肾脏疾病诊断和疗效预测的生物标志物

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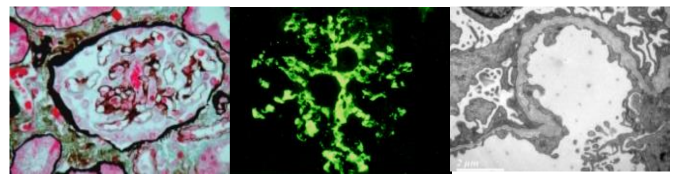
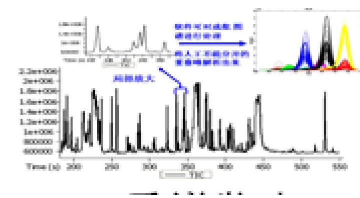
全军肾脏病研究所

肾脏疾病的诊断和治疗





临床综合症诊断

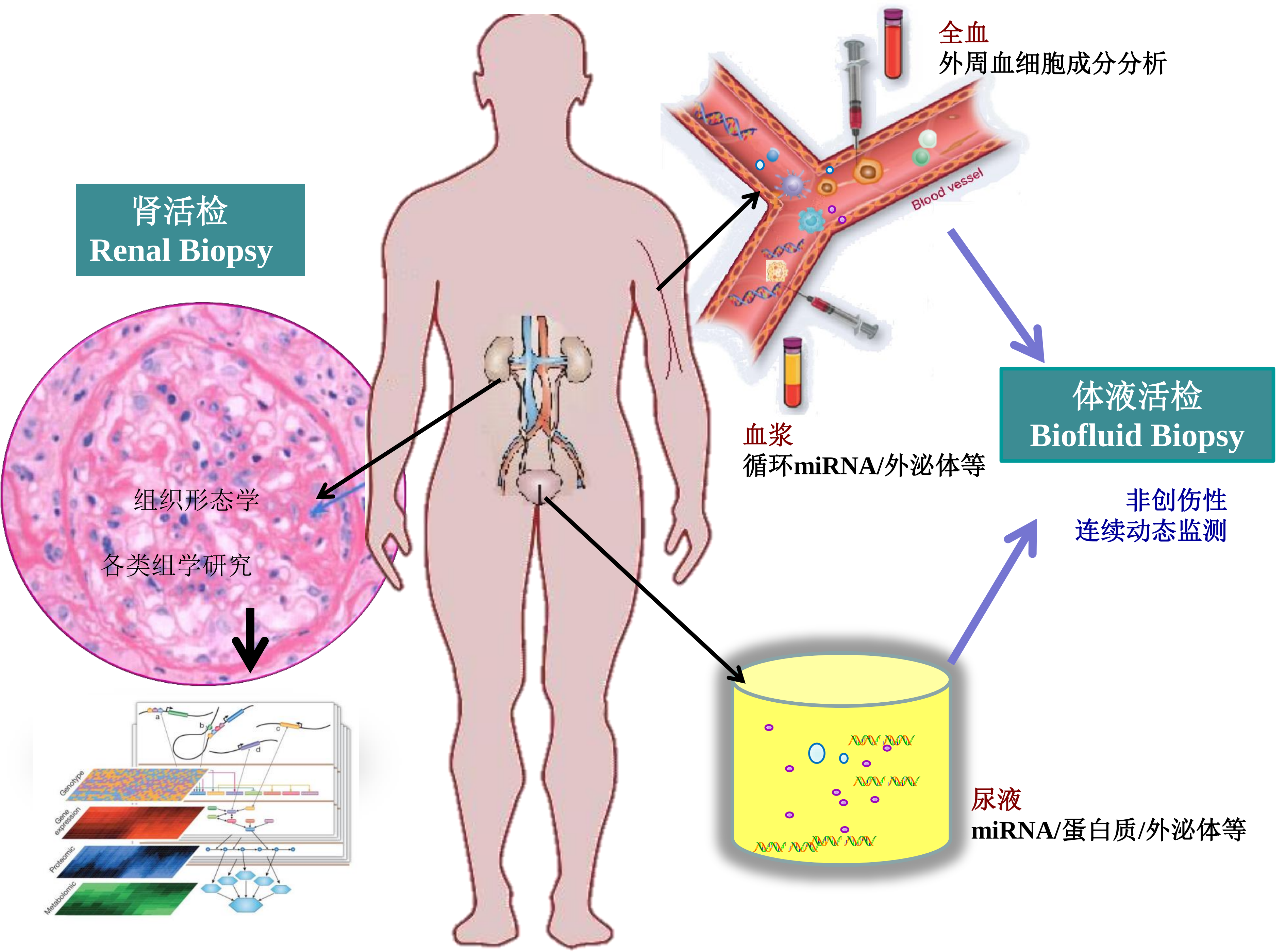
组织形态学诊断

分子表型诊断

病因诊断，分子分型，基于发病机制的靶向治疗

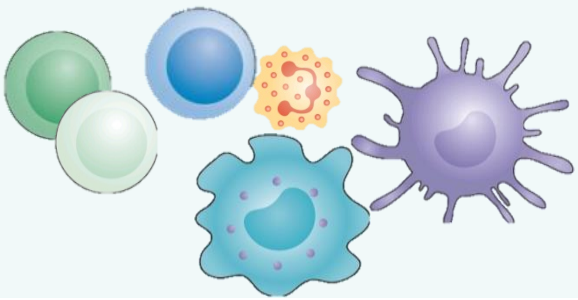
精准医学

（ Precision Medicine）

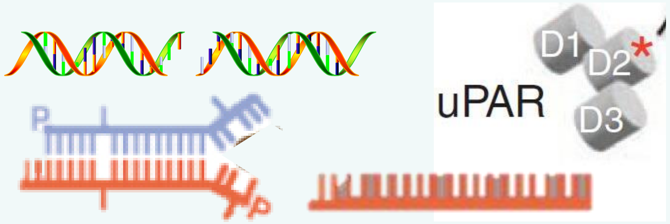

全血
外周血细胞成分分析
Blood vessel
肾活检
Renal Biopsy
组织形态学
各类组学研究
体液活检
Biofluid Biopsy
血浆
循环miRNA/外泌体等
非创伤性
连续动态监测
尿液
miRNA/蛋白质/外泌体等
Genotype
Gene expression
Proteomic
Metabolomic

体液中的生物标志物
Biofluid-based Biopsies

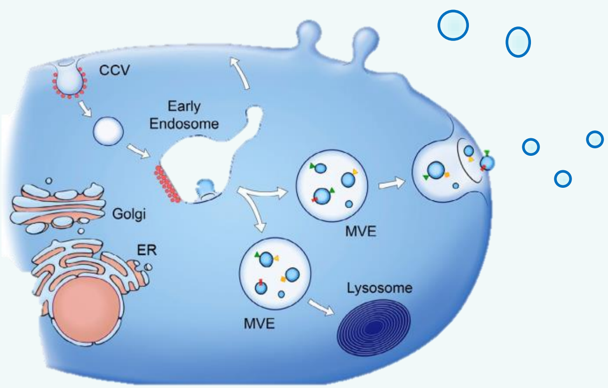
细胞：例如循环肿瘤细胞，髓系来源抑制性细胞，调节性T细胞，T细胞，B细胞



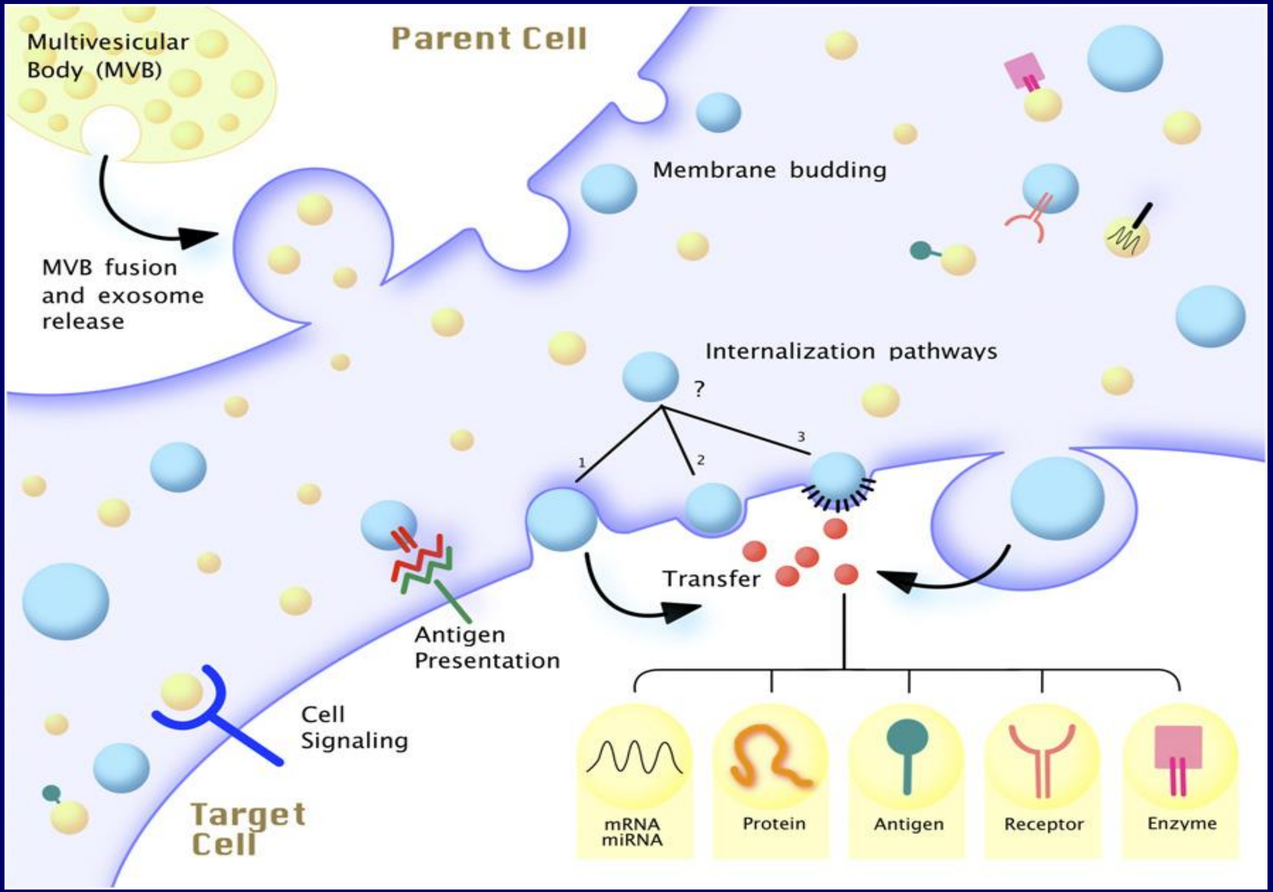
循环中的核苷酸，microRNA 蛋白质，酶，膜受体等



外泌体(Exosome)：装载miRNA、DNA膜受体、酶等



Bartel (2004). Cell ; Stoorvogel (2013). Journal of Cell Biology



miRNAs对维持肾脏的生理和病理过程至关重要。循环和尿液miRNAs可能作为一种无创的生物标志物帮助诊断和监测疾病。

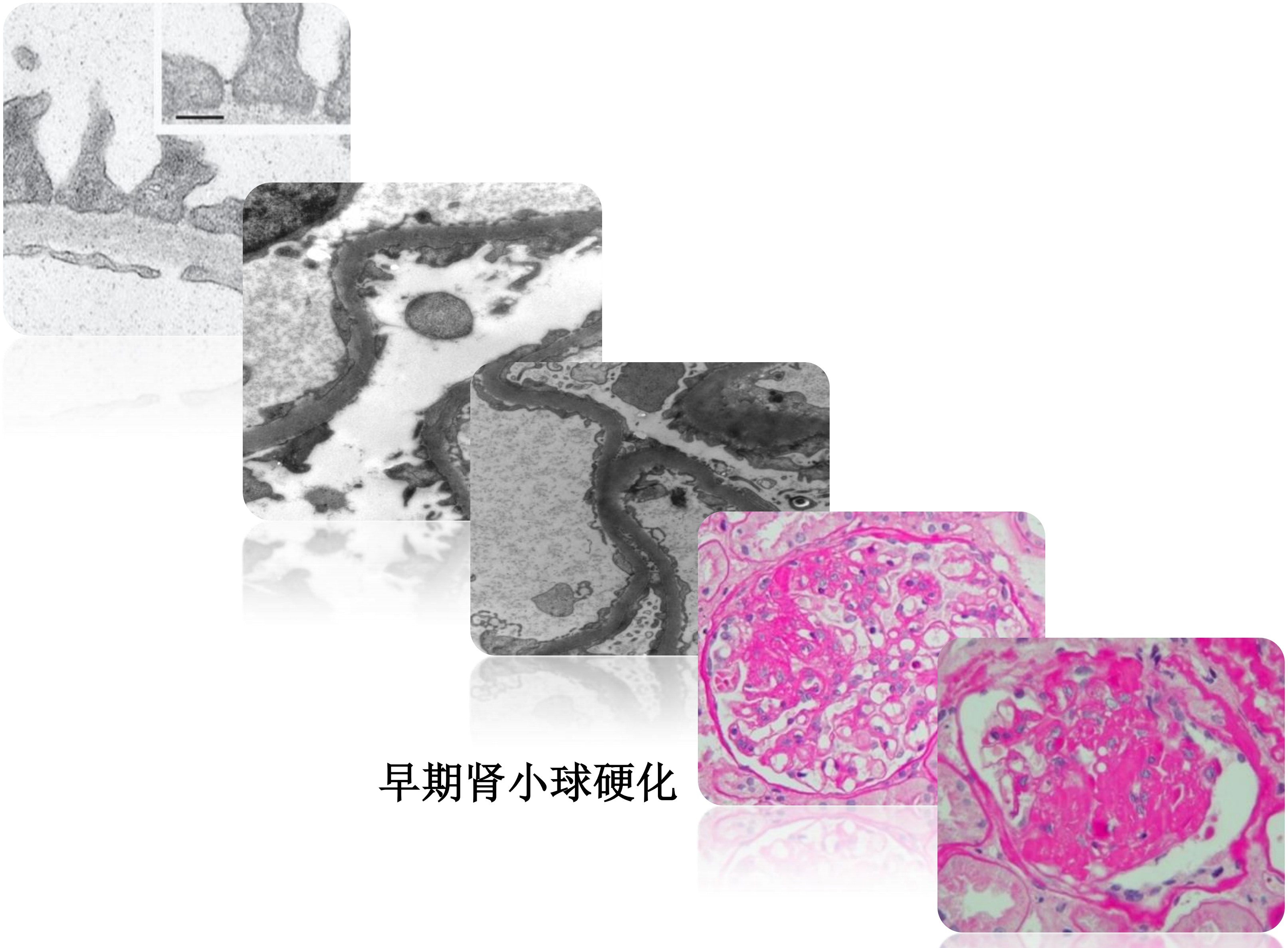
J Am Soc Nephrol 27: 2015

原发性肾小球疾病
(18813 cases, 2000-2010，南京)

	n	%
IgAN	8580	45.61
FSGS	2400	12.87
MN	2422	12.76
IgMN	240	1.28
MPGN	242	1.29
EnPGN	213	1.13

FSGS accounts for 20%–25% of adult patients undergoing biopsy for evaluation of idiopathic GN in western countries.

足细胞损伤：FSGS的中心环节



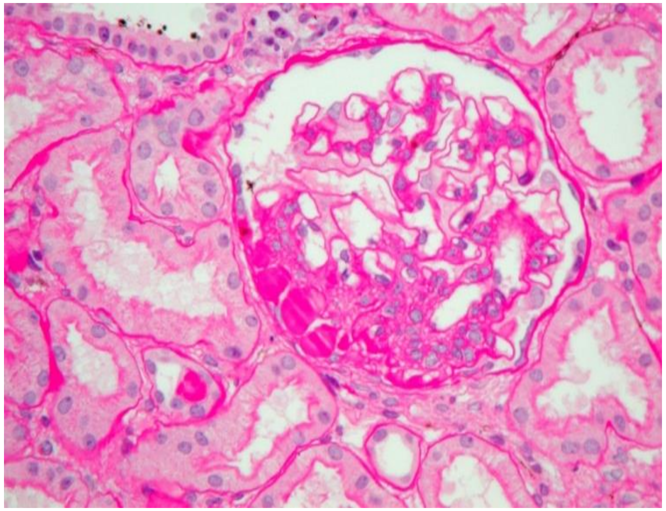
早期肾小球硬化

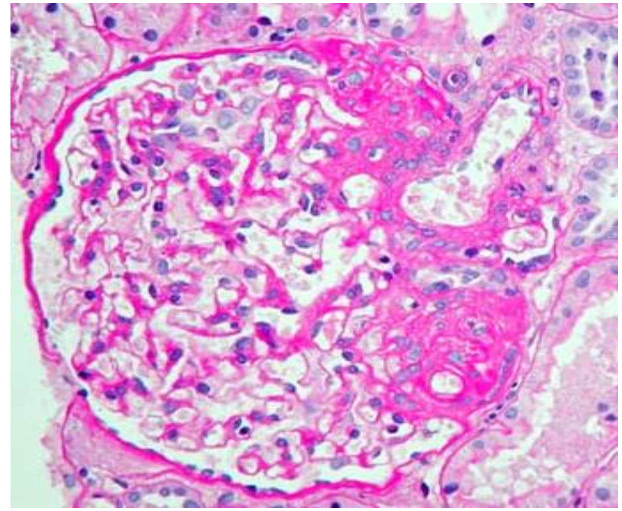
FSGS 病因分类

Primary alterations of glomerular epithelial cell
Primary (idiopathic) FSGS
Viral diseases (HIV-associated nephropathy, parvovirus B19, hepatitis C)
Drugs (heroin, pamidronate, lithium, anabolic steroids)
Genetic disorders (podocin, α-actinin 4, transient receptor potential action channel 6)
Familial
Sporadic
Secondary to reduced nephron mass/glomerular adaptations
Reflux nephropathy
Renal dysplasia
Oligomeganephronia
Obesity-related glomerulopathy
Sickle cell disease
Primary glomerular diseases
Secondary to focal proliferative GN
Secondary to hereditary nephropathies (Alport syndrome)

J Am Soc Nephrol 2012;23: 1769–1776

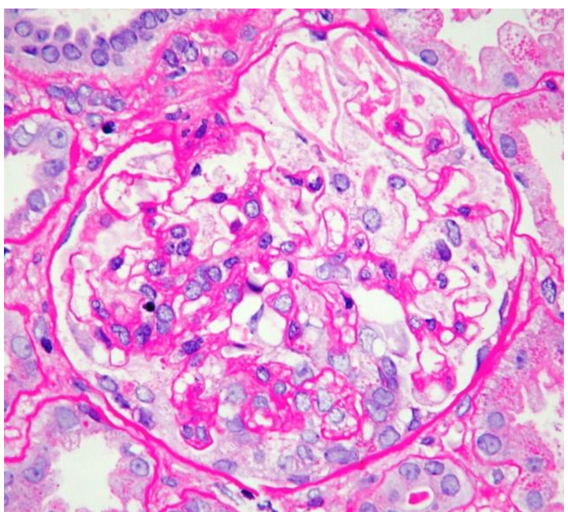
经典型



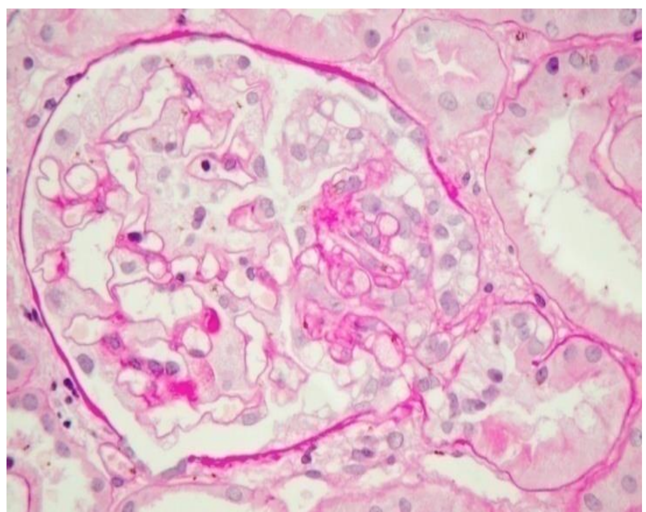


门周型

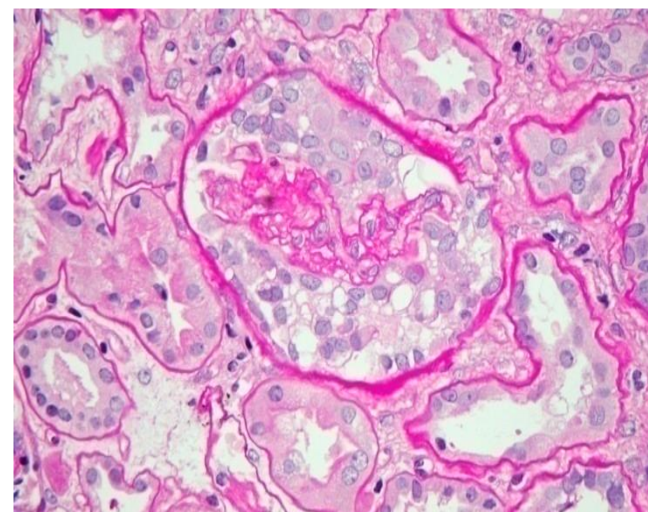
FSGS的病理分型



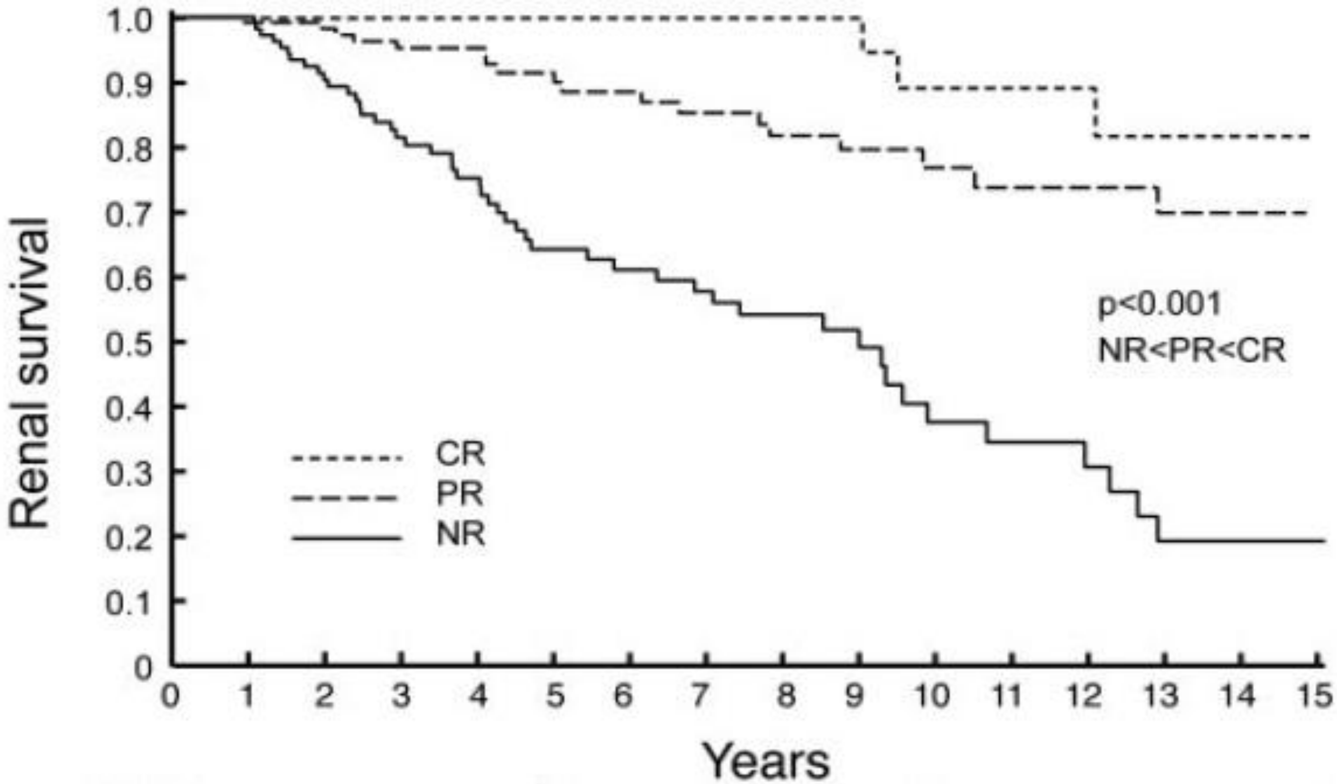
细胞型



顶部型



塌陷型



治疗后完全缓解的患者预后明显优于无缓解的患者，即使部分缓解者也呈现较好的预后。

J Am Soc Nephrol 2005;16: 1061-1068

生物标记物 (Biomarkers)

Detection of disease

Monitor of the disease in

- Severity
- Progression
- Regression

Predict response to therapy

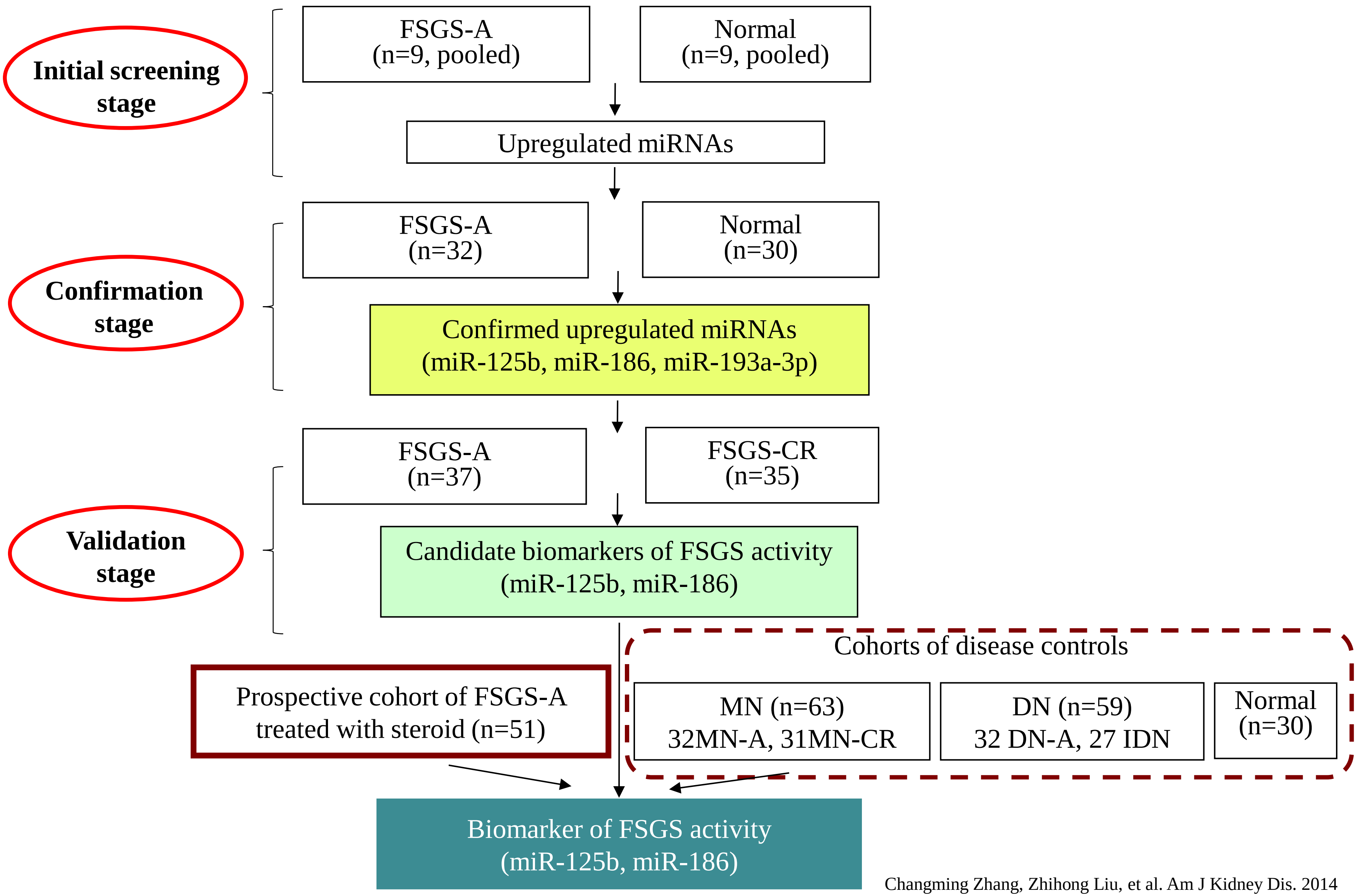
生物标记物 (Biomarkers)

从哪里来？ （Donor）

到哪里去？ （Recipient）

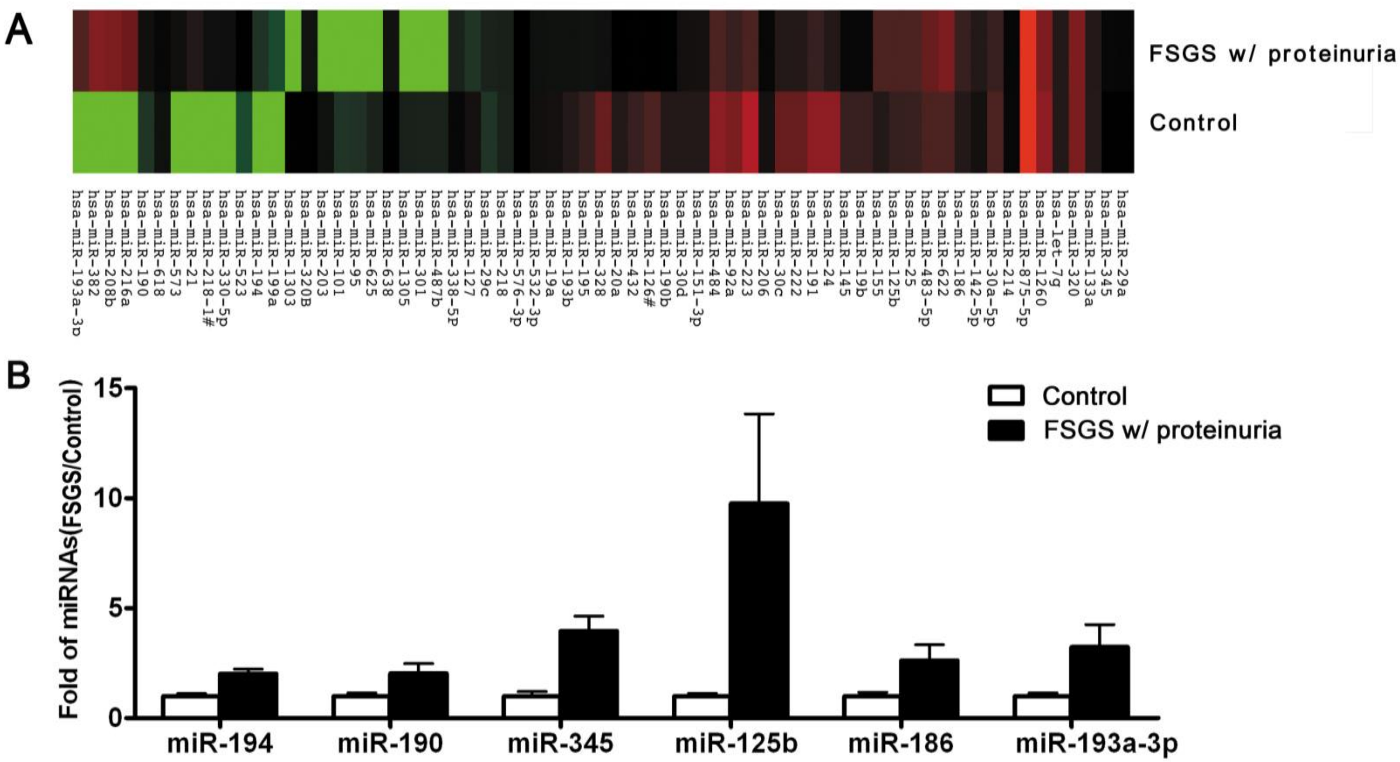
从血浆 miRNAs 中寻找 FSGS标志物

研究技术路线



Changming Zhang, Zhihong Liu, et al. Am J Kidney Dis. 2014

寻找并验证在活动性FSGS患者中升高的血浆miRNAs

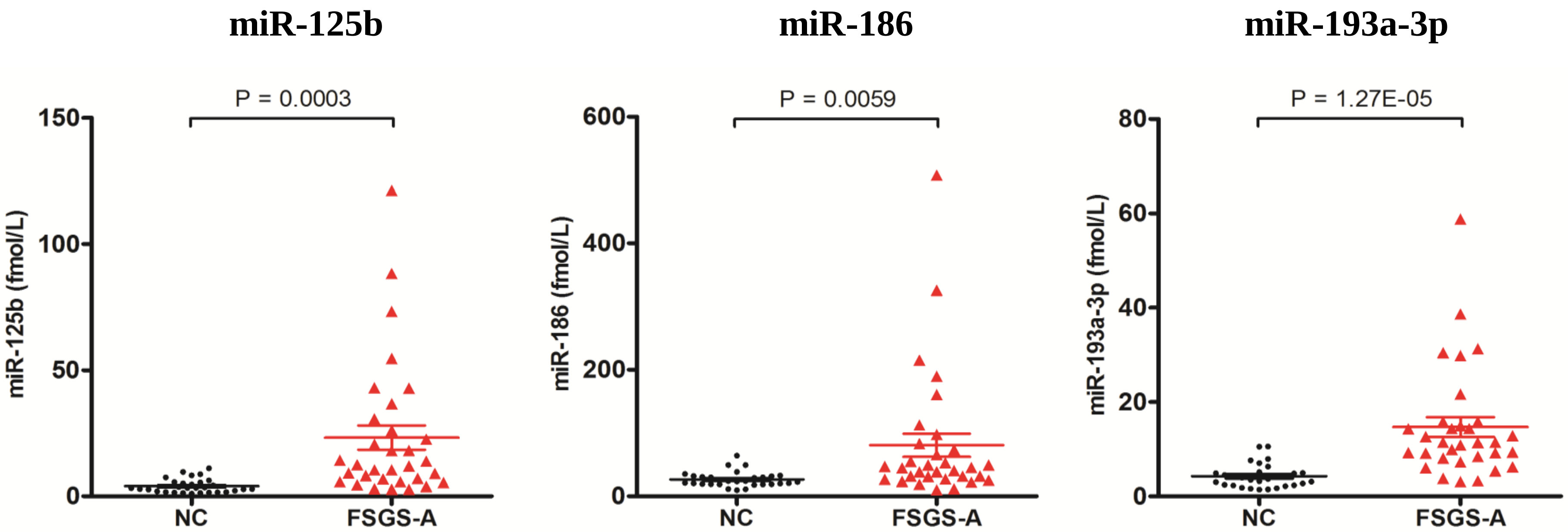


The FSGS with nephrotic proteinuria had a plasma miRNA profile that was different from controls (miRNAs 45)

Individual qRT-PCR analyses confirmed the upregulation of 6 miRNAs in FSGS.

Changming Zhang, Zhihong Liu, et al. Am J Kidney Dis. 2014

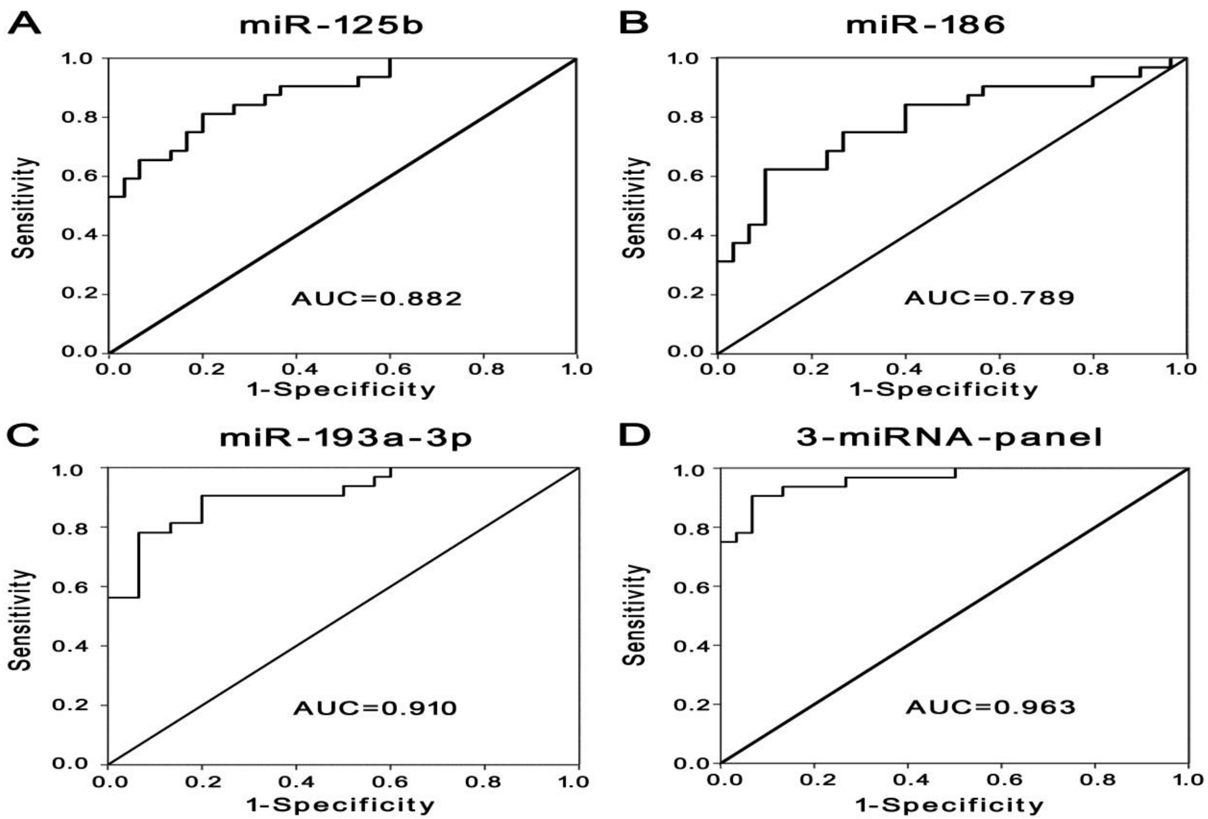
独立队列样本验证中血浆miRNAs的表达



活动性FSGS患者血浆miR-125b, miR-186和miR-193a-3p较正常对照明显升高

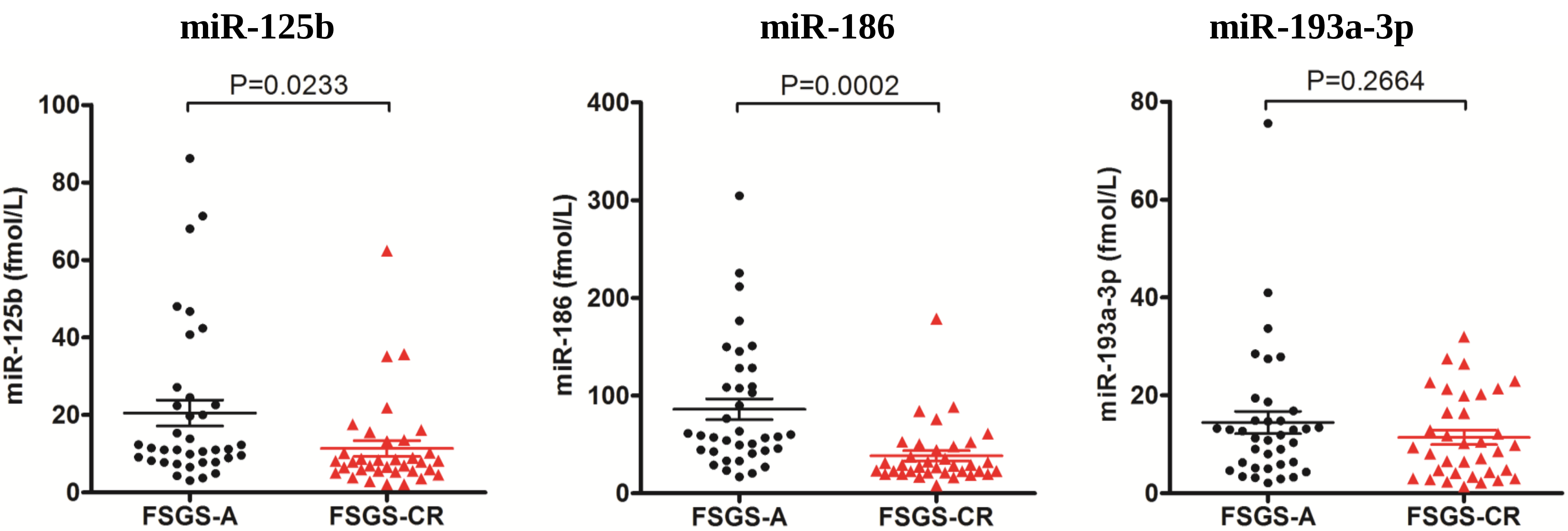
Changming Zhang, Zhihong Liu, et al. Am J Kidney Dis. 2014

血浆miR-125b, miR-186和miR-193a-3p区分活动性FSGS和正常对照的价值



三种miRNAs均可用于区分活动性FSGS和正常对照，三者联合则显示更好的预测效力。

不同疾病状态下FSGS患者血浆miR-125b, miR-186和miR-193a-3p表达



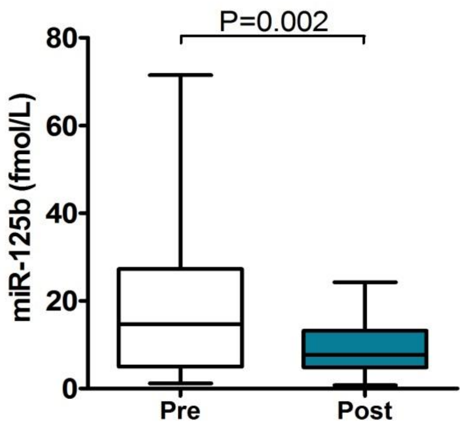
活动性FSGS患者血浆miR-186, miR-125b水平显著高于完全缓解FSGS

Changming Zhang, Zhihong Liu, et al. Am J Kidney Dis. 2014

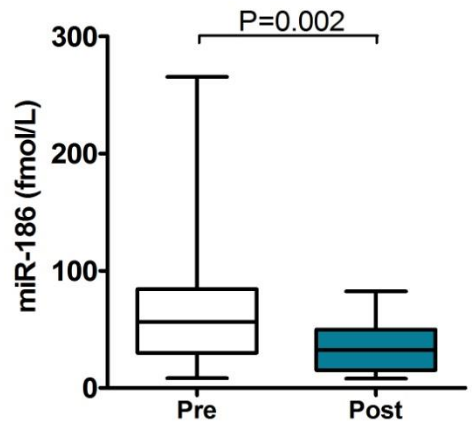
FSGS患者接受激素治疗前后血浆miR-125b, miR-186水平的变化

Steroid-sensitive (n=29)

miR-125b

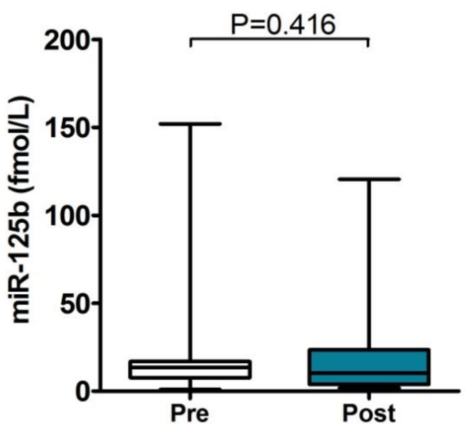


miR-186

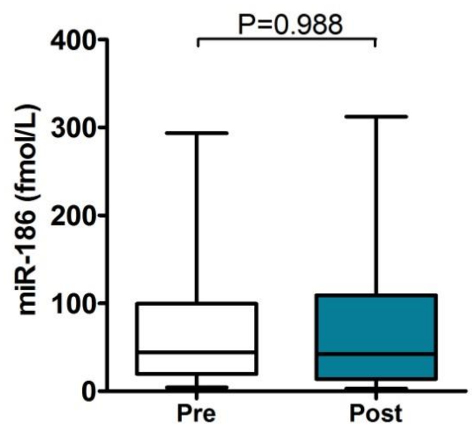


Steroid-resistant (n=21)

miR-125b

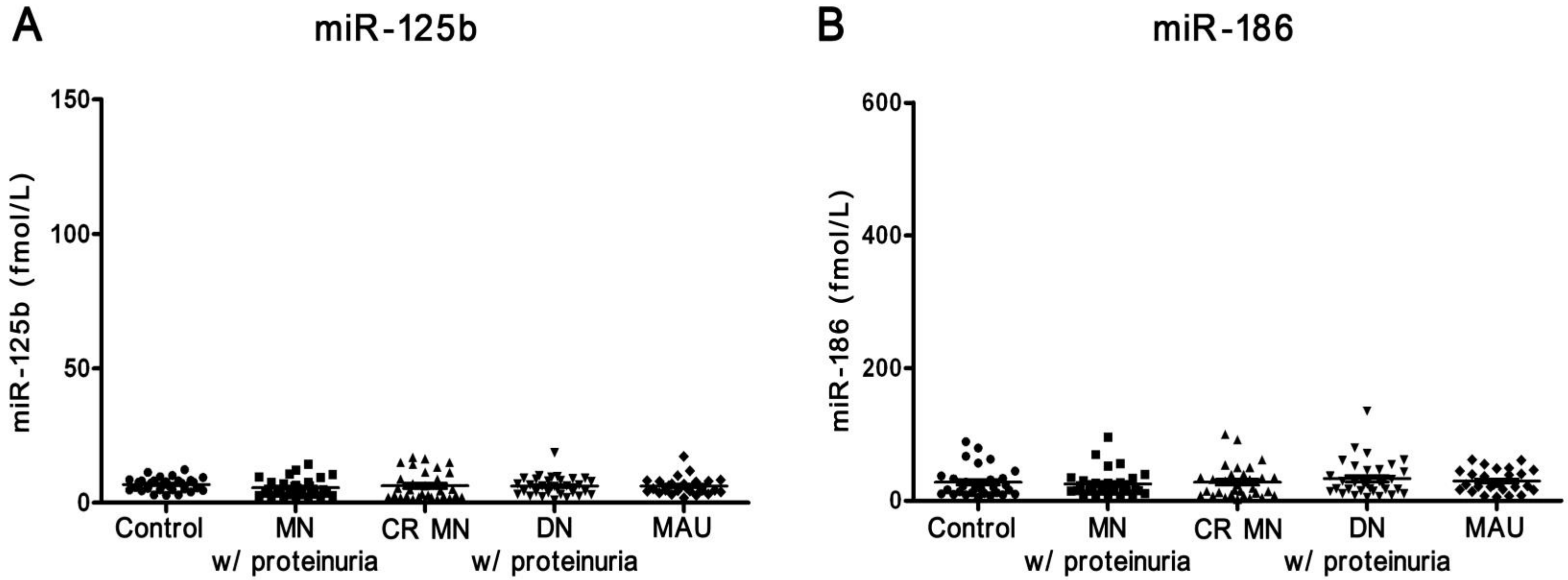


miR-186



激素敏感的FSGS患者血浆miR-125b和miR-186水平在治疗后显著下降

膜性肾病和糖尿病肾病患者血浆miR-125b和miR-186表达

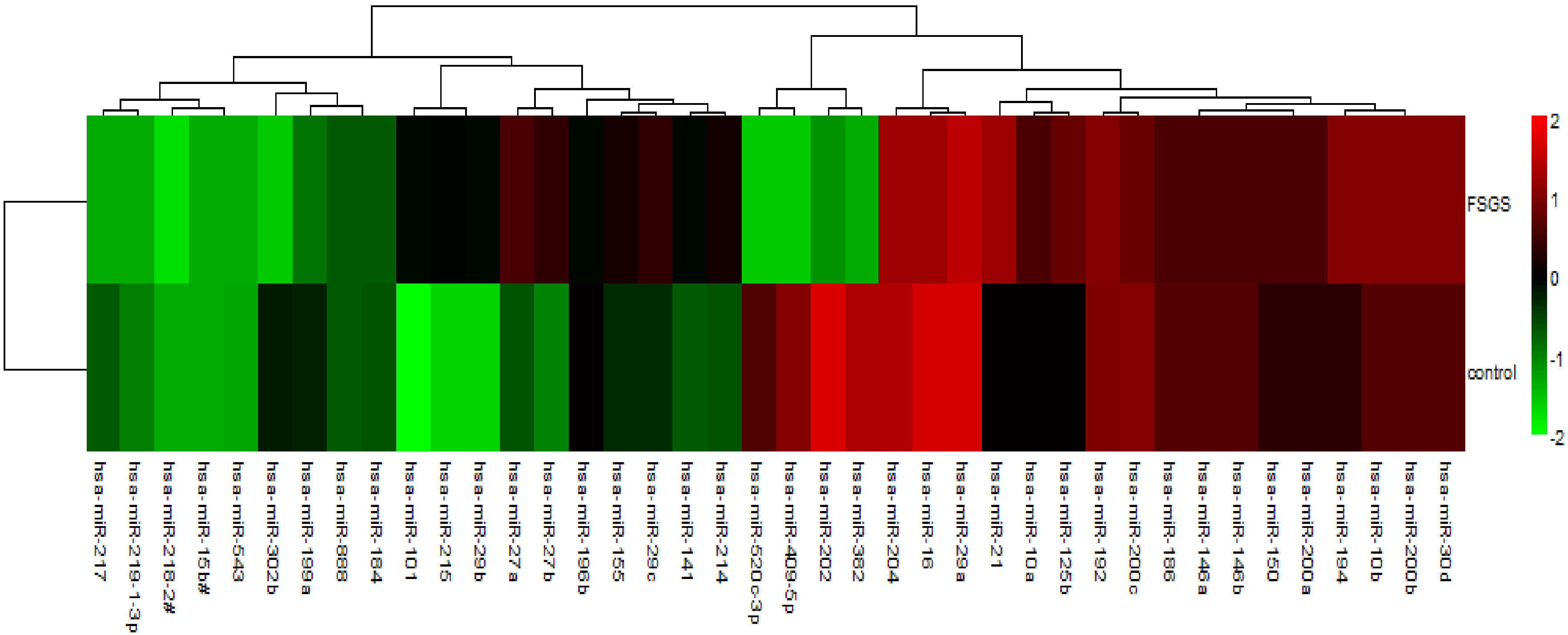


不论蛋白尿水平如何，MN和DN患者血浆miR-125b和miR-186水平与正常对照相比均无明显变化

血浆miR-186水平与FSGS疾病活动密切相关。激素敏感患者，血浆miR-125b和miR-186水平在治疗后较治疗前显著下降，激素抵抗患者则不然，表明二者可能作为监测FSGS疾病活动和预测激素疗效的生物标志物。

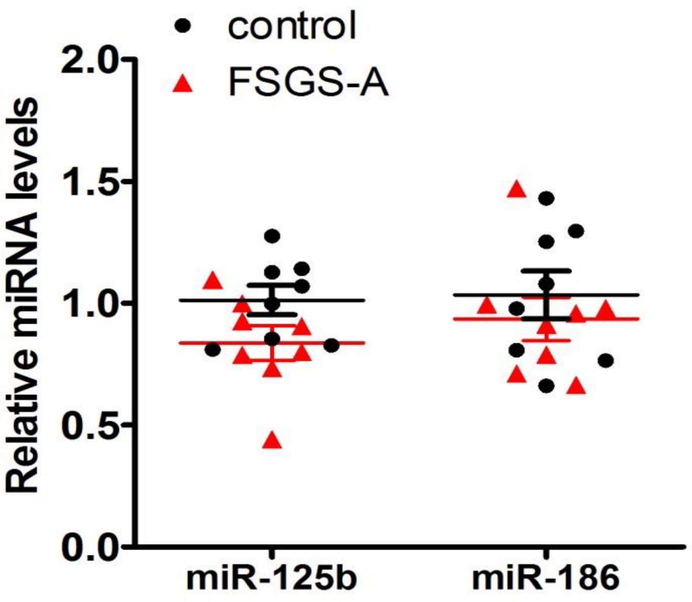
FSGS患者血浆miR-125b和miR-186变化的意义

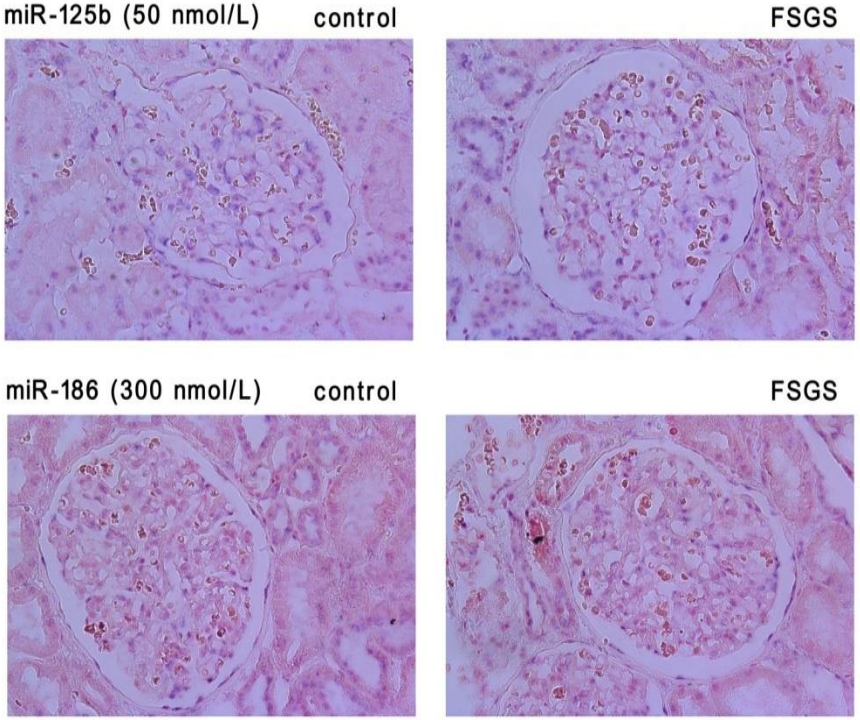
FSGS患者肾组织miRNAs表达谱



与正常对照相比，FSGS患者肾组织miR-125b和miR-186表达均无明显变化。

FSGS患者肾组织miR-125b和miR-186表达





qRT-PCR及原位杂交结果均显示：与正常对照相比，FSGS患者肾组织miR-125b和miR-186表达均无上调。

PATHOGENESIS OF LIPOID NEPHROSIS: A DISORDER OF T-CELL FUNCTION

ROBERT J. SHALHOUB

Veterans Administration Hospital, 50 Irving Street N.W Washington D.C. 20422, U.S.A.

Summary Clinical observations suggest that lipoid nephrosis is produced by a systemic abnormality of T-cell function resulting in the secretion of a circulating chemical mediator toxic to an immunologically innocent glomerular basement membrane. The lack of evidence of a humoral antibody response, remission induced by measles which modifies cell-mediated immunity, the therapeutic benefits of steroids and cyclophosphamide which also abate cell-mediated responses, and the occurrence of this syndrome in Hodgkin's disease support this hypothesis. The susceptibility of untreated patients to pneumococcal infections may be of primary or secondary pathogenetic importance. Taken together, the data suggest that this syndrome is a clinical expression of a self-limited primary immune-deficiency disease.

The Lancet
September 7,1974

BASIC RESEARCH www.jasn.org

Role of Myeloid-Derived Suppressor Cells in Glucocorticoid-Mediated Amelioration of FSGS

Limin Li,* Tao Zhang,† Wenli Diao,* Fangfang Jin,* Lei Shi,*‡ Jiao Meng,† Huan Liu,* Jing Zhang,* Cai-Hong Zeng,† Ming-Chao Zhang,† Shaoshan Liang,† Yuan Liu,‡ Chen- Yu Zhang,* Zhihong Liu,† and Ke Zen*†

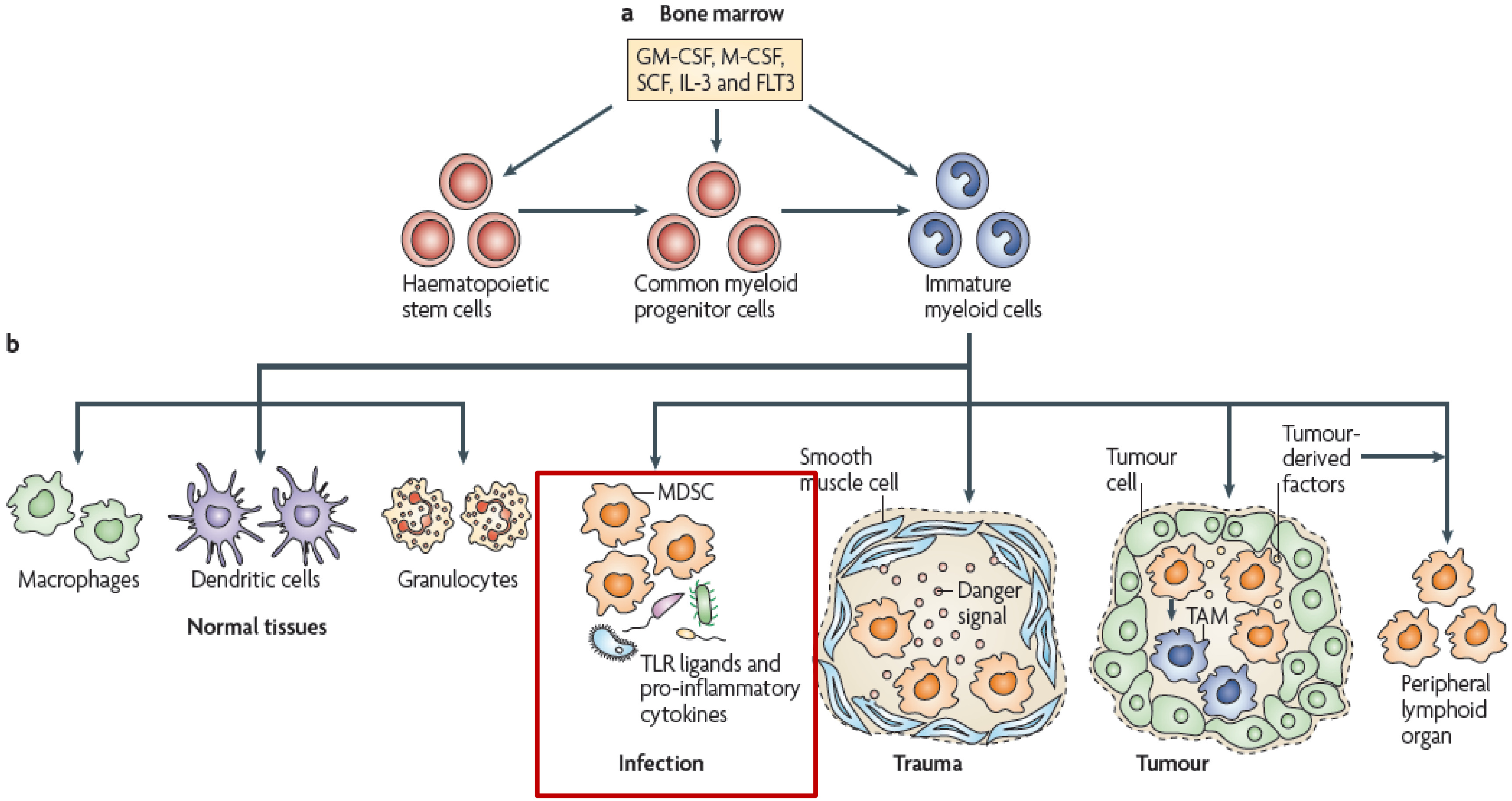
*State Key Laboratory of Pharmaceutical Biotechnology, Jiangsu Engineering Research Center for MicroRNA Biology, Nanjing, China; †National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China; and ‡Center for Inflammation, Immunity and Infection, Department of Biology, Georgia State University, Atlanta, Georgia

ABSTRACT

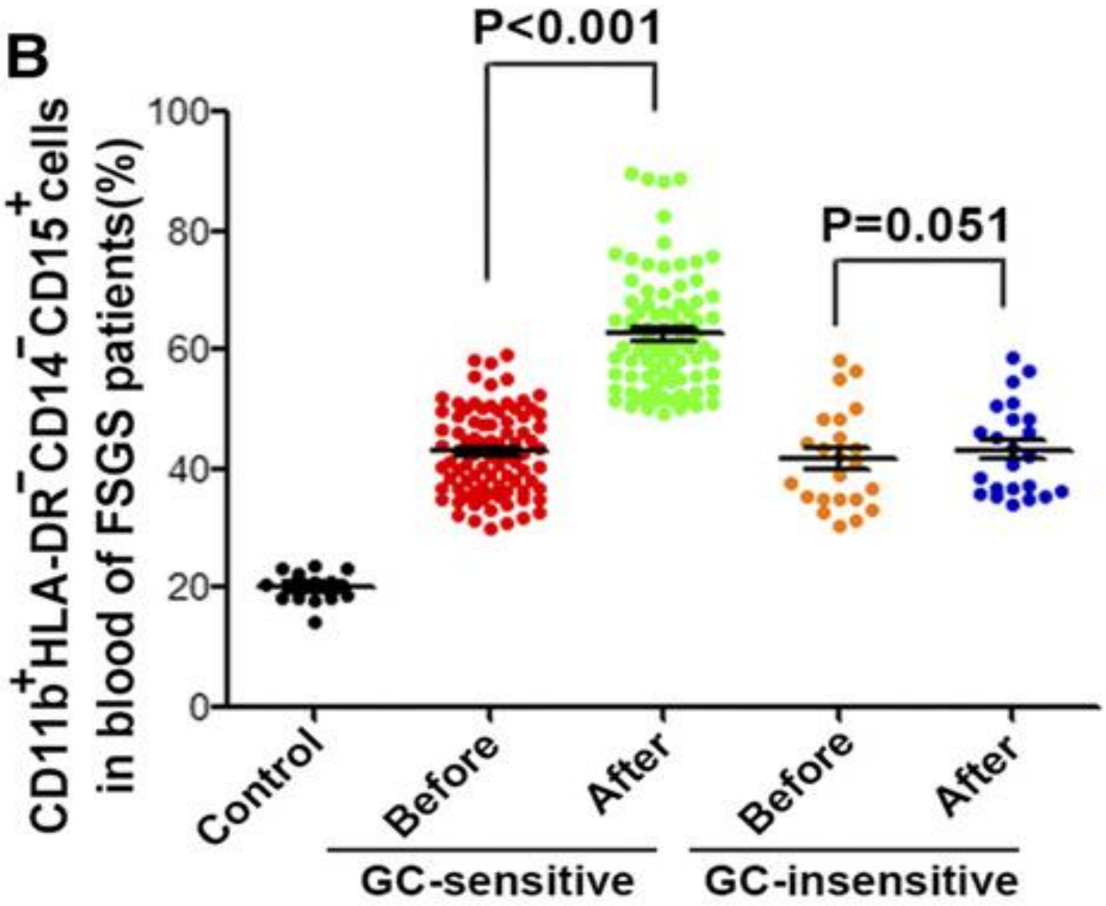
These rapidly induced MDSCs after steroid treatment would suppress the proliferation and activation of T cells and thus attenuate the inflammation and renal injury in steroid sensitive patients.

Liming Li , Zhihong Liu, et at. J Am Soc Nephrol 26: 2183, 2015

Myeloid-derived suppressor cells (MDSCs)



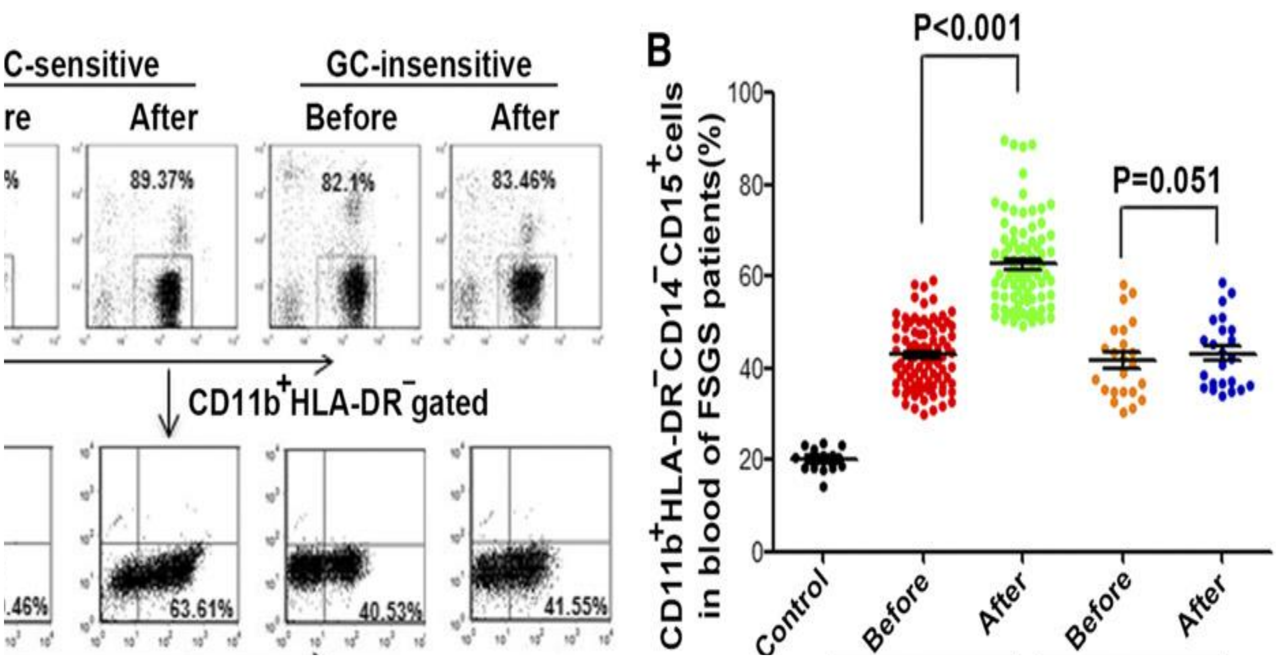
- ➢MDSC, a heterogeneous population of immature myeloid cells, which have an ability to suppress T cell responses.



激素敏感FSGS患者治疗后MDSC增多，激素抵抗患者则不然。

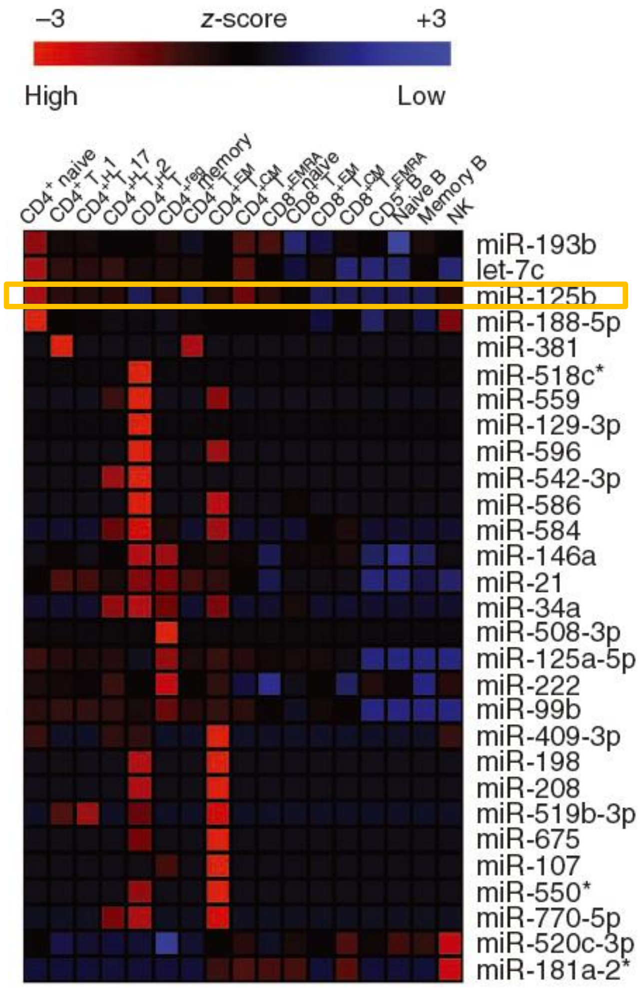
Liming Li , Zhihong Liu, et at. J Am Soc Nephrol 26: 2183, 2015

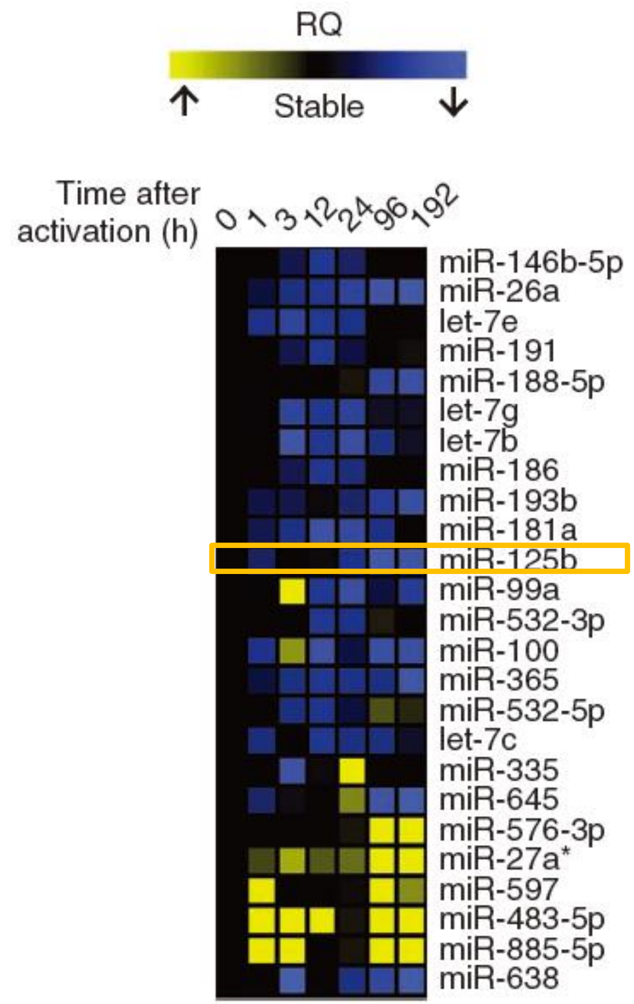
激素敏感FSGS患者MDSC能显著抑制T细胞增殖



Liming Li , Zhihong Liu, et at. J Am Soc Nephrol 26: 2183, 2015

MiR-125b特异性的表达于静息CD4^{+} T细胞



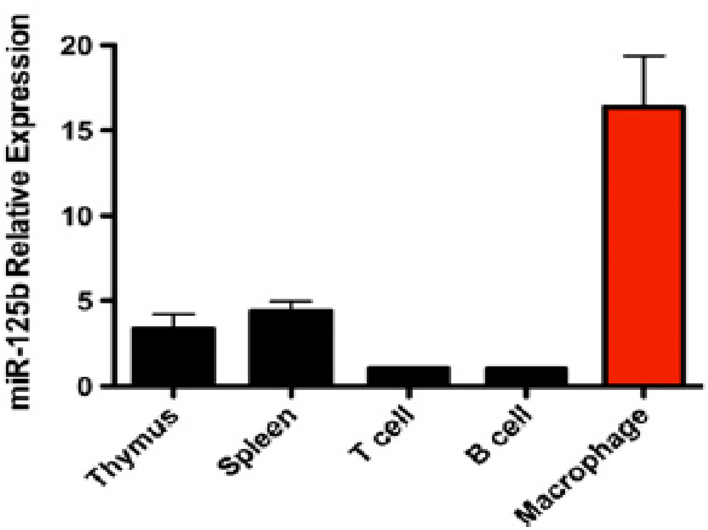


miR-125b as signature of human lymphocyte subsets , selectively expressed in naive CD4^{+} T cells

miR-125b with high expression specifically in resting naive CD4^{+} T cells were downregulated after activation with CD3 and CD28

Rossi et al. *Nature Immunol*, 2011

巨噬细胞富含MiR-125b

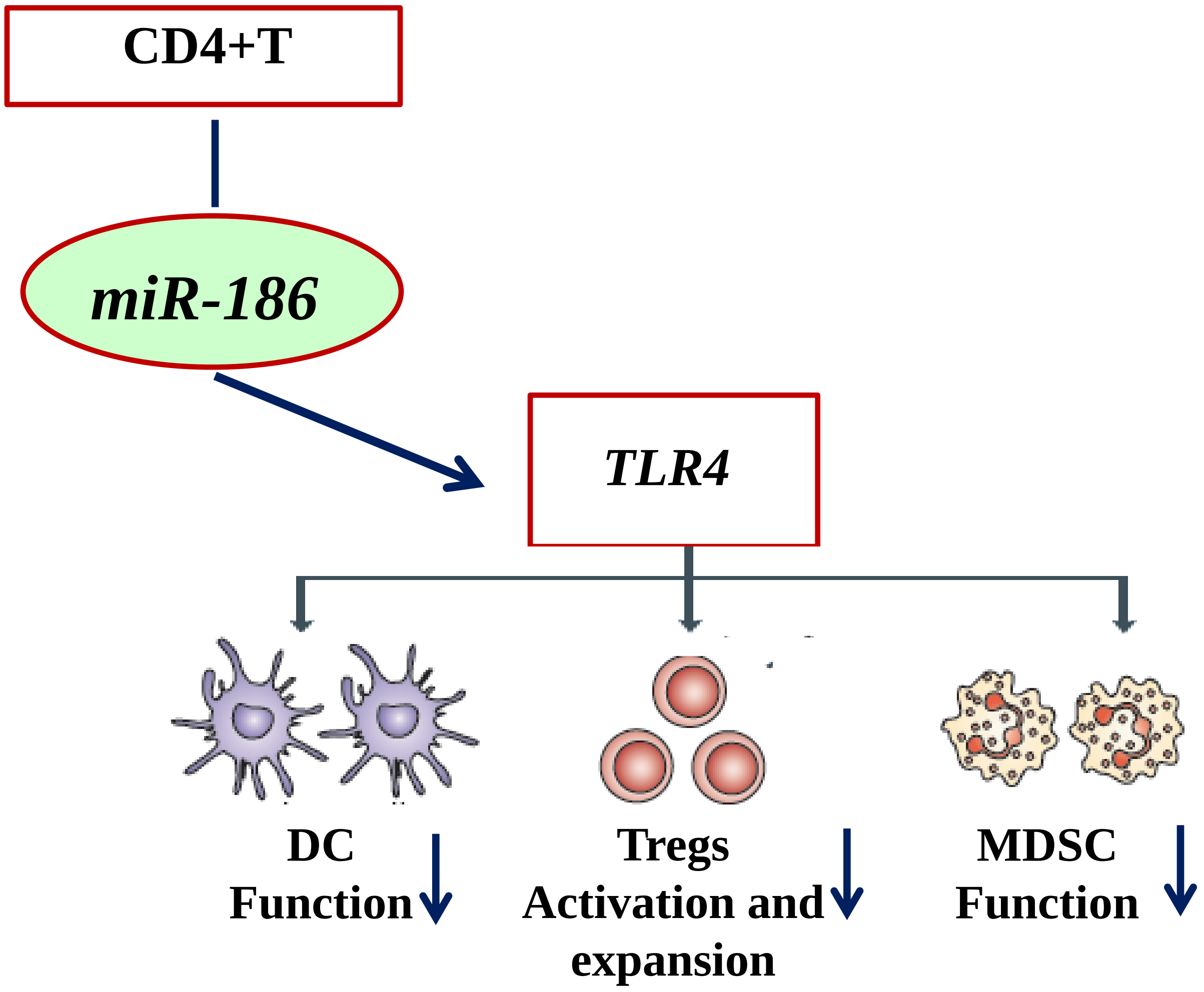


miR-125b level in total splenocytes, thymocytes, splenic T cells, splenic B cells, and peritoneal macrophages from C57BL/6 mice.

miR-125b is enriched in macrophages compared with lymphoid cells and whole immune tissues. Enforced expression of miR-125b induces increased costimulatory factor expression and elevated responsiveness to IFN-g

Chaudhuri et al. *J Immunol*, 2011

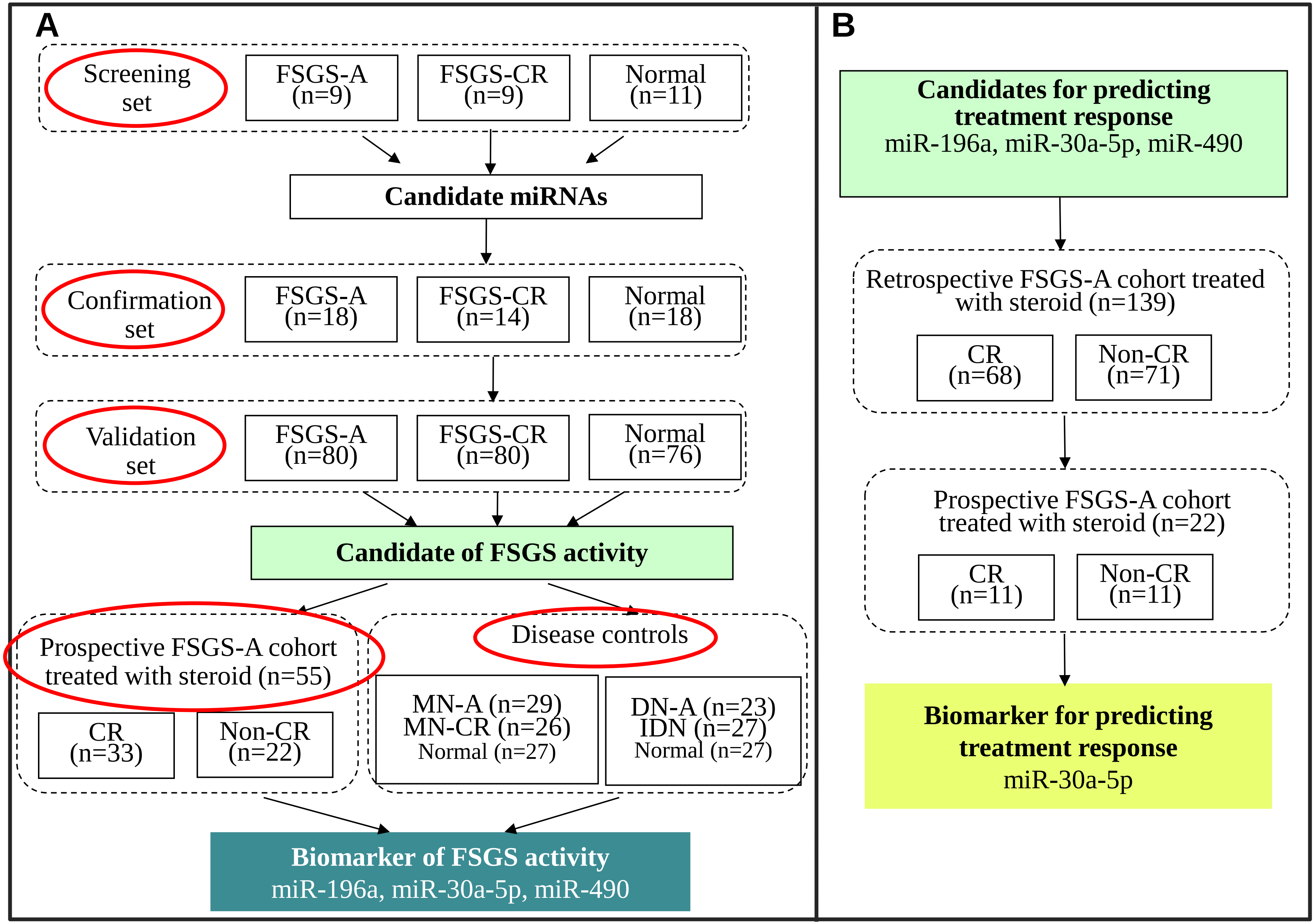
miR-125b能促进巨噬细胞的抗原提呈和诱导T细胞活化，增强巨噬细胞介导的获得性免疫。活化的T细胞又能分泌IFN-g，进一步增强巨噬细胞的活化。



Cell Immunol,2015
Journal of Immunology, 2002

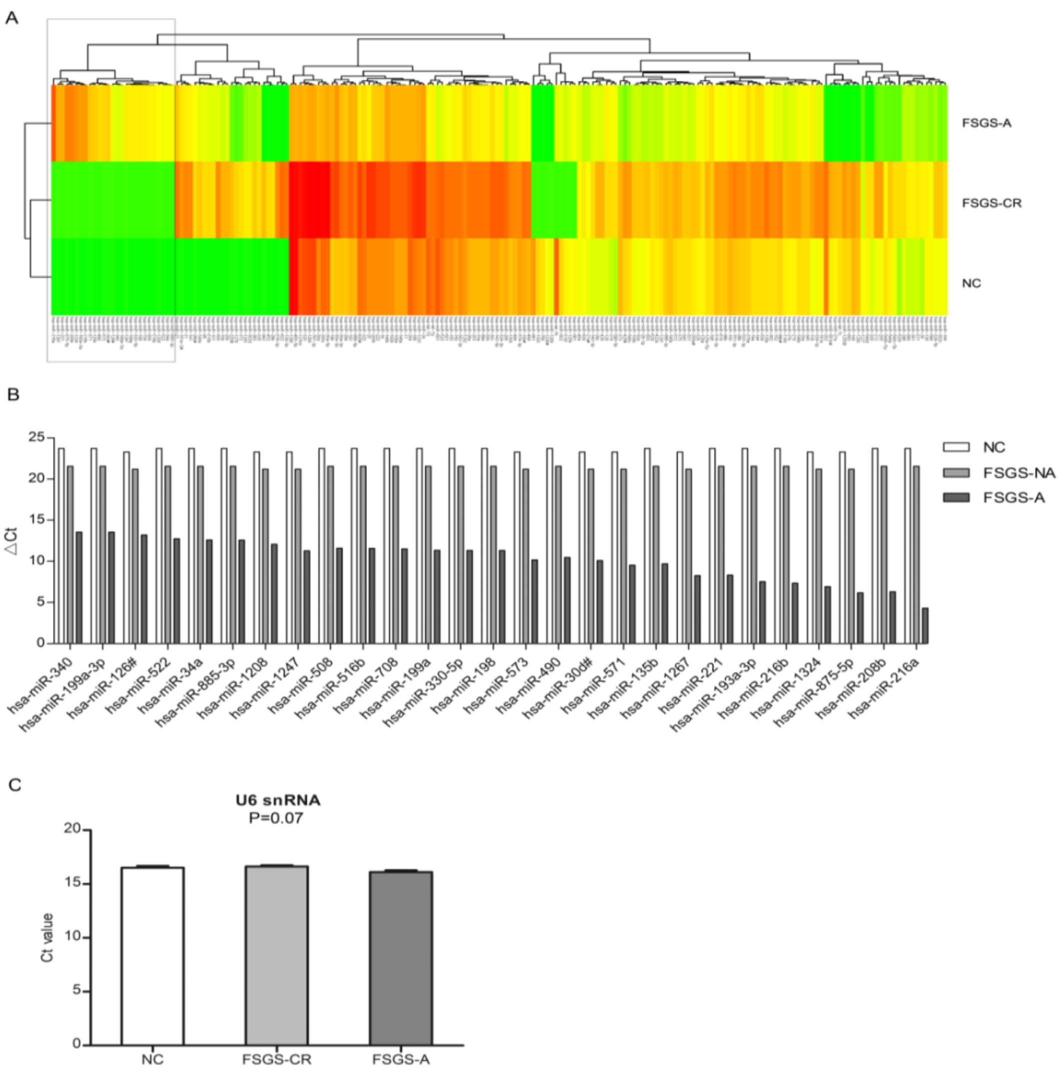
从尿液 miRNAs中寻在FSGS 标志物

研究技术路线



A
Screening set
FSGS-A (n=9)
FSGS-CR (n=9)
Normal (n=11)
Candidate miRNAs
Confirmation set
FSGS-A (n=18)
FSGS-CR (n=14)
Normal (n=18)
Validation set
FSGS-A (n=80)
FSGS-CR (n=80)
Normal (n=76)
Candidate of FSGS activity
Prospective FSGS-A cohort treated with steroid (n=55)
CR (n=33)
Non-CR (n=22)
Disease controls
MN-A (n=29)
MN-CR (n=26)
Normal (n=27)
DN-A (n=23)
IDN (n=27)
Normal (n=27)
Biomarker of FSGS activity
miR-196a, miR-30a-5p, miR-490
B
Candidates for predicting treatment response
miR-196a, miR-30a-5p, miR-490
Retrospective FSGS-A cohort treated with steroid (n=139)
CR (n=68)
Non-CR (n=71)
Prospective FSGS-A cohort treated with steroid (n=22)
CR (n=11)
Non-CR (n=11)
Biomarker for predicting treatment response
miR-30a-5p

筛查候选 miRNAs



A distinct miRNA profiles among patients with FSGS-A, FSGS- CR, and normal controls

Individual qRT-PCR analyses confirmed the upregulation of 27 miRNAs in FSGS-A than that from patients with FSGS-CR and controls

.

Wanfen Zhang, Zhihong Liu, et al. Clin J Am Soc Nephrol 9: 1545–1552, 2014

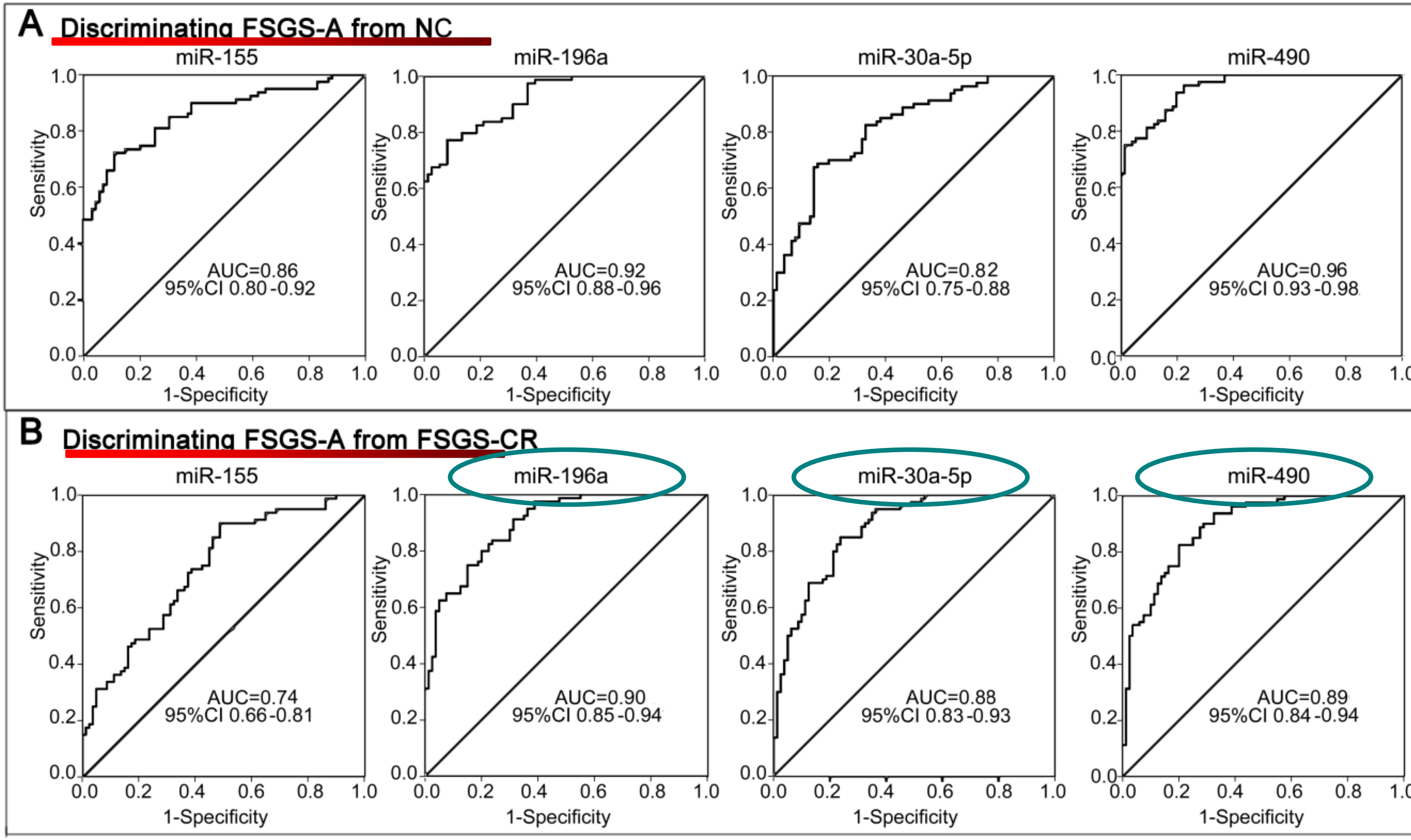
独立队列验证FSGS患者尿液miRNAs表达

microRNAs	Concentration of Urinary miRNAs (fmol/ L)				Fold Change for FSGS-A versus Controls		Fold Change for FSGS-A versus FSGS-CR	
	Controls	FSGS-CR	FSGS-A	P Value	Mean	P Value	Mean	P Value
Confirmation set								
hsa-miR-135b	46.56 45.1	48.86 28.4	1066 85.6	0.01	2.30	0.004	2.22	0.01
hsa-miR-155	25906 1970	69906 5750	13,3006 11,100	0.001	5.14	, 0.001	1.91	0.04
hsa-miR-196a	2086 99.3	1906 80.6	13506 1040	0.01	6.51	0.004	7.11	0.01
hsa-miR-30a-5p	2816 196	3976 164	8556 673	0.001	3.05	, 0.001	2.15	0.01
hsa-miR-320	2386 123	7636 615	15906 1060	, 0.001	6.70	, 0.001	2.09	0.004
hsa-miR-490	33.16 28.3	27.56 12.5	1076 96.0	0.03	3.24	0.03	3.89	0.03
Validation set								
hsa-miR-135b	1056 159	1176 152	1406 165	0.70		0.43		0.58
hsa-miR-155	23806 2080	49206 4770	12,3006 10,900	, 0.001	3.33	, 0.001	2.49	, 0.001
hsa-miR-196a	1656 140	2056 195	12506 1090	, 0.001	6.74	, 0.001	6.08	, 0.001
hsa-miR-30a-5p	4896 326	3906 328	12306 1110	, 0.001	2.81	, 0.001	3.15	, 0.001
hsa-miR-320	19806 1890	19606 1950	20606 2280	0.27		0.11		0.17
hsa-miR-490	42.96 33.0	69.36 56.0	2626 219	, 0.001	4.68	, 0.001	3.80	, 0.001

与正常对照和完全缓解FSGS患者相比，活动性FSGS患者尿液miR-30a-5p, miR-196a, miR-155和miR-490水平显著升高。

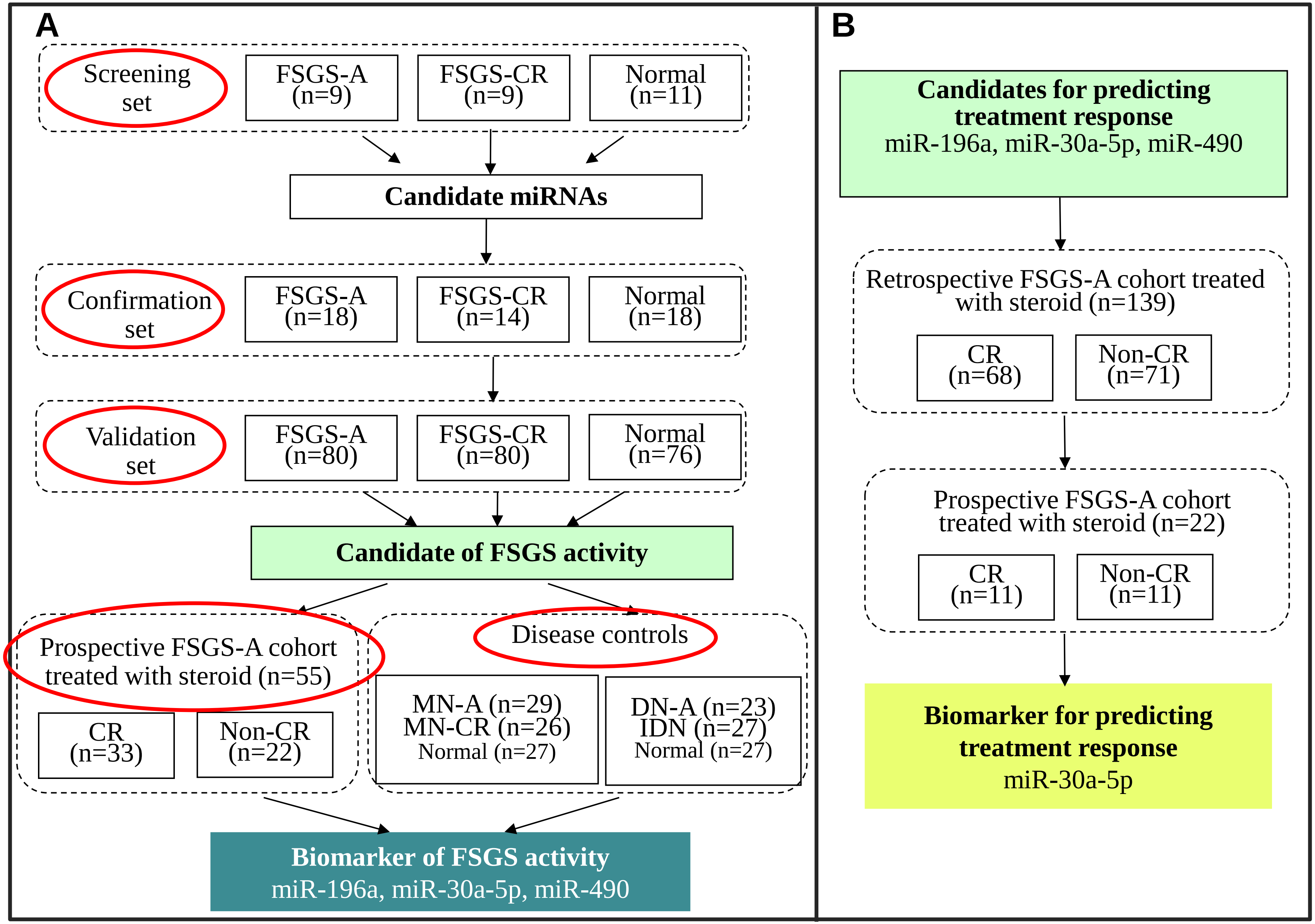
Wanfen Zhang, Zhihong Liu, et al. Clin J Am Soc Nephrol 9: 1545–1552, 2014

尿液miR-196a, miR-30a-5p和miR-490与FSGS疾病活动

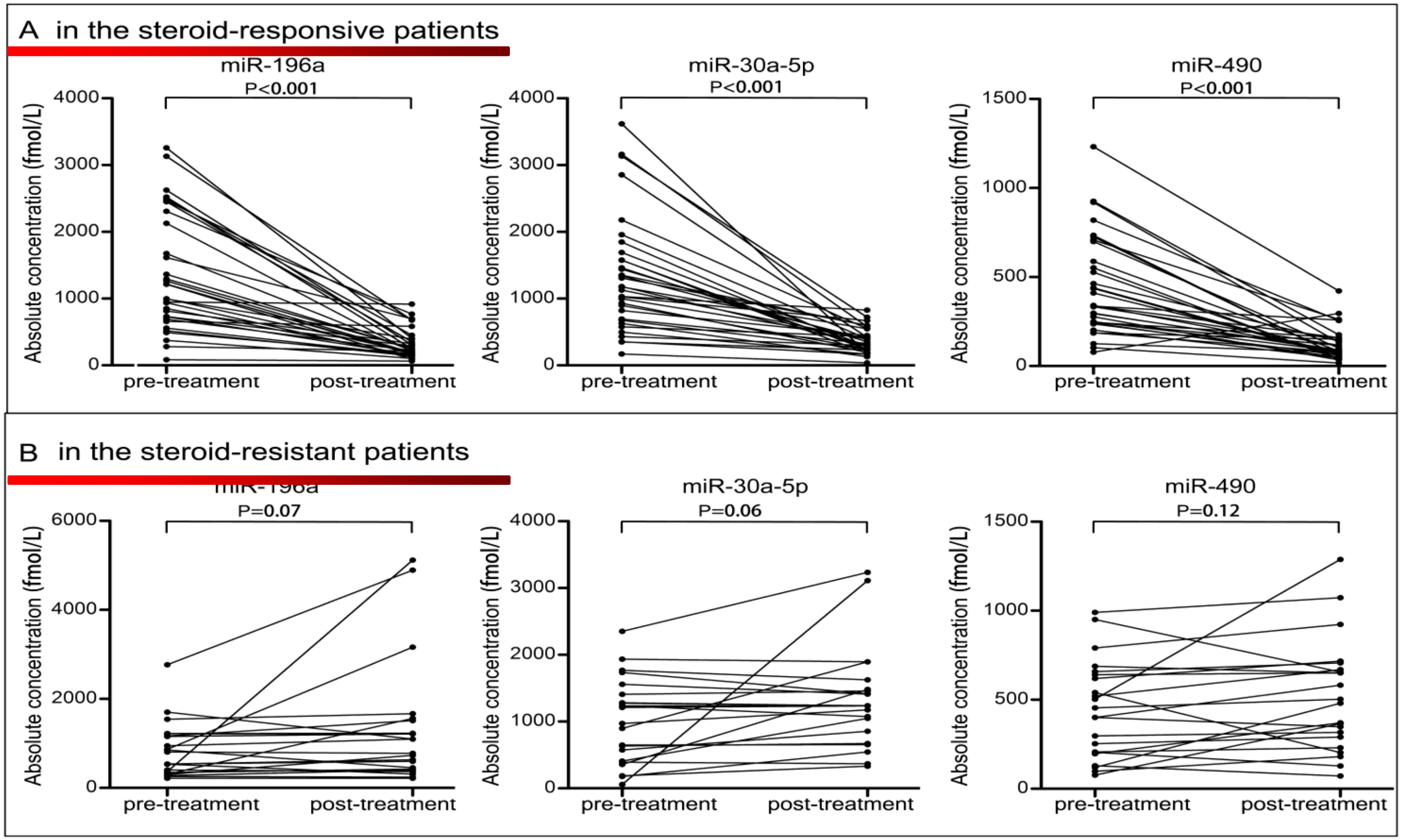


尿液miR-196a, miR-30a-5p and miR-490能很好的区分活动性FSGS和完全缓解FSGS

研究技术路线


A
Screening set
FSGS-A (n=9)
FSGS-CR (n=9)
Normal (n=11)
Candidate miRNAs
Confirmation set
FSGS-A (n=18)
FSGS-CR (n=14)
Normal (n=18)
Validation set
FSGS-A (n=80)
FSGS-CR (n=80)
Normal (n=76)
Candidate of FSGS activity
Prospective FSGS-A cohort treated with steroid (n=55)
CR (n=33)
Non-CR (n=22)
Disease controls
MN-A (n=29)
MN-CR (n=26)
Normal (n=27)
DN-A (n=23)
IDN (n=27)
Normal (n=27)
Biomarker of FSGS activity
miR-196a, miR-30a-5p, miR-490
B
Candidates for predicting treatment response
miR-196a, miR-30a-5p, miR-490
Retrospective FSGS-A cohort treated with steroid (n=139)
CR (n=68)
Non-CR (n=71)
Prospective FSGS-A cohort treated with steroid (n=22)
CR (n=11)
Non-CR (n=11)
Biomarker for predicting treatment response
miR-30a-5p

激素治疗前后尿液miR-196a, miR-30a-5p和miR-490表达变化

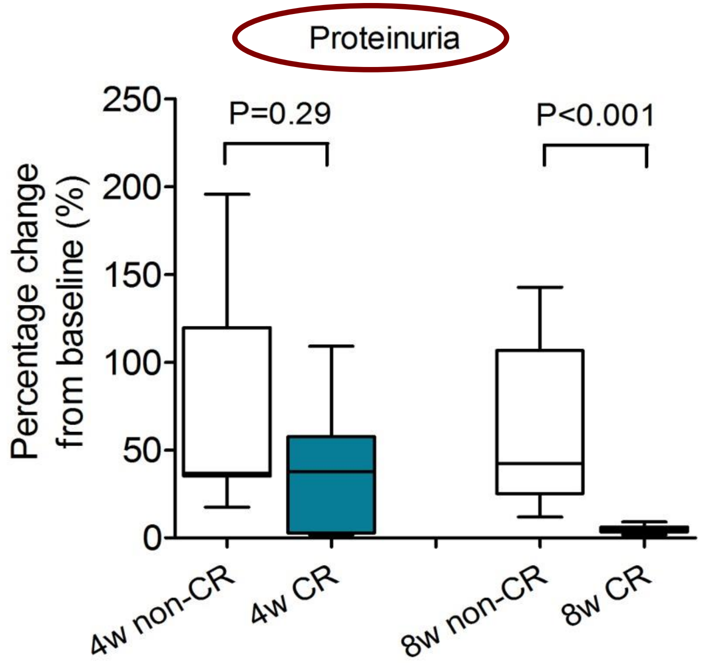


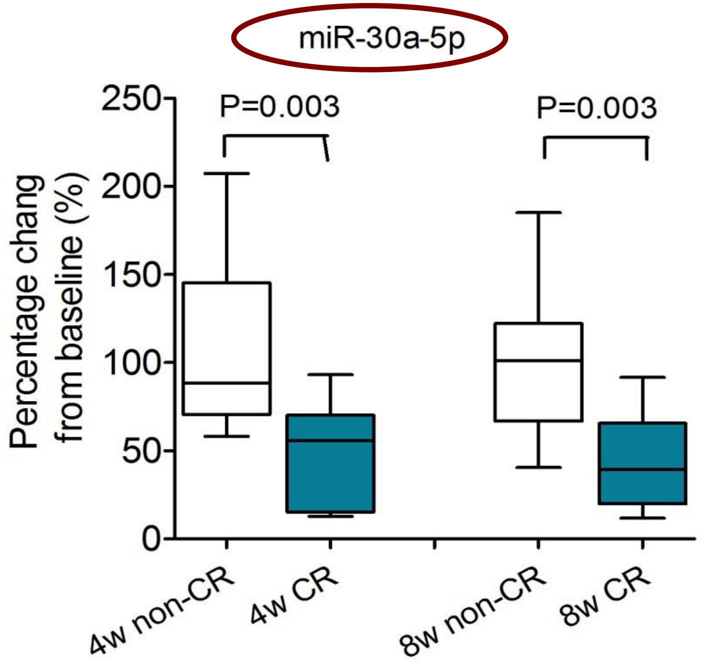
激素治疗后，激素敏感患者尿miR-196a, miR- 30a-5p和miR-490显著下降。

Wanfen Zhang, Zhihong Liu, et al. Clin J Am Soc Nephrol 9: 1545–1552, 2014

尿液miR-30a-5p预测FSGS患者对激素治疗的反应

In a prospective study (n=22)





激素敏感患者，激素治疗4周后尿miR-30a-5p水平即显著下降，早于尿蛋白的缓解。

Wanfen Zhang, Zhihong Liu, et al. Clin J Am Soc Nephrol 9: 1545–1552, 2014

尿液miR-30a-5p，miR-196a和miR-490可能作为FSGS疾病活动的潜在标志物，并且尿液miR-30a-5p的变化可能预测FSGS患者对激素治疗的反应。

FSGS患者尿液miR-30变化的意义

肾组织中miR-30的表达

各组织中miR-196a和miR-30a拷贝数

miRNA	liver	brain	spleen	kidney	esophagus	intestine	lung	uterus	heart	colon
miR-196a	0.32	0.19	26.78	2061.8	12.38	55.35	1.9	605.13	0.64	2.55
miR-196b	1.1	0.38	28.66	4706.24	19.99	4.61	8.54	1569.33	2.97	75.09
miR-30a	14532.42	10437.57	1561.69	35190.85	1526.49	1822.76	23887.77	2124.95	8607.81	76.13

BASIC RESEARCH www.jasn.org

Downregulation of MicroRNA-30 Facilitates Podocyte Injury and Is Prevented by Glucocorticoids

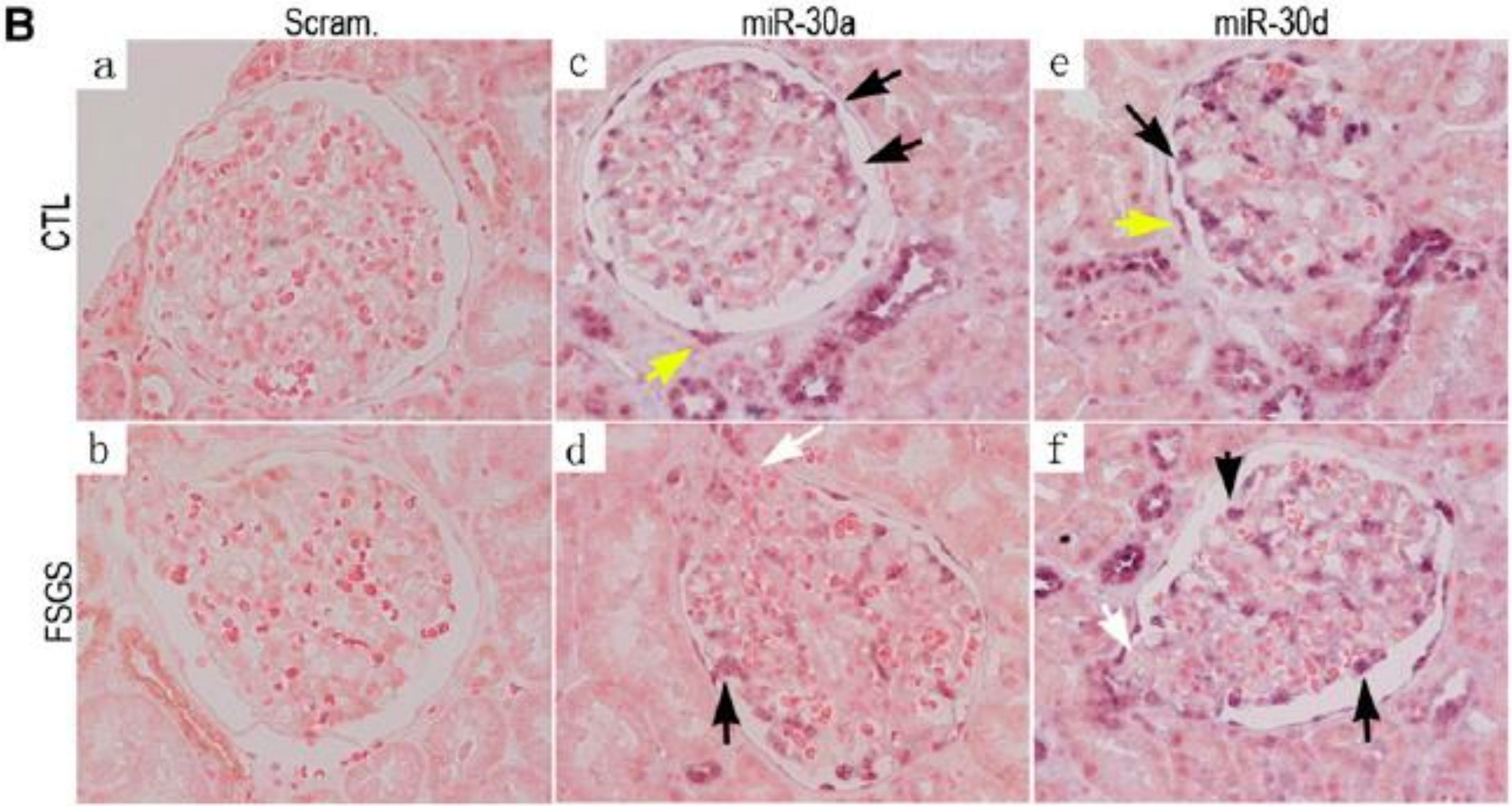
Junnan Wu, Chunxia Zheng, Yun Fan, Caihong Zeng, Zhaohong Chen, Weisong Qin, Changming Zhang, Wanfen Zhang, Xiao Wang, Xiaodong Zhu, Mingchao Zhang, Ke Zen, and Zhihong Liu

Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu, China

ABSTRACT

Downregulation of miR-30s in podocyte can induce proteinuria and podocyte injury. We found that miR-30s protect podocyte from injury by targeting Notch1 and p53. Glucocorticoid treatment sustains miR-30 expression in cultured podocytes treated with TGF-b or PAN and in the podocytes of PAN-treated rats.

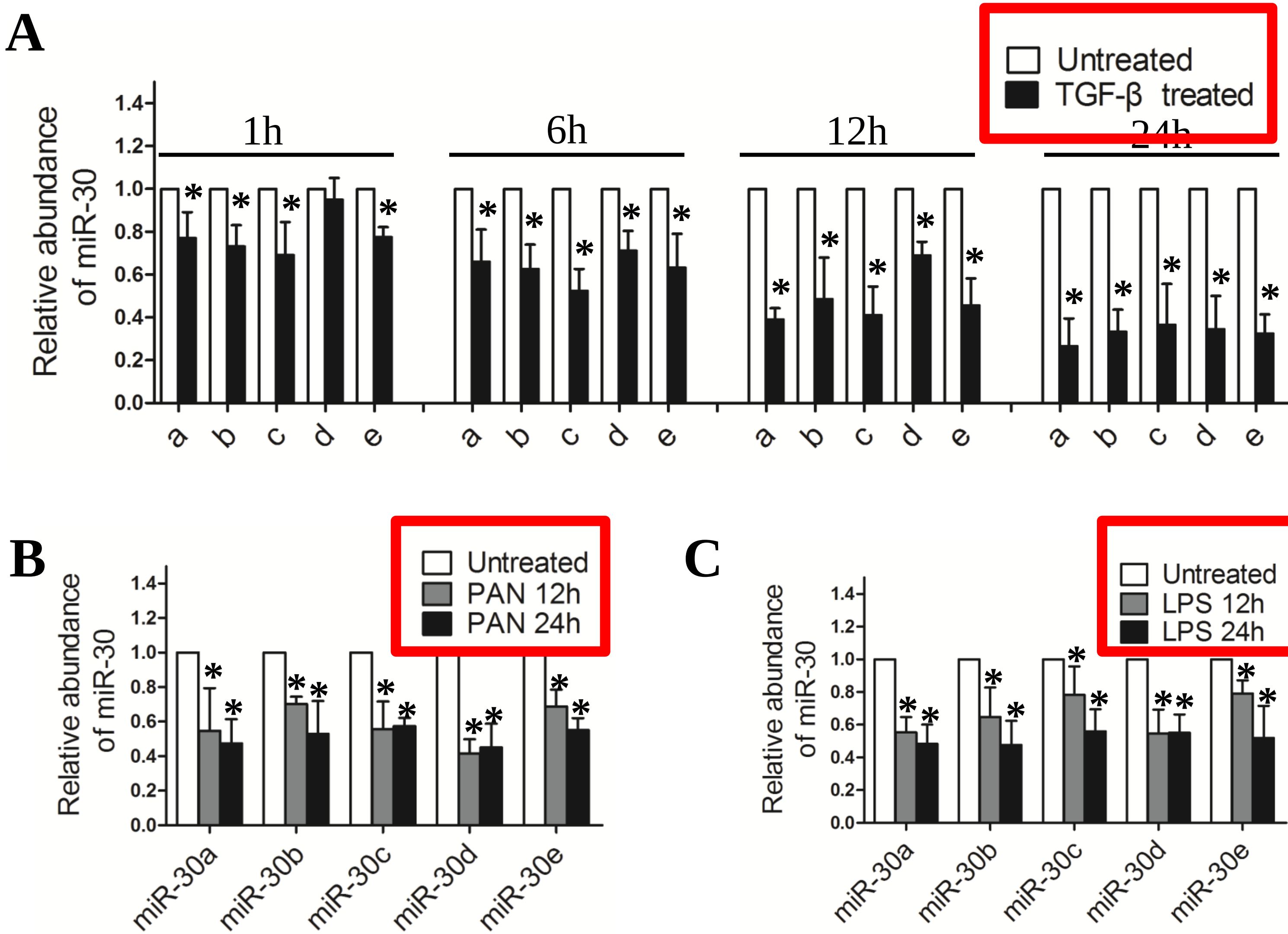
J Wu, ZH Liu, et al. J Am Soc Nephrol 25: 92–104, 2014



miR-30s表达于人足细胞，并且在FSGS患者肾组织中表达下调

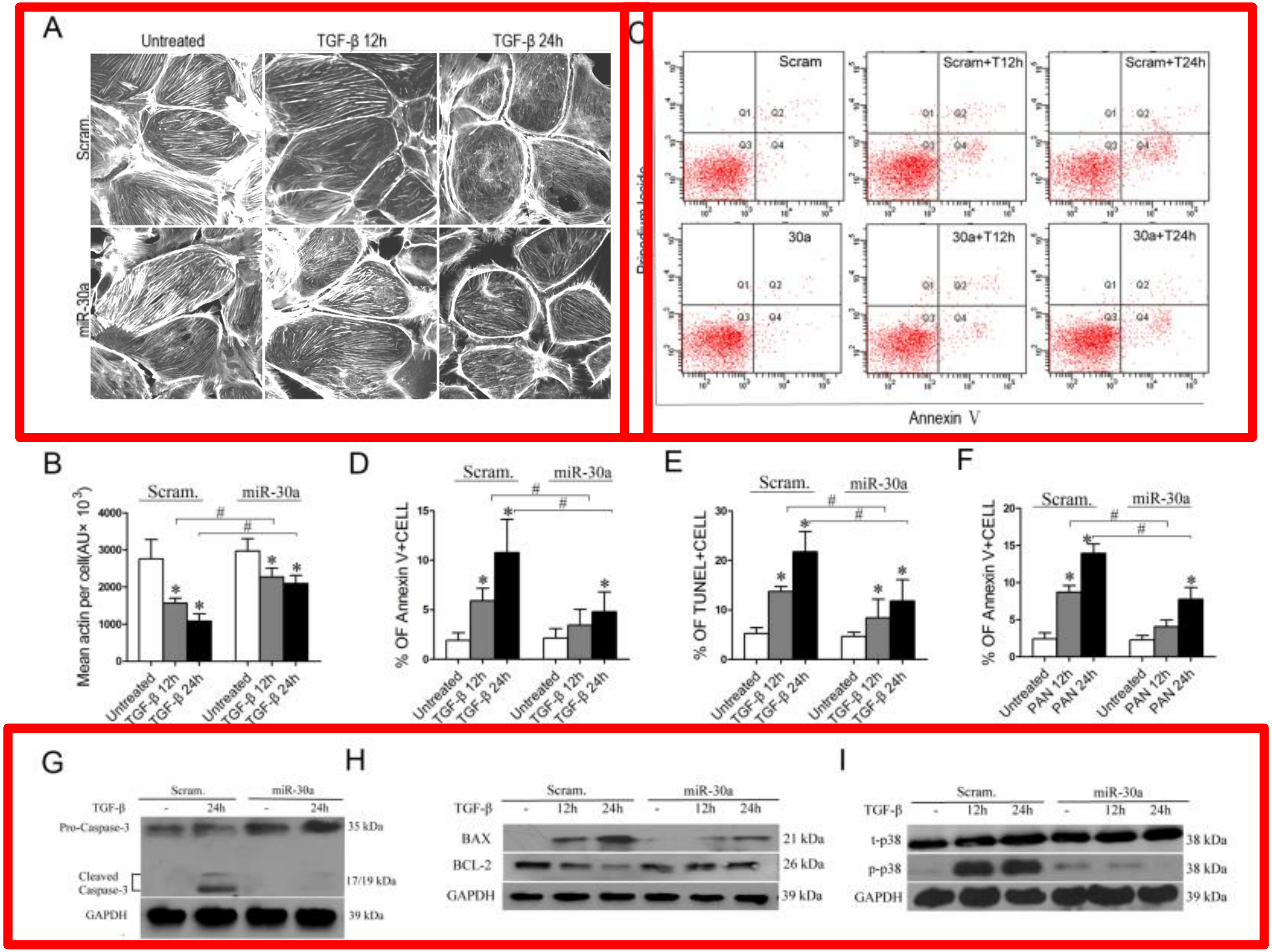
J Wu, ZH Liu, et al. J Am Soc Nephrol 25: 92–104, 2014

损伤因素刺激后足细胞miR-30s表达下降



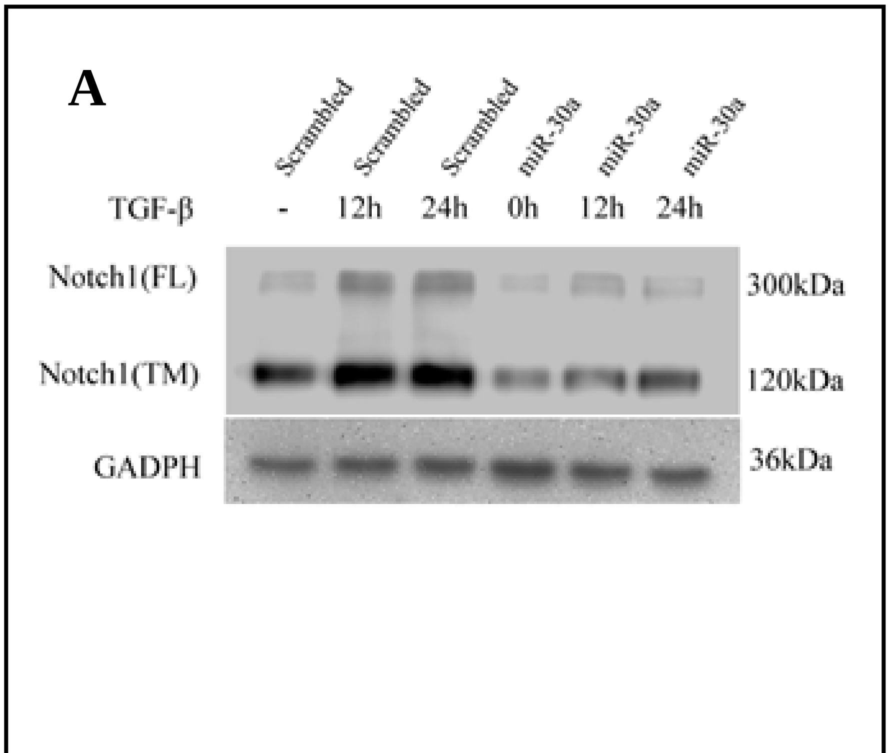
J Wu, ZH Liu, et al. J Am Soc Nephrol 25: 92–104, 2014

miR-30s表达下降参与足细胞损伤



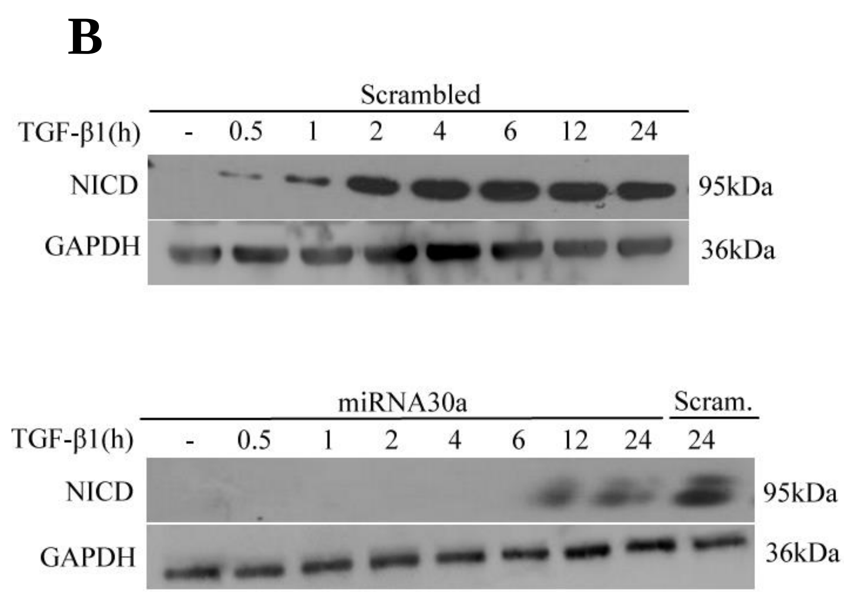
足细胞转染miR-30a质粒通过抑制Notch1受体表达从而抑制Notch1信号通路活化

Notch1 production



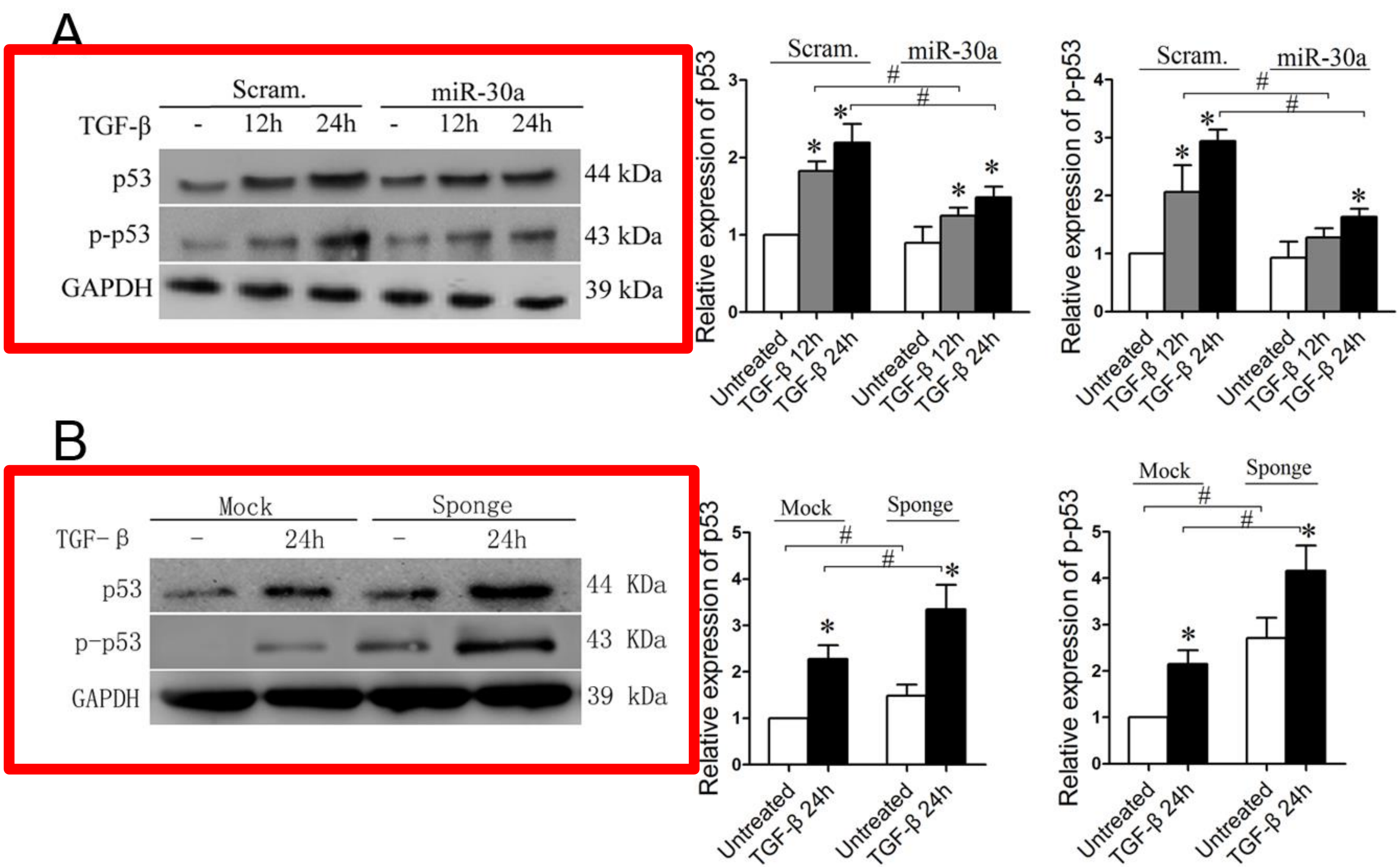
Western blot：足细胞Notch1受体检测（图A）

Notch1 activation



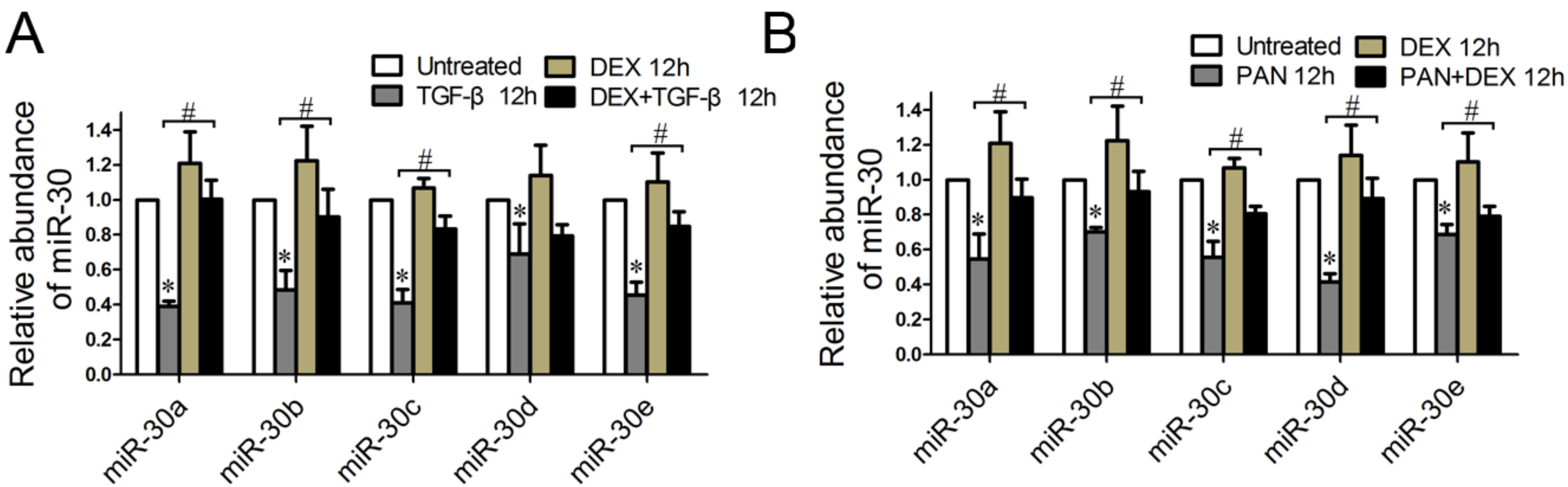
Western blot：足细胞NICD检测（图B）

足细胞转染miR-30a质粒后可显著拮抗TGF-β所致的p53通路激活



J Wu, ZH Liu, et al. J Am Soc Nephrol 25: 92–104, 2014

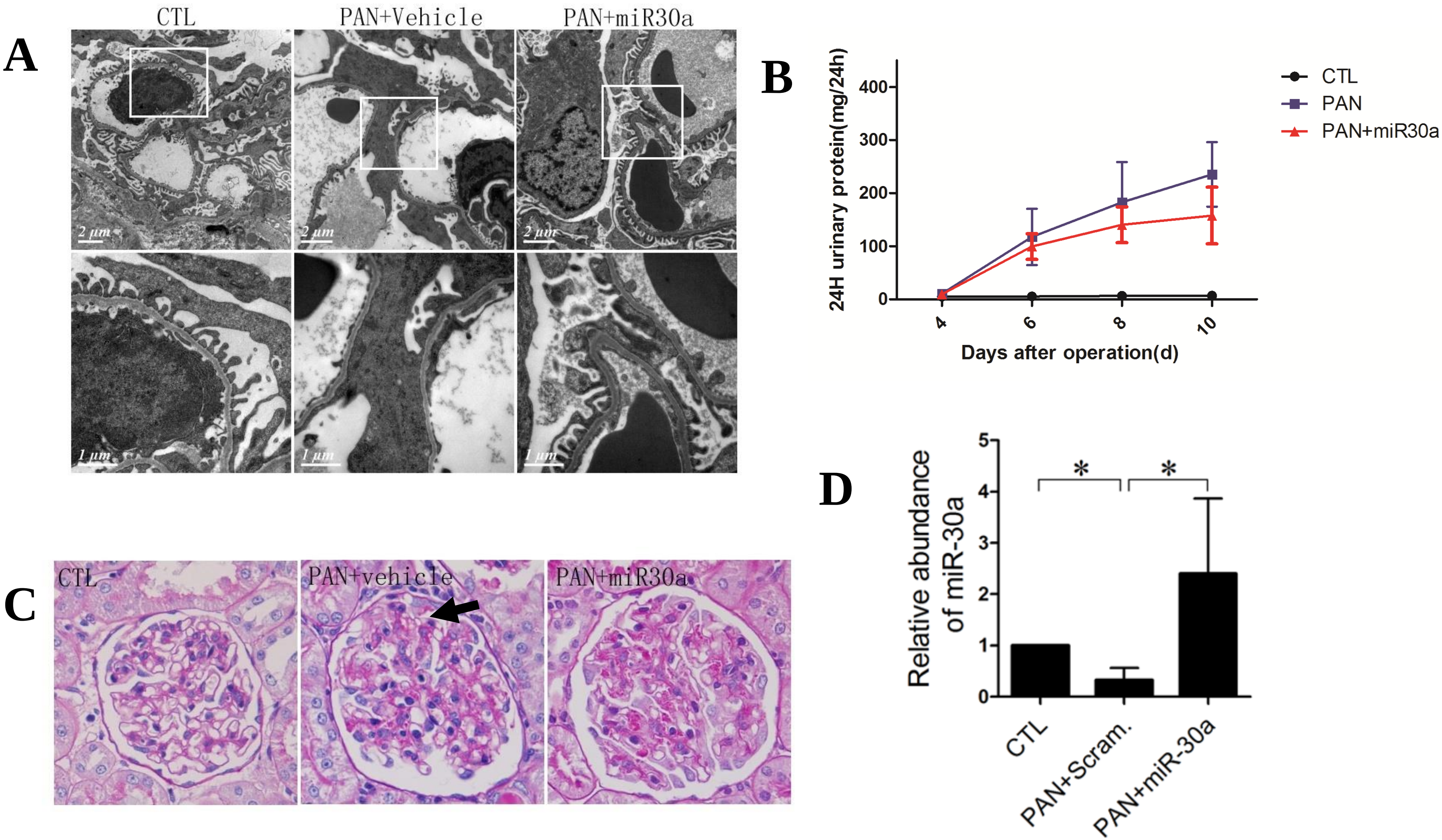
糖皮质激素（DEX）可以逆转TGF-β /PAN诱导的足细胞miR-30s表达下降



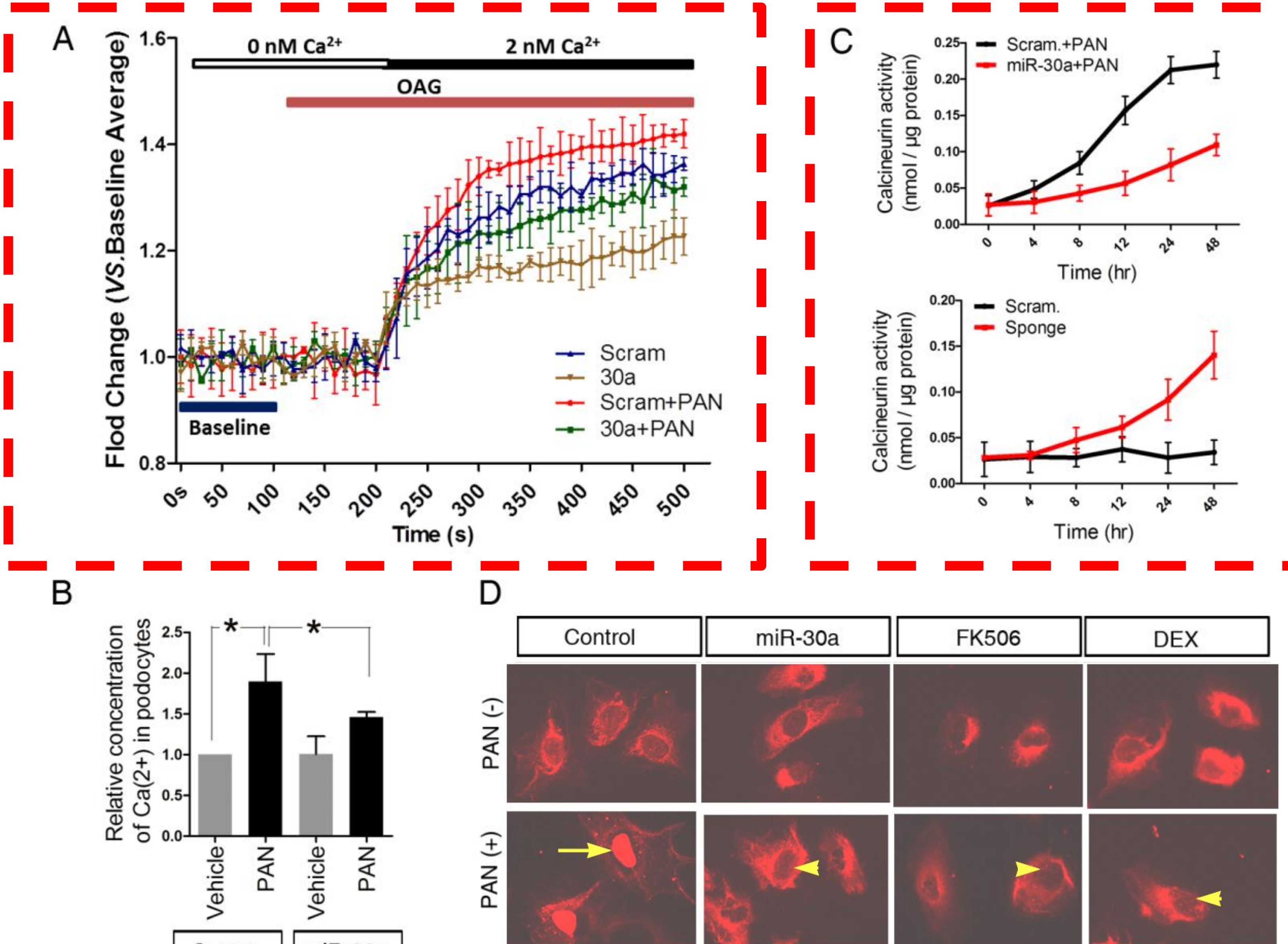
qPCR：足细胞miR-30s表达（图A、B）

J Wu, ZH Liu, et al. J Am Soc Nephrol 25: 92–104, 2014

miR-30a治疗可逆转PAN大鼠肾小球损伤

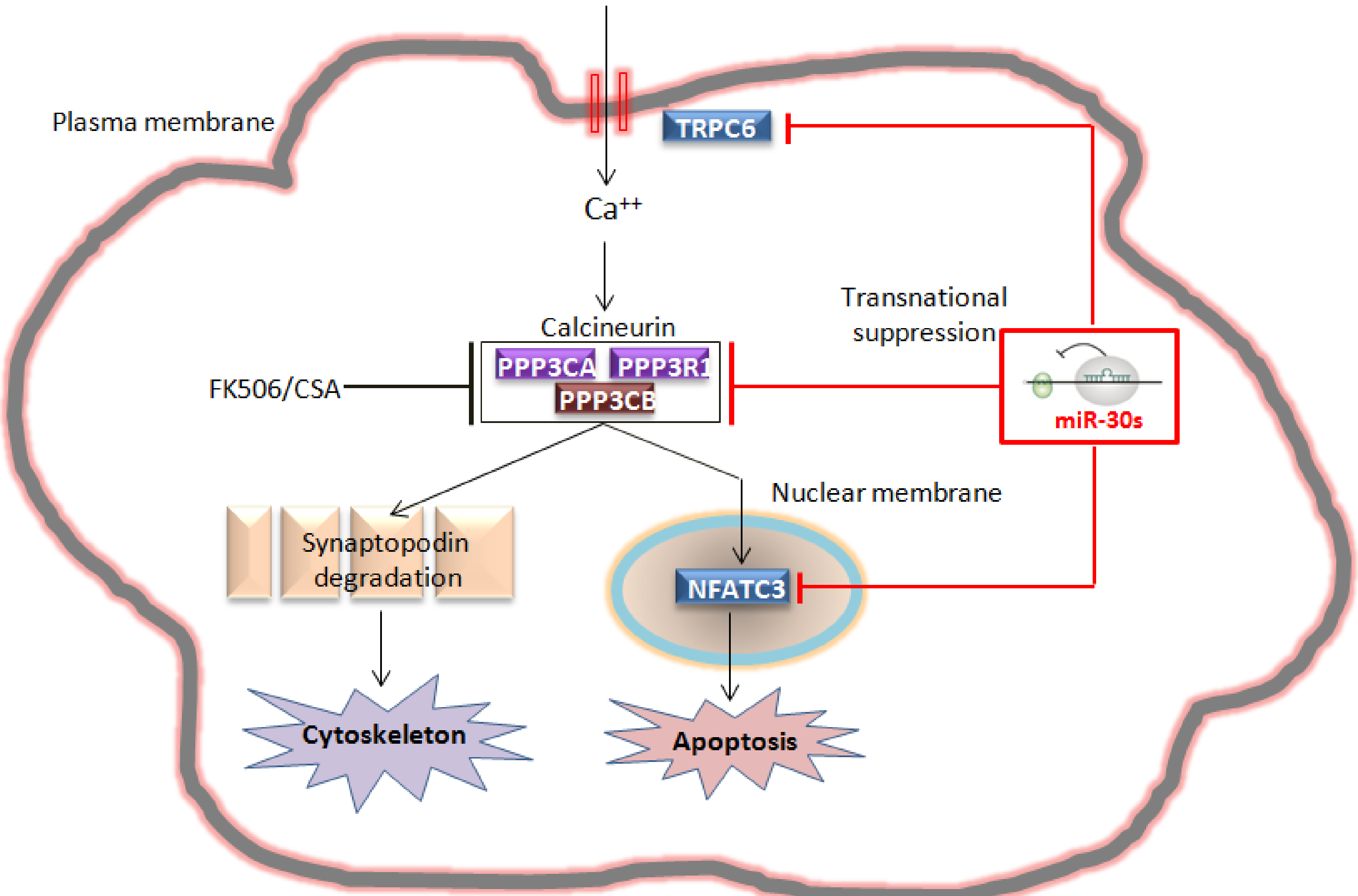


miR-30s调控足细胞钙内流及calcineurin活性



J Wu, ZH Liuet al , *J Clin Invest* 2015

miR-30s调控Ca2+-Calcineurin信号通路

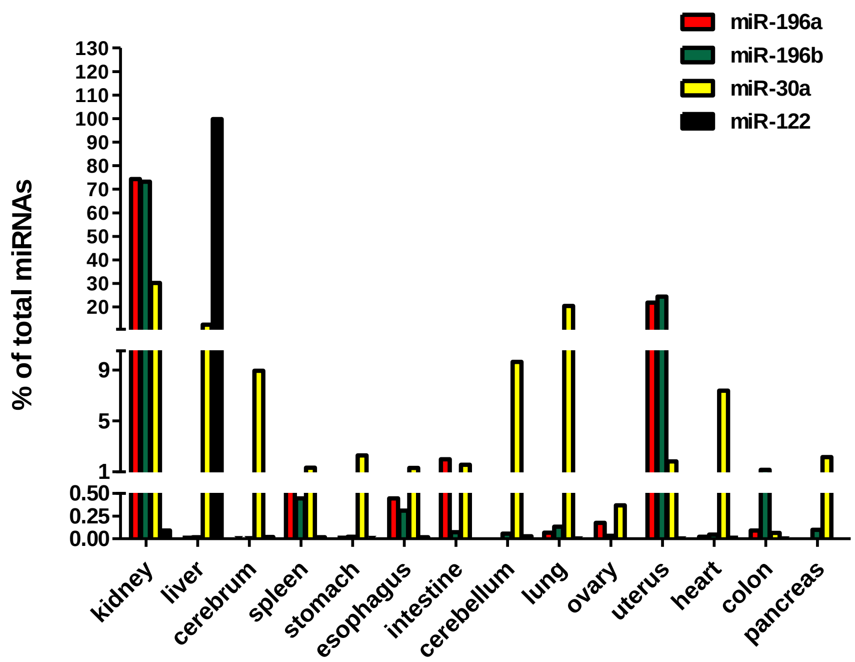


J Wu, ZH Liuet al , *J Clin Invest* .2015

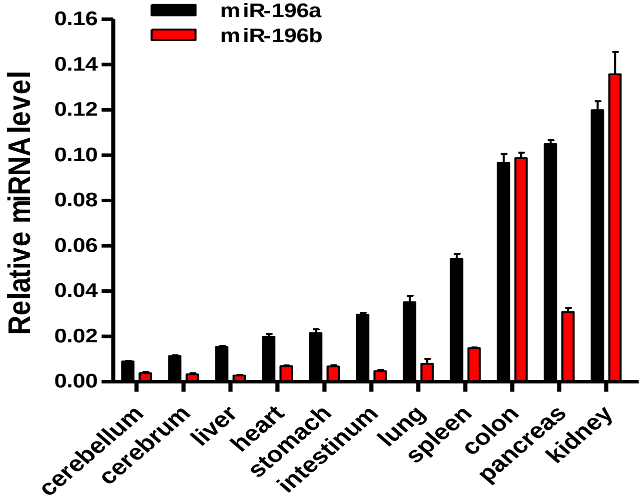
FSGS患者尿液miR-196变化的意义

miR-196a和miR-196b在小鼠肾脏中高表达

A



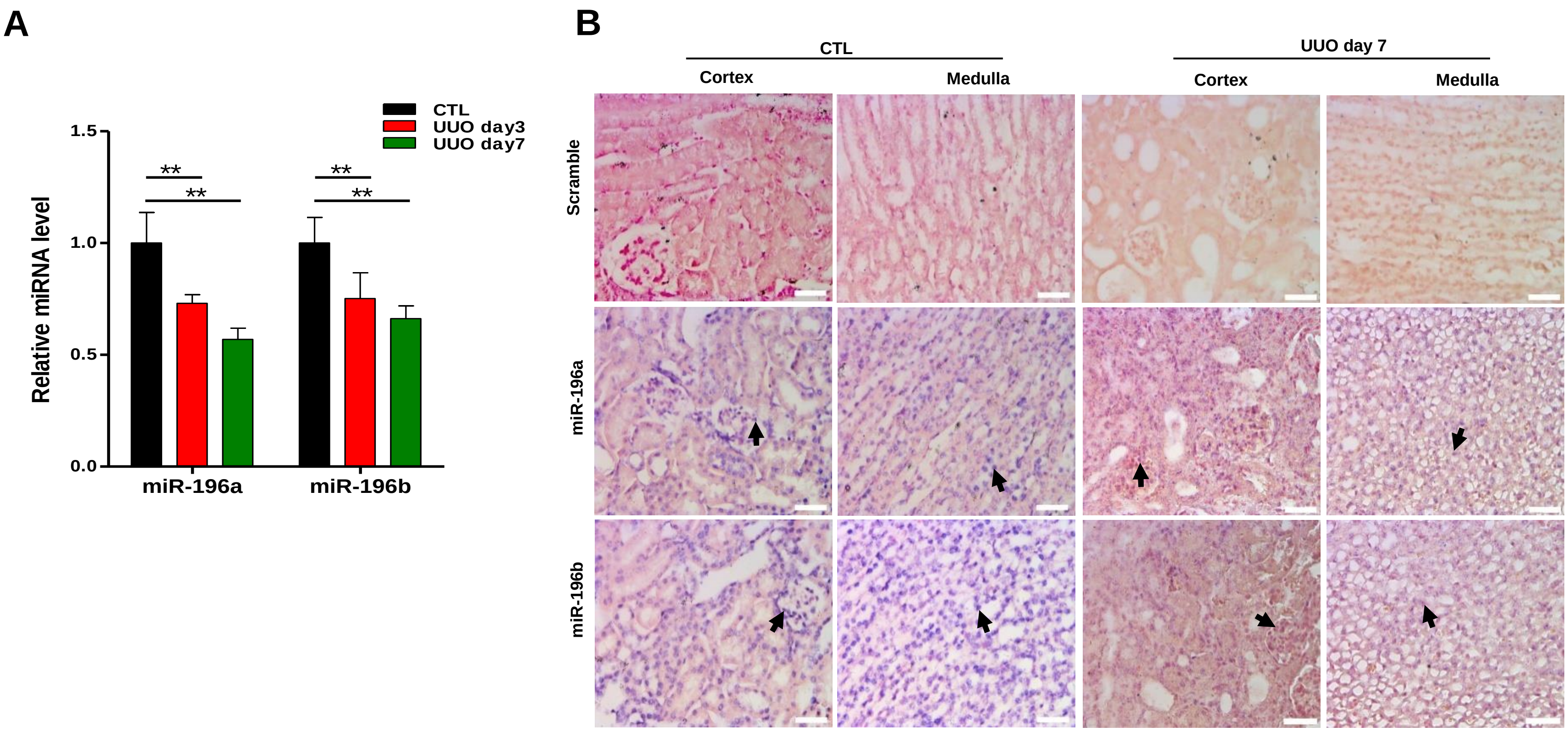
B



Using Solexa deep sequencing and identified that miR-196a is highly expressed in kidney

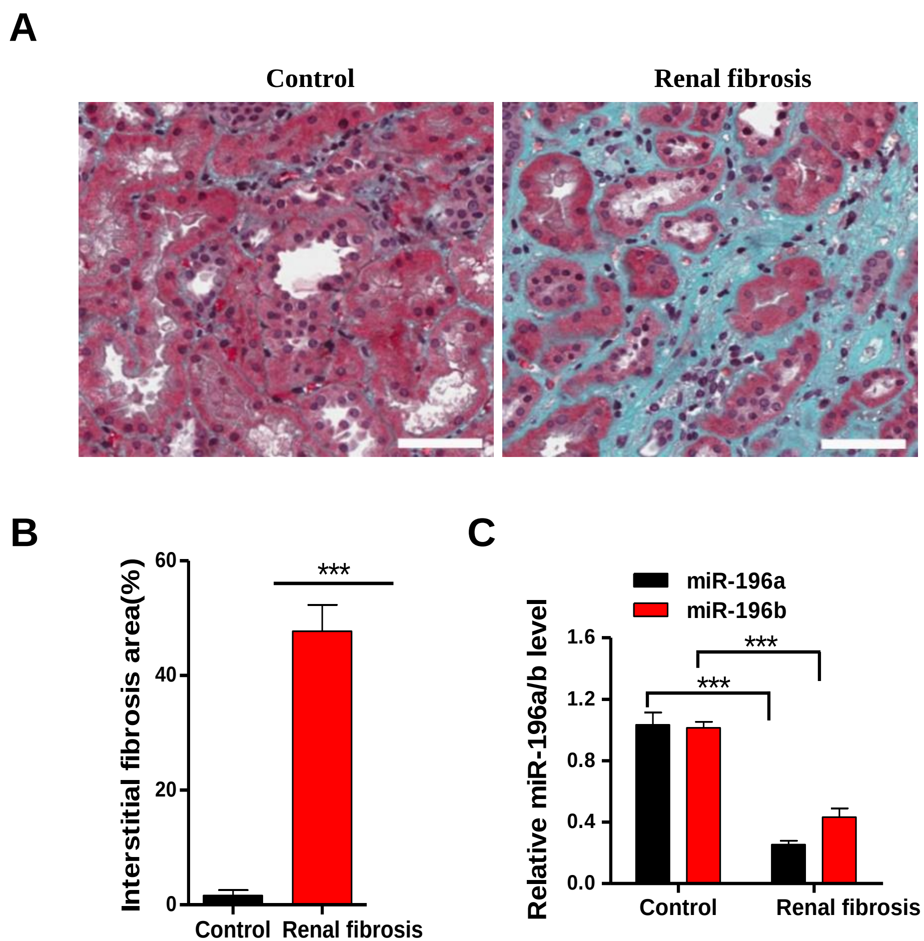
Meng et al. JASN revised

UUO模型小鼠肾脏miR-196a和miR-196b表达下调



Meng et al. JASN revised

肾脏纤维化患者肾组织中miR-196a和miR-196b表达下调



Meng et al. JASN revised

体内外研究证实miR-196a/b通过TGFβ-Smad信号通路抑制肾脏纤维化。

肾脏富含miR-196，FSGS患者尿液中高表达的miR-196可能源于损伤肾脏的被动泄漏或主动分泌。

血浆和尿液miRNAs作为FSGS生物标志物的联合检测

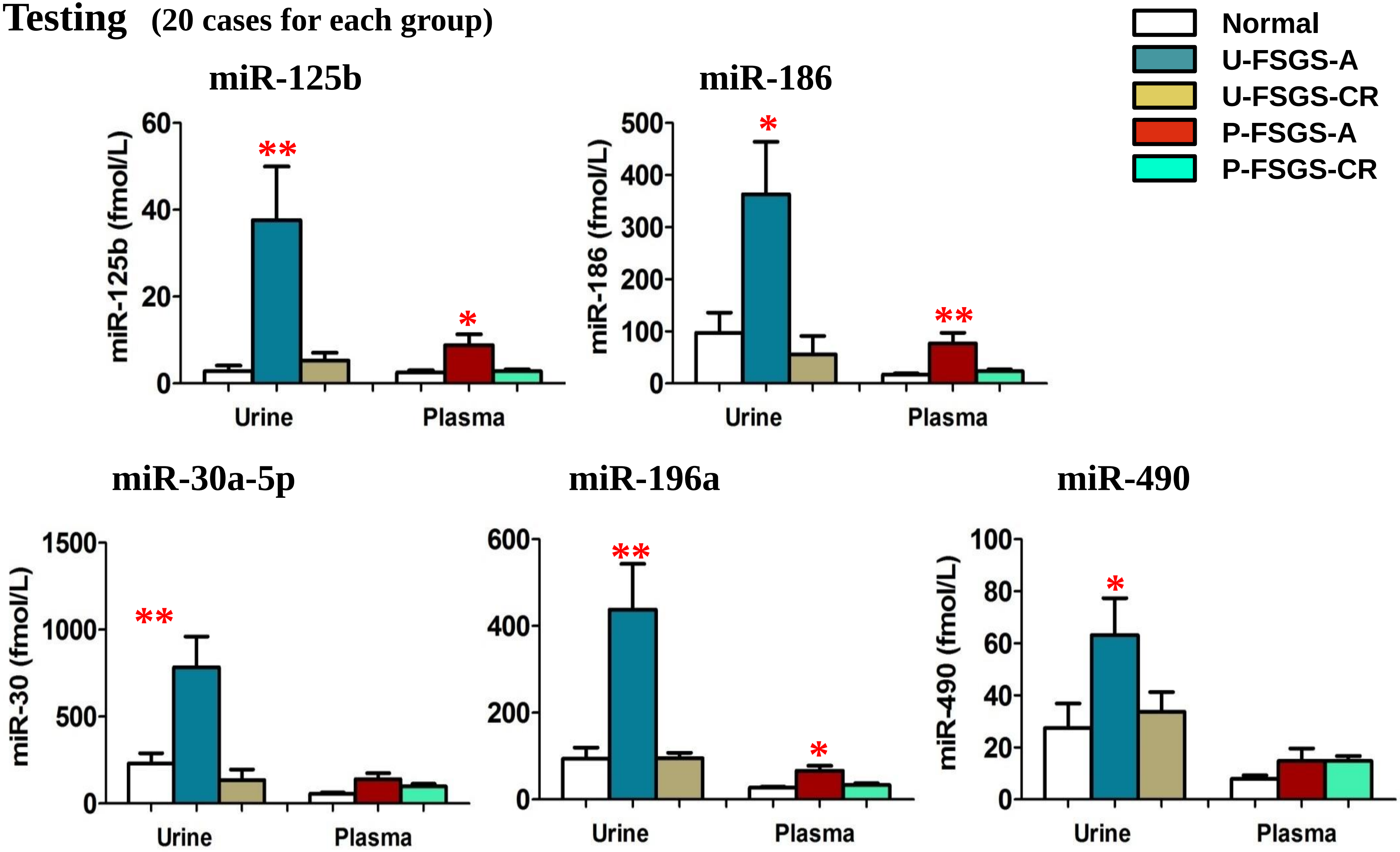
Plasma
miR-125b
miR-186

Urine
miR-30a-5p
miR-196a
miR-490

Plasma and urine

miR-125b, miR-186, miR-30a-5p, miR-196a, miR-490

Testing (20 cases for each group)



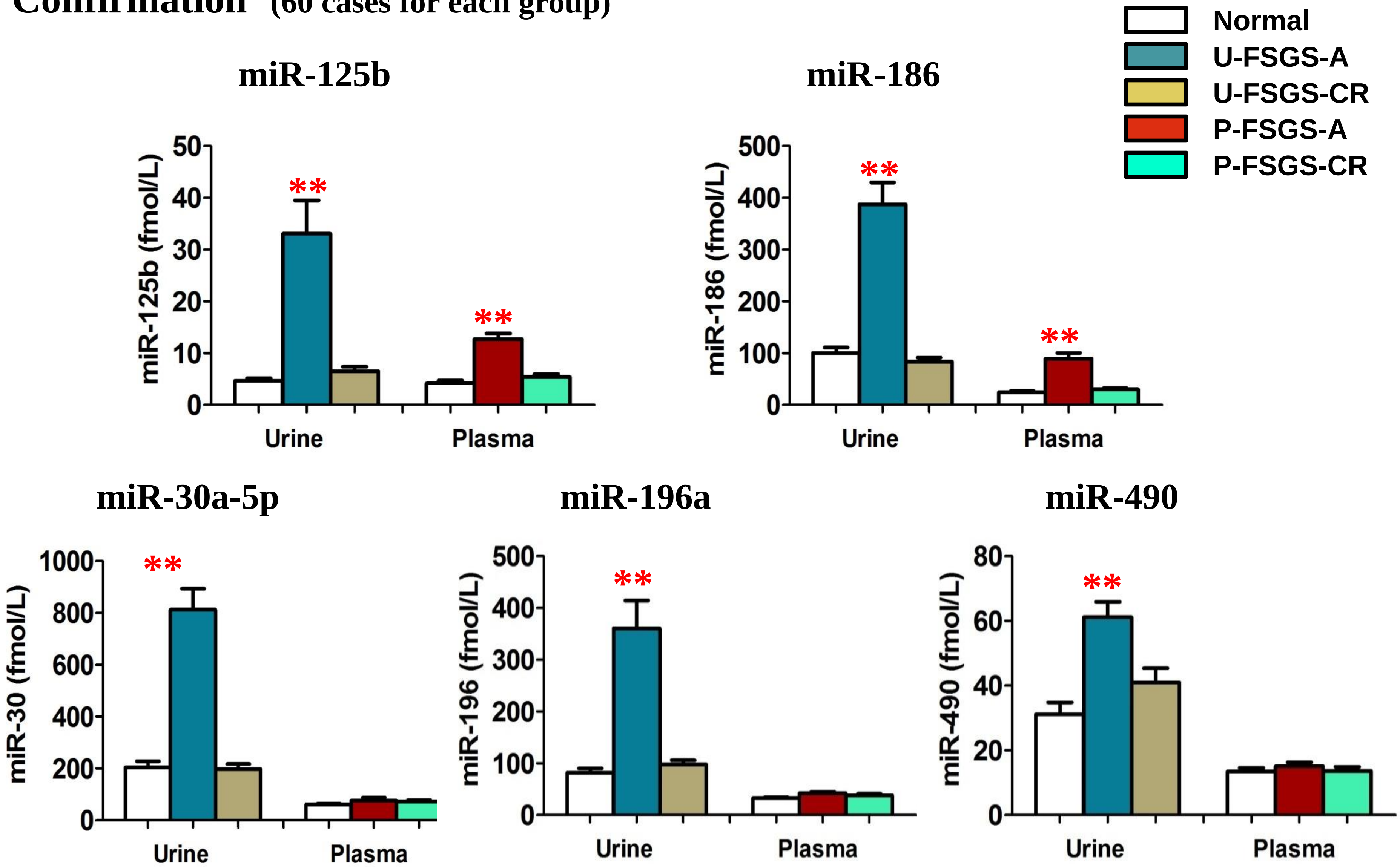
➢ **Plasma: miR-125b, miR-186, miR-196a**

➢ **Urine: miR-30a-5p, miR-196a, miR-490, miR-125b, miR-186**

*$p < 0.05$ vs Normal, FSGS-CR

**$p < 0.01$ vs Normal, FSGS-CR

Confirmation (60 cases for each group)

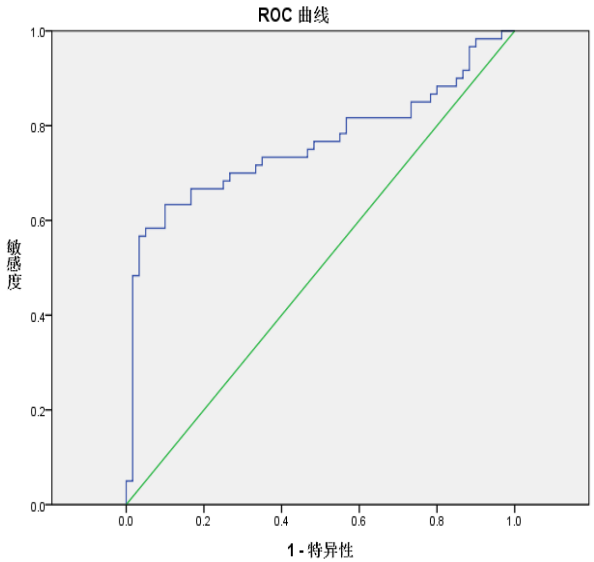


- **Plasma: miR-125b, miR-186**
- **Urine: miR-30a-5p, miR-196a, miR-490, miR-125b, miR-186**

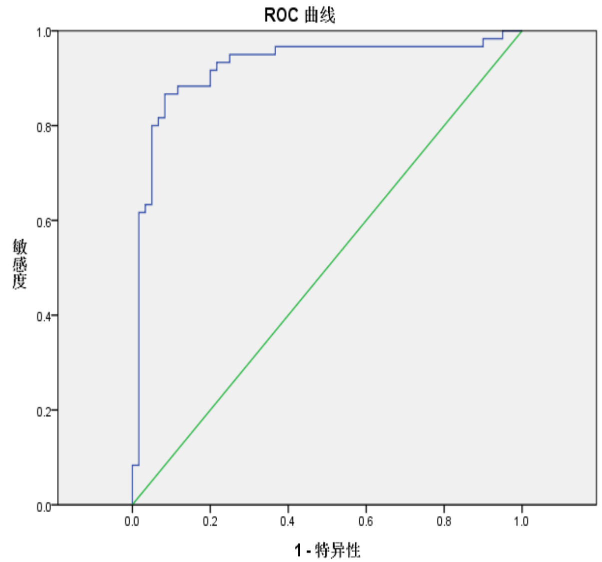
**$p < 0.01$ vs Normal, FSGS-CR (**)

血浆和尿液miR-125b区分活动性FSGS和完全缓解FSGS

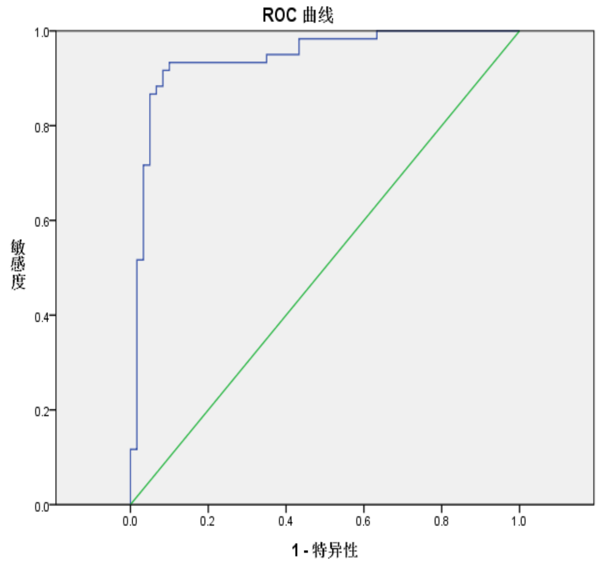
Plasma miR-125b



Urinary miR-125b



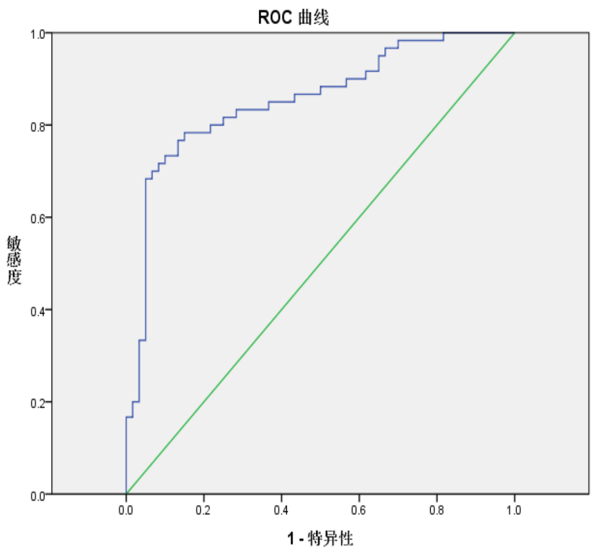
2 panel



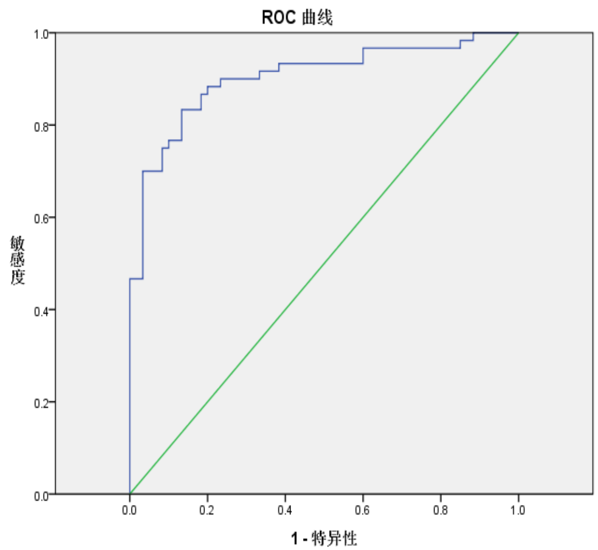
	AUC	95% CI	Specificity	Sensitivity	P value
P-miR-125b	0.760	0.669-0.851	0.967	0.567	8.98*10^-7
U-miR-125b	**0.924**	0.869-0.978	0.917	0.867	1.20*10^-15
2 panel	0.943	0.899-0.987	0.917	0.917	5.94*10^-17

血浆和尿液miR-186区分活动性FSGS和完全缓解FSGS

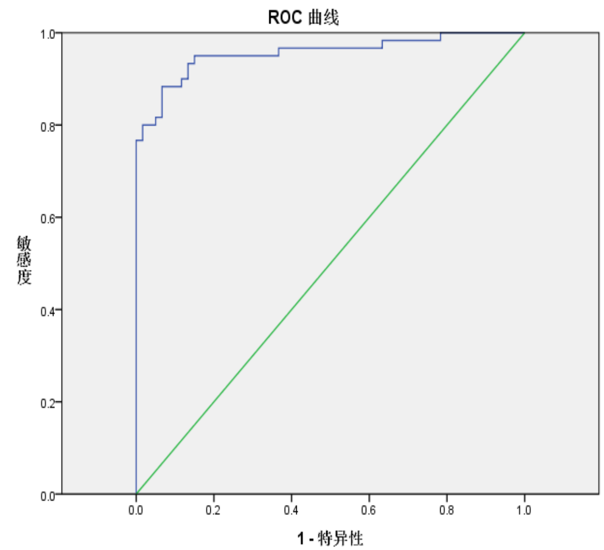
Plasma miR-186



Urinary miR-186



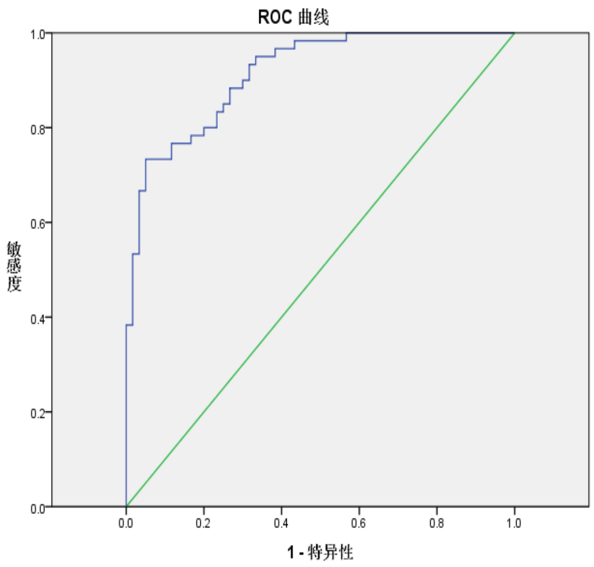
2 panel



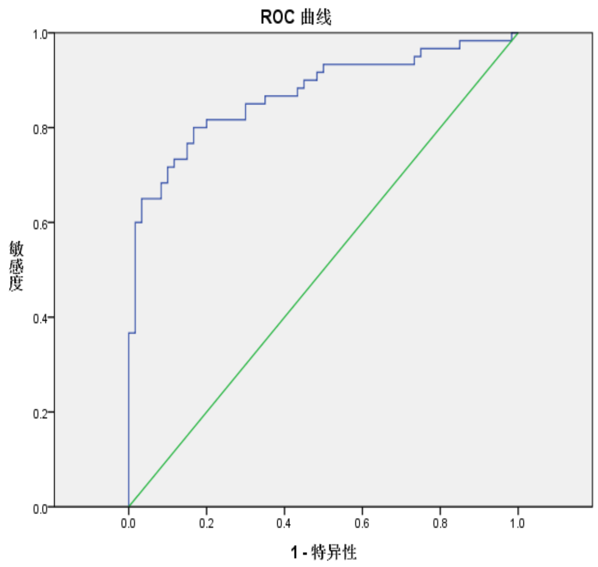
	AUC	95% CI	Specificity	Sensitivity	P value
P-miR-186	0.854	0.785-0.924	0.867	0.767	$2.12*10^{\wedge-11}$
U-miR-186	**0.903**	0.847-0.960	0.867	0.833	$2.52*10^{\wedge-14}$
2 panel	0.956	0.918-0.993	0.933	0.883	$7.45*10^{\wedge-18}$

尿液miR-30a, miR-196a 和miR-490区分活动性FSGS和完全缓解FSGS

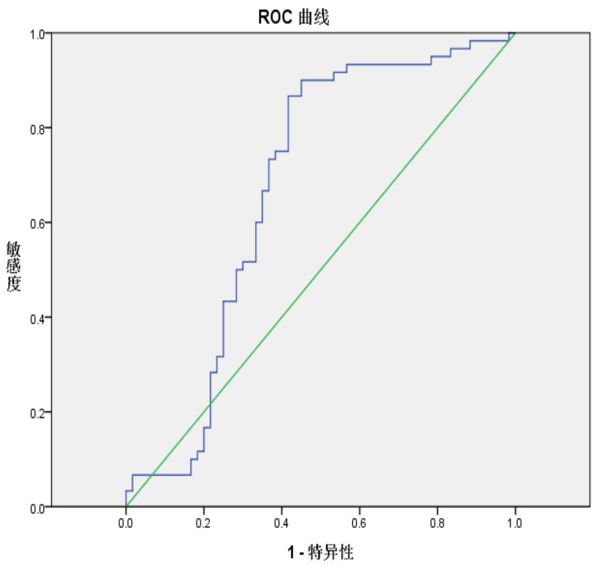
Urinary miR-30a



Urinary miR-196a



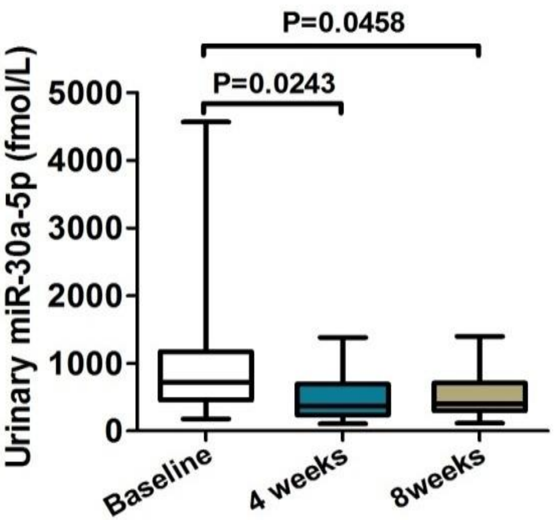
Urinary miR-490



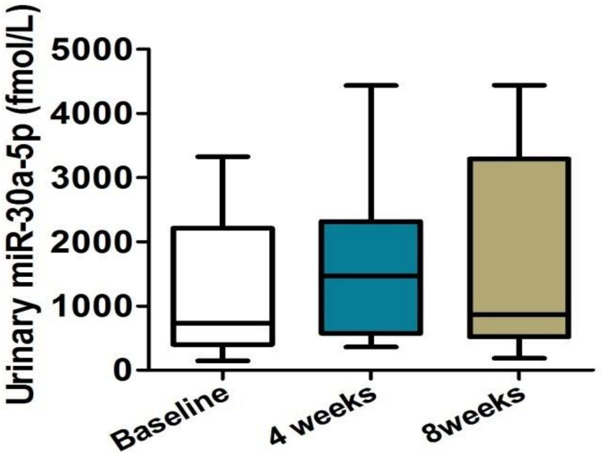
	AUC	95% CI	Specificity	Sensitivity	P value
U-miR-30a	**0.915**	0.867-0.962	0.950	0.733	$4.64*10^{\wedge-15}$
U-miR-196a	0.870	0.805-0.936	0.833	0.800	$2.63*10^{\wedge-12}$
U-miR-490	0.673	0.571-0.775	0.550	0.900	0.001

激素治疗前后尿液miR-30a-5p水平

Steroid-sensitive patients



Steroid-resistant patients



激素敏感患者，治疗4周后尿液miR-30a-5p水平较治疗前已有明显下降，早于尿蛋白的变化，激素抵抗患者在治疗前后则无明显差异。

miR-125b

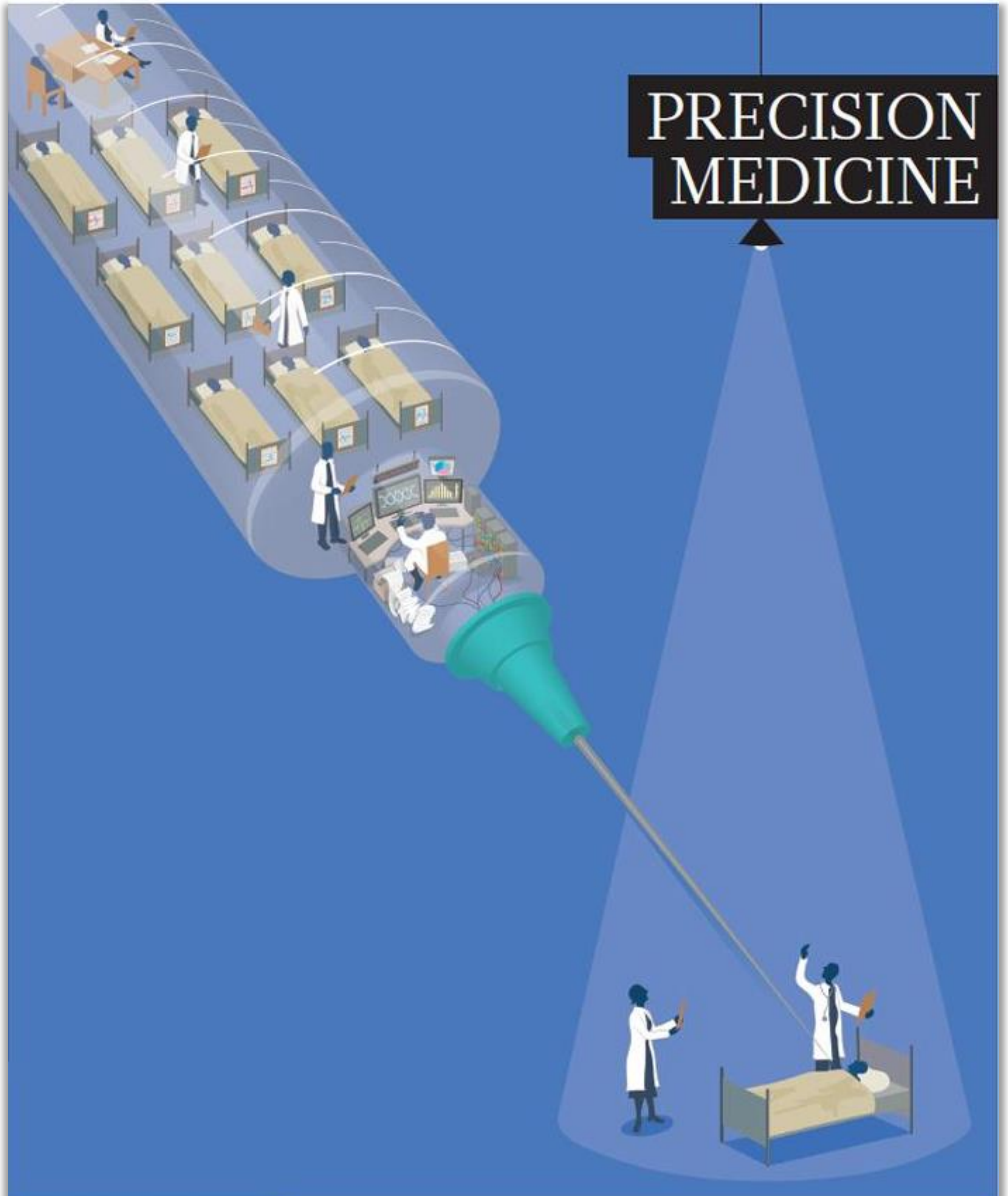
血浆和尿液miR-125b均与FSGS疾病活动相关，尿液miR-125b呈现更好的预测效力，尿液miR-125b可以作为FSGS诊断和监测的生物标志物。

miR-186

血浆和尿液miR-186均与FSGS疾病活动相关，尿液miR-186呈现更好的预测效力，尿液miR-186可以作为FSGS诊断和监测的生物标志物。

miR-30a

监测尿液miR-30a-5p的变化可用于预测FSGS患者对激素治疗的反应。



Bahcall (2015). Nature

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体液活检

前瞻性研究队列

生物样本库

基于组学的系统研究

大数据的生物信息学分析

鸣谢

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