Urinary microRNAs and Renal Scar in Acute Pyelonephritis

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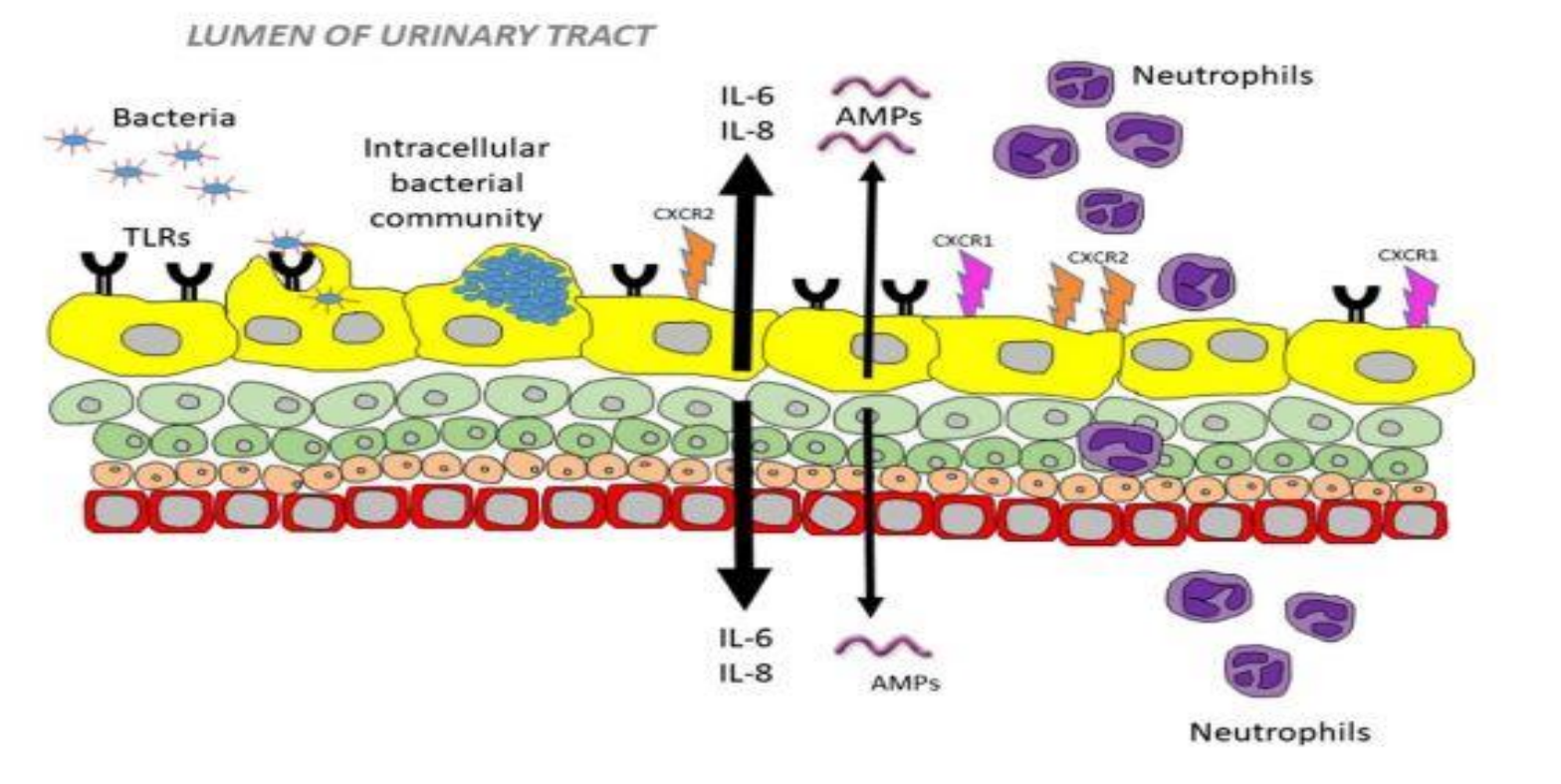
- Can urinary microRNAs be markers to determine infection severity and renal scar formation in pediatric patients with acute pyelonephritis?

Introduction

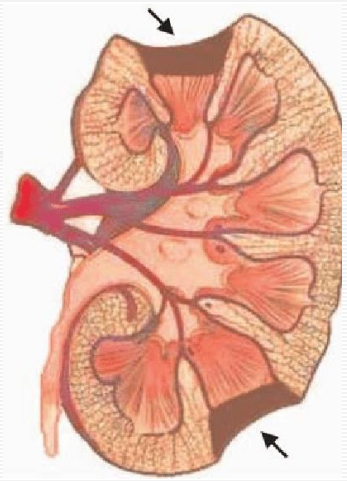
- Acute pyelonephritis is one of the most serious bacterial diseases in childhood.
- Affects the renal parenchyma and is usually associated with systemic signs of inflammation .



- Host and microorganism- related factors play an important role in this pathogenesis. Especially the innate immune system of the host is important in inflammatory responses

Asymptomatic bacteriuria and lower urinary tract infection

Acute pyelonephritis with systemic immune system activation



The innate immune system responds rapidly and firstly to the microorganism.

If the innate immune system is dysregulated and insufficient, the infection becomes visible with progressive inflammation

Factors affecting the innate immun system

Table 1 Epithelial antimicrobial peptides and proteins produced in the urinary tract

Name	Classification	Cellular source	Biological relevance
α- and β-defensins	AMP	Bladder urothelium and kidney intercalated and principal cells	*Defb1*$^{-/-}$ mice exhibit increased rates of spontaneous bacteriuria [42].
Cathelicidin	AMP	Bladder irothelium and kidney intercalated cells	Increased kidney UPEC burden in *Camp*$^{-/-}$ mice after experimental UTI [43].
Ribonucleases	AMP	Bladder urothelium and kidney intercalated cells	RNase 4 and 7 neutralization promotes UPEC growth in human urine [44, 45].
Lipocalin 2	Siderophore	Bladder urothelium and kidney intercalated cells	Increased bladder UPEC burden in *Lcn2*$^{-/-}$ mice after experimental UTI [46, 47].
Hepcidin	Iron regulation	Nephron and collecting duct	Increased bladder/kidney UPEC burden in mice after experimental UTI [48, 49].
Uromodulin	Glycoprotein	Loop of Henle	Increased bladder UPEC burden in *Thp*$^{-/-}$ mice after experimental UTI [50].

Ching C et al. Innate immunity and urinary tract infection. Pediatric Nephrology (2020) 35:1183–1192

Factors affecting the innate immun system

Table 2 Cellular effectors of innate immunity during UTI

Cell	Mechanism
Urothelial cells	•Detect UPEC by expressing PRRs [58] •UPEC expulsion [59] •Secrete AMP [5] •Release chemokines that promote neutrophil chemotaxis [38] •Exfoliation and regeneration [60, 61]
Intercalated cells	•Detect ascending bacteria in a TLR4 and TLR5 dependent manner [16, 17] •Secrete AMP, some of which are regulated by insulin/PI-3 kinase signaling [5, 44, 46]
Monocyte-derived phagocytes	•Regulate neutrophil recruitment during pyelonephritis [62] •Phagocytose and kill bacteria [62] •Regulated by medullary sodium gradient in an NFAT5-dependent manner [62]
Neutrophils	•Phagocytosis and bactericidal activity [63] •Prolonged recruitment and/or activation may promote tissue injury and infection chronicity [38, 39, 64]
Inflammatory monocytes	•Recruited to the infected bladder and kidney from the peripheral blood and bone marrow •Secrete TNF-α, which indirectly promotes neutrophil recruitment during acute cystitis [65]
Resident macrophages	•Secrete Cxcl2 in a TNF-α dependent manner, which promotes secretion of matrix metalloproteinase essential for neutrophil transmigration across the urothelium [65]
Natural killer cells	•Promote bacterial clearance by secreting TNF-α [66]
Natural killer T cells	•Promote bacterial clearance by cytokine secretion in response to activating glycolipids [67]
Mast cells	•Recruit neutrophils by producing TNF-α [68, 69] •Release cytolytic enzymes that trigger exfoliation of bladder umbrella cells [60]

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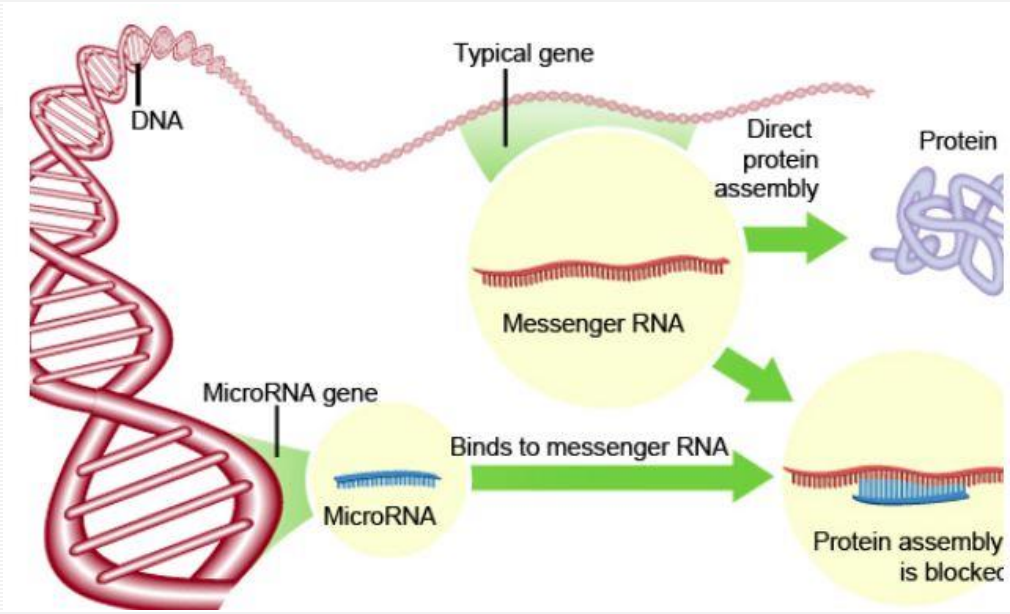
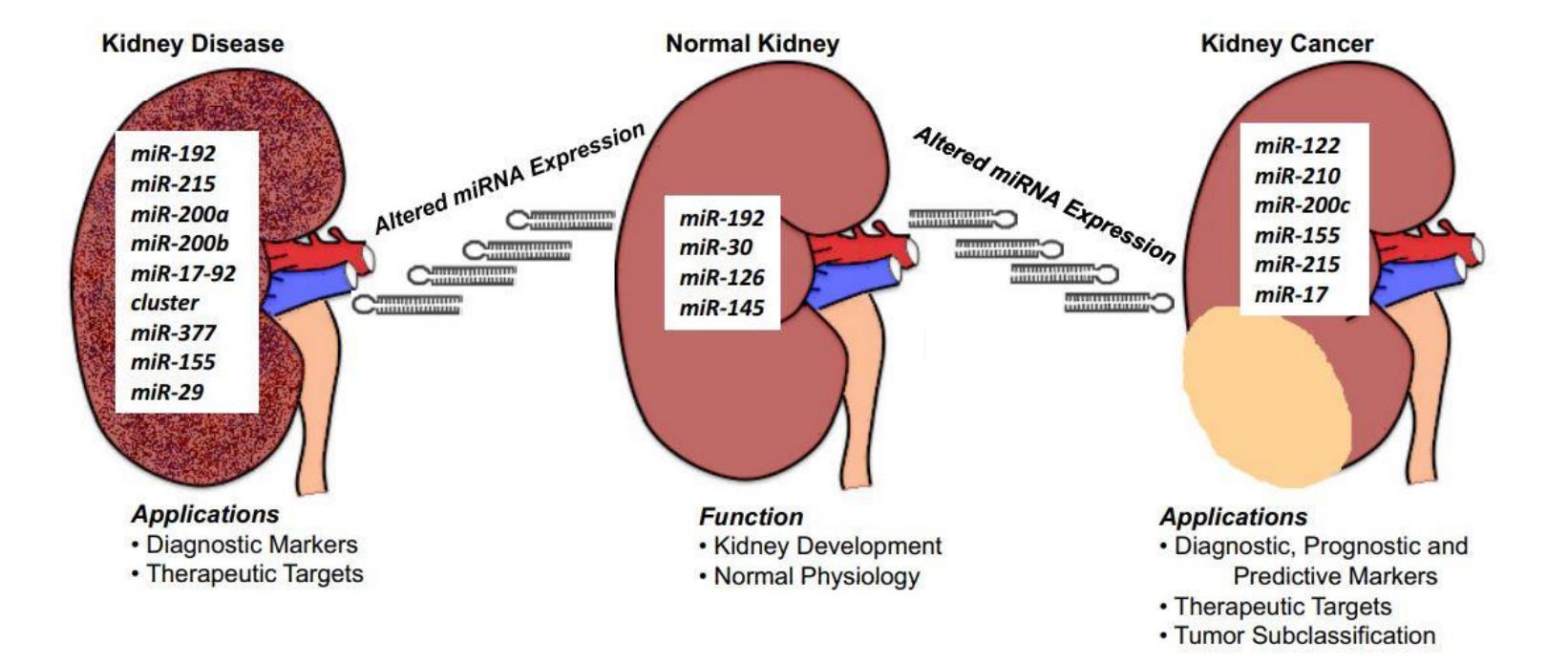
What is a MicroRNA?



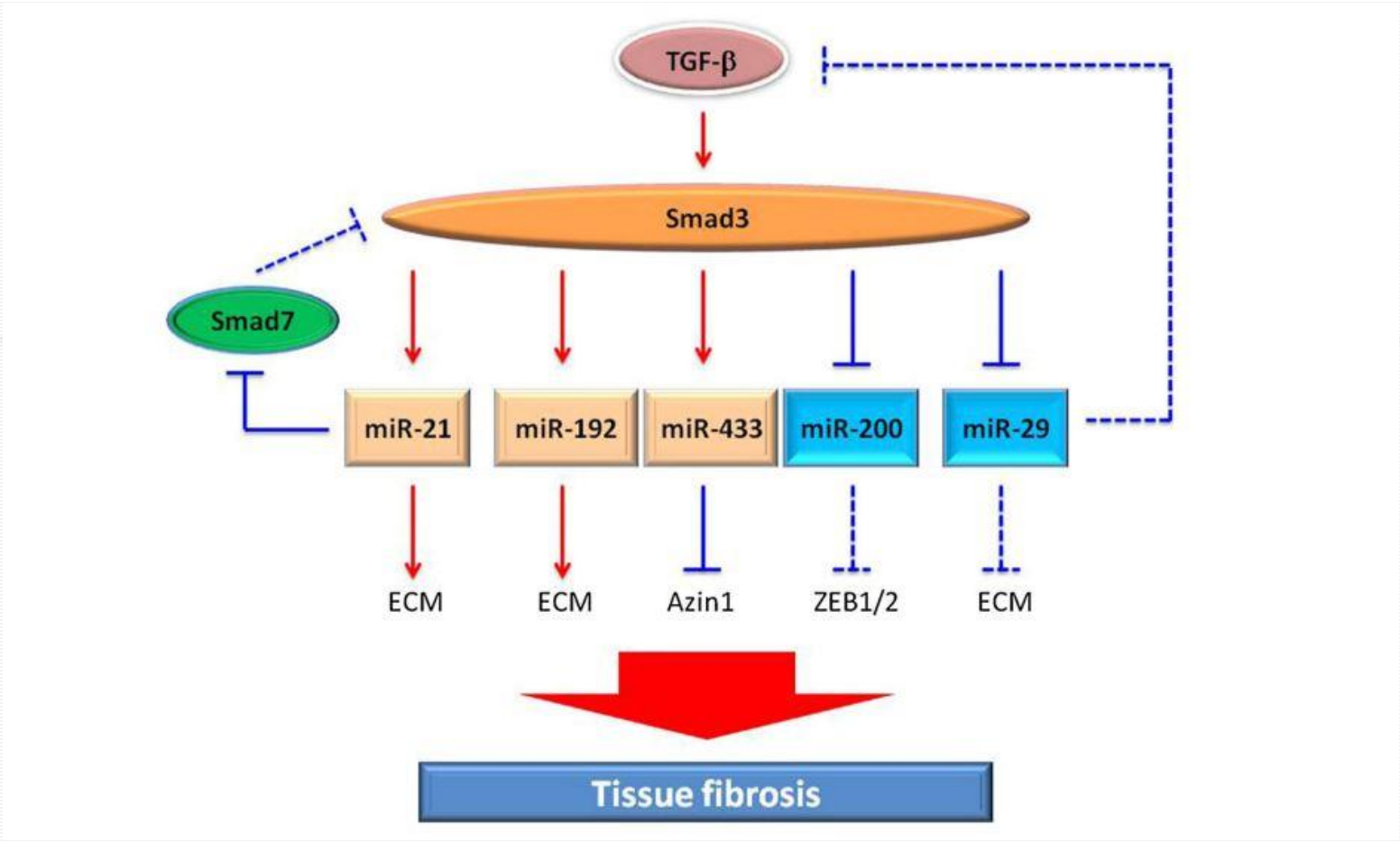
- Short, non-coding genetic material that regulates gene expression and play a role in cellular and developmental processes
- MicroRNA are implicated in diseases like cancer, multiple sclerosis, Parkinson's disease and Alzheimer's disease

MicroRNAs & Kidney



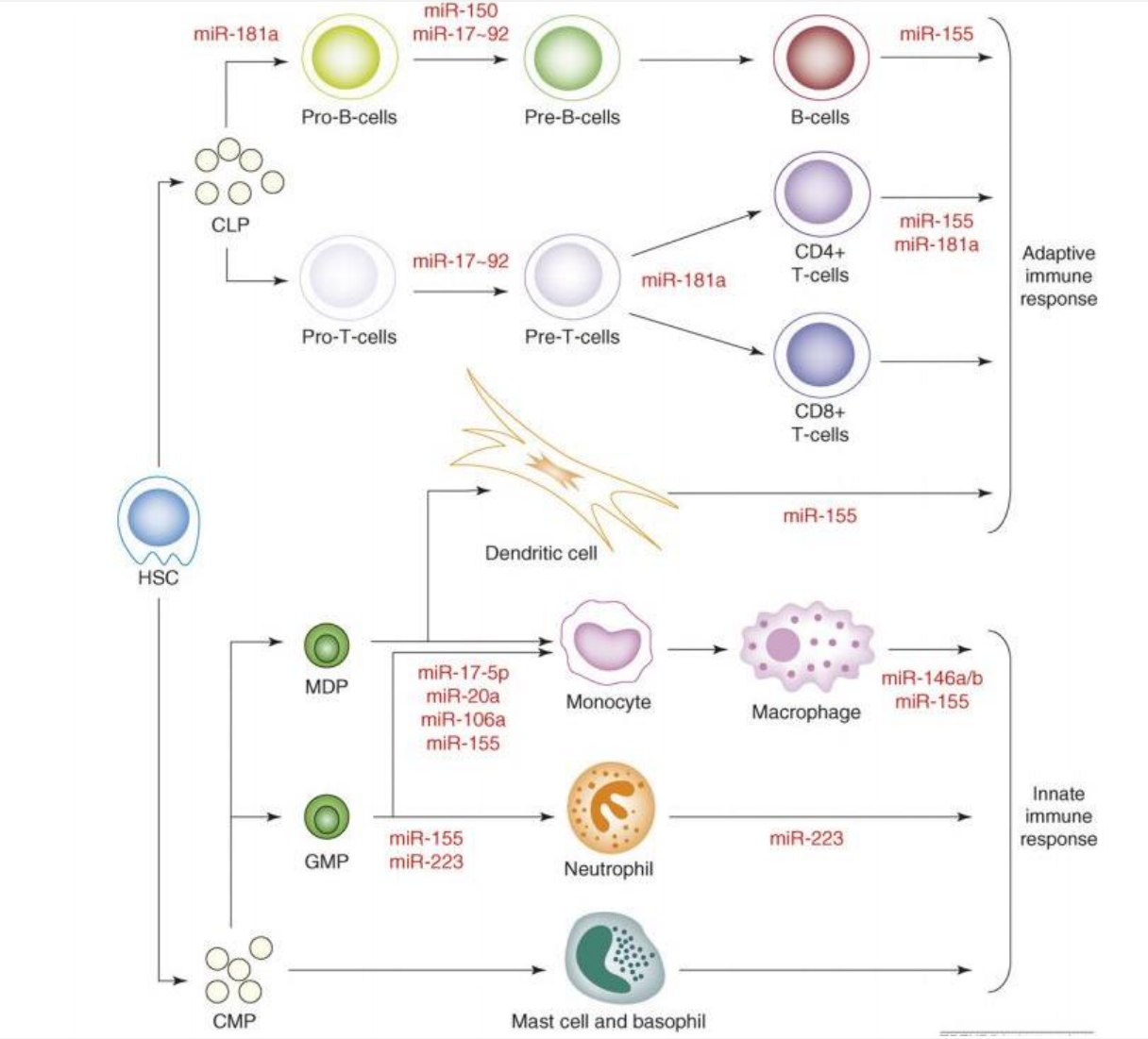
Altered miRNA expression can result in kidney diseases, including diabetic nephritis, hypertension, glomerulonephritis, and cancer.

The detection of exons containing miRNAs in the urine suggests that they are also released from different parts of the nephron.



It is known that miRNAs are highly expressed in the kidneys and play a role as effectors of TGF-beta.

MicroRNAs &Immun system



Some of those are…

MicroRNA	Effect or role
miR 21	Interstitial fibrosis and tubular damage in the kidney
miR132	Anti-inflammatory role
miR 223	Myeloid precursors proliferation, regulating functions of neutrophils
miR155	Proinflammatory role
miR27a	Effects on TLR4 reduce TLR4-associated ischemia reperfusion injury
miR146b-5p	Decreases IL6 and IL8 levels

Material & Method

Patient selection

- The study will be carried out with a prospective evaluation of children aged 0-18 years diagnosed with acute pyelonephritis.
- Midstream urine samples will be obtained in children > 2 years of age or children with toilet training, and using a catheter in infants
- Must be an age and gender-matched healthy control group
- DMSA should be administered to the patient group for renal scar development 4 months after infection.

Material & Method

Patient selection

Exclusion criteria:

- meningomyelocele,
- renal hypo-dysplasia,
- cystic kidney disease,
- chronic diseases (diabetes mellitus, immunodeficiency, epilepsy, bronchial asthma, chronic renal failure, hypertension),
- obesity or malnutrition,
- permanent urinary catheter, urinary stent or nephrostomy catheter, and neurogenic bladder

will be excluded from the study.

Material & Method

Collection of samples

- Min 10 ml urine sample (mid-stream / catheter)
- Collected urine samples will be centrifuged at 2500 rpm for 5 minutes
- Must be stored at -80 ^{0}C without delay

Material & Method

Isolation of RNA

- RNA will be isolated from urine samples obtained from both groups.
- The samples will be treated with DNase I against DNA contamination.
- RNA concentration and purity will be evaluated by spectrophotometer.
- Determination of expression differences of miR21, miR-132, miR-223 and miR-155, miR27a and miR-146b-5p by Quantitative Real Time PCR (qRT-PCR)

Conclusion

- After the discovery of miRNAs, information about their function and potential uses is rapidly increasing.
- They are potential new biological markers, and play a role in the pathogenesis of many diseases.
- Demonstration of their relationship with urinary miRNA and renal scar development in childhood will a step for the new miRNA-based therapies in the future.

Thank you for your attention…