Evaluating miRNA expression integrity in FFPE samples with qRT-PCR by using patient-matched formalin-fixed and fresh-frozen tissues from Renal Cell Carcinoma (RCC) patients

Van Le[1], David Juan[1], Derek Beaton[2], Travis Antes[3], Benjamin Kennan[1], Tanmay Parekh[1], Joon Hee Lee[1], Yasmin Akbari[1], and Louis Liou[4]

[1] Department of Pathology, Boston University School of Medicine, Boston, MA 02118

[2] University of Massachusetts, Dartmouth, MA, [3] System Biosciences, Mountain View, CA

[4] Harvard Medical School, Boston, MA; Cambridge Health Alliance, Cambridge, MA

Background

MicroRNAs (miRNAs) are a class of endogenous gene regulators that play an important role in oncogenesis through the regulation of oncogenes and tumor suppressors. Profiling miRNA expressions may provide new tools for cancer diagnosis and therapy.

The majority of the published clinical studies are performed with formalin-fixed, paraffin-embedded (FFPE) samples. Using FFPE tissue is preferable because of its abundance and clinical follow up is known. However, the challenge in using FFPE tissues for molecular profiling is the volatility of the nucleic acids, although this seems less crucial with miRNA.

Given the promise of miRNA profiling in FFPE tissues and the potential alterations in the original molecular signature, we sought to investigate the effect of formalin fixation on miRNA expression in renal cell carcinoma.

Methods

IRB approval was obtained from our institution. Kidney tissues taken at the time of surgeries for each patient were made into FFPE blocks and flash frozen. Pathology specimens were assessed and documented by a pathologist.

RNA from FFPE samples and fresh-frozen tissues of six patients were extracted using RecoverAll Total Nucleic Acid Isolation Kit (Ambion, Texas) and *mirVana*™ miRNA Isolation Kit (Ambion, California), respectively. All samples were one to three years old. Extracted total RNA was then quantified with NanoDrop ND-1000 Spectrophotometer and reverse transcribed to cDNA using Quantimir (Systems Bioscience, California).

Real-time qRT-PCR was performed on 60 miRNAs of interest in triplicate using the Oncomir kit (Systems Bioscience, California). Statistical analysis was performed with Matlab where p-values < 0.01 were considered statistically significant.

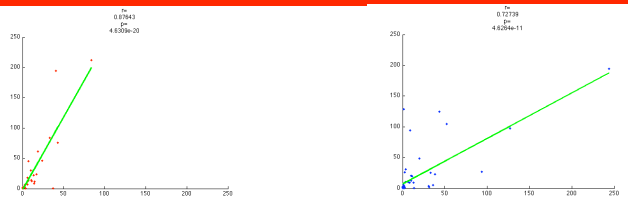

Figure Ia and Ib. Demonstrative examples of ΔCt ($2^{-(Ct\ target - Ct\ control)}$) correlation between FFPE and Frozen samples in two groups: Tumor (left) and Normal (right). The green line is a polynomial fit of the data points, using polyfit() in MatLab. The control miR is 106b.

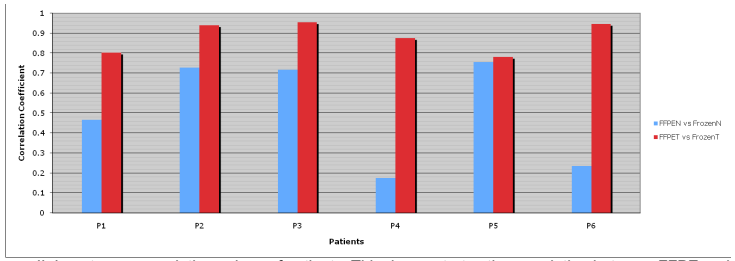

Figure II. In-category correlation values of patients. This demonstrates the correlation between FFPE and Fresh/Frozen tissues in a tissue matched patient (1-6).

Transform Consistency

During statistical analysis, two methods of transformation were used, $2^{-(Ct\ target - Ct\ control)}$ and $2^{-Ct\ target}$. The DeltaCT method, $2^{-(Ct\ target - Ct\ control)}$,used 3 different controls: 106b, 421, and RNU43-Control. 106b is a control miR from previous research, 421 was the most stable miR in this dataset, and RNU43-Control is a standard control miR. In all methods of transformation, identical correlation values were observed, indicating data was consistent.

		FFPEN NONE	FFPEN 106b	FFPEN 421	FFPEN RNUC
FFPEN	NONE	1	1	1	1
FFPEN	106b	1	1	1	1
FFPEN	421	1	1	1	1
FFPEN	RNUC	1	1	1	1
FFPET	NONE	0.74	0.74	0.74	0.737
FFPET	106b	0.74	0.74	0.74	0.737
FFPET	421	0.74	0.74	0.74	0.737
FFPET	RNUC	0.74	0.74	0.74	0.737
FRESHN	NONE	0.18	0.18	0.18	0.177
FRESHN	106b	0.18	0.18	0.18	0.177
FRESHN	421	0.18	0.18	0.18	0.177
FRESHN	RNUC	0.18	0.18	0.18	0.177
FRESHT	NONE	0.67	0.67	0.67	0.665
FRESHT	106b	0.67	0.67	0.67	0.665
FRESHT	421	0.67	0.67	0.67	0.665
FRESHT	RNUC	0.67	0.67	0.67	0.665

Table I. A partial table of patient 4, demonstrating the 4 methods of normalization for FFPE Normal to FFPE and Fresh/Frozen Tumor and Normal. Precision differences occur only because of column width in Microsoft Excel.

Stats

- Data was transformed via $2^{-(Ct\ target - Ct\ control)}$ and $2^{-Ct\ target}$
- Correlation coefficient was calculated between the expression of patient matched (in group [tumor or normal]) FFPE and Fresh/Frozen tissue

Results

There was a high correlation (Spearman r: 0.78 0.95, mean = 0.88, p-value < 0.01) between formalin-fixed and fresh-frozen tumor specimens. Formalin-fixed normal and corresponding frozen tissues did not correlate as well (Spearman r: 0.17 – 0.75, mean = 0.50, p-value < 0.01). Fold change comparison between pair matched cohorts showed very weak correlation.

Conclusions

- Formalin fixation did not significantly alter miRNA expression in tumor kidney samples, while the non-tumor samples were not as consistent.
- We suggest that the normal samples' cellular heterogeneity might be the reason for low correlations. Tumor masses likely contain more homogenous cells.
- Further studies utilizing laser-captured microdissection (LCM) to obtain homogenous normal cell population can be done to verify our hypothesis.

References

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