

ABSTRACT

Introduction and Objective: Previous studies have shown that ischemia alters gene and protein expression in several tissue types. This, however, has not been studied in renal tumors. Our work evaluates the impact of ischemia and tissue procurement conditions on the RNA integrity and gene expression in renal cell carcinoma.

Methods: Solid renal tumors from patients with von Hippel Lindau who underwent partial nephrectomy at the National Cancer Institute were included if they were resected without clamping of the renal hilum and had greater than 80% homogeneity on immediate gross examination. The procurement of the tumor was performed in the operating room. Immediately upon surgical resection, a piece of tumor was snap frozen to represent the zero time point. Remaining tissue samples were then stored in PBS at 4C, 22C and 37C and frozen at 5, 30, 60, 120, and 240 mins after surgical resection. All tissue samples were stored in liquid nitrogen until RNA extraction. Histopathologic evaluation was performed by a single pathologist on Hematoxylin & Eosin stained frozen sections obtained from each time point. Only tissue samples with at least 80% tumor on H & E were selected and used for RNA extraction, analysis, and gene expression microarrays. RNA integrity was confirmed by the presence of prominent 18S and 28S ribosomal peaks. Gene expression microarrays were performed using the Affymetrix platform. Class comparison paired t-test was performed between the zero time point and tissue samples from all other conditions obtained from the same tumor.

Results: A total of 10 tumors satisfied the inclusion criteria over the last 18 months. Significant RNA degradations were observed 240 mins after resection at both 22C and 37C. A hundred and twenty-six microarrays were performed. We identified over 4000 genes that were susceptible to ischemia times or storage conditions. Importantly, these genes include prognostic genes such as vimentin, jun-oncogene and hypoxia-inducible factor 1A. The greatest gene expression changes were observed with longer ischemia time and warmer tissue procurement conditions.

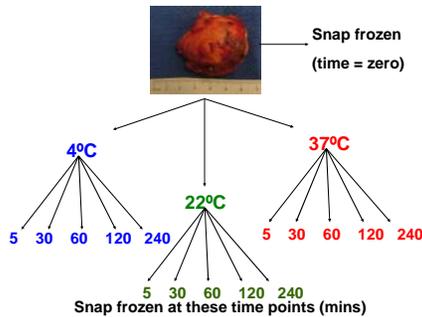
Conclusions: Our data demonstrates that RNA from kidney cancer remains intact for up to 4 hours post surgical resection when stored on ice. Significant degradations were seen at later time points in warmer conditions. Despite excellent RNA preservation sufficient for gene expression analysis, prolonged and warm procurement conditions, such as often encountered with laparoscopic surgery, are associated with significant changes in gene expression profiles.

BACKGROUND

- The concept of individualized medicine depends on the accurate expression profiling of surgical specimen
- Standardized guidelines on human tissue processing after surgical extirpation are lacking
- Methods of tissue procurement have been shown to alter gene and protein expression in several tissue types
- Prolonged *ex-vivo* time before freezing is known to affect RNA integrity and gene expression
- Tissue processing conditions in ice or room temperature are also known to influence expression pattern
- Little is known about RNA quality and gene expression in tumors removed via the laparoscopic approach
- The impact of ischemia time and procurement conditions has not been analyzed in renal tumors

METHODS

- Solid tumors from patients with VHL who underwent partial nephrectomy at NCI were evaluated
- Tumors were procured only if:
 - Resected without clamping of the renal hilum
 - >80% homogeneity on preoperative CT, intraoperative US, and gross examination
- Tissue was frozen in liquid nitrogen at various temperature conditions and time points:



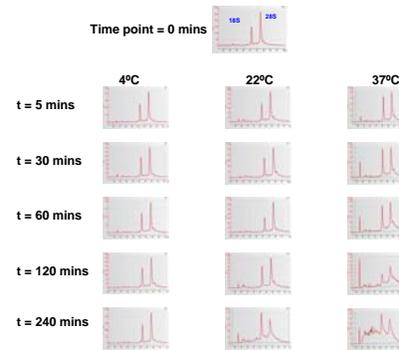
- The tissue was cut on a cryostat with "initial" and "last" slide obtained and stained with H&E to confirm the presence of tumor
- All tissue samples were stored in liquid nitrogen until RNA extraction
- One hundred and twenty-six Affymetrix HG-U133 2.0 microarrays were used to analyze gene expression pattern
- Differentially expressed genes were identified by using BRB Array Tools v3.7 (<http://linus.nci.nih.gov/BRB-ArrayTools.html>)
- Real-Time Polymerase Chain Reaction (RT-PCR) was done to validate microarray results

OBJECTIVE

- To study the impact of ischemia time and procurement conditions on RNA quality and gene expression in renal cell carcinoma

RESULTS

➤ RNA Integrity



➤ Number of Differentially Expressed Genes – Paired Comparison (Snap vs Condition)

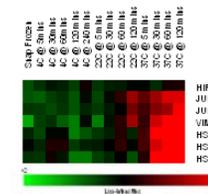
Temperature	Time	Number of Genes	P-value	FDR
4C	5	33	<0.001	0.99
	30	8	<0.001	0.75
	60	29	<0.001	0.87
	120	29	<0.001	0.93
	240	616	<0.001	<0.08
22C	5	8	<0.001	0.96
	30	75	<0.001	<0.53
	60	269	<0.001	<0.20
	120	289	<0.001	<0.18
	240	3835	<0.001	<0.01
37C	5	298	<0.001	<0.18
	30	246	<0.001	<0.22
	60	700	<0.001	<0.07
	120	3835	<0.001	<0.01

➤ Number of Differentially Expressed Genes sorted by Temperature Conditions

Temperature	Number of Genes	P-value	FDR
4C	107	<0.001	<0.48
22C	193	<0.001	<0.18
37C	4452	<0.001	<0.01

RESULTS

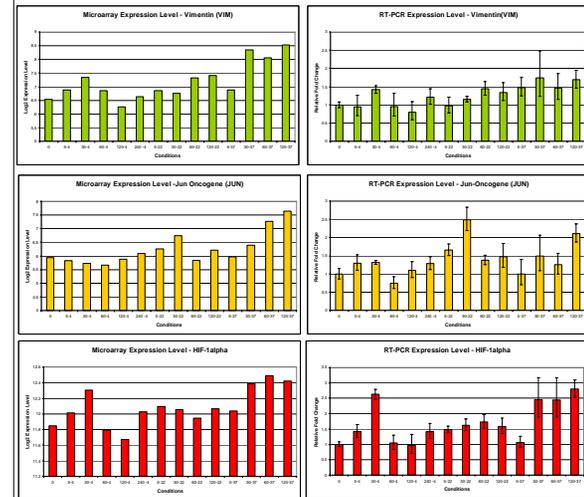
➤ Gene Expression vs Ischemia Time (Heat Map)



Prognostic Renal Cell Carcinoma Biomarkers Affected by Warm Ischemia at 37C

Over Expressed Genes	Under Expressed Genes
VIM, TYMP, JUN, CCL5, CD44, DPYD, HIF1A, RB1, SPP1, TIMP2	TIMP1, PRDX2, PTEN, CTSD, CDH6, CA9, DIABLO, CLU, NUDT6, CTNNA1, NME1, IMP3, AQP1, BAX

➤ Microarray Expression of VIM, JUN and HIF-1a Validated by RT-PCR



CONCLUSIONS

- RNA from kidney cancer remains intact for up to 4 hours post surgical resection when stored on ice
- Significant degradations were seen at later time points in warmer conditions.
- Prolonged and warm procurement conditions, such as often encountered with laparoscopic surgery, are associated with significant changes in gene expression profiles.