Developing and validating the use of microRNA biomarkers for risk stratification of Non Muscle Invasive bladder cancer (NMIBC) patients

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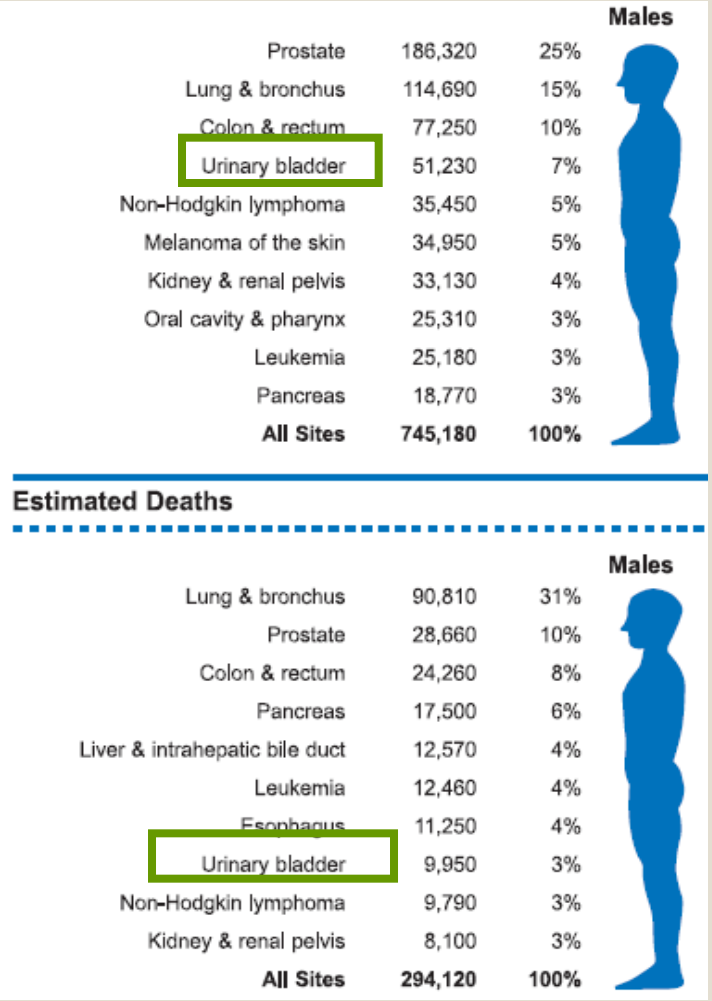
MicroRNA biomarkers for NMIBC

- Prostate cancer
- Bladder cancer
- Kidney cancer
- Testis cancer
- Soft tissue sarcomas
- Adrenal cancer

MicroRNA biomarkers for NMIBC

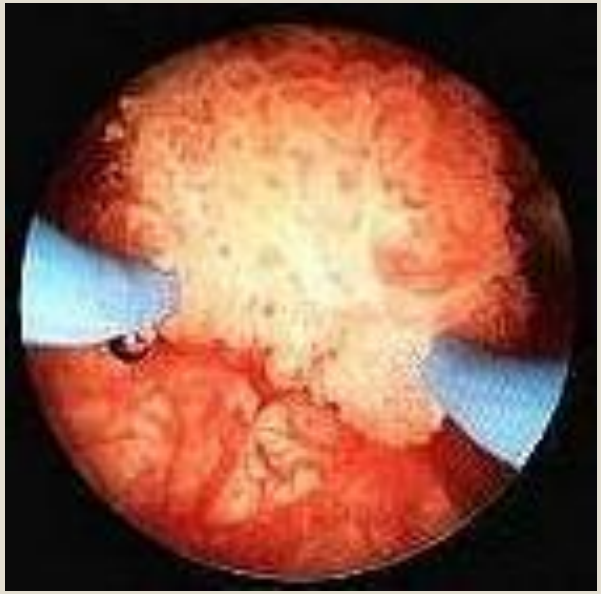
- Bladder cancer is the 4th most common cancer among males and accounts for 7% of all cancer cases
- Bladder cancer is the 8nd cause of cancer mortality among males and account for 3% of all cancer mortality cases among males



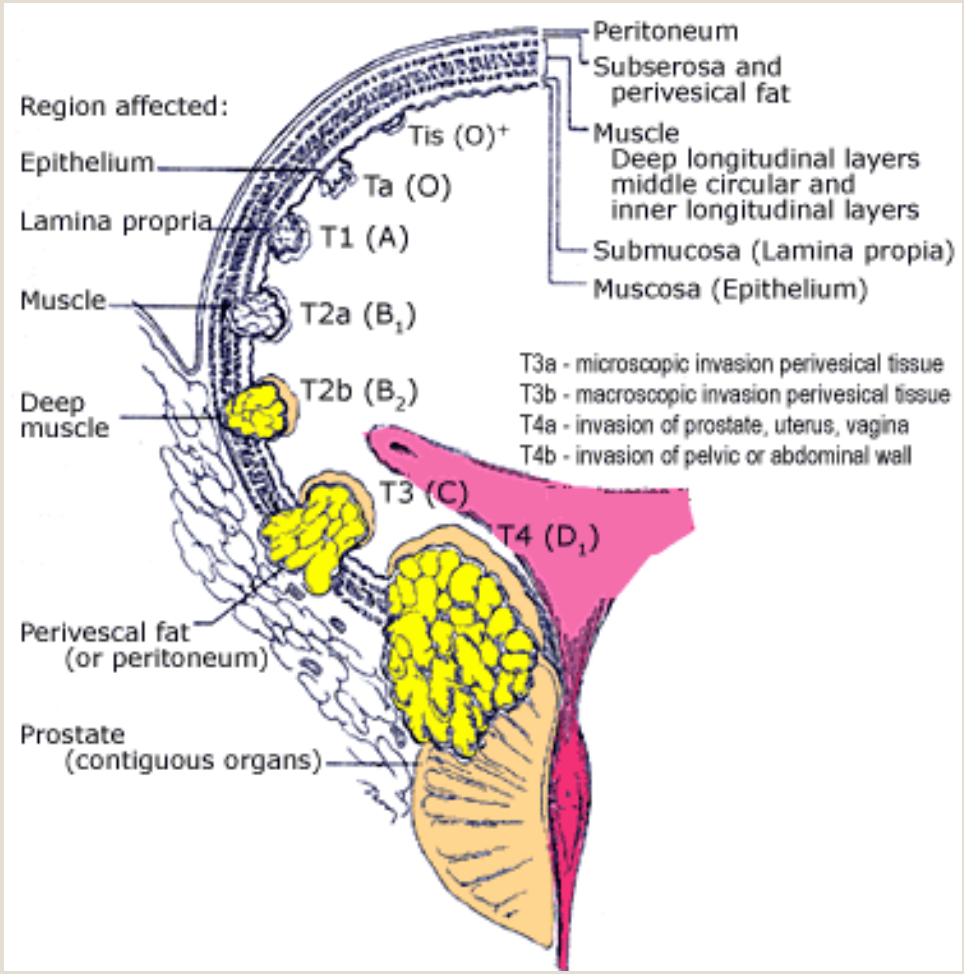
MicroRNA biomarkers for NMIBC

Mortality Rate of Cancer/100,000 males			
Disease	1990	2004	Change
Prostate	38	25	-34%
Lung	90	70	-22%
Colon	30	21	-29%
Larynx	3	2	-24%
Stomach	8	5	-37%
Bladder	8	7.6	-5%
All	278	228	-18%

MicroRNA biomarkers for NMIBC



MicroRNA biomarkers for NMIBC



MicroRNA biomarkers for NMIBC

Staging		
Non muscle invasive	Ta, T1, CIS	70%
Non muscle invasive	T2-4	25%
Metastatic	M+	5%

MicroRNA biomarkers for NMIBC

Author	Year	#	% of positive LN			
			pT1	pT2	pT3	Pt4
Goheneim	1997	1026	6	16	42	36
Leissner	2000	447	10	20	49	75
Stein	2001	1054	7	22	44	42
Madersbacher	2003	507	3	17	35	41

MicroRNA biomarkers for NMIBC

MicroRNA biomarkers for NMIBC

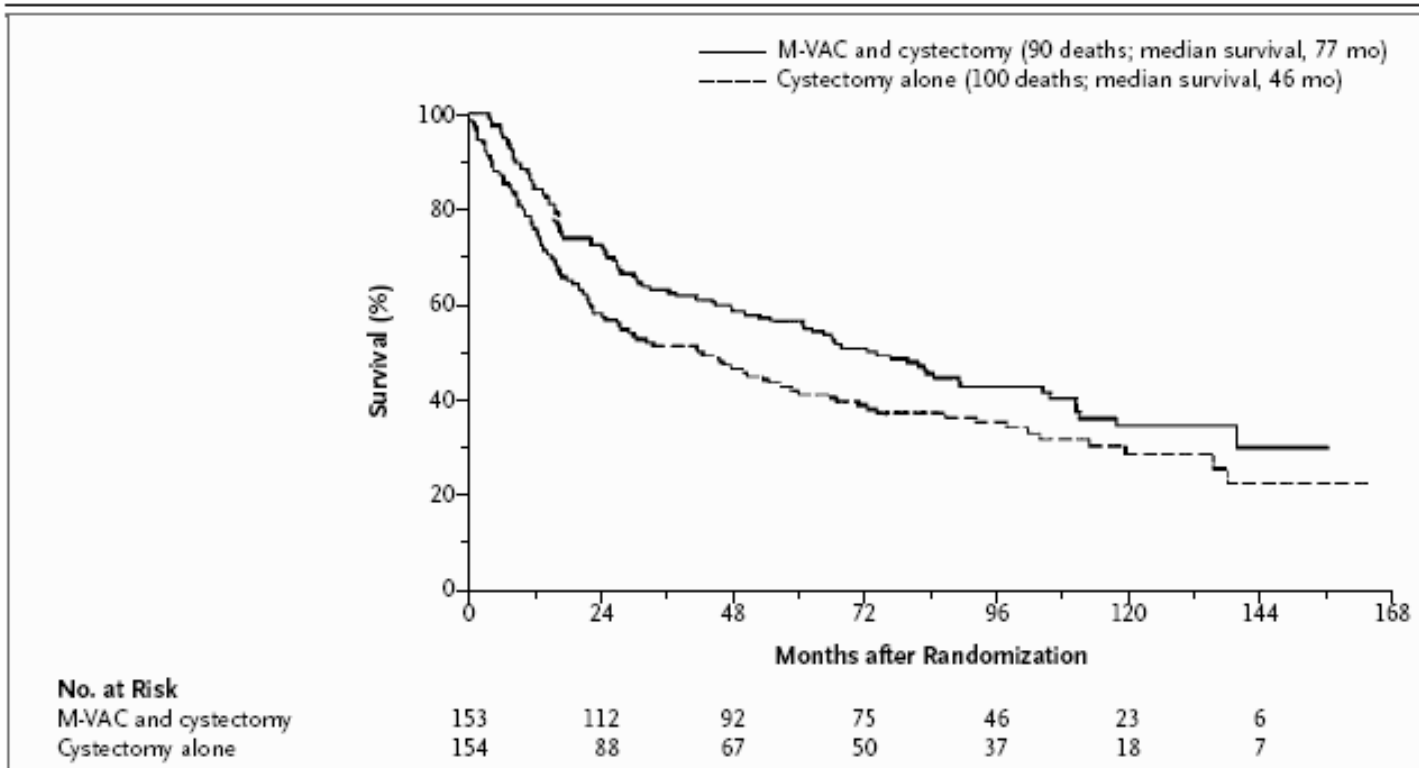


Figure 1. Survival among Patients Randomly Assigned to Receive Methotrexate, Vinblastine, Doxorubicin, and Cisplatin (M-VAC) Followed by Cystectomy or Cystectomy Alone, According to an Intention-to-Treat Analysis.

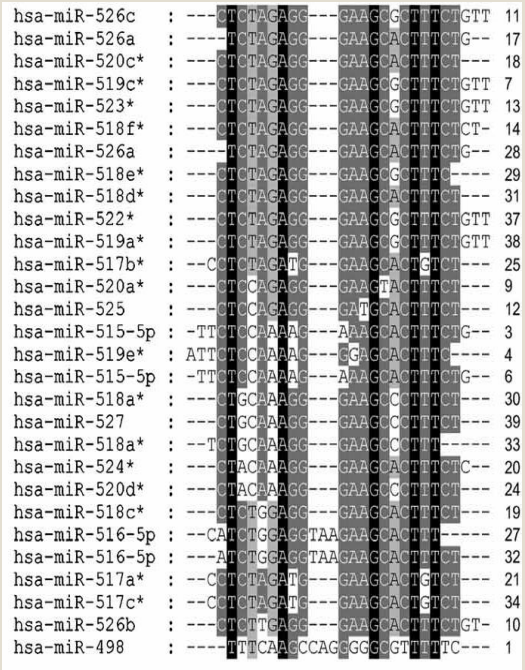
MicroRNA biomarkers for NMIBC

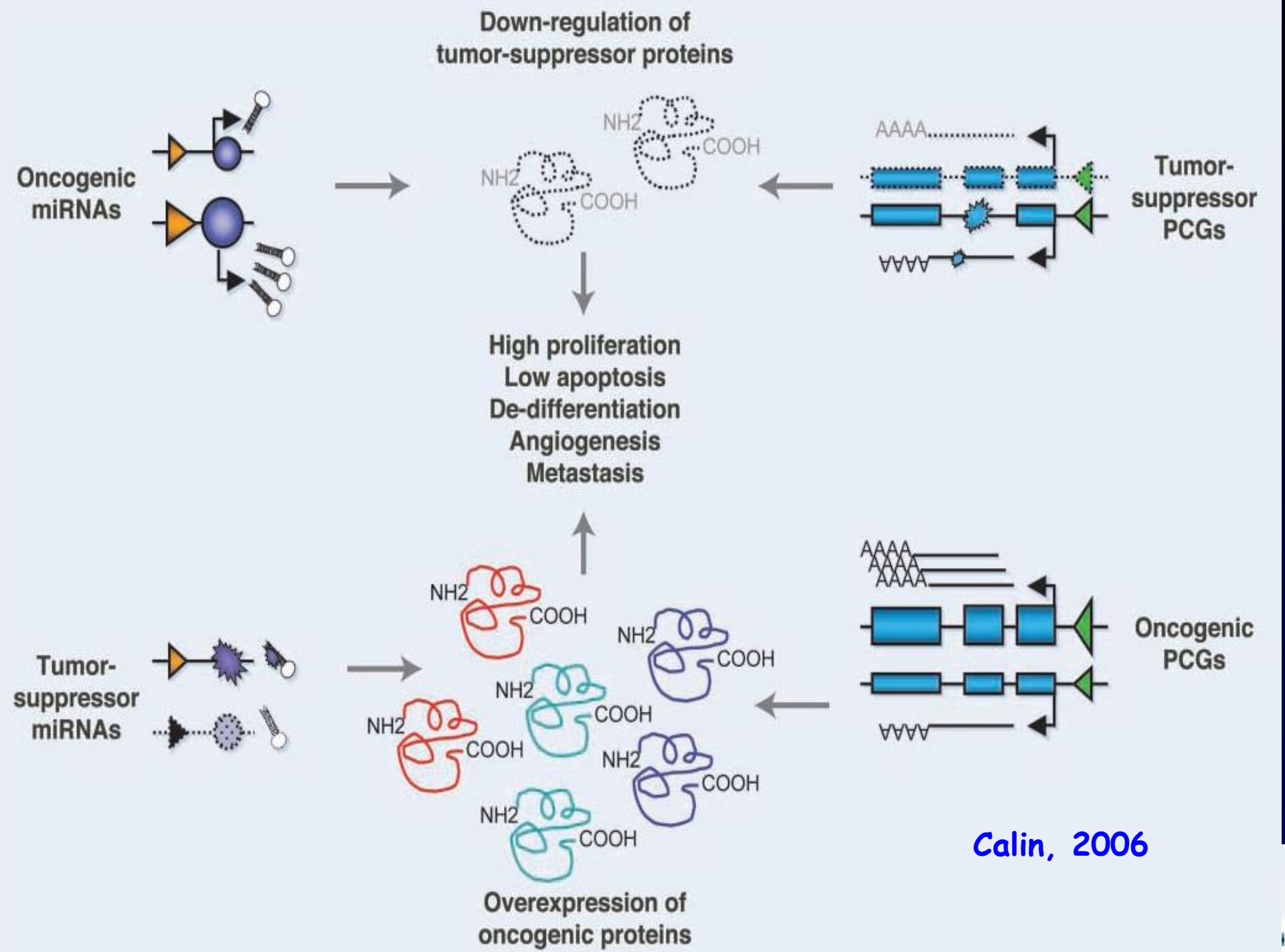
Possible improvements in bladder cancer:

- Early diagnosis
- Better staging – surgical/radiology
- Molecular markers
  - Progression
  - Death of disease
- Improve surgical and medical therapy

MicroRNA biomarkers for NMIBC

- MicroRNAs, a family of small non-coding regulatory RNAs (18-25 nucleotides)
- Specific overexpression or underexpression has been correlated with different tumor types
- Micro- RNAs can act as tumor suppressors and oncogenes



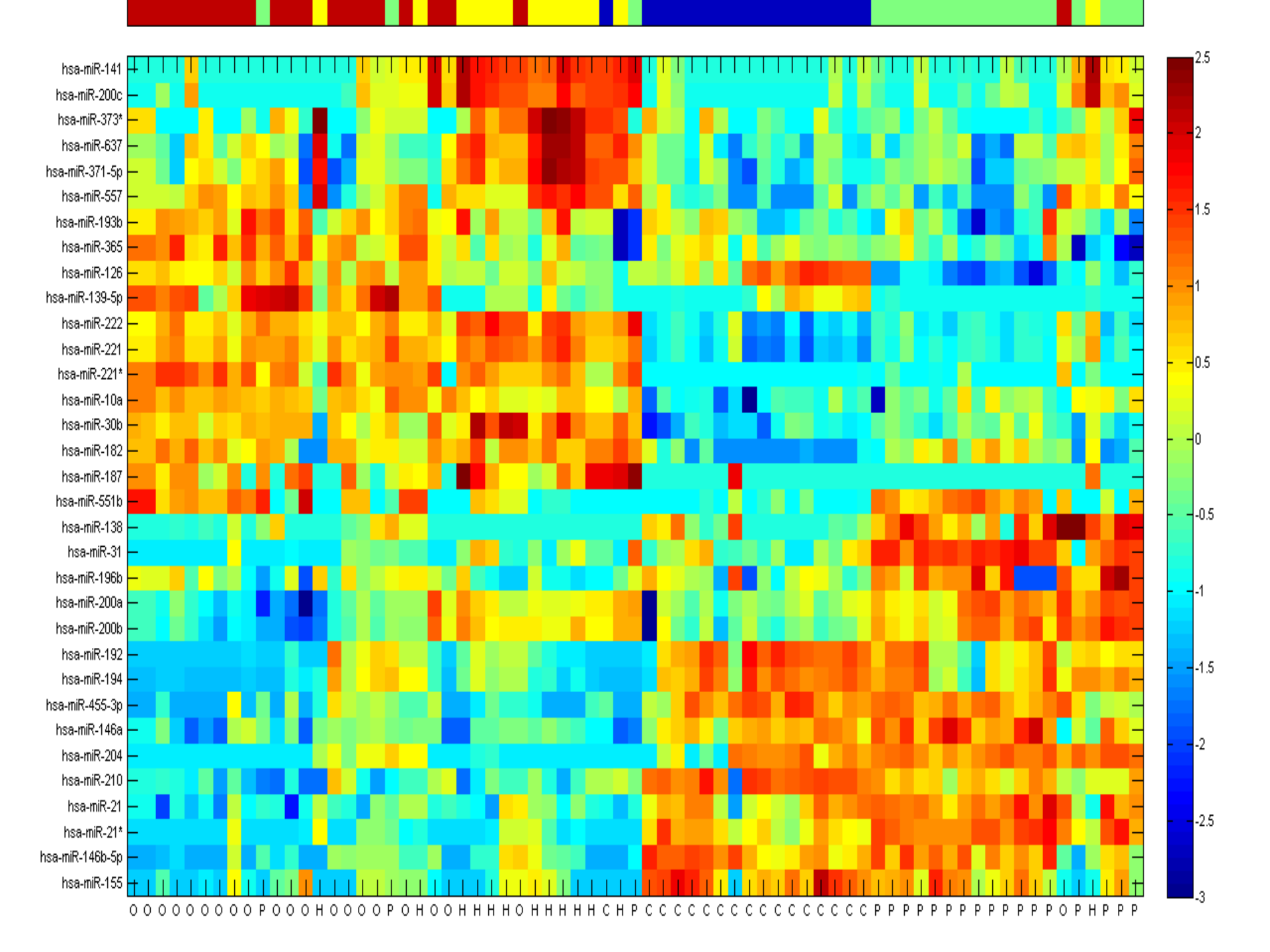

Down-regulation of
tumor-suppressor proteins
Oncogenic
miRNAs
NH2
COOH
NH2
COOH
AAAA
Tumor-
suppressor
PCGs
High proliferation
Low apoptosis
De-differentiation
Angiogenesis
Metastasis
Tumor-
suppressor
miRNAs
NH2
COOH
NH2
COOH
NH2
COOH
NH2
COOH
NH2
COOH
AAAA
Oncogenic
PCGs
Overexpression of
oncogenic proteins

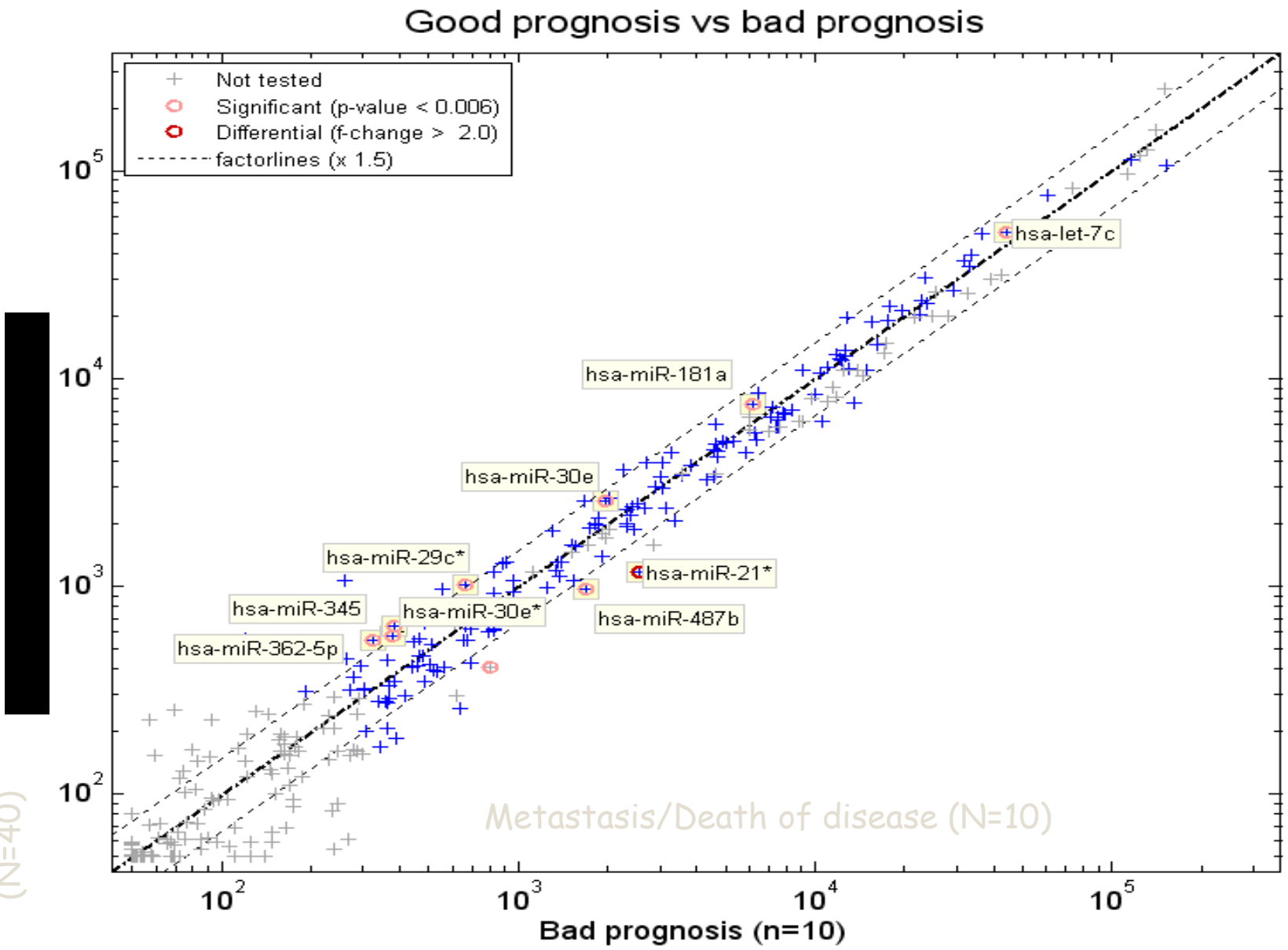
Calin, 2006

MicroRNA biomarkers for NMIBC

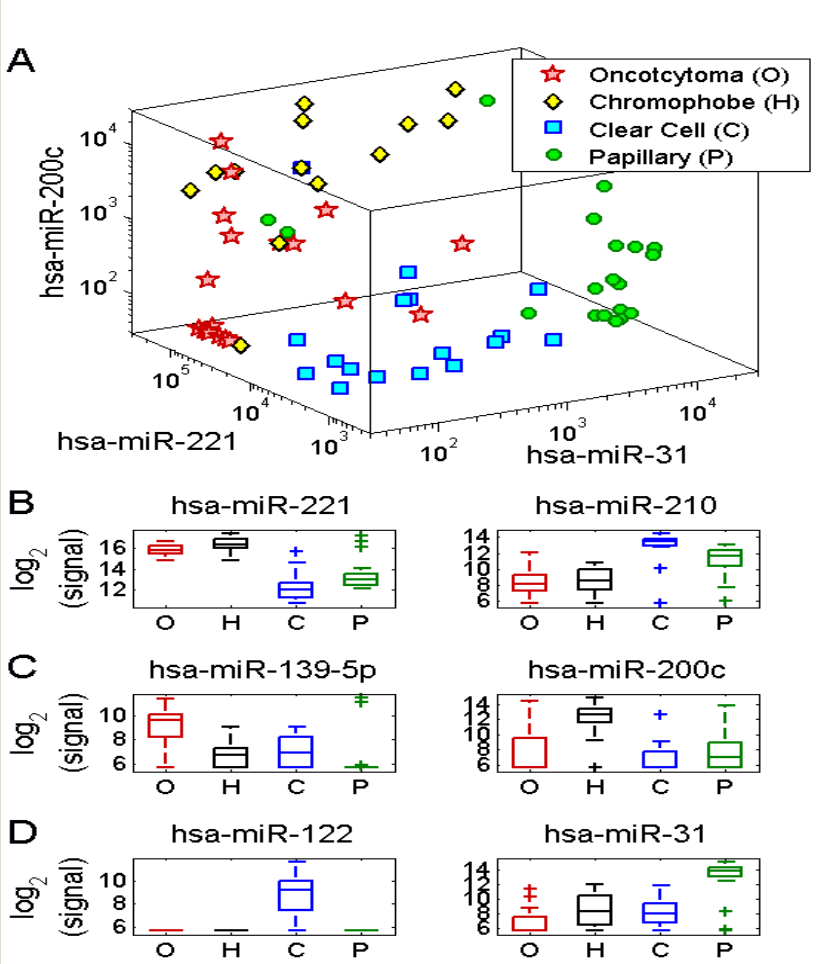
MicroRNA and renal cancer:

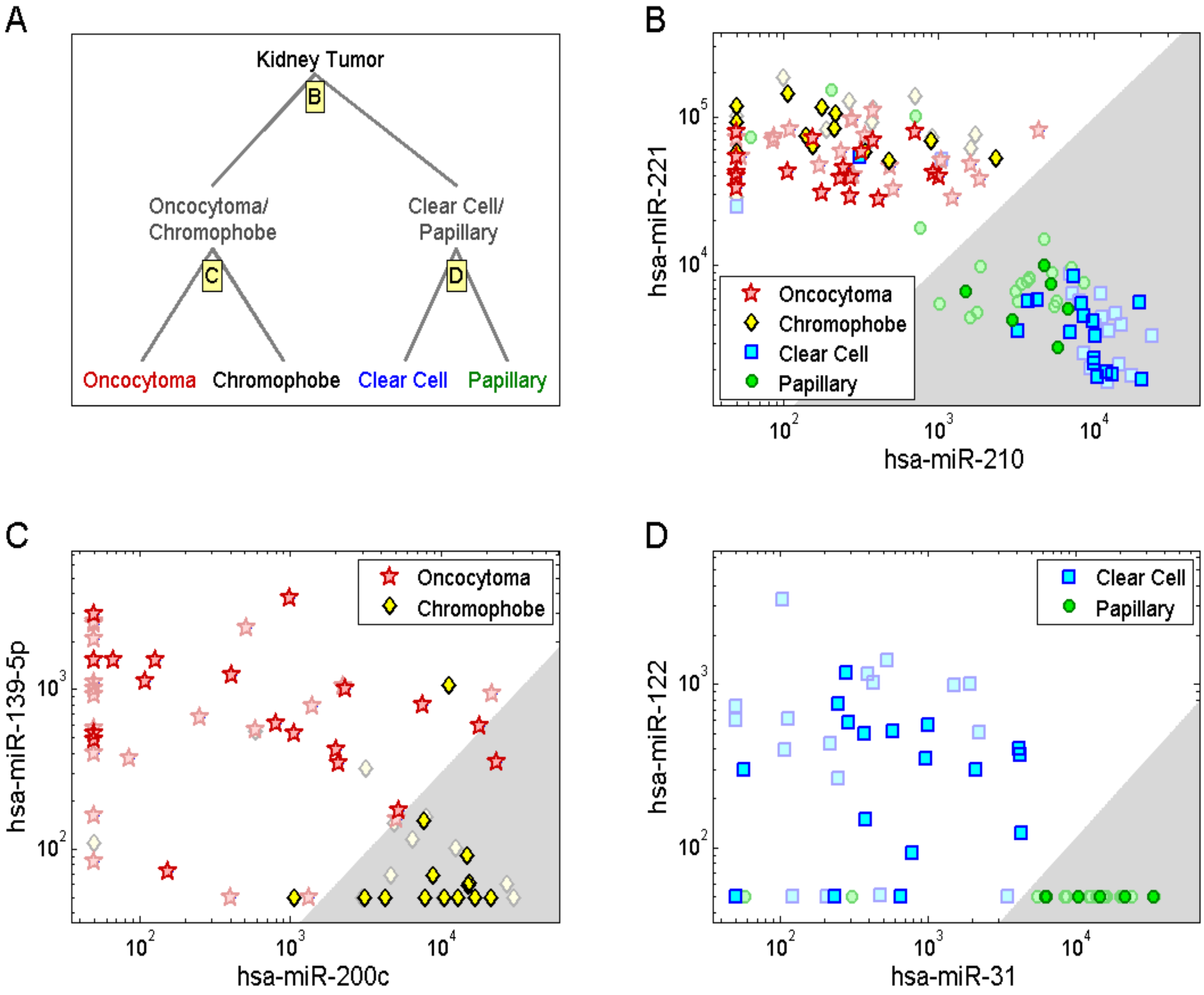
- Renal cell cancer – subtypes
- Renal cell cancer
  - Conventional type
  - pT2-4
  - End points
    - Metastasis progression
    - Death of disease

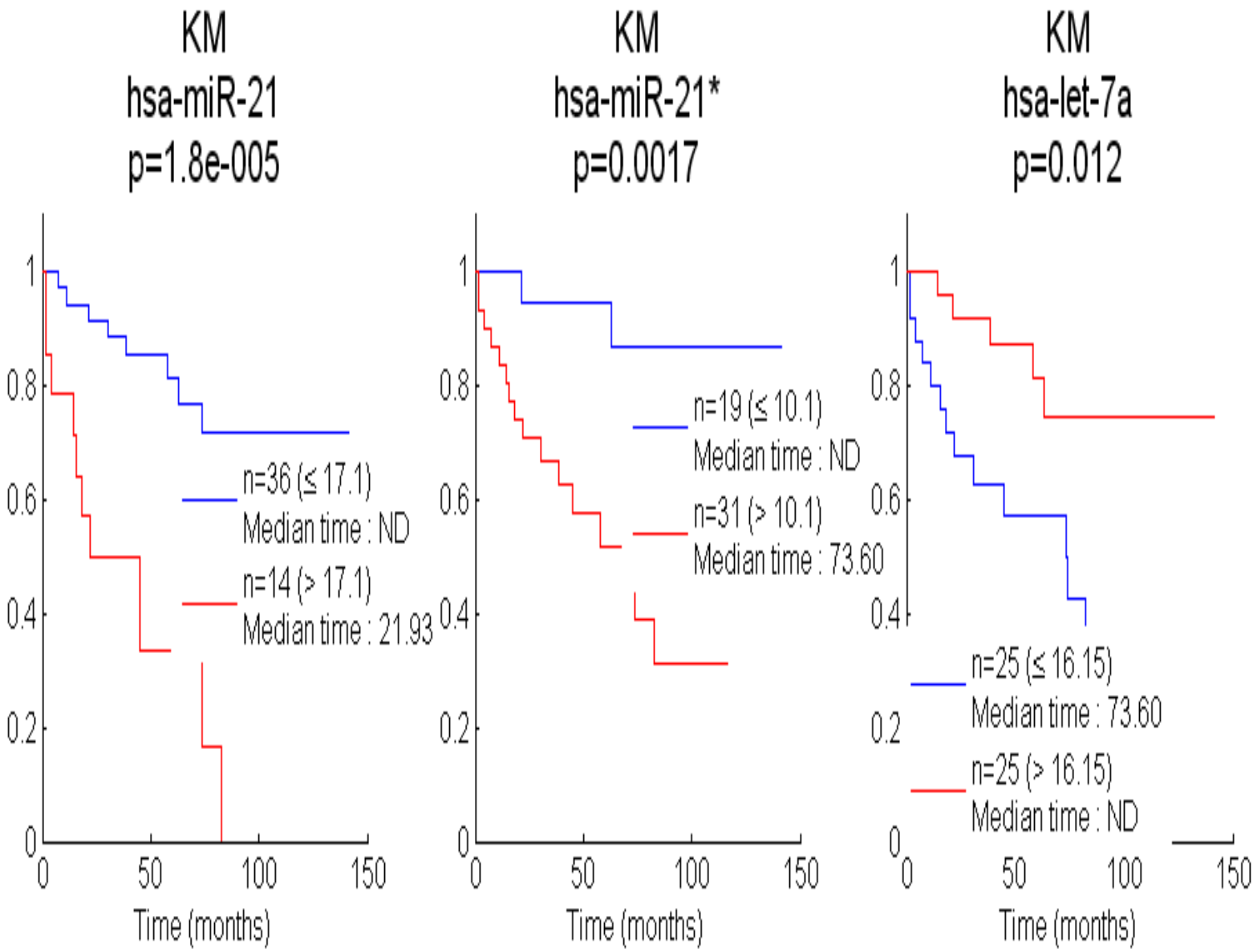

hsa-miR-141
hsa-miR-200c
hsa-miR-373*
hsa-miR-637
hsa-miR-371-5p
hsa-miR-557
hsa-miR-193b
hsa-miR-365
hsa-miR-126
hsa-miR-139-5p
hsa-miR-222
hsa-miR-221
hsa-miR-221*
hsa-miR-10a
hsa-miR-30b
hsa-miR-182
hsa-miR-187
hsa-miR-551b
hsa-miR-138
hsa-miR-31
hsa-miR-196b
hsa-miR-200a
hsa-miR-200b
hsa-miR-192
hsa-miR-194
hsa-miR-455-3p
hsa-miR-146a
hsa-miR-204
hsa-miR-210
hsa-miR-21
hsa-miR-21*
hsa-miR-146b-5p
hsa-miR-155
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3


Good prognosis vs bad prognosis
Not tested
Significant (p-value < 0.006)
Differential (f-change > 2.0)
factorlines (x 1.5)
hsa-let-7c
hsa-miR-181a
hsa-miR-30e
hsa-miR-29c*
hsa-miR-21*
hsa-miR-345
hsa-miR-30e*
hsa-miR-487b
hsa-miR-362-5p
Metastasis/Death of disease (N=10)
Bad prognosis (n=10)
No Evidence of Metastasis/Death of disease (N=40)

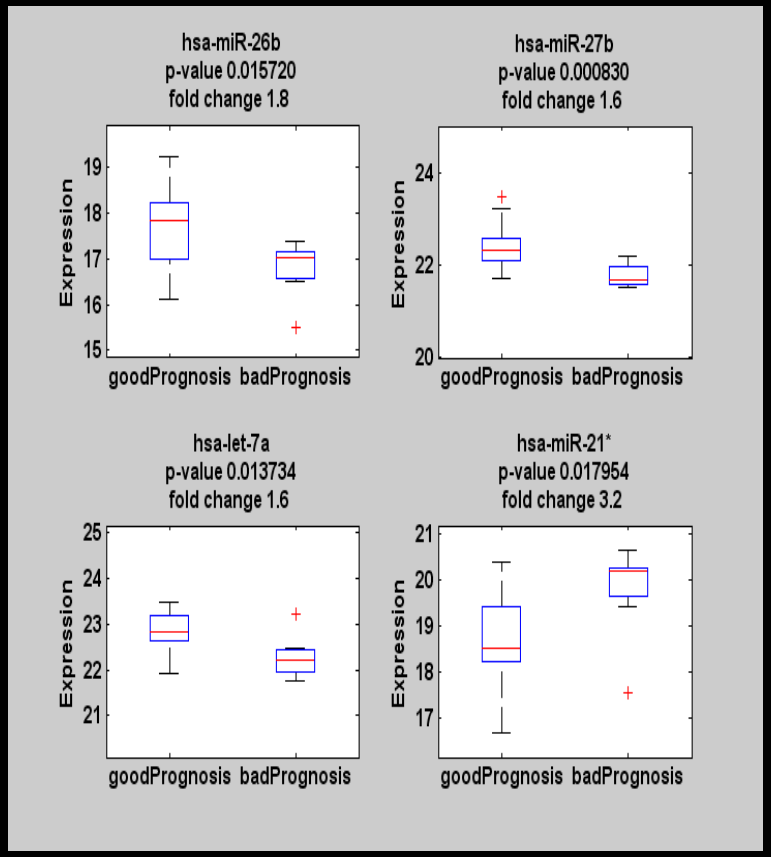
MicroRNA biomarkers for NMIBC

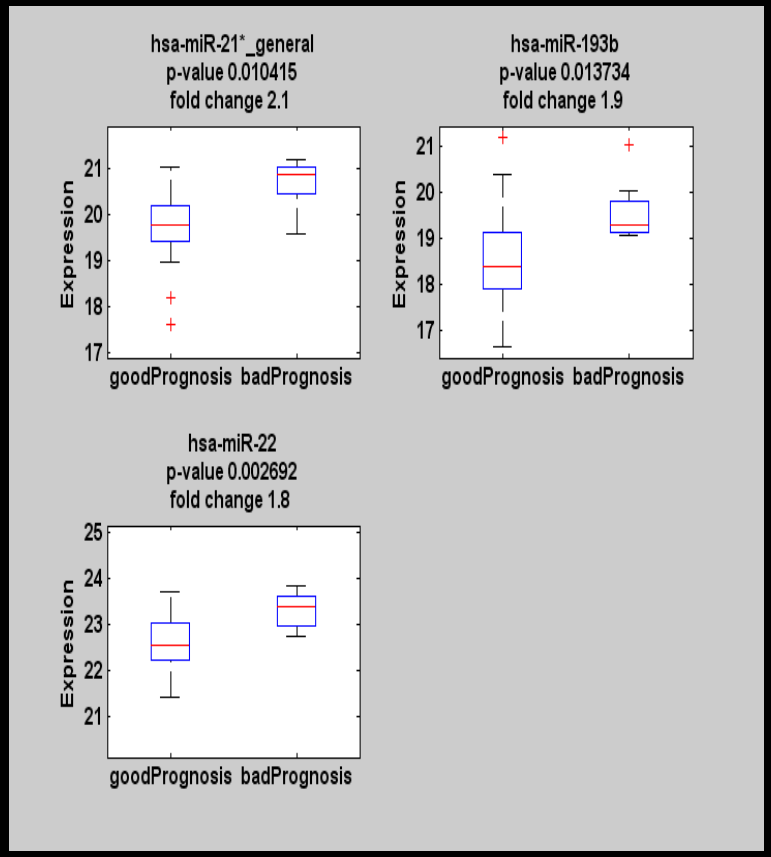



A
Kidney Tumor
B
Oncocytoma/
Chromophobe
Clear Cell/
Papillary
C
D
Oncocytoma
Chromophobe
Clear Cell
Papillary
B
hsa-miR-221
hsa-miR-210
Oncocytoma
Chromophobe
Clear Cell
Papillary
C
hsa-miR-139-5p
hsa-miR-200c
Oncocytoma
Chromophobe
D
hsa-miR-122
hsa-miR-31
Clear Cell
Papillary


KM
hsa-miR-21
p=1.8e-005
n=36 (≤ 17.1)
Median time : ND
n=14 (> 17.1)
Median time : 21.93
Time (months)
KM
hsa-miR-21*
p=0.0017
n=19 (≤ 10.1)
Median time : ND
n=31 (> 10.1)
Median time : 73.60
Time (months)
KM
hsa-let-7a
p=0.012
n=25 (≤ 16.15)
Median time : 73.60
n=25 (> 16.15)
Median time : ND
Time (months)

Expression of different miR in the 2 Clinical Categorizes (Mets/DOD vs. no)





MiR expression by prognosis using PCR

Up regulated in good Prognosis vs. bad Prognosis:

miR name	p-value	fold-change	median values
hsa-miR-26b	0.016	1.76 (+)	17.8
hsa-miR-27b	0.001	1.57 (+)	22.3
hsa-let-7a	0.014	1.55 (+)	22.8

Down regulated in good Prognosis vs. bad Prognosis:

miR name	p-value	fold-change	median values
hsa-miR-21*	0.018	3.16 (-)	18.5
hsa-miR-193b	0.014	1.89 (-)	18.4
hsa-miR-22	0.003	1.81 (-)	22.5

MicroRNA biomarkers for NMIBC

Variable	b	se	P value
Stage – T2 vs T3	0.217	0.713	0.761
Maximal tumor size	0.209	0.098	0.034
hsa-miR-29c*	-1.055	0.417	0.011
hsa-miR-21*	0.915	0.406	0.024

MicroRNA biomarkers for NMIBC

Current study:

- Bladder cancer – T1HG
  - Primary
  - Progression
- Pathology analysis
- Initial group
  - Progression/DOD – 50
  - Cure/no progression - 50
- Validation multicentric

MicroRNA biomarkers for NMIBC

Current study:

- Identify – T1HG
- Datebase – clinical and pathology variables
- Pathology blocks
- Isolation of micro-RNA
- Markers identification
- Validation