THE UNIVERSITY of EDINBURGH

MicroRNAs in renal fibrosis and transplantation

- mediators and therapeutic targets.

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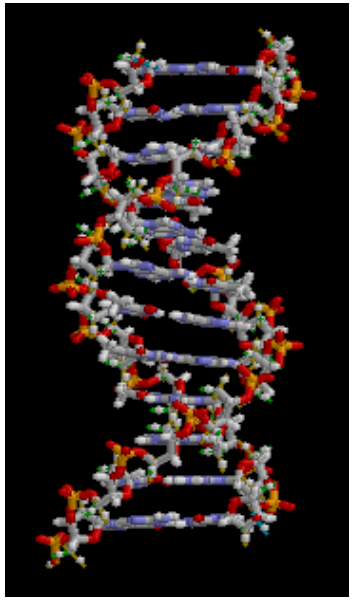
Non-coding RNA

DNA → Gene (Transcription) → mRNA (Translation) → Protein

The central tenet of molecular biology :-

RNA functions mainly as an informational intermediate between a DNA sequence i.e. gene and its encoded protein.



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DNA Sequencing revolution - Human Genome Project

- **22 287** known or predicted protein-coding gene loci.
- coding regions occupy **1.2%** of the euchromatic genome.
- total fraction of bases occupied by known protein-coding transcripts is only about **2%**.
- summation of the sequences covered by known genes, 'mRNAs' and spliced expressed sequence tags (ESTs) indicated that (at least) **60 –70%** of the mammalian genome is transcribed on one or both strands.

Non-coding RNA

Types of non coding RNA

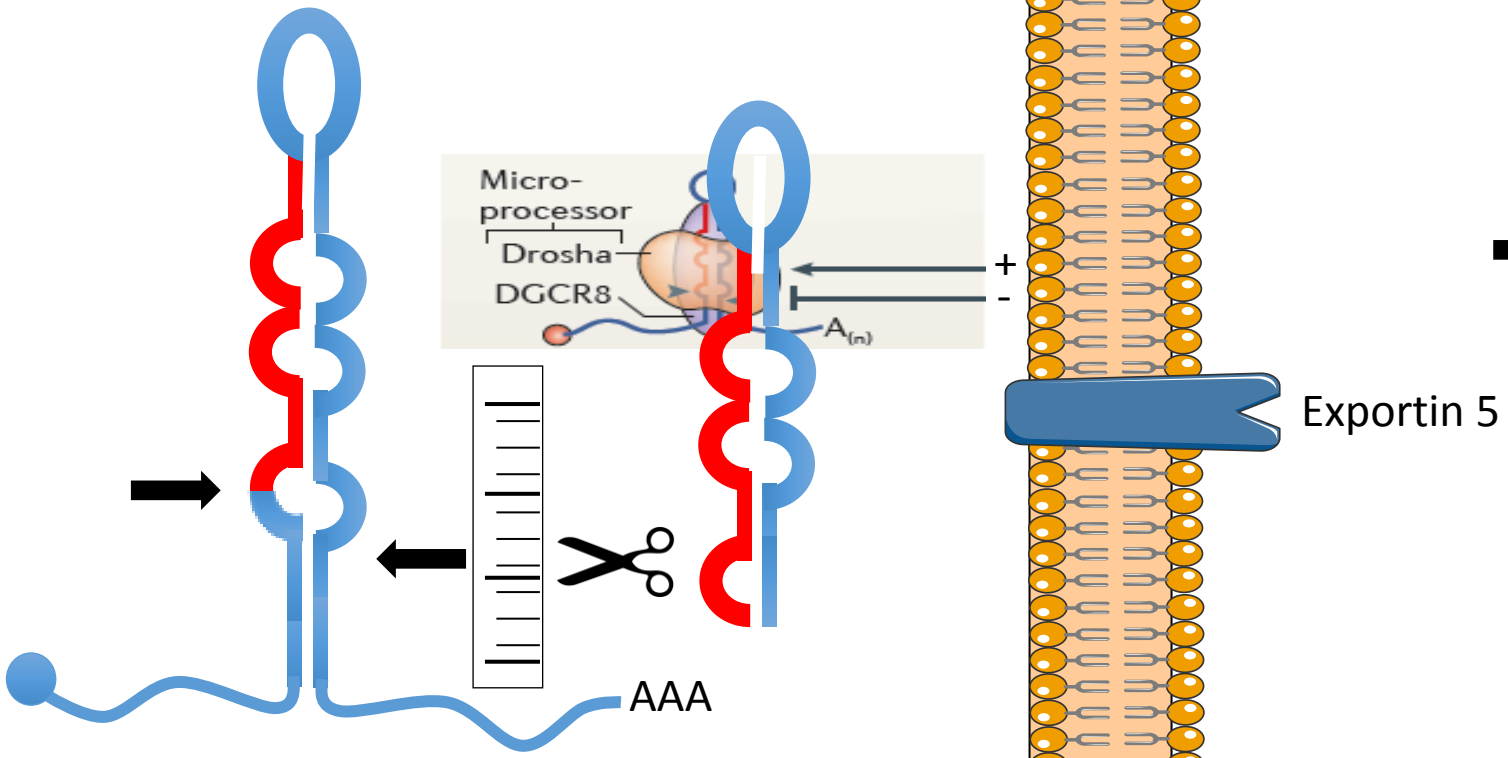
- Ribosomal RNA (rRNA)
- tRNA
- Long non-coding RNA (lncRNA)
- Small non-coding RNA
- ✓ microRNA (miRNA)
- ✓ snoRNA
- ✓ siRNA
- ✓ piRNA
- Circular RNA

- **miRNAs** are small (<25nt) non-coding RNA molecules.
- 1st discovered in 1993 in C.elegans.
- 1st detected in humans 2000.
- Over a 1000 miRNA in humans.
- At least 60% of protein coding genes coding miRNA complementary sites.
- Canonical and non-canonical biogenesis.

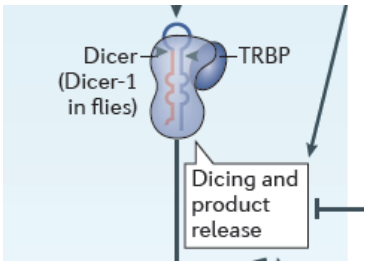
miRNA Biogenesis

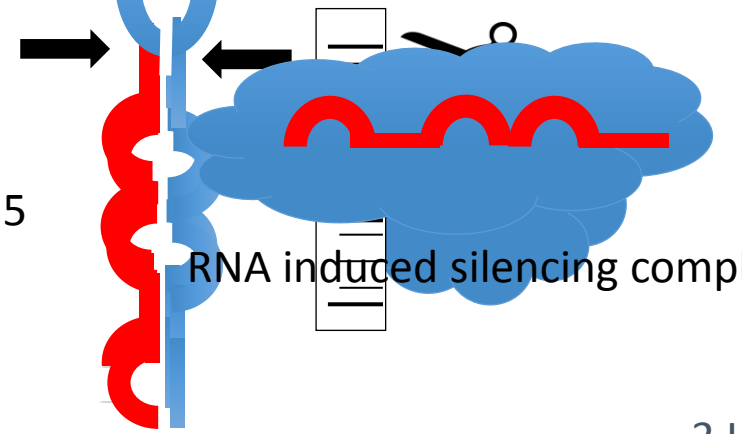
Nucleus

Cytoplasm

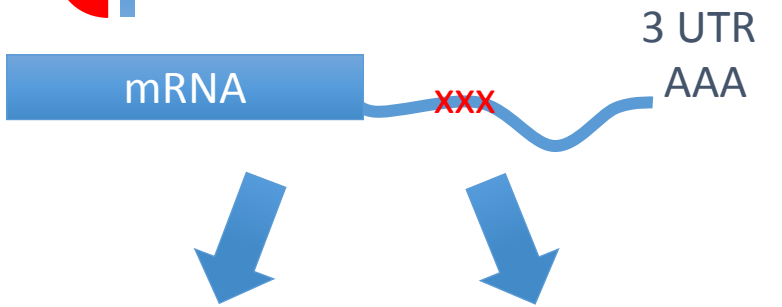


Exportin 5





RNA induced silencing complex



Pri-miRNA transcript

Pre-miRNA transcript

- Transcribed by Pol II
- Contain one or more stem loops

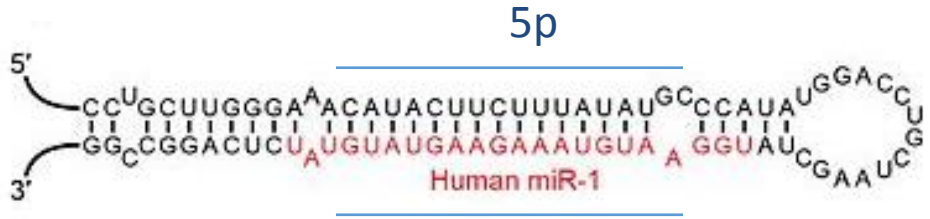
Translational repression / mRNA decay

mRNA cleavage

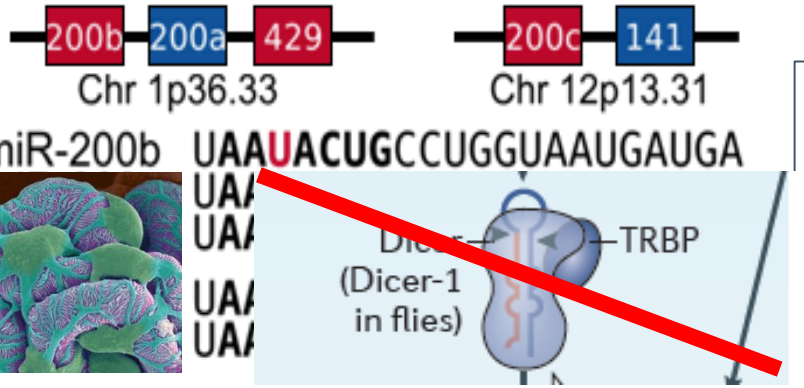
Ha and Kim, Nature Reviews 2014

miRNAs

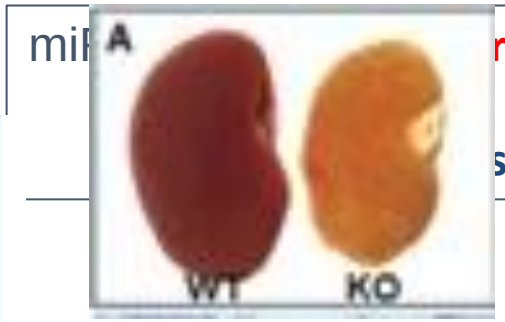
- Each pre-miRNA stem loop encodes **2** miRNA.
- Nomenclature agreement
  - 5p and 3p designation based on position in stem loop.
- miRNA interact with mRNA with specific region called seed region.
- A single miRNA has the ability to hit multiple genes and influence many pathways.
- Frequently miRNA complementary sequences are found in functionally related but distinct mRNAs.
- Essential for normal kidney function.
- Phase I/II clinical trials underway with Anti-miRs for HCV, Alport's Syndrome.



miR-200 family – TGFβ regulated



Podocyte

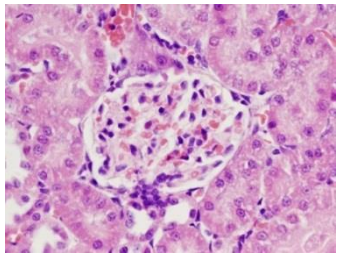


Shi et al, 2008

Seed Region – interacts with 3'UTR (5' UTR and CDS)

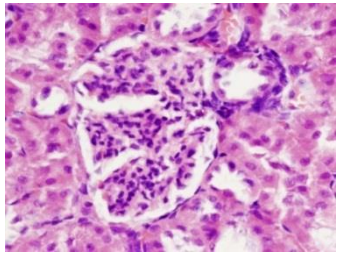
Are miRNAs involved in glomerulonephritis??

Anti-Thy1.1 model – rat model of glomerulonephritis

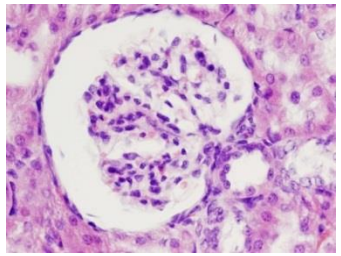


Normal glomeruli

ER-4
(2mg/kg)



Global
mesangial proliferation

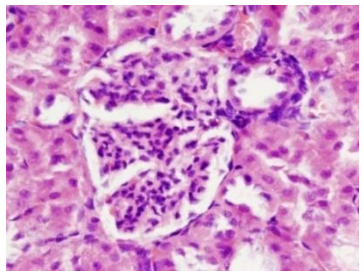


Segmental
mesangial proliferation

Histologic Evaluation of Kidney Damage in Anti-Thy1.1 Animals

Group	Glomerular score	Tubular score	Total
Control	0.25	0.25	0.5
1 × ER4, 7 days	1	0.5	1.5
3 × ER4, 7 days	2	2	4
3 × ER4, 14 days	2.1	1.3	3.4

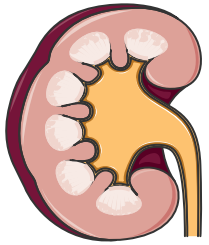
25% of glomeruli had a glomerular lesion. Evidence of tubular damage (5-25%)



From **Denby** et al., 2011. *Am J Pathol*. **179**:661-72.

Are miRNAs involved in glomerulonephritis??

ER-4
(2mg/kg)



RNA

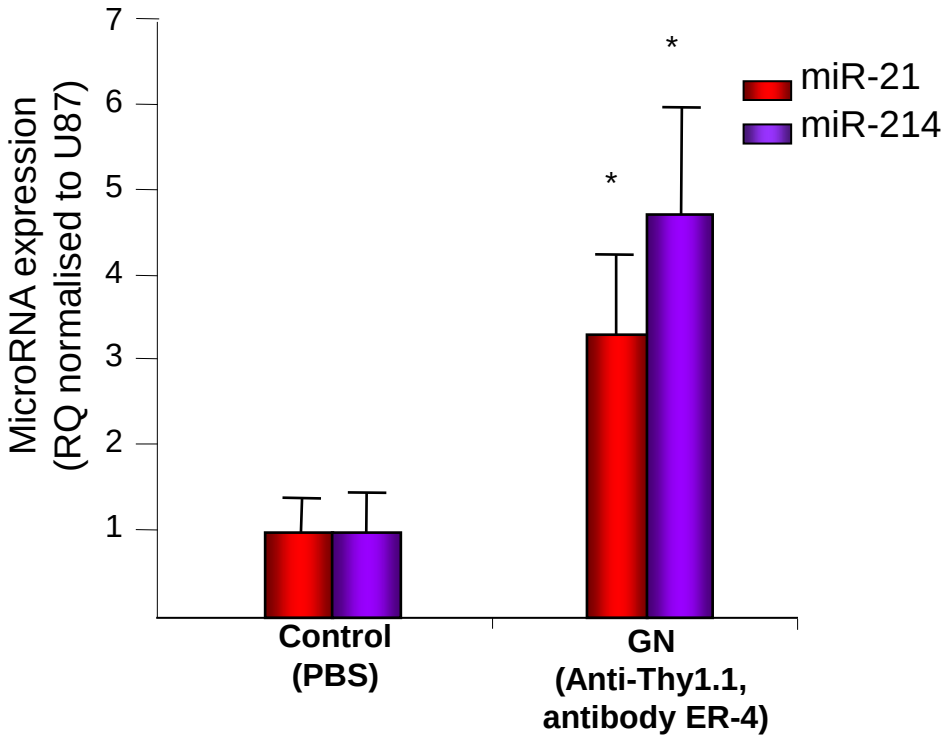
N=3/gp
3 injections
1 week apart
Sacrificed at 7 days post last injection

MiRNA Microarray

3xGlomerulonephritis (D) vs Control (C) 7days

rno-miR-214
*p<0.05

rno-miR-21
p=0.05



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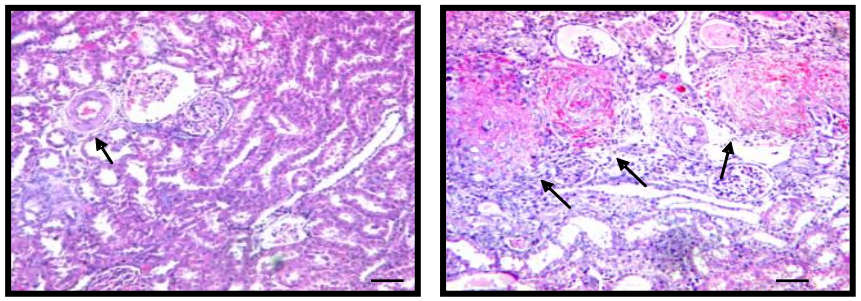
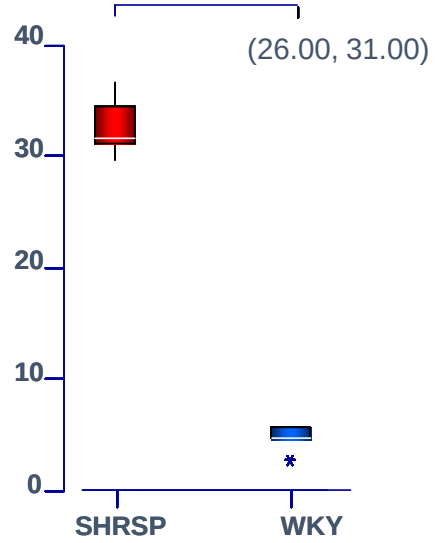
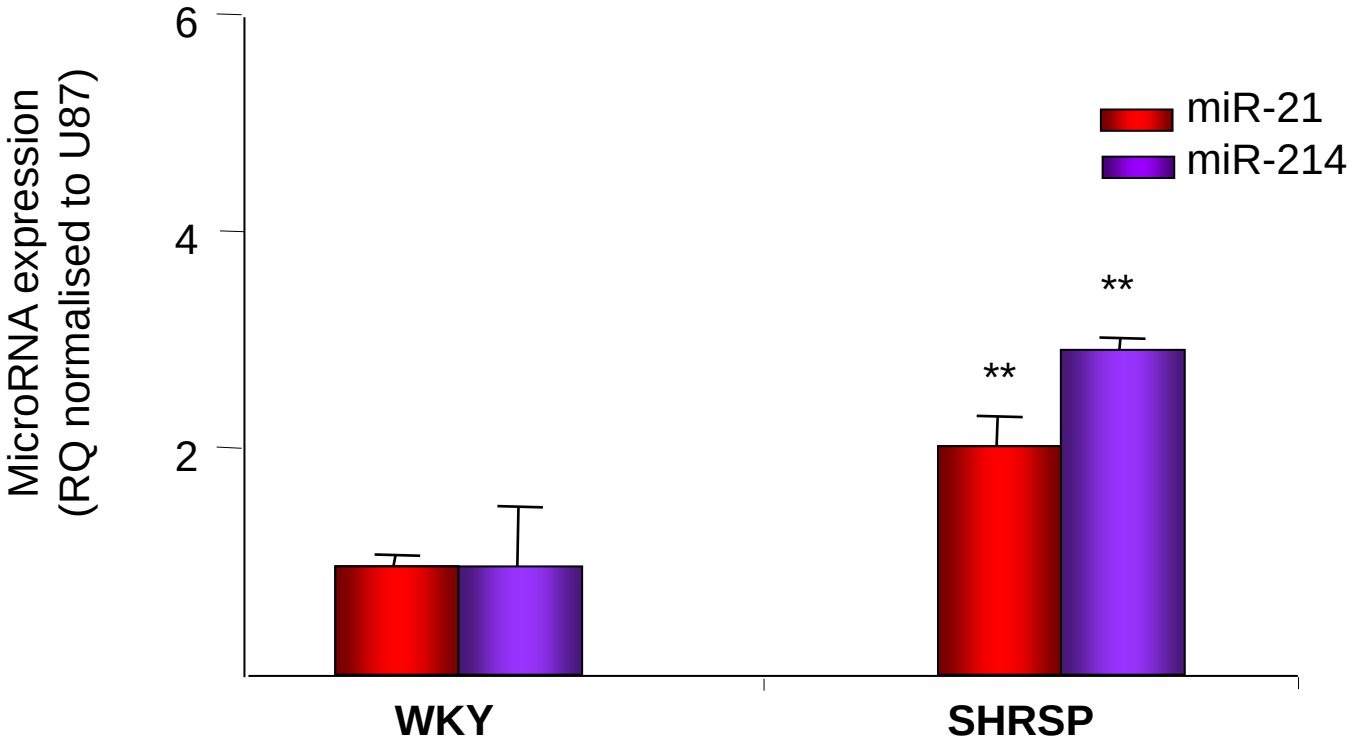
Hypertension induced nephropathy



WKY SHRSP

Malignant hypertension - Gross vascular lesions (onion skin pattern)





From **Denby** et al., 2011. *Am J Pathol*. **179**:661-72.

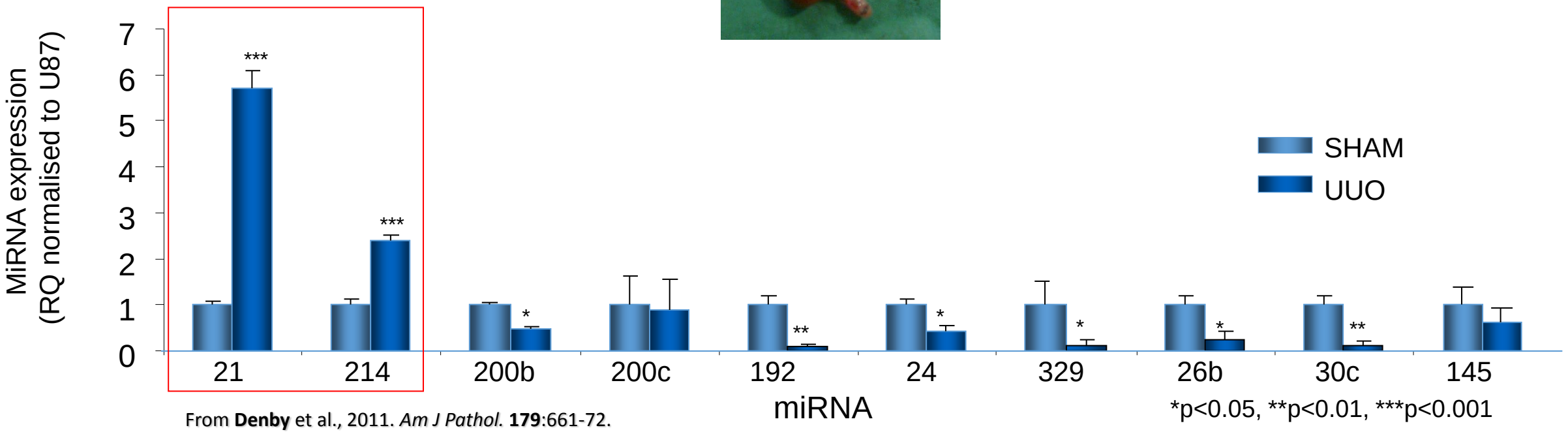
Renal Fibrosis

- is the final common pathway for most forms of progressive renal disease.

- involves glomerular sclerosis and/or tubulointerstitial fibrosis.

- tubulointerstitial fibrosis is best histological predictor of progression.

Unilateral ureter obstruction (UUO) model of fibrosis



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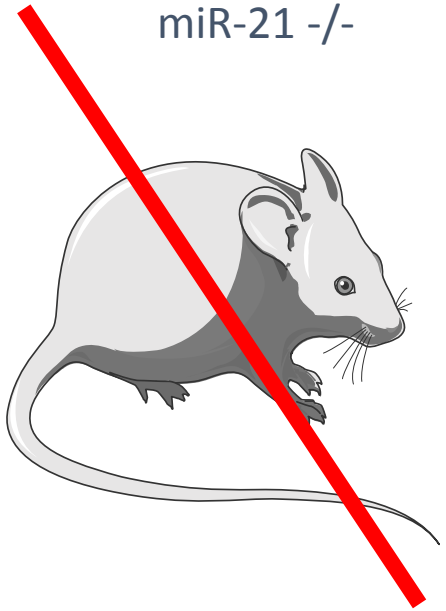
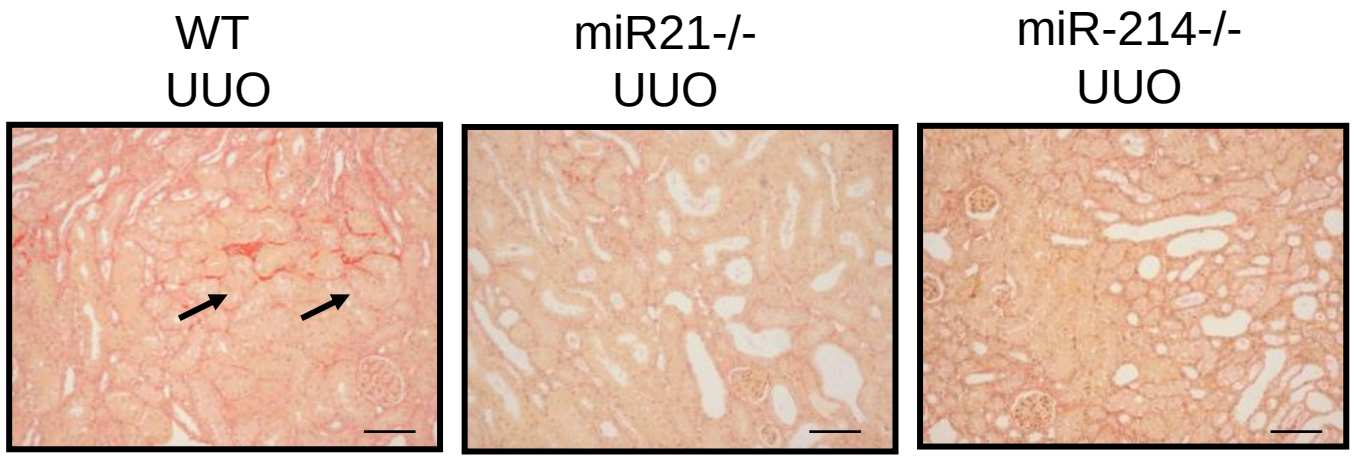
miR-21 and miR-214 are mediators of renal fibrosis.

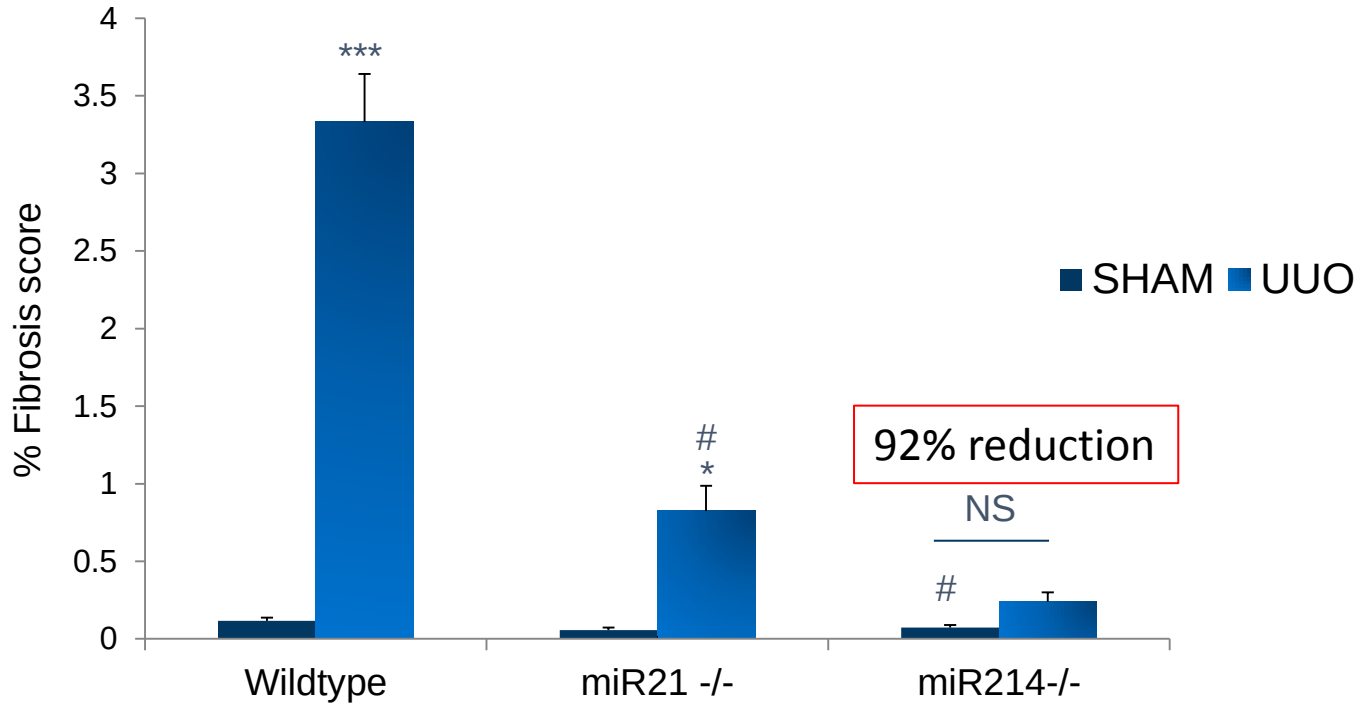
Global

miR-214 -/-

miR-21 -/-







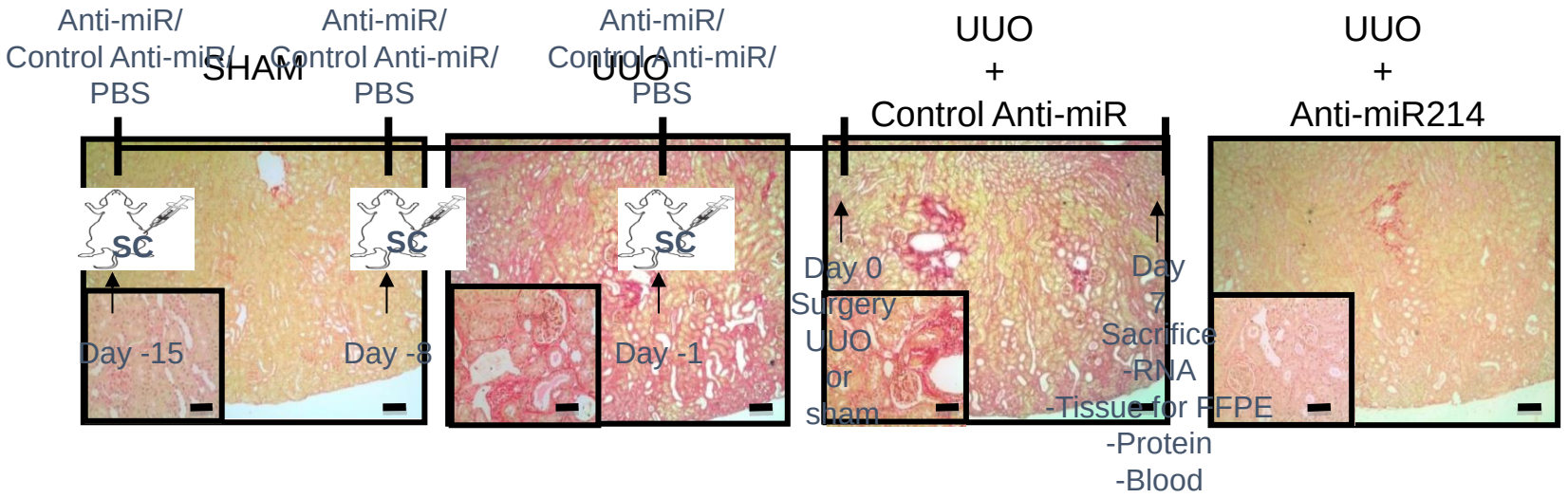
From **Denby** et al., 2014. *J Am Soc Nephrol.* **25**:65-80.

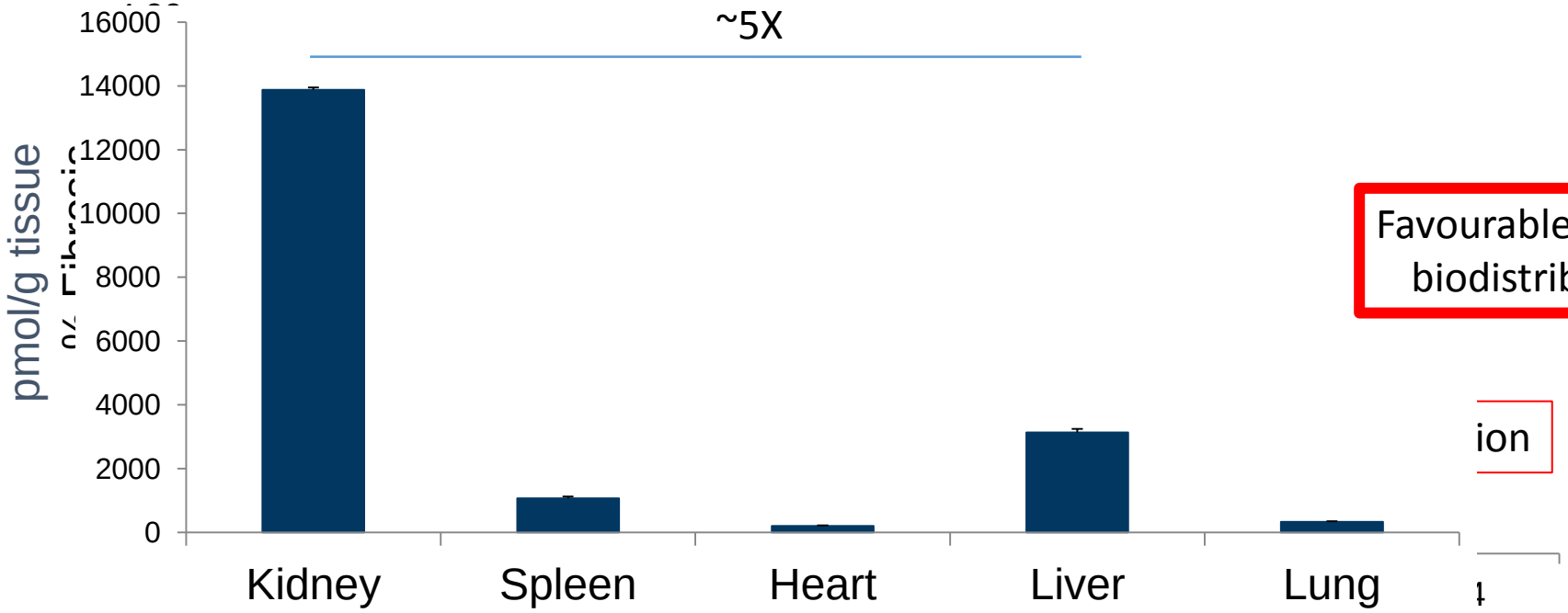
*p<0.05, p<0.001 vs SHAM; #p<0.001 vs WT UUO

Anti-miR-214-3p as a potential therapeutic for renal fibrosis.

5mg/kg

miRagen



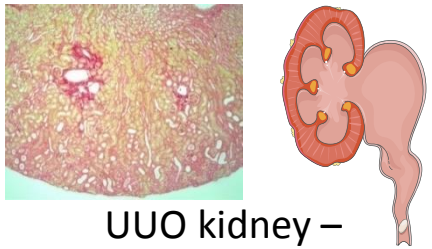


Favourable kidney biodistribution

From **Denby** et al., 2014. *J Am Soc Nephrol.* **25**:65-80.

Mechanism of action of miR-214

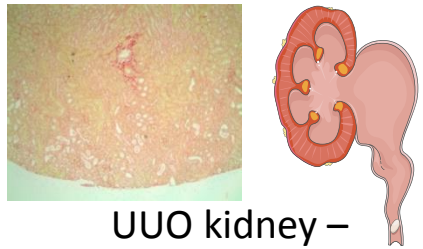
- miRNA targets can be organ/cell type/injury specific!
- Algorithms give false positives – regulation of biogenesis and expression exist!



UUO kidney – Control anti-miR

Vs

KIDNEY
Illumina microarray



UUO kidney – Anti-miR-214

1160 genes downregulated

DAVID Functional Annotation
Enriched Functional Annotations

Extracellular Matrix p-value 1E^{-13}
Including
Collagen type 3, α 1
Collagen type 4, α 1
Collagen type 7, α1
MMP2
MMP14
TIMP2
Alpha SMA

KIDNEY
Illumina microarray
UUO kidney (n=4)
Control Anti-miR treated vs Anti-miR-214 treated

1911 differentially expressed genes analysed by Rank Products

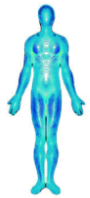
>5000 differentially expressed genes analysed by LIMMA

751 genes upregulated

~20%
3'UTR predicted targets of miR-214

From **Denby** et al., 2014. *J Am Soc Nephrol.* **25**:65-80.

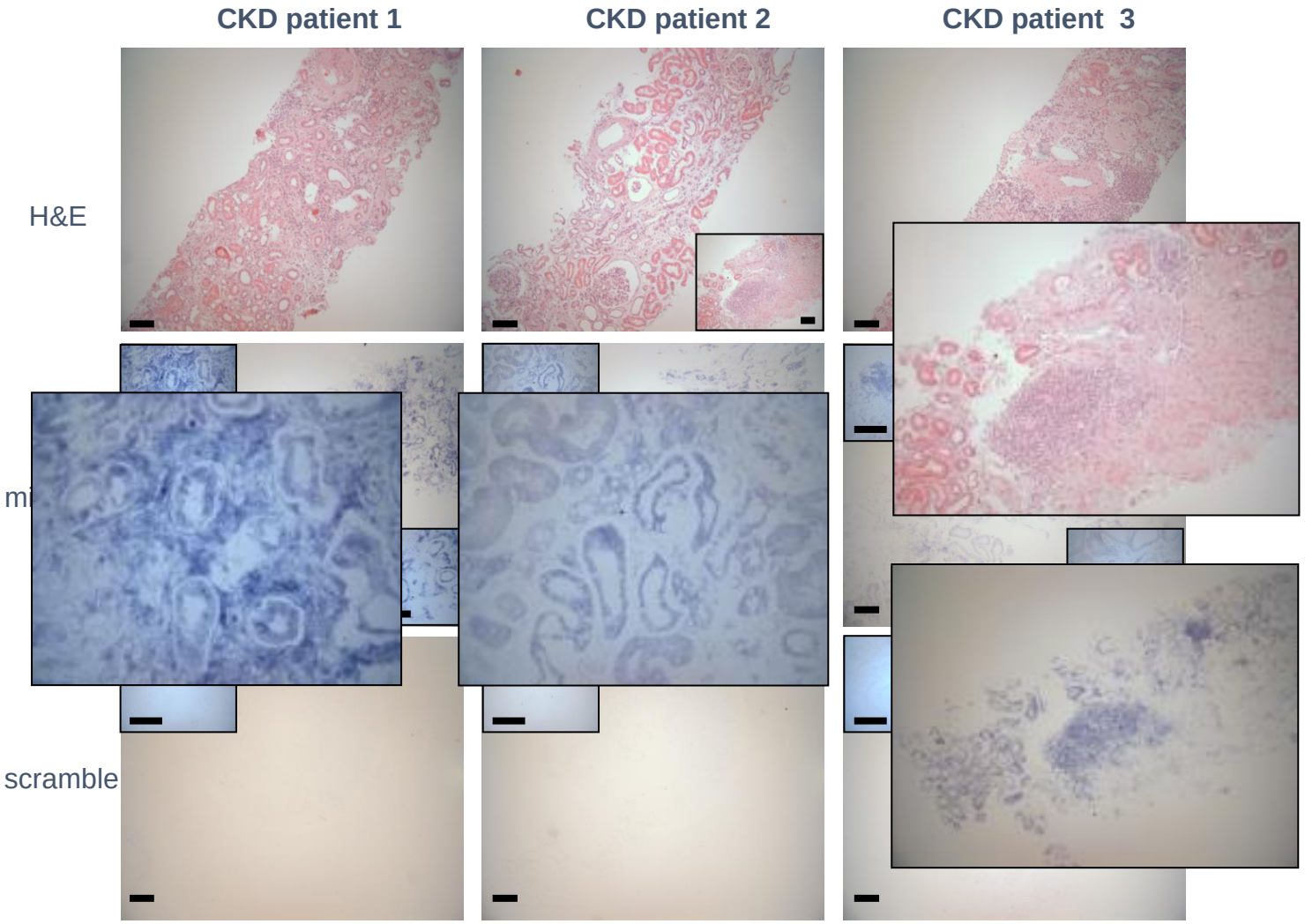
Is miR-214-3p a translatable target?



Chronic kidney disease

monocytes

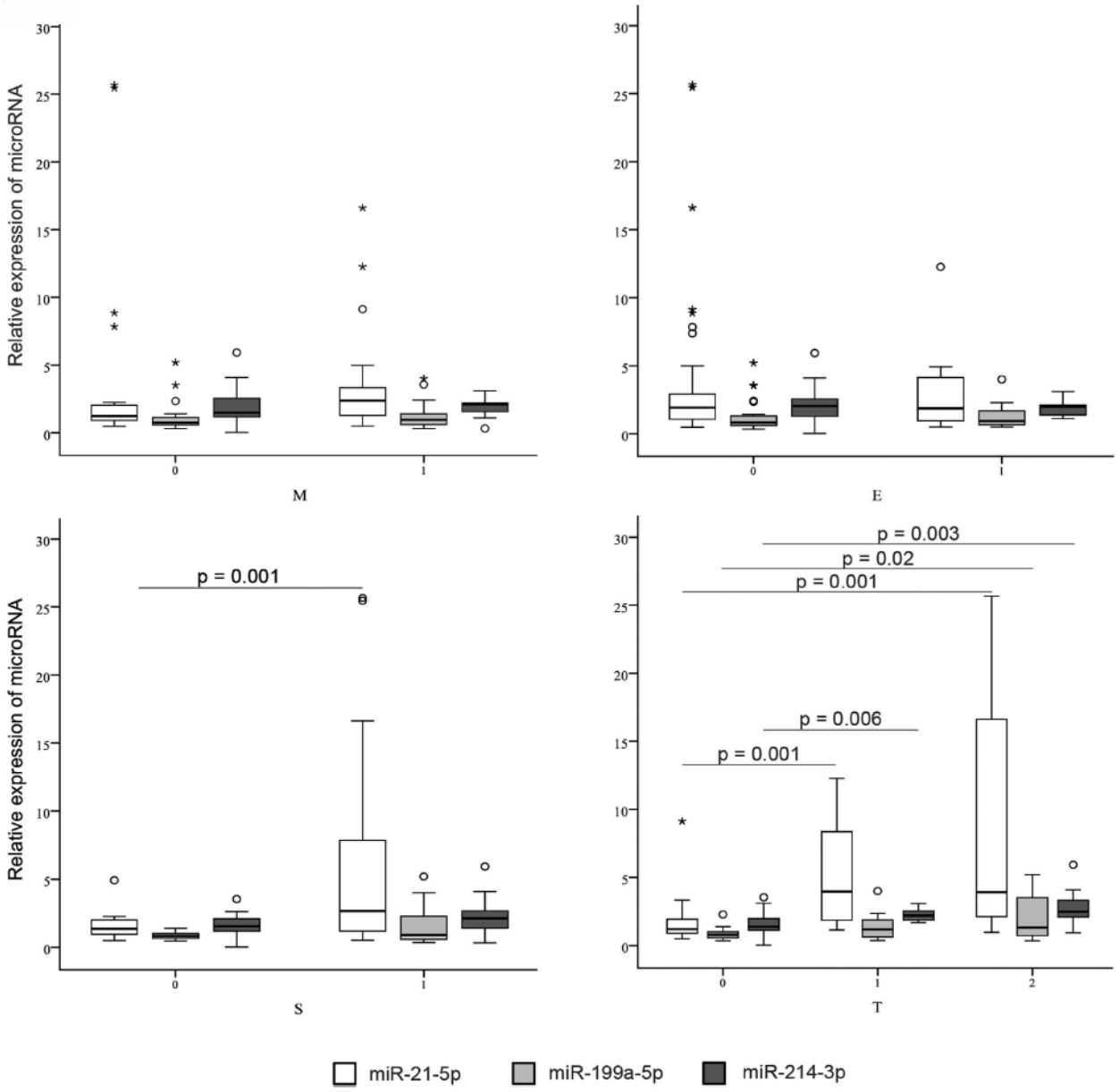
miR-214



From **Denby** et al., 2014. *J Am Soc Nephrol.* **25**:65-80.

Li, L.-M., et al., The Journal of Immunology, 2011. **186**(4): p. 2552-2560.

miRNAs in biopsy proven IgAN



miR-214-3p and miR-21-5p

are overexpressed in renal tissue of patients with moderate-severe fibrosis.

For example:

miR-214-3p

- with moderate fibrosis (T1: median RQ = 2.20 [1.67–3.07])

- with severe fibrosis (T2: median RQ = 2.48 [2.03–5.93])

Hennino et al, Scientific Reports, 2016

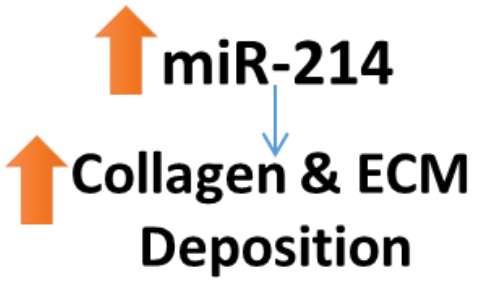
miRNAs in transplantation

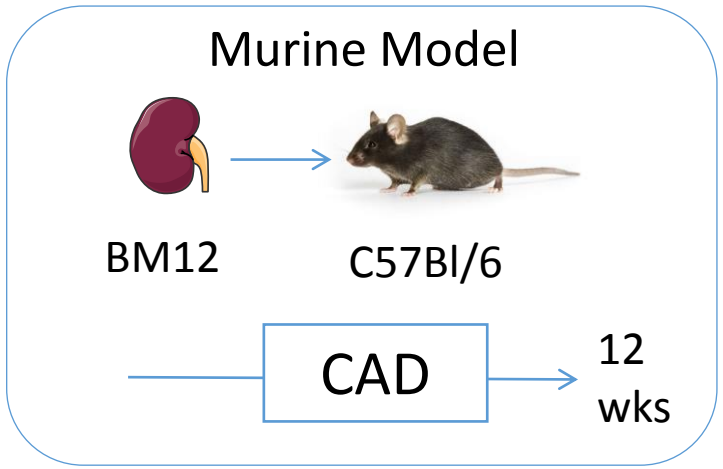
Victoria Banwell

Is there a role of miR-214 in chronic allograft damage (CAD) ?

CAD

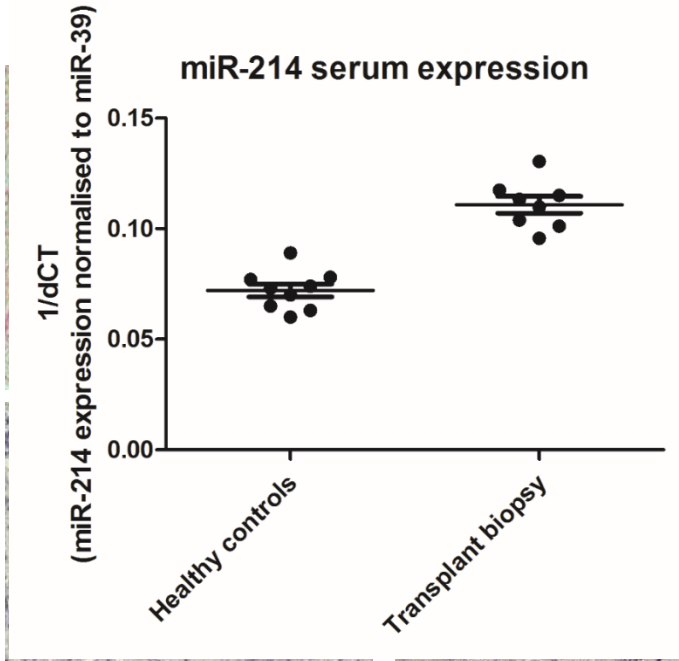
- significant cause of graft failure following renal transplantation.
- characterised by **interstitial fibrosis** and **tubular atrophy** (IFTA).
- leads to the failure of up to 5% grafts annually.
- there are no specific therapies available.

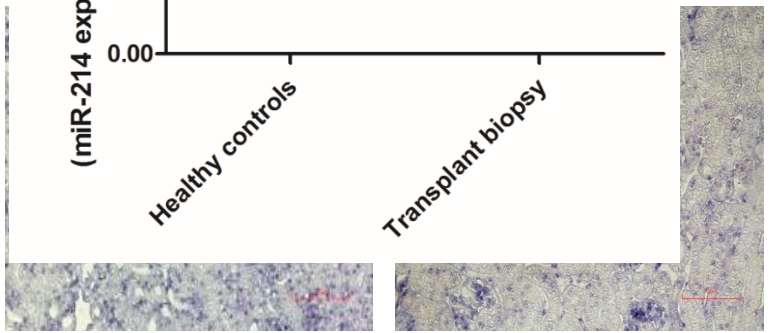


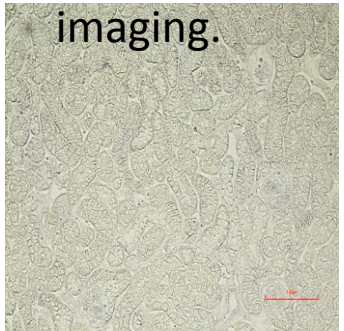


PSR

miR-214 expression







??Potential Non-invasive biomarker of CAD in combination with imaging.

miRNA biomarkers

- Identified a miRNA signature in IgAN patients that correlated with fibrosis on biopsy and eGFR.

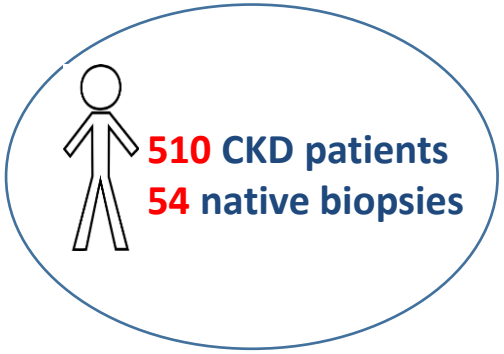
Non-invasive biomarkers of renal disease

AIM:

- **predicts functional outcome in IgAN patients (including in a validation cohort).**
  n=100 historical with followup; n=400 validation cohort

- **is limited to IgAN or is present in other renal diseases.**
  n=300 generic age, sex and eGFR matched CKD patients with IgAN pts n=200.

- **reflects the severity of renal pathological changes.**
  n=75 newly diagnosed IgAN



? Treatment effect- disease modifiable biomarker would be useful.

Kidney Research UK
Funding research to save lives

miRNAs in transplantation

Original Clinical Science—General

Hemin Preconditioning Upregulates Heme Oxygenase-1 in Deceased Donor Renal Transplant Recipients: A Randomized, Controlled, Phase IIB Trial

Rachel A. B. Thomas, MBChB,[1] Alicja Czopek, PhD,[1] Christopher O. C. Bellamy, MBChB, PhD,[2] Stephen J. McNally, MBChB, PhD,[1,3] David C. Kluth, MBBS, PhD,[1] and Lorna P. Marson, MBBS, MD[1]

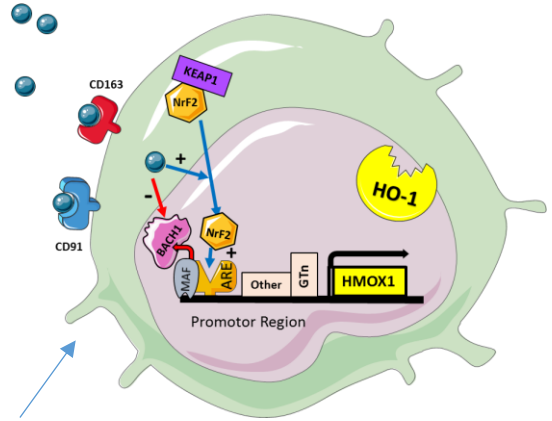
Katie Connor

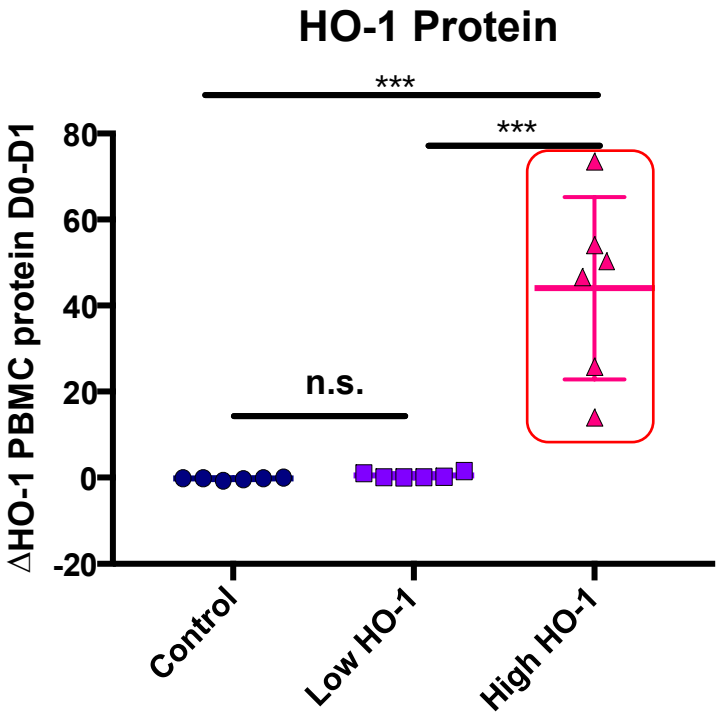
PBMCs

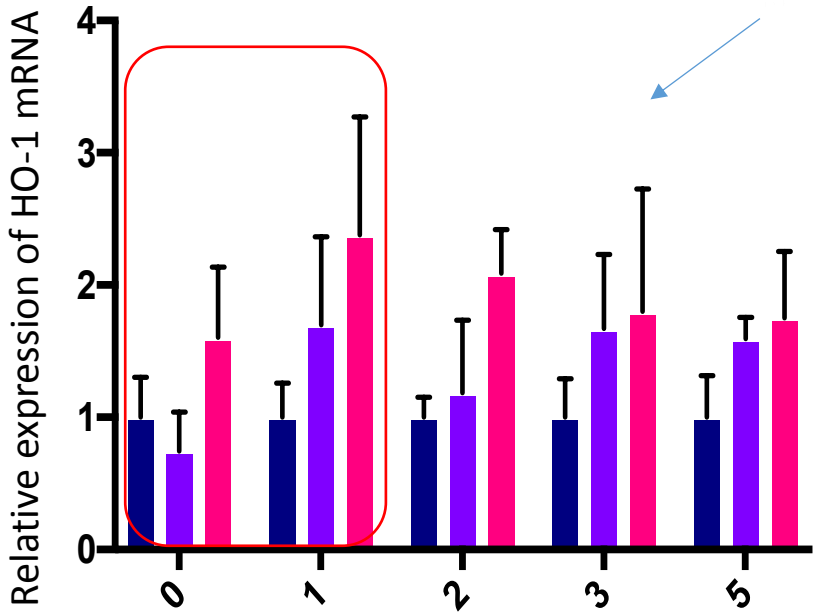
HA
+
Surgery

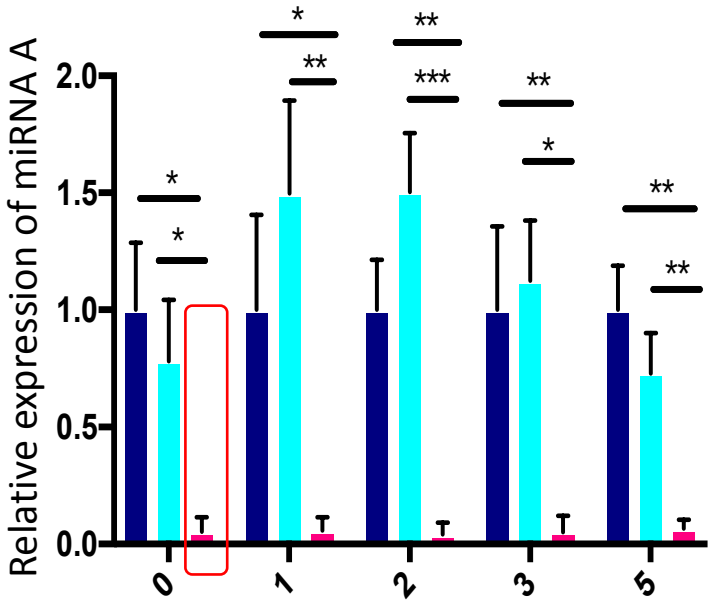
HA

Heme Arginate
(Infusion)



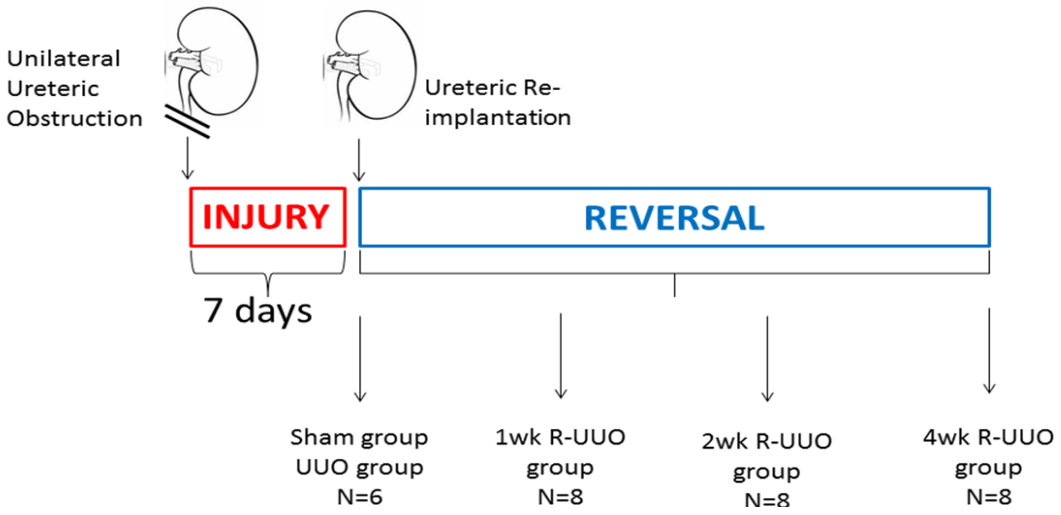




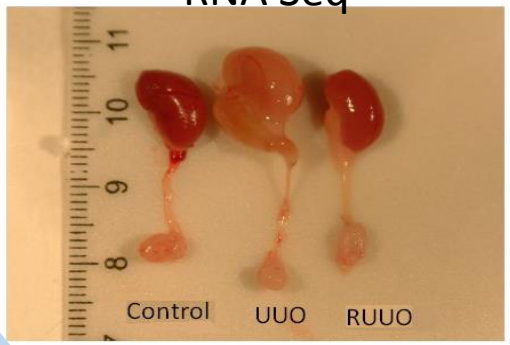


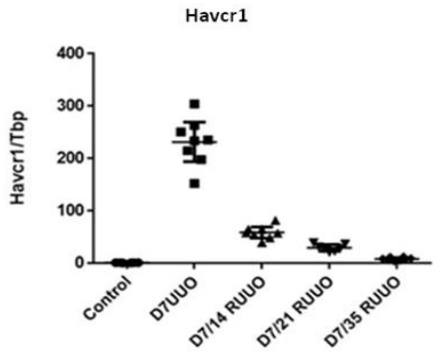
miRNA as mediators of repair?

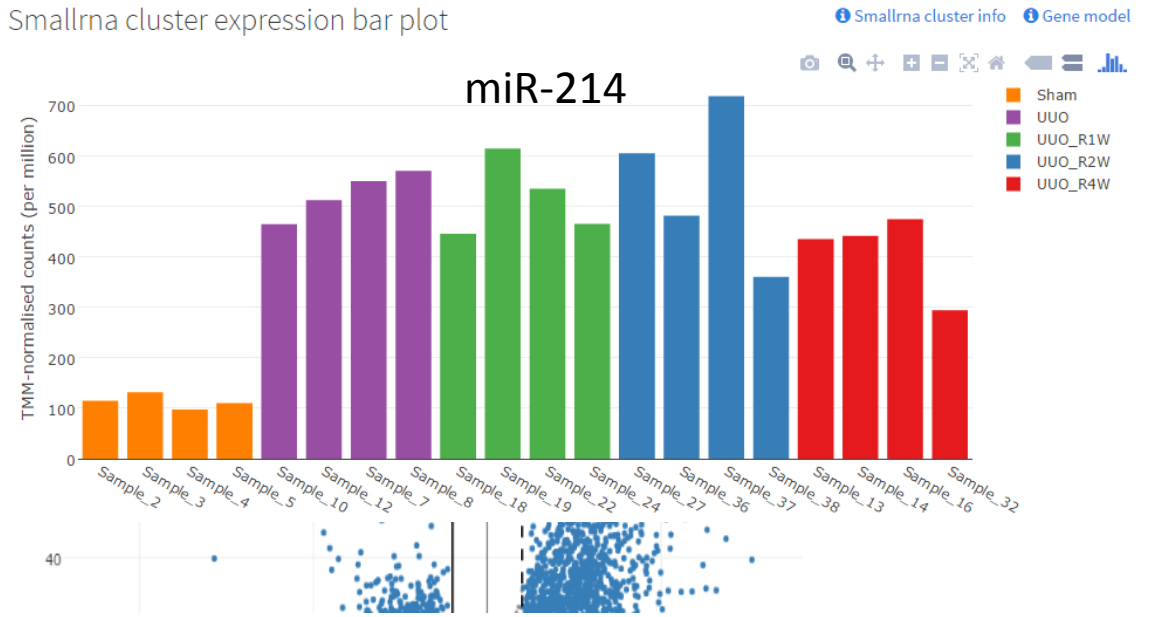
Reversible Unilateral ureteric obstruction model (rUUO)

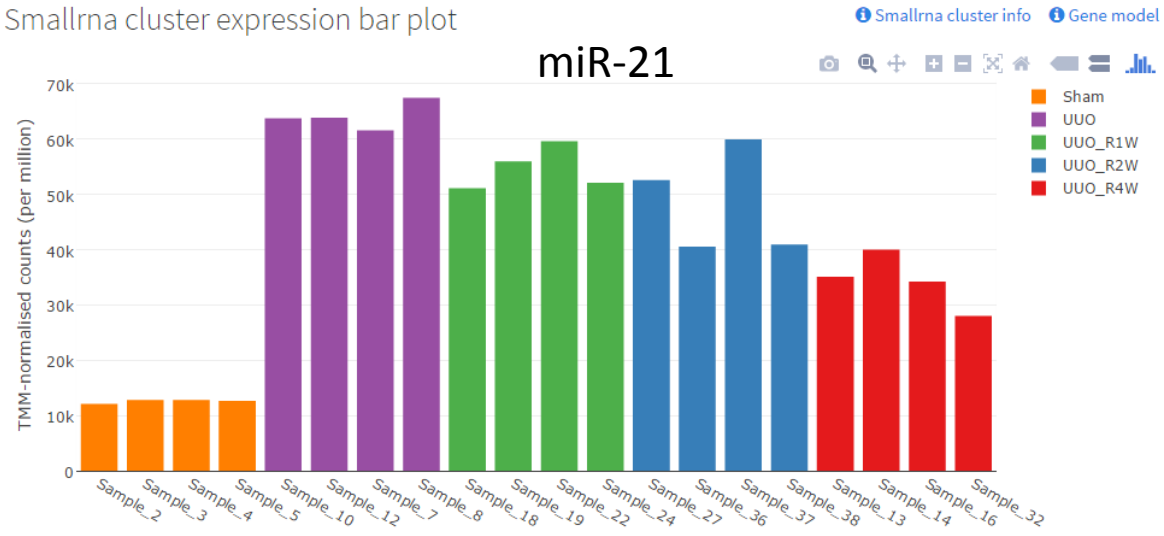


RNA Seq





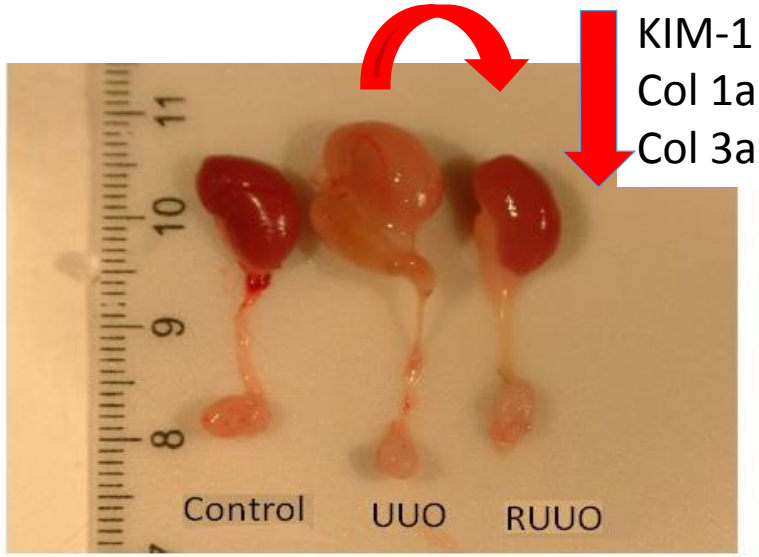


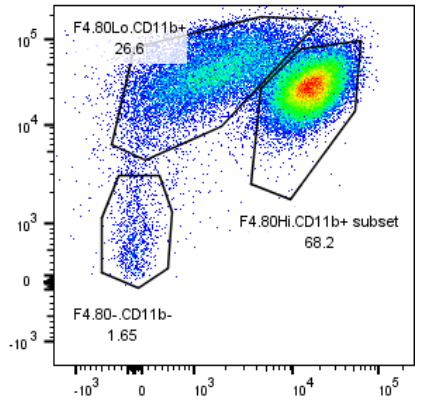


Picture – Spike Clay; https://www.jove.com/video/52559/a-murine-model-irreversible-reversible-unilateral-ureteric

miRNA as mediators of repair?

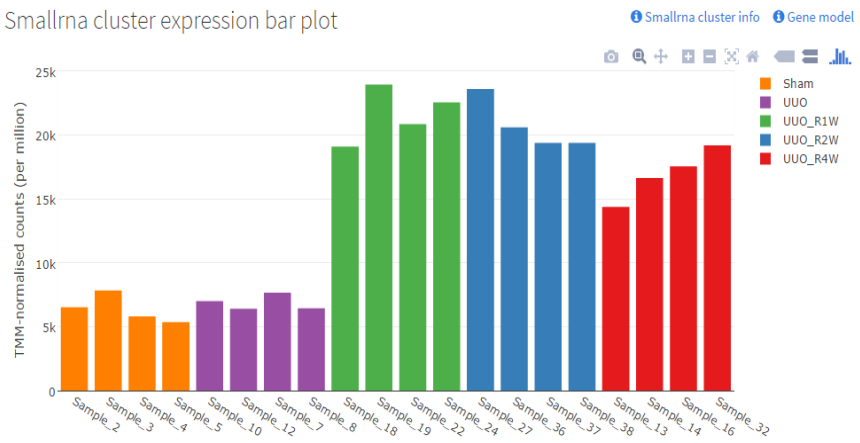
Cell type ?

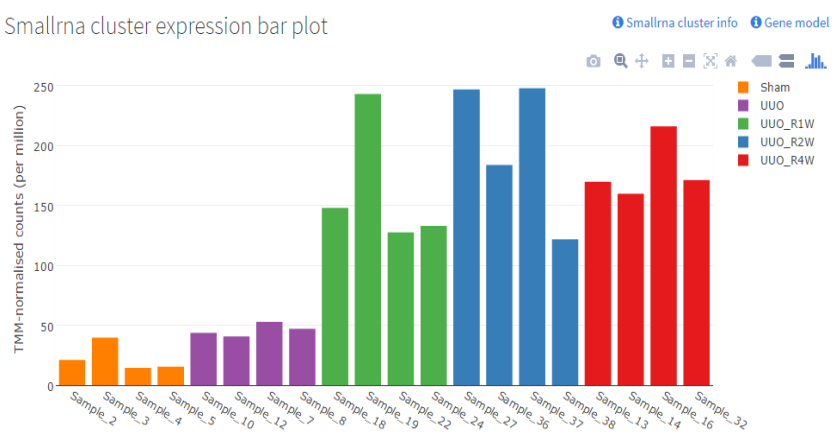


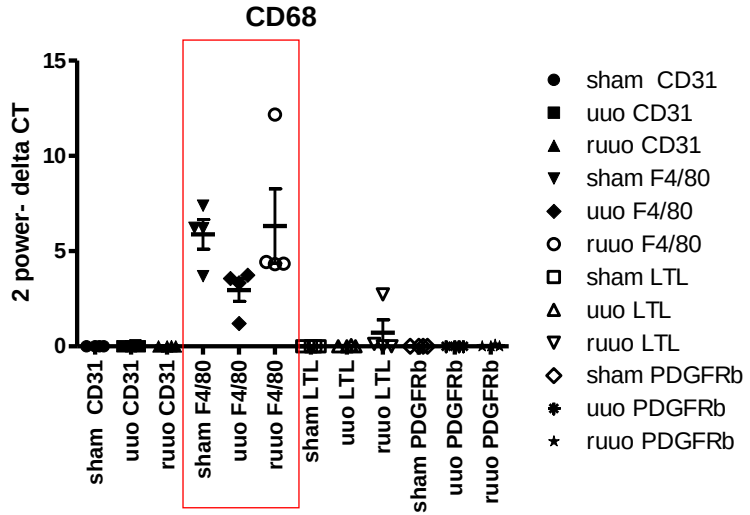


Kidney FACS

- Endothelial cells
- PDGFBR+
- Proximal Tubules
- Gli +ve
- Myeloid cells e.g. Macrophages





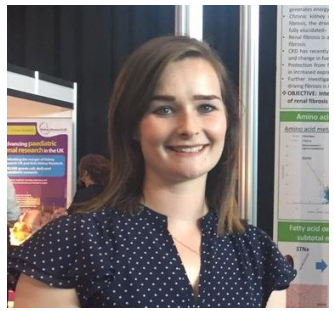
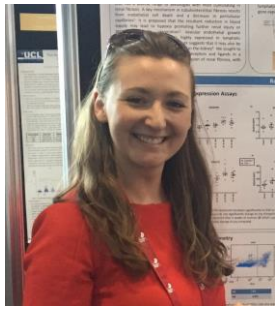


Acknowledgements

Team Denby






Renal and Transplant Patients of Glasgow and Edinburgh.

Liz, Hayley, Deborah, Rowan – Clinical Research Nurses

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Carolynn Cairns
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