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Assessing the Potential of the Htert Cell Line as a Model of Normal Bladder Urothelium Via Analysis of microRNA Expression

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Background

MicroRNAs (miRNAs) are short, noncoding RNA molecules involved in human gene regulation, showing differential expression levels in cancer cells.

The Htert cell line is designed to model normal bladder urothelium for comparison to cell lines for high grade and low grade urothelial carcinoma (UC). We intend to develop a panel of miRNAs with a consistent expression pattern in UC to serve as a diagnostic marker for cancer, using the Htert line as a control.

Objective

This study was conducted to determine whether the Htert cell line could serve as a model of normal bladder urothelium by comparing the miRNA expression pattern in Htert cells with those collected from normal male urine cytology.

Methods

- ❖ Cells were grown and collected to form two sets of 10 patients each:
 - 5 high grade UC (cell lines 5637 and T24)
 - 2 low grade UC (cell line RT4)
 - 3 normal bladder cells (cell lines Htert001 and Htert002 in Set 1; normal male cytology in Set 2)
- ❖ MiRNAs were extracted, reverse transcribed to cDNA, and quantified using Real-Time PCR (with primers for 384 total human miRNAs; patients run in triplicate)

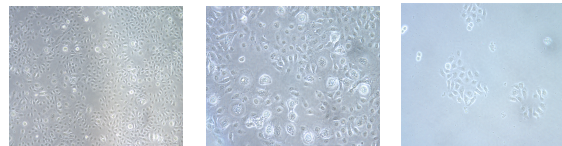
Right: Htert NU cell lines

MiRNAs that Distinguish Cancer Lines from Hterts

miRNA	p-value	Fold change, high grade	Fold change, low grade
hsa-let-7a	0.013	6.366	3.130
hsa-let-7e	0.003	5.527	3.449
miR-107	0.002	5.394	2.769
miR-128a	0.007	1.241	2.502
miR-130a	0.049	3.032	1.770

MiRNAs that Distinguish Cancer Lines from Normal Cytology

miRNA	p-value	Fold change, high grade	Fold change, low grade
hsa-let-7a	0.003	208.231	102.385
hsa-let-7e	<0.001	127.821	79.770
hsa-let-7i	<0.001	46.087	23.144
miR-105	0.002	28.287	18.900
miR-106a	0.001	862.517	521.940
miR-106b	<0.001	450.761	117.611
miR-107	<0.001	220.606	113.241
miR-10a	0.032	180.300	191.322
miR-122a	0.018	10.686	7.617
miR-128b	0.004	60.745	25.485



Results

- ❖ All statistical analysis using MATLAB
- ❖ MiRNA expression data was normalized using miR-U6 as endogenous control
- ❖ Two-tailed t-test (p<0.05) used to select miRNAs differentially expressed in cancer
- ❖ Hierarchical clustering used to select miRNAs from above list that distinguished cancer from normal cells
- ❖ Out of 5 miRNAs in the Htert set and 10 miRNAs in the normal urothelium set, 3 miRNAs were common to both sets (highlighted in tables):
 - Hsa-let-7a, hsa-let-7e, and miR-107
 - Show comparable trends in fold changes between both groups (upregulated in both high grade and low grade UC, more so in high grade UC)

Conclusion

- ❖ Htert cell line may serve as a suitable model for normal bladder urothelium
- ❖ Compared to high grade and low grade UC cell lines, Htert line showed similar miRNA expression profile to that of normal bladder cytology
- ❖ Further experiments will use the Htert cell line in miRNA transfection studies to assess the diagnostic potential of miRNA panels for UC

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