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.

Method

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 one of the specimens was normal architecture. Three 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 Universal reverse primer and 500 nM microRNA-specific forward assay primer in a total reaction volume of 20ul. The sequence of the microRNA-specific forward assay primers used are the target microRNA being measured. The entire qPCR plate containing 96 wells (two microRNA assay panel) was subjected to standard real-time PCR. All reactions were performed in triplicate using an ABI 7900HT Sequence Detection System with PowerSYBR reagents (Applied Biosystems, Foster City, CA).

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)

MicroRNA Signature Distinguishing HG/LG Bladder Cancer From Normal

HG

LG

References


Patient Study

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Conclusion

• Top 15 differentially expressed microRNAs are down regulated in HG and LG bladder cancer.
• 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.