Medizinische Hochschule Hannover

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Source and function of miR-17 in murine kidney ischemia-reperfusion injury

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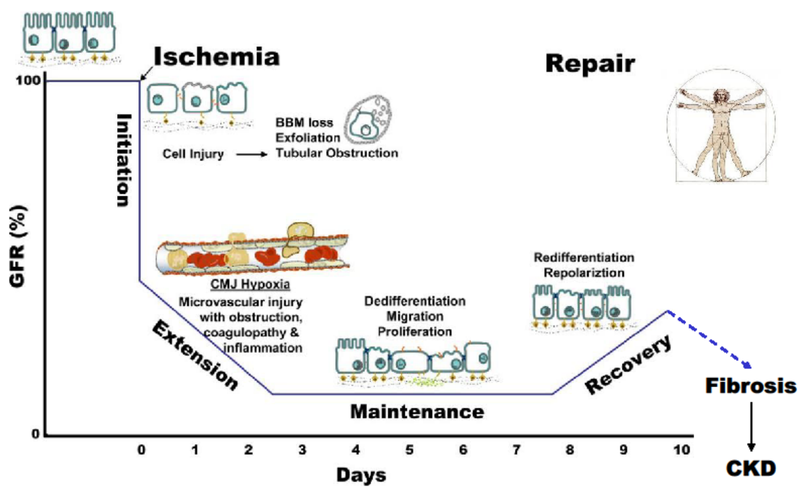
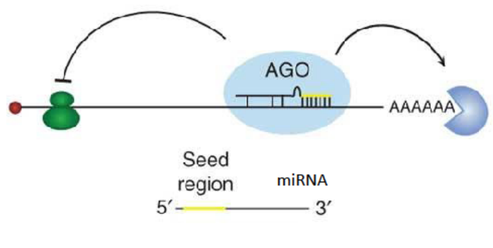
Background

- ischemia-reperfusion (I/R) → main cause of acute kidney injury (AKI)
- renal replacement therapy is mainly supportive → alternative therapeutic approaches are needed.

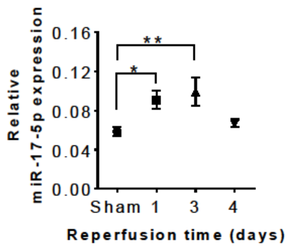


(Basile DP, et al. *Compr Physiol.* 2012)

- MicroRNAs (miRNA) → regulate gene expression
- We showed, that renal expression of the anti-apoptotic and pro-proliferative miR-17-5p increases after ischemic AKI:



Obad S, et al. *Nat Genet.* 2011



Kaucsár T, et al. *Nucleic Acid Ther.* 2013

- AIMS: → **source** (cell-sorting) and → **role** (functional analysis) of miR-17-5p expression in ischemia induced AKI.

Conclusions

Cell sorting:

- miR-17-5p is activated in early tubular response to renal I/R injury
- Injured tubular cells overexpress miR-17-5p only later, 7 days after ischemia

Functional analysis:

- miR-17-5p antagonism impaired renal regeneration, 3 days after ischemia
- I/R induced fibrosis is more pronounced after miR-17-5p antagonism
- miR-17-5p mimicry could be beneficial in I/R induced AKI and fibrosis

Acknowledgements

- Cell Sorting Core Facility of the Hannover Medical School
- Else-Kröner-Fresenius Foundation
- US NIH Research grant #R03 TW07069
- Hungarian Scientific Research Fund (OTKA: K81972, NF69278)
- Health Science Council (ETT: 011-07/2009)
- Catholic Academic Exchange Service (KAAD)

Abbreviations

LNA: Locked Nucleic Acid;
LTL: Lotus Tetragonolobus Lectin – proximal tubular marker;
KIM1: Kidney Injury Molecule-1;
FITC: Fluorescein isothiocyanate;
PE: Phycoerythrin;
APC: Allophycocyanin;
FN1: fibronectin-1;
COL1a1: alpha-1 type I collagen.

Results

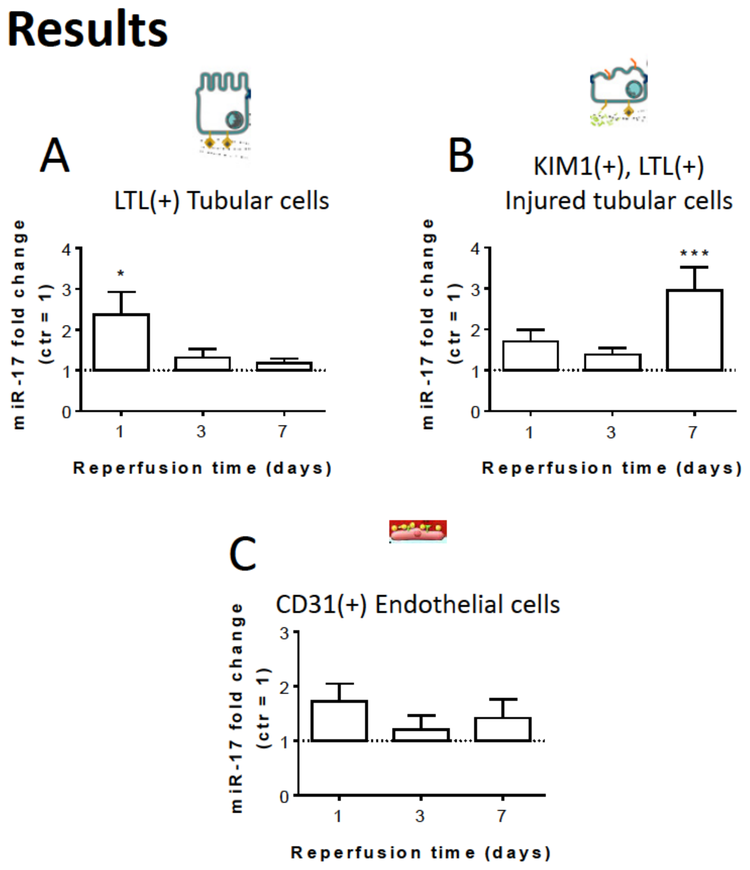


Figure 1. MiR-17-5p fold change in different cellular fractions from the ischemic kidneys.

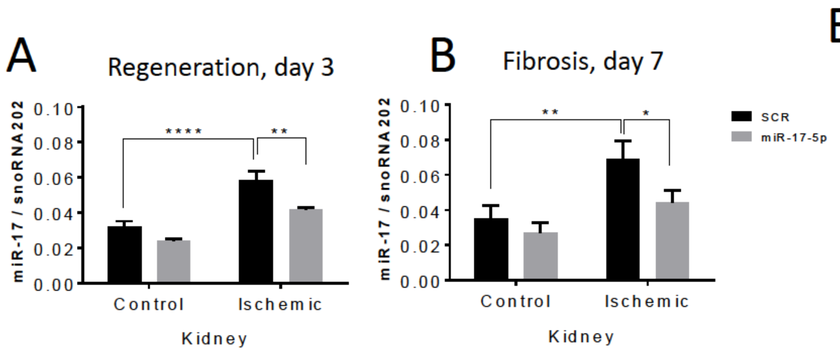


Figure 2. Anti-miR-17-5p LNA downregulated miR-17-5p expression in the ischemic kidneys to 71% and 64% in the regeneration (A) and fibrotic (B) model, respectively.

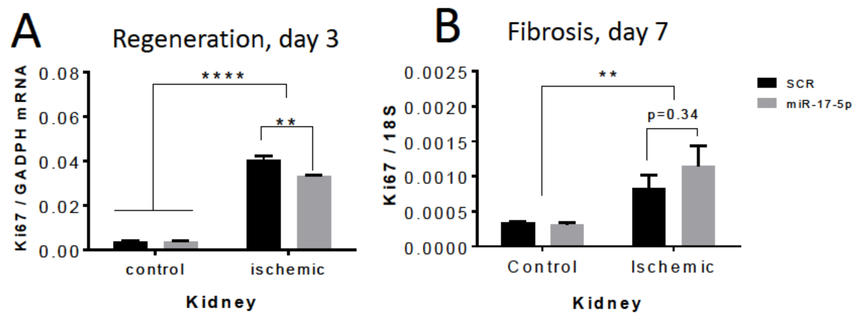
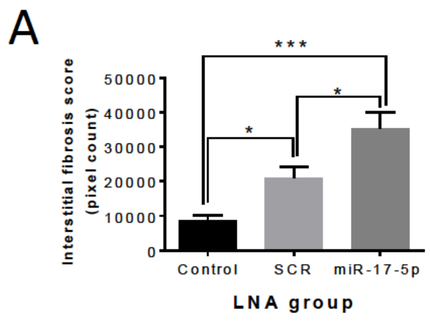


Figure 3. Ki67 mRNA expression change after kidney ischemia and miR-17-5p antagonsim in the regeneration (A) and fibrosis (B) model.
Though plasma urea and KIM1 mRNA levels were increased after ischemia, they were not affected by miR-17-5p antagonism.



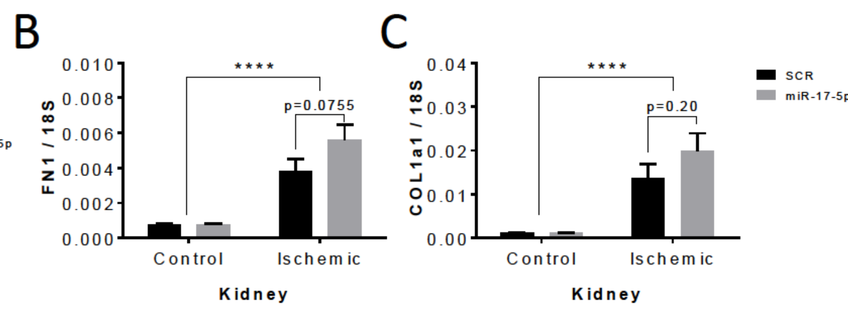
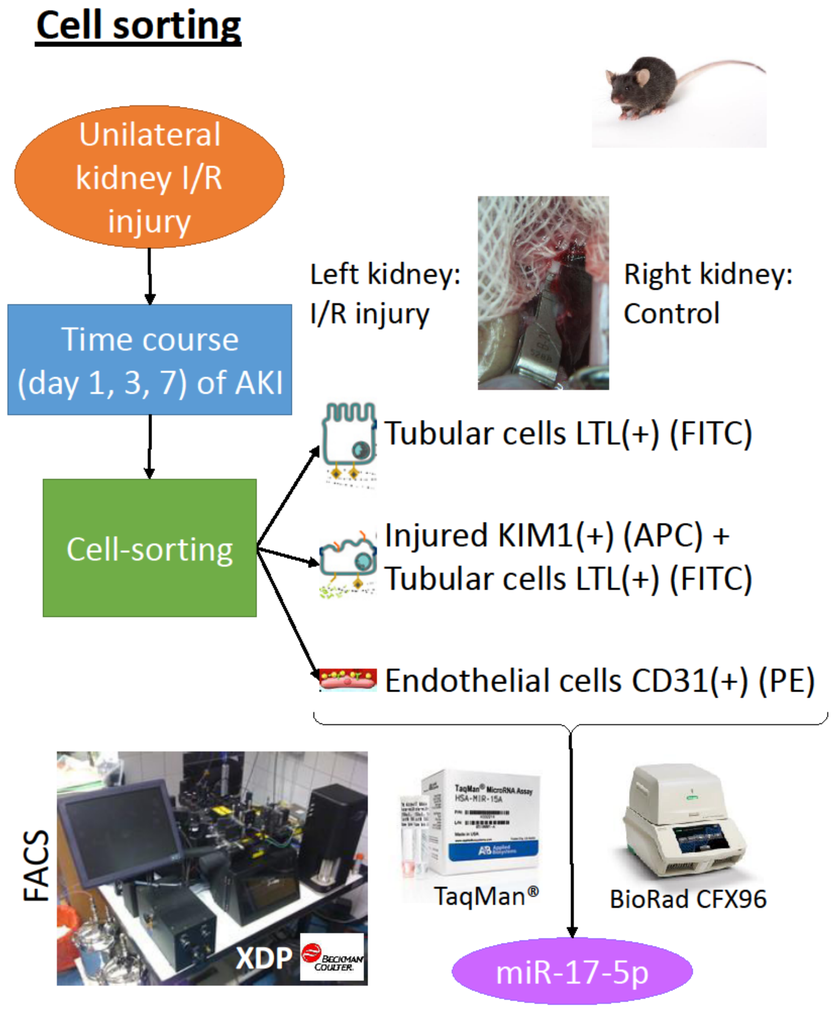


Figure 4. Interstitial fibrosis score (A) and fibrotic gene-upregulation (B, C) in kidneys 7 days after I/R injury (fibrosis model) and miR-17-5p antagonsim.

Methods

Cell sorting



Functional analysis

Model	Regeneration	Fibrosis
DAY -1: miR-17-5p antagonism	i.p. injection 10 mg/kg LNA + Control LNA: SCR (Scrambled, non-complementary sequence)	
DAY 0: I/R Op.	Unilateral kidney I/R injury (20 min ischemia)	
Contralateral nephrectomy	Yes	No
Kidney function	Plasma urea (daily; enzimatic)	N/A
Harvest	Day 3	Day 7
Efficiency of miR-17-5p antagonism	miR-17-5p (qPCR)	
Tubular injury	KIM1 mRNA (qPCR)	
Proliferation	Ki67 mRNA (qPCR)	
Fibrosis	N/A	Histology (Masson's Trichrome)
		FN1, COL1a1 mRNA (qPCR)

DOI: 10.3252/pso.eu.53era.2016