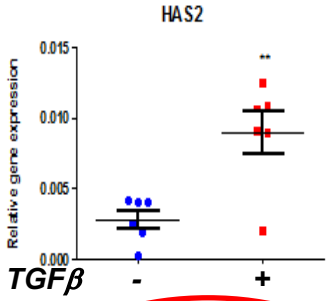
Common/unique patterns of gene expression

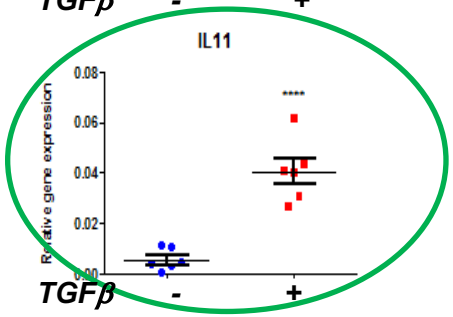
NHK -/+ TGFβ

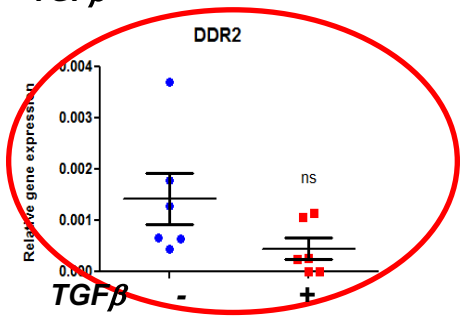


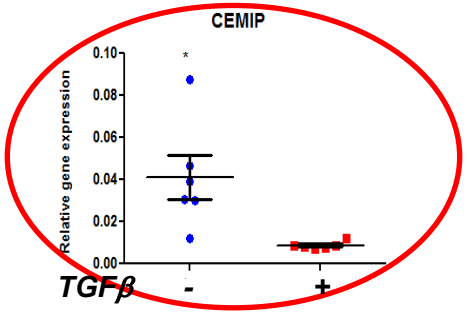




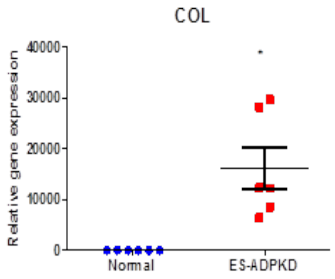


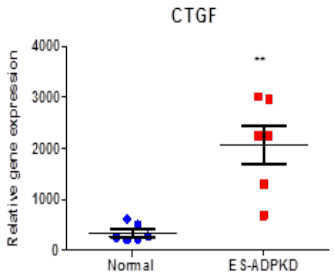


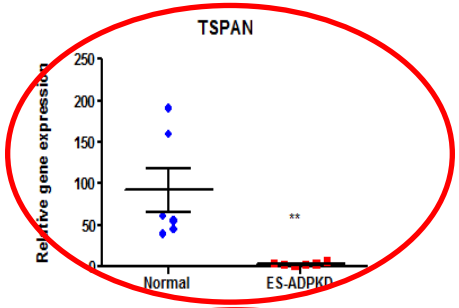


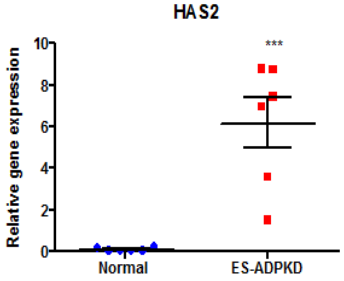


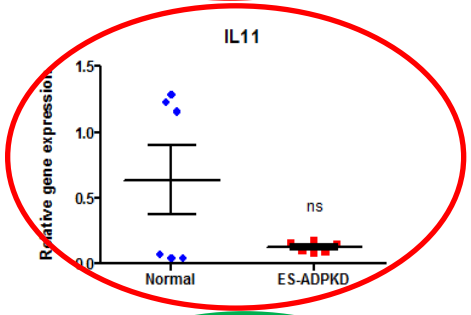
ADPKD

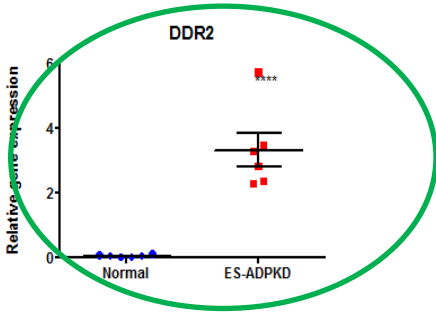


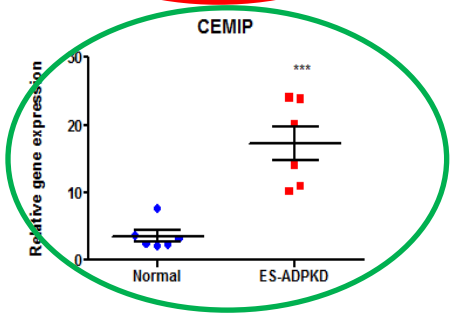












Gene profiling of ADPKD vs normal fibroblasts

- Compare gene expression in NHK and ES-ADPKD fibroblasts
- Human Gene 1.0ST Affymetrix chip (UCL Genomics)
- Analysis Integromics® Biomarker Discovery software

Up-regulated genes:507 **Down-regulated genes:556** *(>2 fold)*

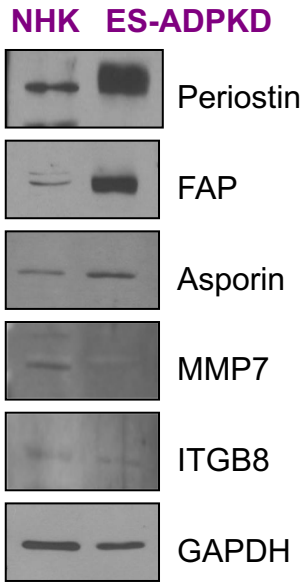
Top 20

Name	Fold Change
Periostin - osteoblast specific factor	8.03468375
Matrix Gla protein	7.601936667
Protocadherin 18	7.063819167
UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 2	6.797520833
Oxidized LDL receptor 1	6.539281667
Fibroblast activation protein, alpha	6.456475833
Serglycin	6.45483
Sulfatase 1	6.4291425
Microfibrillar-associated protein 4	6.2683275
Lipid phosphate phosphatase-related protein type 4	6.167144583
Vestigial like 3	6.0196925
Sodium channel, voltage-gated, type IX, alpha subunit	6.001779167
Discoidin domain receptor tyrosine kinase 2	5.9929425
Asporin	5.9757225
Serpin peptidase inhibitor, clade B (ovalbumin), member 2	5.942604167
Anoctamin 3	5.938825833
Biglycan	5.933563333
Collagen, type I, alpha 2	5.903893333
Regulator of G-protein signaling 4	5.896416667
micro RNA 145	5.860660833

Top 20

Name	Fold Change
Matrix metallopeptidase 7	-7.8520425
Tumor necrosis factor (ligand) superfamily, member 10	-7.423973333
Prominin 1 (CD133)	-7.293641667
CD24	-7.194954167
C-type lectin domain family 4, member E	-6.83895
Mal, T-cell differentiation protein 2 (gene/pseudogene)	-6.771321667
Hepatitis A virus cellular receptor 1	-6.700473333
Ets homologous factor	-6.503751667
iltegrin, beta 8	-6.381093333
Potassium inwardly-rectifying channel, subfamily J, member 16	-6.20179
Secreted phosphoprotein 1	-6.19344
Olfactory receptor, family 12, subfamily D, member 2	-6.0944
Integrin, beta 6	-6.038710833
Solute carrier family 17 (sodium phosphate), member 1	-6.0147
Epithelial cell adhesion molecule	-5.9800175
FAM134B	-5.972165833
Olfactory receptor, family 12, subfamily D, member 2	-5.9550325
Doublecortin domain containing 2	-5.861695

Target validation



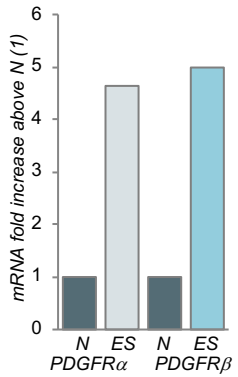
Gene ontology (GO) enrichment analysis

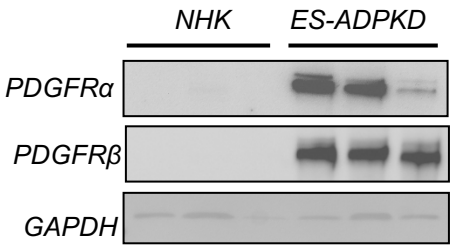
- PDGFRs most common genes regulated in the array (65 relevant GO annotated biological processes, up-regulated PDGFRα and PDGFRβ feature in 11)

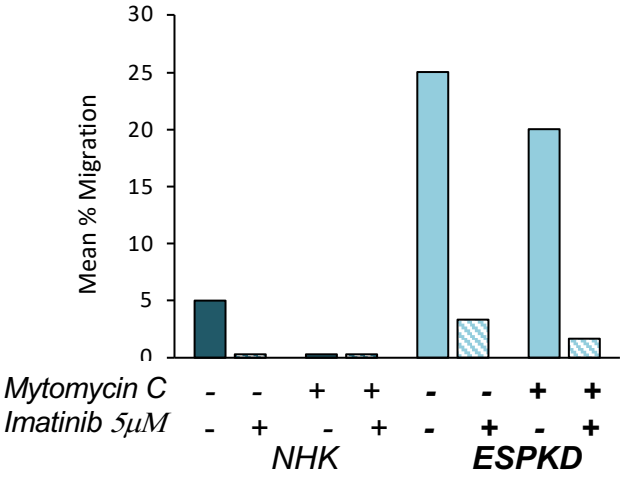
PDGF/PDGFR receptors in ADPKD fibrosis

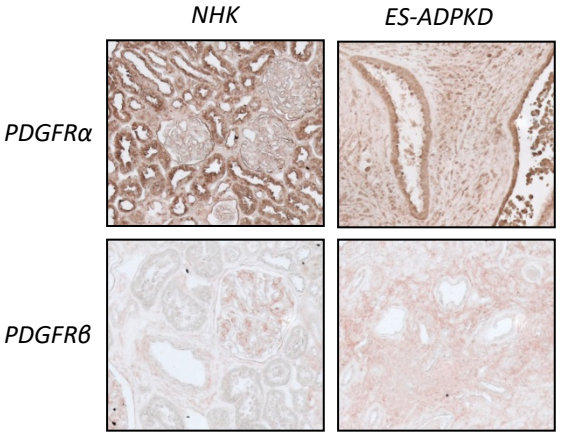
- PDGFRα and β tyrosine kinase receptors interact with ligands (A, B, C, D)
- PDGF/PDGFR widely implicated in fibrosis;
- Up-regulated in a number of renal diseases
- Responses to PDGF co-ordinated by primary cilium

- ADPKD fibroblasts in vitro hyper-proliferative to PDGF
- PDGFRs elevated in ADPKD fibroblasts in vitro and in vivo
- Inhibition of PDGFR/signaling (imatinib, siRNA) attenuates fibrotic characteristics of ADPKD fibroblasts in vitro









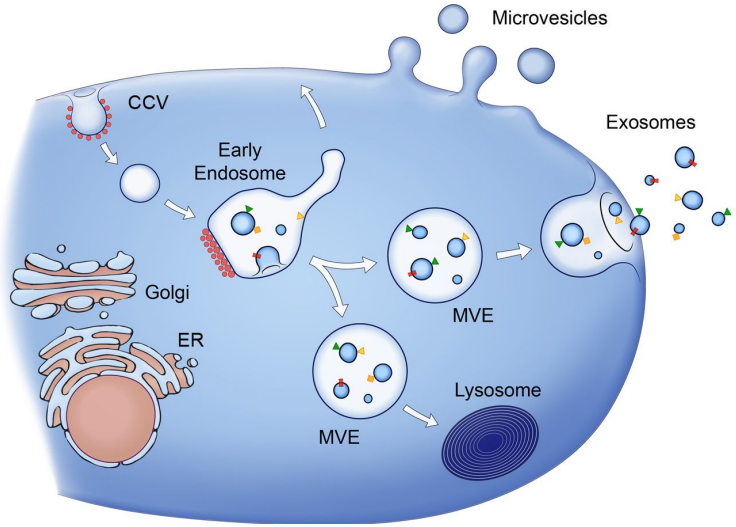
- PDGFR pathway target to slow progression of ADPKD?
- In vivo studies: Pharmacologic inhibition
  Fibroblast-specific deletion
  Inducible Coll1a2 Cre x PDGFR floxed mice x Pkd1$^{nl/nl}$

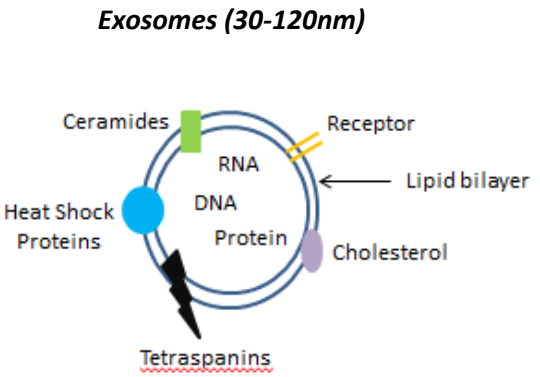
Re-purposing of PDGFR TKIs in clinical use for ADPKD?

Biomarkers of renal fibrosis

- Challenges in fibrosis: to identify at-risk individuals and to predict rate of progression
- Biomarkers are under intense investigation
- Advantage of the kidney is the availability of urine as a non-invasive source of biomarkers (urinary RNAs, miRNAs, proteins, microvesicles)

Exosomes as source of biomarkers in ADPKD





Adapted from Ko et al., Analyst 2016

Exosomes

- *30-120nm vesicles*
- *Originating from multivesicular bodies*
- *Contain a subset of proteins, miRNAs and RNAs*
- *Released into body fluids (urine, blood)/cell medium*
- *Involved variety of cellular processes; cell-cell communication*
- *Altered in disease*

Patient cohort

- Royal Free – Specialist PKD clinic with ~350 patients
- Range of stage of disease:

CKD Stage	Number of patients
1	70 (20%)
2	88 (25%)
3	140 (40%)
4	52 (15%)

- Urine and blood samples collected and stored (PKD Charity-sponsored Biobank)
  - Longitudinal sampling of patients over time (~6 years)
- Linked to detailed clinical data

Urinary exosome preparation

- Small volumes of urine
- Optimisation of exosome isolation from 5ml urine samples

Ultracentrifugation protocol

Add protease inhibitor to urine aliquot Vortex.

↓

Centrifuge samples at 300xg 15mins at 4°C. Discard pellet

↓

Centrifuge samples at 20,000xg 30mins. Discard pellet

↓

Filter supernatant to remove uromodulin network

→

Centrifuge samples at 197,000xg for 2.5hrs.

↓

Add Isolation Solution to pellet and resuspend.

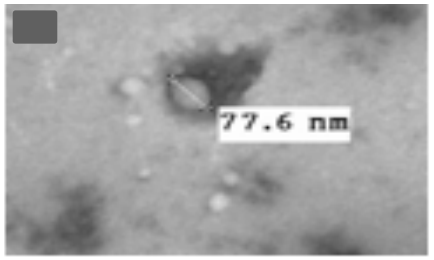
↓

Centrifuge at 197,000xg for 2.5hrs to purify exosomes.

↓

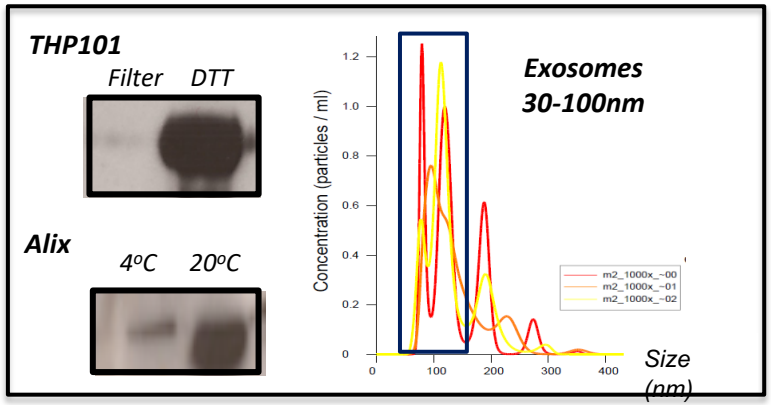
Remove supernatant without disturbing exosomes

Exosome purification



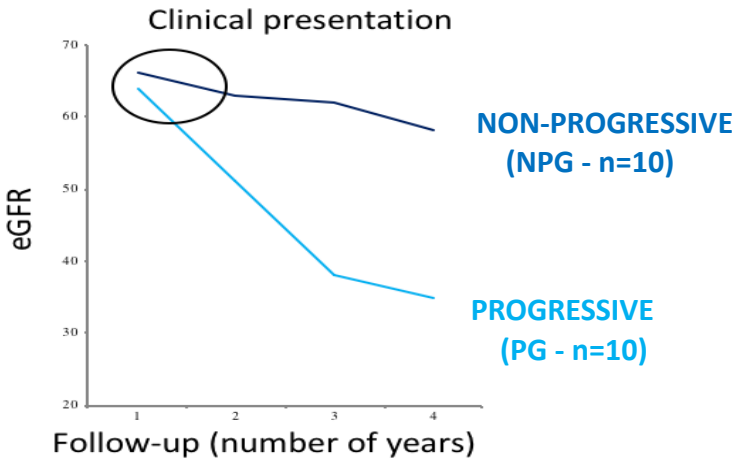
Transmission electron micrograph

I. Clathworthy, RFH EM unit



Urinary exosome protein profiling

- Longitudinal urine samples from patients who had similar function (eGFR) at presentation but (based on clinical data) declined at different rates over 4 year follow-up



1) eGFR >70

Sample	eGFR range
NPGs	**90-74**
PGs	**88-70**

2) eGFR 69-50

Sample	eGFR range
NPGs-17	**63-59**
PGs	**64-55**

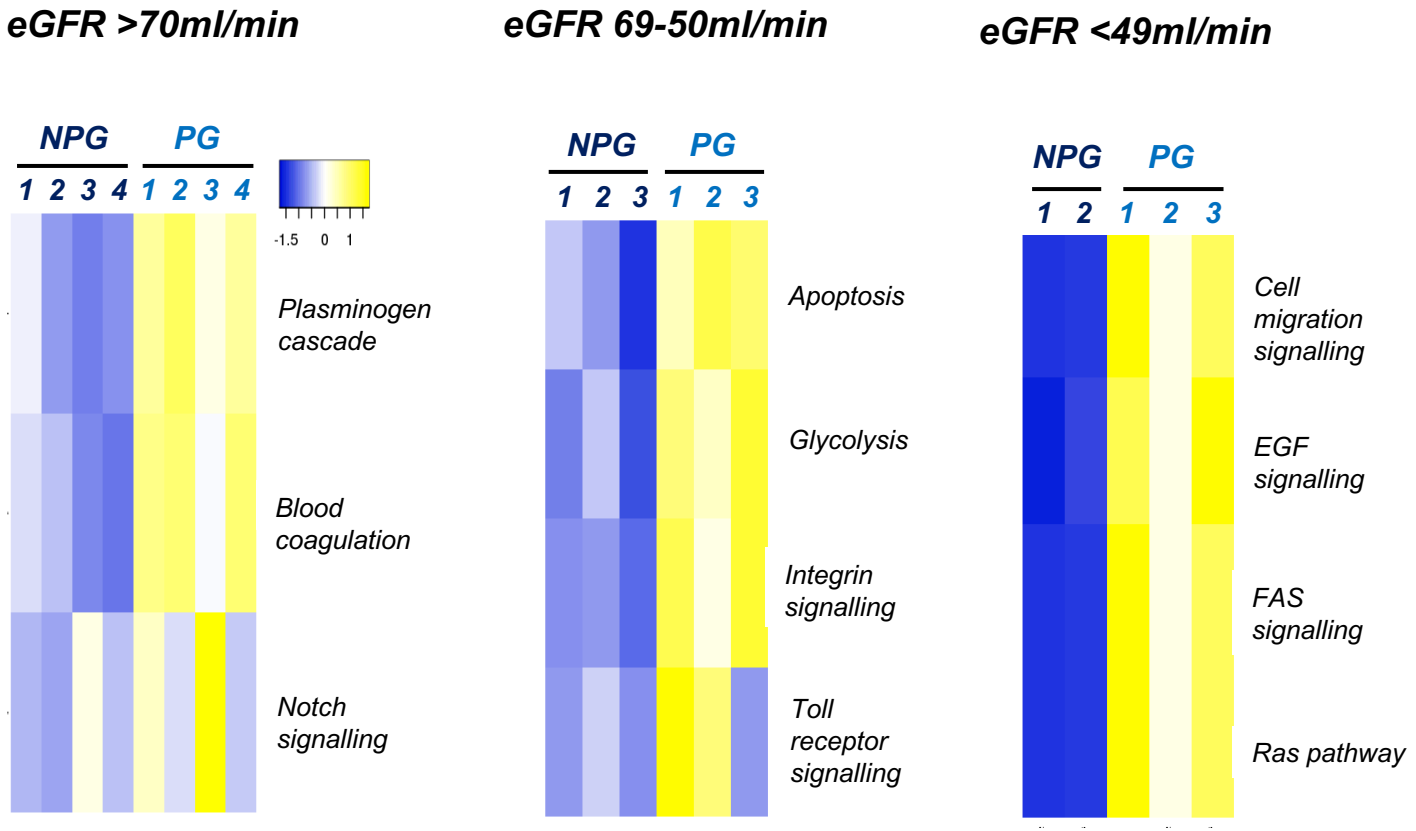
3) eGFR <49

Sample	eGFR
NPGs	**49-23**
PGs	**41-34**

- Proteomics of exosomes isolated from presentation urine samples (KCL Proteomics)
- Compared protein profiles
- >2-fold difference cut-off:
  - 291 proteins up-regulated in **PGs** compared to **NPGs**
  - 30 proteins down-regulated in **PGs** compared with **NPGs**

Pathways altered in progressors vs non-progressors

- Pathway analysis (>2-fold upregulated) identified distinct patterns between those with rapid (**PG**) vs slow progression (**NPG**)
- Can distinguish PG and NPG at different starting eGFR (levels of renal function)



- Develop protein panel to distinguish rapid and slow progressors at presentation
- Use of urinary exosome profiles to determine response to treatment
- Potential to predict response to Tolvaptan (Otsuka)

New Treatments for renal fibrosis?

1. Develop drugs/biologics (antibodies) targeting pathways altered in renal fibrosis
    - New drug discovery
    - Repurposing (SGLT2 inhibitors for diabetes)

2. Developing and implementing strategies to enhance endogenous renal repair and promote generation of new nephrons

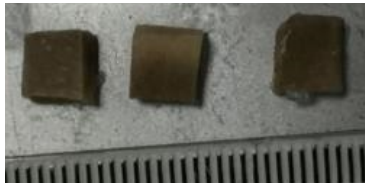
3. Engineer new organs for transplantation
    Supplement remaining tissue or replace damaged organ
    - Organoids
    - Re-seeded scaffolds (synthetic/natural)

- Studies *in vivo* and *in vitro* models of AKI/CKD have identified numerous factors and pathways dysregulated in renal fibrosis (TGFβ)

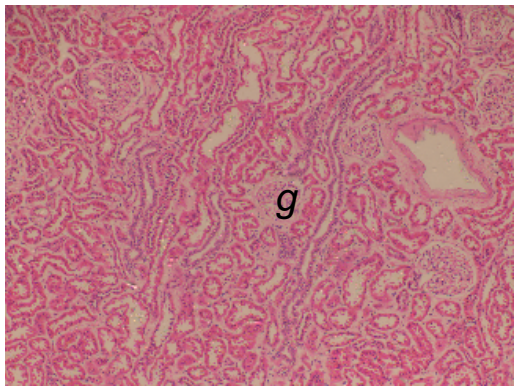
- Poor translation to the clinic

- Improved models?
    Human cell-based models

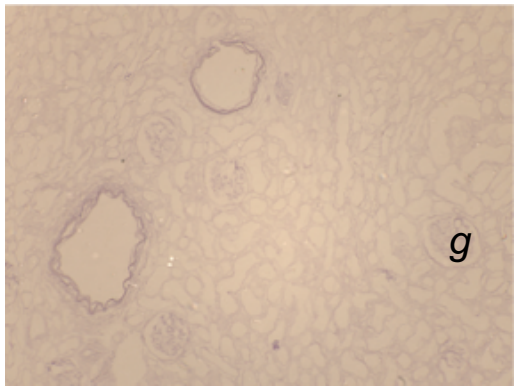
Human kidney ECM scaffolds



SDS-based Decellularisation

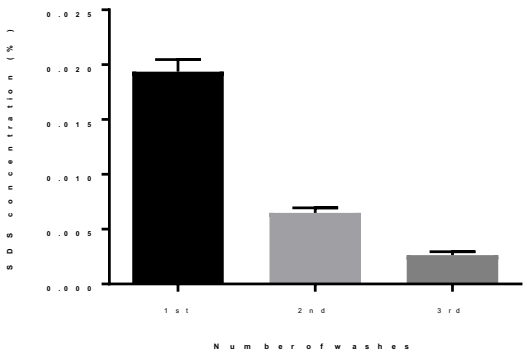


Native kidney

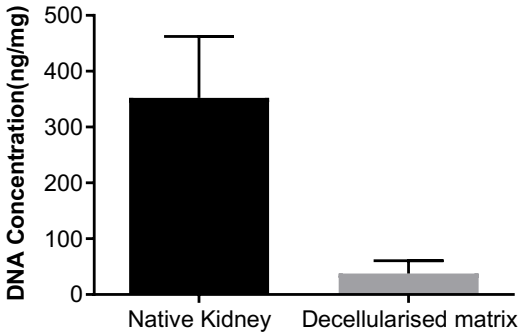


Decellularised ECM scaffold

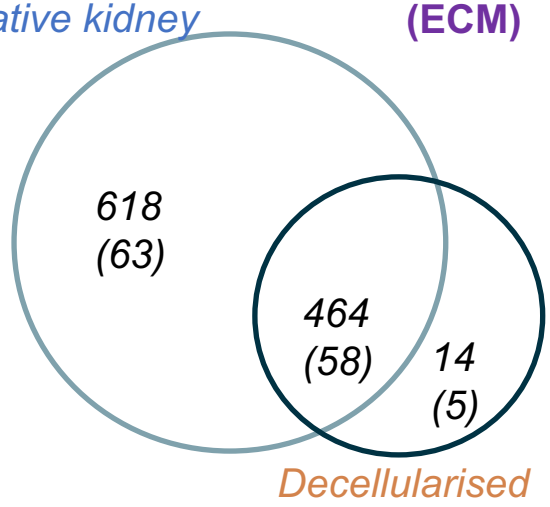
SDS concentration



DNA content

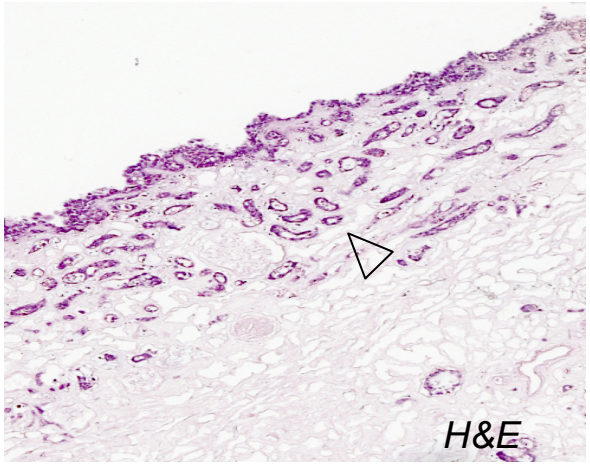


Proteomic analysis: number of proteins



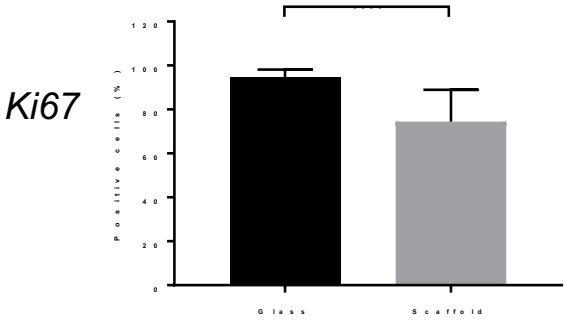
Reseeding scaffolds with human renal cells

- Normal human kidney ECM scaffolds seeded with human PTEC cell line (HK-2)

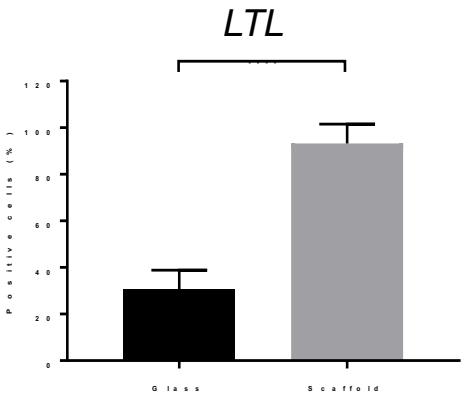


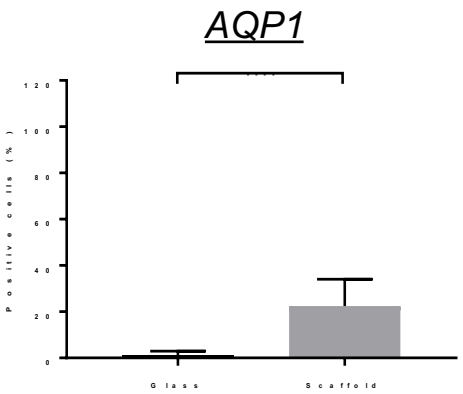
- Epithelial cells repopulate the human kidney ECM scaffold and line tubular lumens

- ECM scaffold suppresses cell proliferation



- Increases expression of cell type-specific differentiation markers





Summary

- Background to CKD and renal fibrosis
- Some insights into some of the mechanisms of renal fibrosis
- The value of in vitro human cell models in understanding the biology of fibrosis and identifying candidate therapeutic targets
- The potential of urinary exosomes as a source of biomarkers to predict progression and response to treatment
- Challenges in developing new therapeutic strategies for renal fibrosis

Acknowledgements

Patricia Wilson
Johanna Donovan
Timo Haschler
Qingyang Kong
Kiron Koshi
Siobhan Moyes
Shilpa Nayuni
Jane Ong
Katie Raby
Surya Rayapureddi
Johanna Verneau
Yu Hsuen Yang
Hadzliana Zainal

Collaborators
UCL
Institute for Child Health
Institute of Opthalmology
King's College London
Imperial College
UCB

Rosetrees Trust
Helping humanity through medical research

pkd Polycystic Kidney Disease Charity

St Peter's Trust
Kidney | Bladder | Prostate | Research

royal free charity

PETER SAMUEL ROYAL FREE FUND

Kidney Research UK
Funding research to save lives

MRC Medical Research Council

AstraZeneca

ucb