MicroRNA Expression Profiling Distinguish Normal From Neoplastic Urothelium

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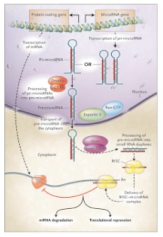
Introduction

- The majority of studies, to date, on the genetic mutations that contribute to bladder cancer formation have focused on alterations to the genome and expression of protein coding genes.
- Increasing evidence indicates, however, that dysregulation of microRNAs is associated with cancer and their expression profile can be correlated with disease pathogenesis and diagnosis.
- The dysregulation of microRNAs has been observed in many cancers, including those in the breast, lung, colon, and pancreas.
- In this report, we describe a procedure designed to identify the global expression pattern of microRNA expression in low and high grade bladder cancer. The goal is to define the microRNAs that can differentiate low and high grade bladder cancer from normal tissue for diagnostic and prognostic reasons.



- Furthermore, recognition of microRNAs that are differentially expressed between the normal and tumor may help to identify those that are involved in bladder cancer and establish the basis to unravel their pathogenic role.

Patient Study

Tissue Specimens
Bladder tissue specimens were obtained from patients at Boston Medical. Institutional Review Board-approved informed consent for the collection of specimens was obtained from all patients. Seven of the tissue specimens were classified as high-grade, eight of the specimens were classified as low-grade, while ten of the specimens were normal urothelium. These fresh operative specimens were then put into five volumes of RNA later, set at room temperature for 1 hour, and stored at -80 C until RNA extraction.

cDNA Synthesis
Total RNA was prepared using the mirVANA microRNA Isolation Kit (Ambion, Austin, TX, USA), and subsequently quantified using a spectrophotometer. Two micrograms of total RNA were reverse transcribed into first-strand cDNA using the QuantiMir Kit (System Biosciences, Mountain View, CA, USA), which tags and converts small RNAs into detectable cDNAs for qPCR.

Real-time quantitative PCR
Each qPCR reaction contained 5.0 ng of synthesized cDNA, 300 nM Univeral reverse primer and 500nM microRNA-specific forward assay primer in a total reaction volume of 20ul. The sequence of the microRNA-specific forward primer was identical to the target microRNA being measured. The entire qPCR plate containing 96 assays (see microRNA assay panel) was subjected to standard real-time qPCR thermocycling and data collection. All reactions were performed in triplicate using an ABI 7900HT Sequence Detection System with PowerSYBR reagents (Applied Biosystems, Foster City, CA).

Method

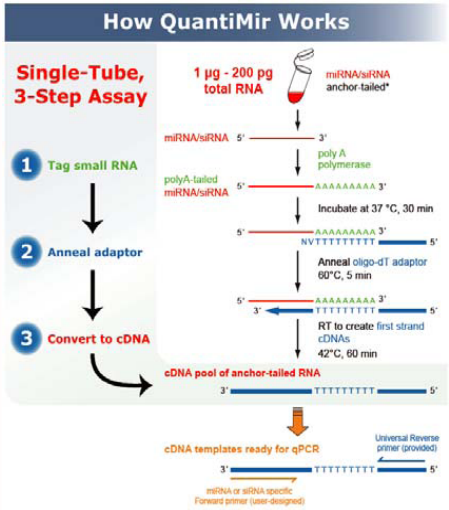
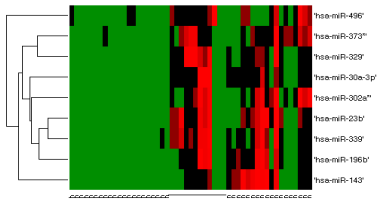


Plate Array Arrangement

	1	2	3	4	5	6	7	8	9	10	11	12
A	let-7-family	miR-7	miR-92	miR-93	miR-9-1	miR-101-1	miR-103	miR-106a	miR-106b	miR-107	miR-10b	miR-1-1
B	miR-122a	miR-125a	miR-125b	miR-126	miR-128b	miR-132	miR-133a	miR-134	miR-135b	miR-136	miR-137	miR-140
C	miR-141	miR-142-5p	miR-143	miR-145	miR-146a	miR-149	miR-150	miR-151	miR-153	miR-154	miR-155	miR-15a
D	miR-15b	miR-16	miR-17-3p	miR-17-5p	miR-181a	miR-181b	miR-181c	miR-181d	miR-183	miR-185	miR-186	miR-188
E	miR-18a	miR-190	miR-191	miR-192	miR-194	miR-195	miR-196a	miR-197	miR-198	miR-199a+b	miR-30b	miR-19a+b
F	miR-95	miR-20a	miR-200a	miR-200b	miR-200c	miR-202	miR-203	miR-204	miR-205	miR-206	miR-21	miR-210
G	miR-214	miR-215	miR-372	miR-373	miR-218	miR-219	miR-22	miR-488	miR-221	miR-222	miR-223	miR-224
H	miR-23a	miR-24	miR-25	miR-26a	miR-26b	miR-27a+b	miR-30c	miR-29a+b+c	miR-30a-3p	miR-30a-5p	miR-296	U6 snRNA

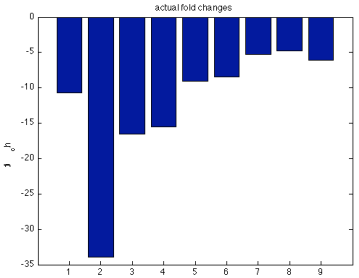
MicroRNA Signature Distinguishing HG/LG Bladder Cancer From Normal

High Grade Bladder Cancer

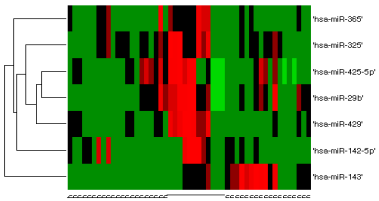


miR	fold change
'hsa-miR-196b'	-10.7424526
'hsa-miR-143'	-33.9199892
'hsa-miR-23b'	-16.5776332
'hsa-miR-30a-3p'	-15.5383422
'hsa-miR-302a*'	-9.03335411
'hsa-miR-329'	-8.46341989
'hsa-miR-339'	-5.30631439
'hsa-miR-373*'	-4.80447516
'hsa-miR-496'	-6.05300353

P < 0.05

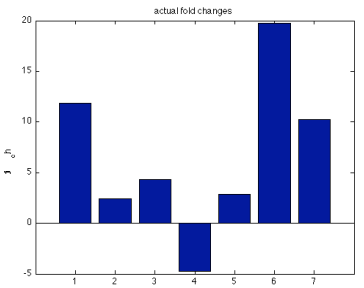


Low Grade Bladder Cancer



P < 0.05

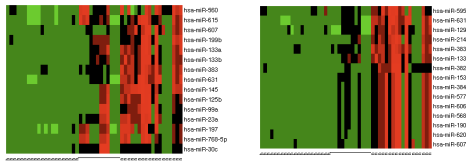
miR	fold
'hsa-miR-142-	11.8381426
'hsa-miR-29b'	2.44437238
'hsa-miR-325'	4.32463512
'hsa-miR-143'	-4.73075626
'hsa-miR-425-	2.84153053
'hsa-miR-365'	19.7338189
'hsa-miR-429'	10.2700075



Statistical Method

- Data transformed via DeltaCt: $2^{-(Ct\_target - Ct\_control)}$
- Control miR: 106b
- Independent samples t-test run for HG miR vs. Normal miR
- Independent samples t-test run for LG miR vs. Normal miR
- Reject when p< 0.05 (i.e. these are miR with different means)
- Find non-overlapping miR from rejected lists, thus creating separate identifiers
- Calculate average fold change for a group (e.g. HG vs. Normal)
- Select literature supported (143) and fold change based discriminators (i.e. with heatmap)

Bladder cancer signature



miR	Fold Change
'hsa-miR-631'	-8.64832444
'hsa-miR-145'	-46.5856007
'hsa-miR-133a'	-19.0326341
'hsa-miR-383'	-8.75437978
'hsa-miR-23a'	-18.8686399
'hsa-miR-125b'	-44.173504
'hsa-miR-197'	-5.53230178
'hsa-miR-133b'	-16.0324883
'hsa-miR-99a'	-46.2086429
'hsa-miR-30c'	-5.44951327
'hsa-miR-607'	-10.3710262
'hsa-miR-615'	-5.34084239
'hsa-miR-768-5p'	-12.0061793
'hsa-miR-560'	-4.38513412
'hsa-miR-199b'	-8.95891163

P < 0.001

miR	Fold Change
'hsa-miR-631'	-4.6211824
'hsa-miR-383'	-3.55477869
'hsa-miR-607'	-4.68154368
'hsa-miR-129'	-3.42240449
'hsa-miR-190'	-5.37522055
'hsa-miR-384'	-5.37522055
'hsa-miR-568'	-5.37522055
'hsa-miR-577'	-5.37522055
'hsa-miR-606'	-5.37522055
'hsa-miR-620'	-5.37522055
'hsa-miR-595'	-4.40108418
'hsa-miR-382'	-6.41931282
'hsa-miR-153'	-4.58550589
'hsa-miR-133b'	-3.64378085
'hsa-miR-214'	-6.16848656

HG LG

Conclusion

- Top 15 differentially expressed microRNAs are down regulated in HG and LG bladder cancer.
- 9 MicroRNA signature set can distinguish HG bladder cancer from normal tissue, while 7 microRNAs can distinguish LG from normal
- Mir143 is common to both
- Further validation on more samples are needed.

References

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