

Impact of Ischemia and Tissue Procurement Conditions on Gene Expression in Renal Cell Carcinoma

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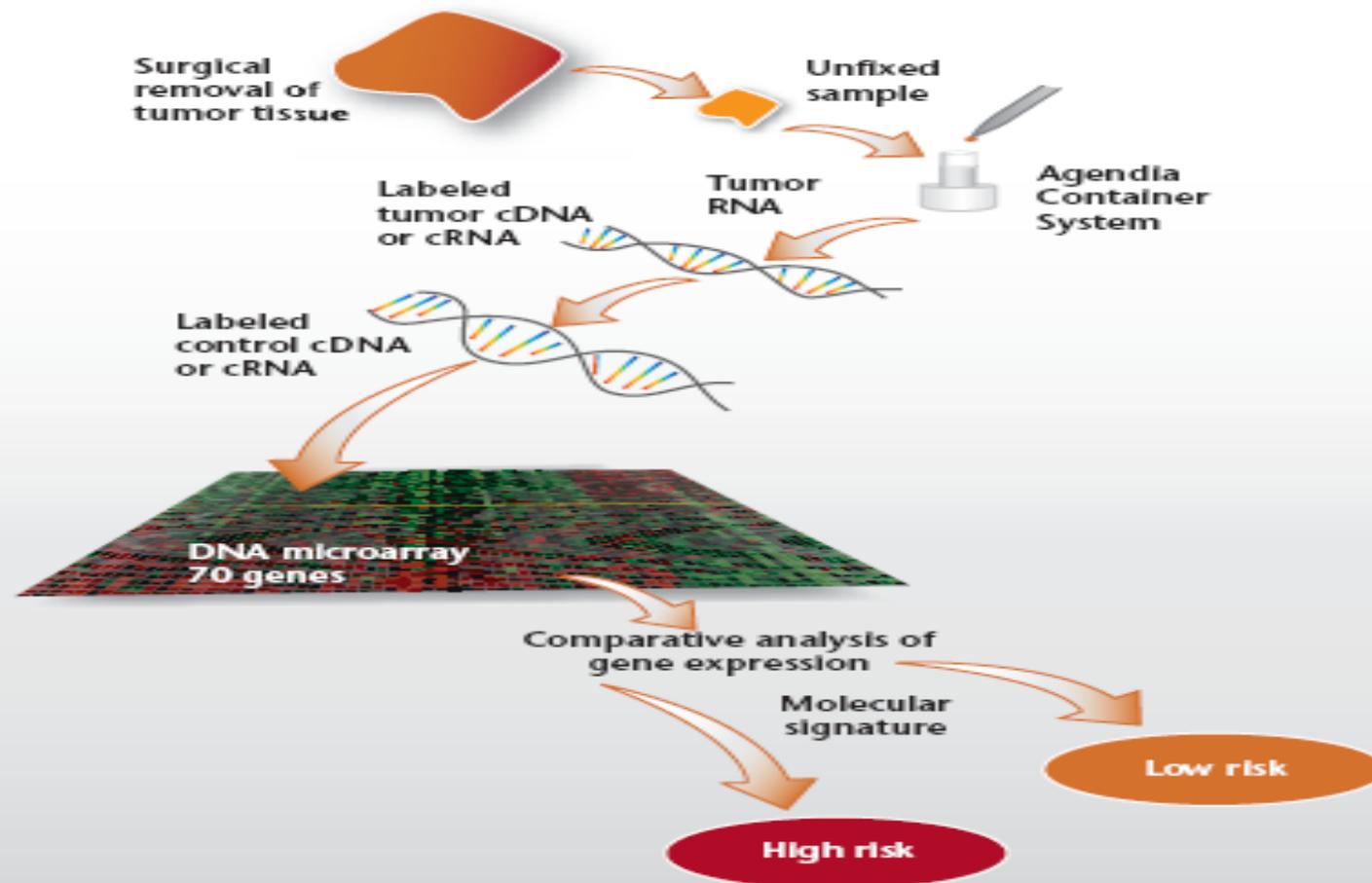
Personalized Medicine

- What is it?
- Ability to diagnose disease, prognosticate, and provide therapy that is particularly suited to a patient

What are the Tools used to Determine Clinical Outcomes in Oncology?

- Disease type
- Tissue histology
- Tumor size/clinical stage
- Grade
- Node status
- Presence of metastasis
- *Unchanged for the past 50 years!*

How is Personalized Medicine Being Practiced Today?



How is Personalized Medicine Being Practiced Today?

- There are currently two commercially available microarray tests for breast cancer.
 - OncotypeDX™ based on a 21 gene signature
 - Mammaprint® based on a 70 gene signature
- There are two prospective clinical trials evaluating the utility of these tests in clinical medicine.
 - TAILORx is evaluating the OncotypeDX™ signature
 - MINDACT is evaluating the Mammaprint® signature

Search PubMed for gene expression signature [Advanced Search \(beta\)](#) [Save Search](#)

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1: [Counts SE, Perez SE, Mufson EJ.](#)

 Galanin in Alzheimer's disease: Neuroinhibitory or neuroprotective?
Cell Mol Life Sci. 2008 May 27. [Epub ahead of print]
PMID: 18500641 [PubMed - as supplied by publisher]

2: [Yamaguchi S, Fujii-Taira I, Katagiri S, Izawa E, Fujimoto Y, Takeuchi H, Takano T, Matsushima T, Homma KI.](#)

Related Article

 Gene expression profile in cerebrum in the filial imprinting of domestic chicks (*Gallus gallus domesticus*).
Brain Res Bull. 2008 Jun 15;76(3):275-81. Epub 2008 Feb 29.
PMID: 18498941 [PubMed - in process]

3: [Loi S, Haibe-Kains B, Desmedt C, Wirapati P, Lallemand F, Tutt AM, Gillet C, Ellis P, Ryder K, Reid J, Daidone MG, Pierotti MA, Berns EM, Jansen MP, Foekens JA, Delorenzi M, Bontempi G, Piccart MJ, Sotiriou C.](#)

Related Article

31,910 Publications on Gene Signatures!

So, Which Genes to Believe?

- Myriad of literature
- Gene expression studies are often done using tissues procured under different conditions
- ***What happened to tumors during and after the surgery?***

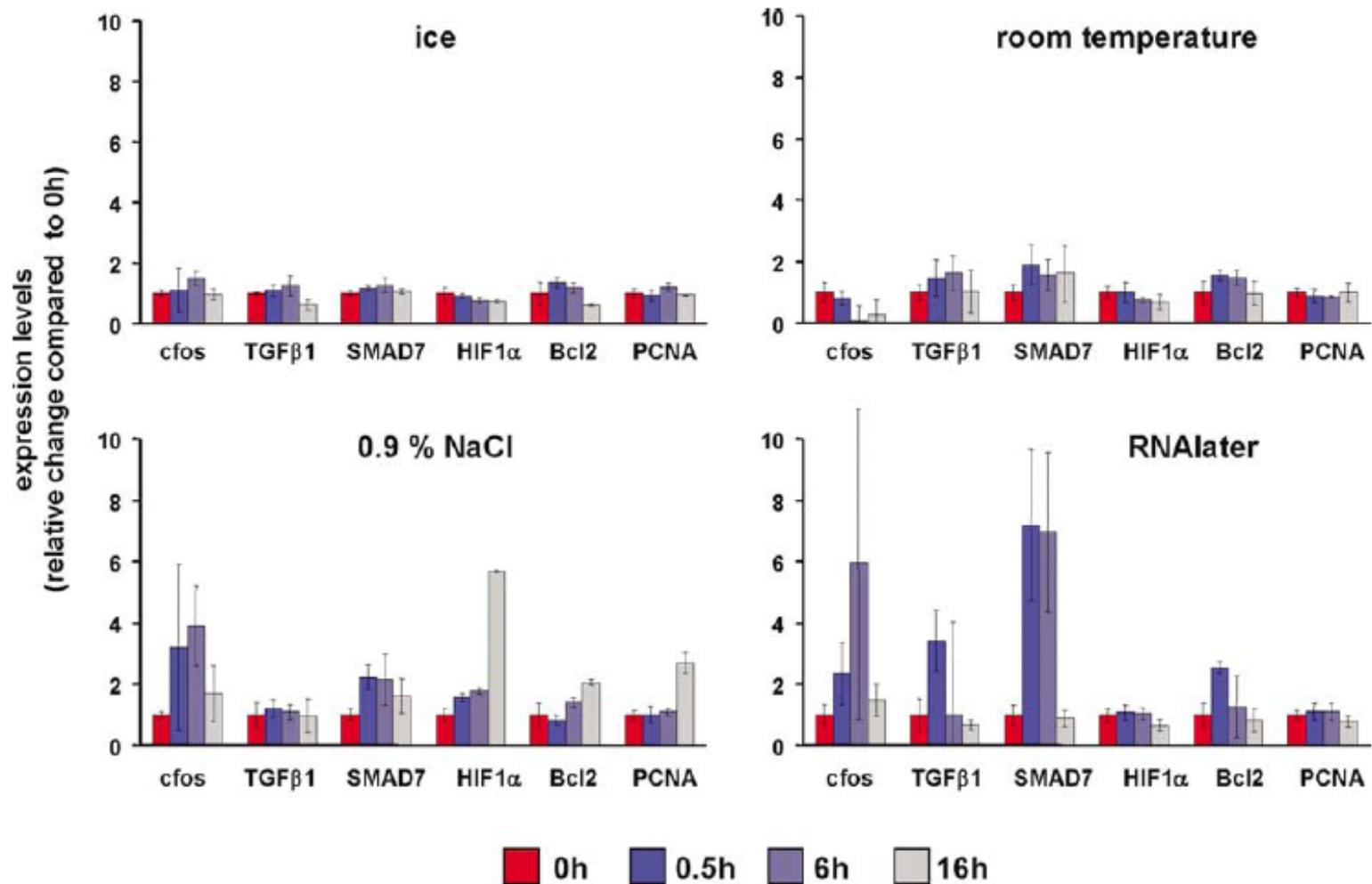
Tissue Procurement Conditions

- Personalized Medicine depends on *accurate expression profiling* of surgical specimens
- Standardized guidelines on tissue processing after surgical extirpation are *lacking*
- Methods of tissue acquisition alone could *significantly impact* tumor gene expression

The Effect of Ischemia on RNA Stability

- Fresh tonsil and colon tissues
- Snap-frozen at 0.5, 1, 3, 6 and 16h after surgical removal
- Interestingly, RNA is stable even after 16h

The Effect of Ischemia on Gene Expression



Influence of Surgical Manipulation on Prostate Cancer Gene Expression

HUGO	DESCRIPTION	PRESURGERY												POSTSURGERY												RELATIVE EXPRESSION
		1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
JUN	Jun sarcoma virus 17 oncogene homolog																								5.6	
VMP1	Likely ortholog of rat vacuole membrane protein 1																									5.1
SOCS3	Suppressor of cytokine signaling 3																									4.6
DUSP1	Dual specificity phosphatase 1																									3.7
KLF6	Kruppel-like factor 6																									3.6
JUNB	Jun B proto-oncogene																									3.1
CXCR4	Chemokine receptor 4																									2.7
CTGF	Connective tissue growth factor																									2.6
BTG2	BTG family member 2																									2.5
CCL4L1	Chemokine ligand 4-like 1 telomeric																									2.5
IER2	Immediate early response 2																									2.4
MCL1	Myeloid cell leukemia sequence 1																									2.3
CLDN4	Claudin 4																									2.2
XBP1	X-box binding protein 1																									2.2
JUND	Jun D proto-oncogene																									2.2
FN	Fibronectin 1																									2.1
H3F3B	H3 histone family 3B																									2.1
NRAA1	Nuclear receptor subfamily 4 group A member 1																									2.1
RHOB	Ras homolog gene family member B																									2.0
TGFB14	Transforming growth factor beta 1 induced transcript 4																									2.0
KLF4	Kruppel-like factor 4																									2.0
MT2A	Metallothionein 2A																									2.0
PTGS2	Prostaglandin-endoperoxide synthase 2																									1.9
DKFZP564S167	DKFZP564S167 protein																									1.9
SLC25A25	Solute carrier family 25 member 25																									1.9
DTR	Diphtheria toxin receptor																									1.9
IRF1	Interferon regulatory factor 1																									1.8
AMD1	Adenosylmethionine decarboxylase 1																									1.8
CDKN1A	Cyclin-dependent kinase inhibitor 1A																									1.8
P4HB	Procollagen-proline 2-oxoglutarate 4-dioxygenase																									1.7
ARF4	ADP-ribosylation factor 4																									1.7
MGC19764	Hypothetical protein MGC19764																									1.7
RAS4A	RAB4A member RAS oncogene family																									1.7

Pre-surgery

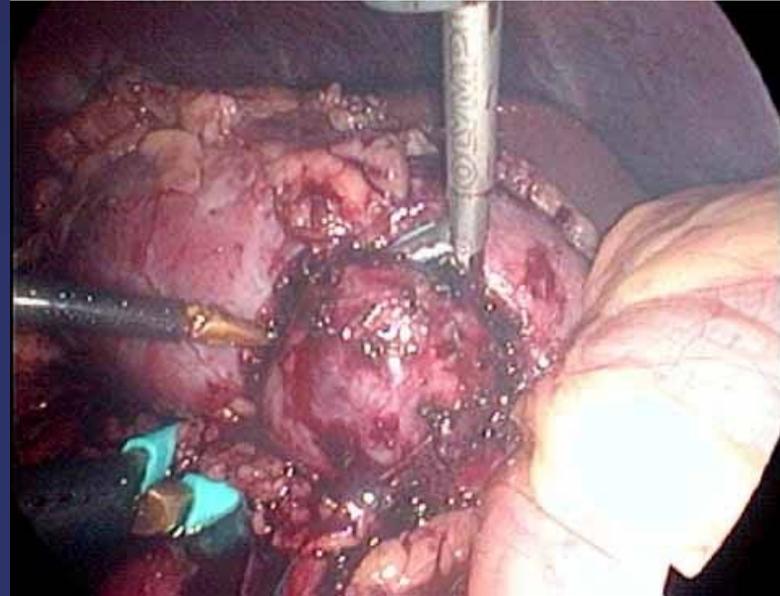
Post-surgery

- Gene expression affected by surgical manipulation may not reflect underlying tumor biology

One More Variable

LAPAROSCOPY

Laparoscopic Surgery



- Tissue procured via laparoscopic procedures is subjected to ischemia time at **body temperature**

Potential Factors Impacting Gene Expression Include:

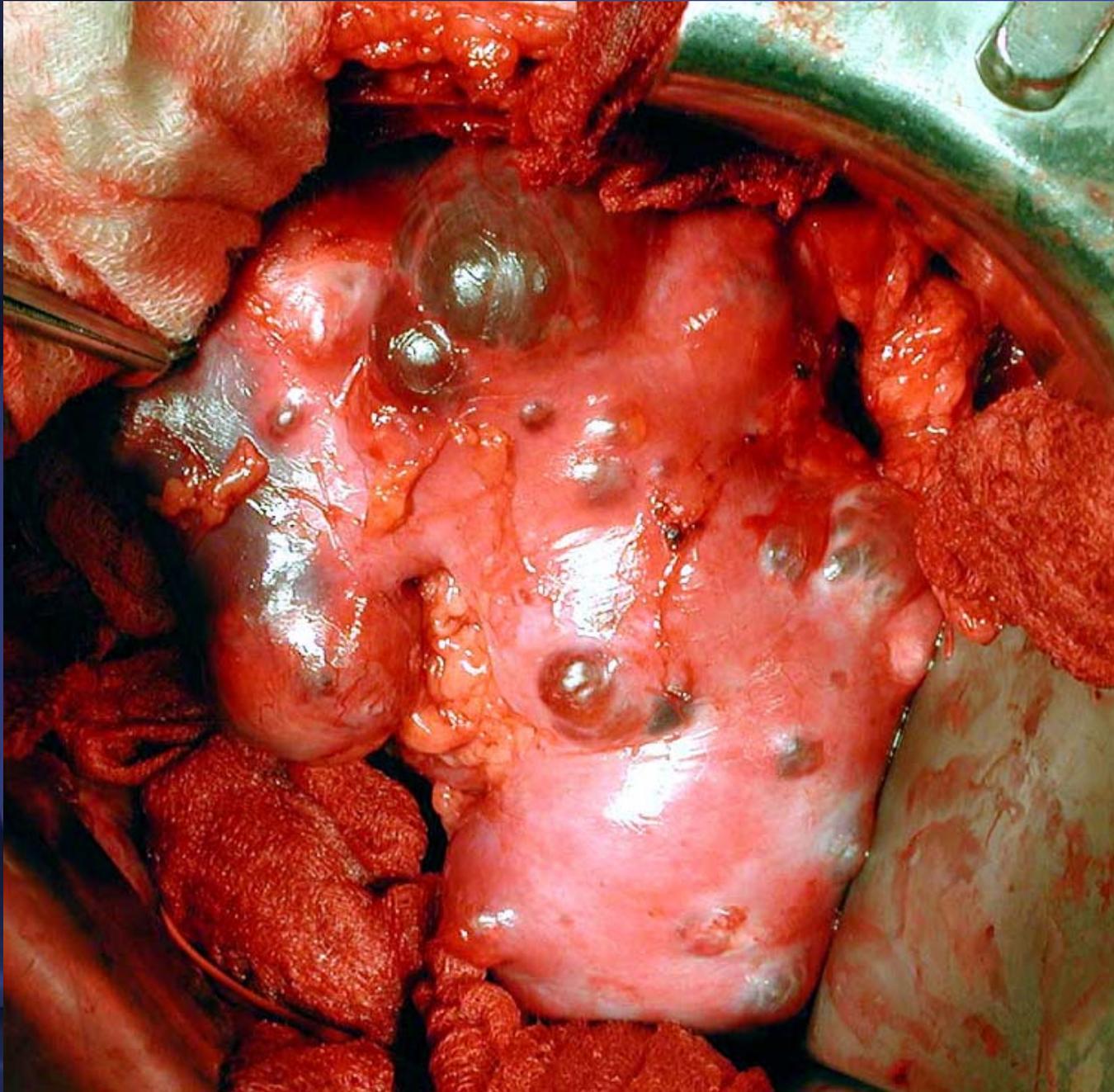
- Surgical manipulation
- Open vs laparoscopic approach
- Tissue Ischemia
 - Length of ischemia
 - Storage of tissue during ischemic period

Objective

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

Study Design – Tumor Selection Criteria

- 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
 - *Predominantly* solid preoperative CT, intraoperative US, and gross examination
 - Confirmed tumor tissue of *greater than 80% RCC* from each sample



Methods – Tissue Procurement

Pathologist



CRTP fellows



16 samples per tumor

Snap frozen a piece
(time = zero)

4°C - Ice

37°C - Laparoscopy

22°C - Room

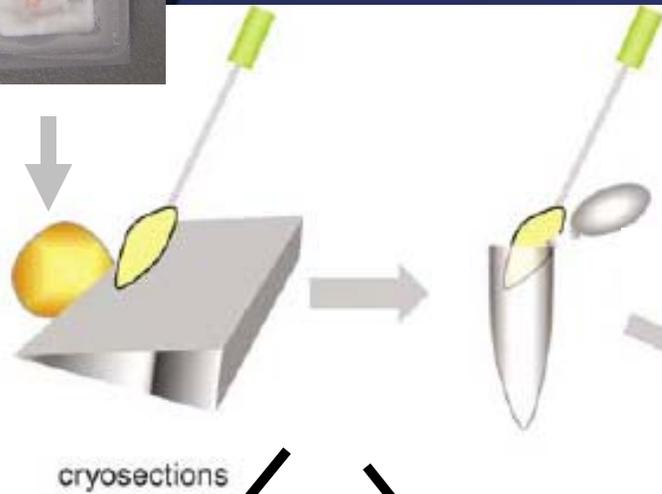
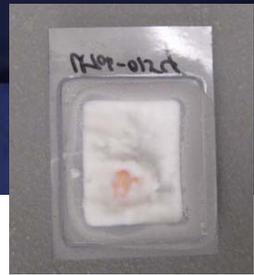
5 30 60 120 240

5 30 60 120 240

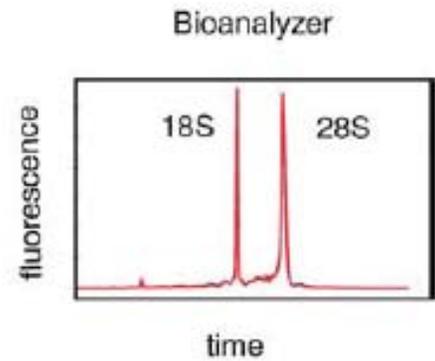
5 30 60 120 240

Snap frozen at these time points (mins)

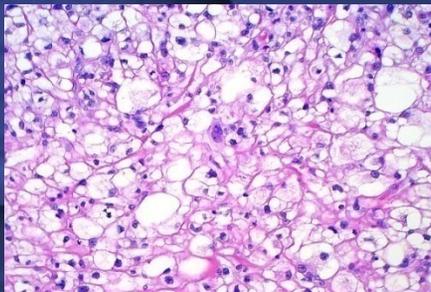
RNA Extraction and Frozen Sections



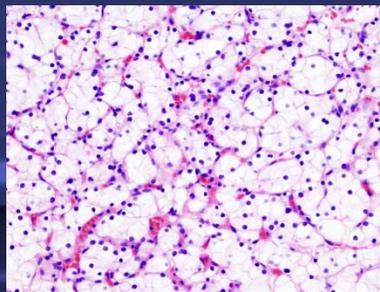
Trizol



Pre



Post

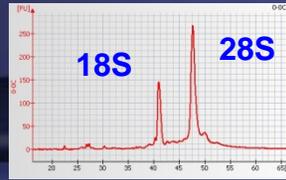


160 tumor pieces
320 H&E frozen sections

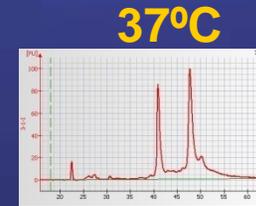
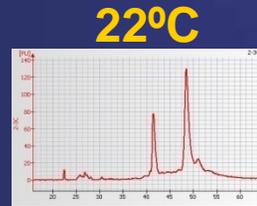
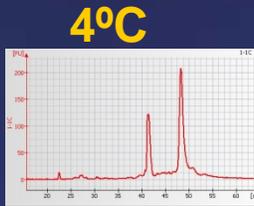
Results

- 10 tumors satisfied the inclusion criteria over the course of 18 months
- RNA were extracted from 160 tumor samples (16 samples per tumor)
- All 160 samples were reviewed by a single pathologist for RCC confirmation

Time point = 0
mins



t = 5 mins



t = 30 mins



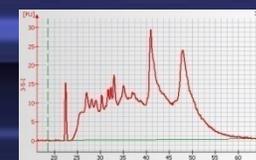
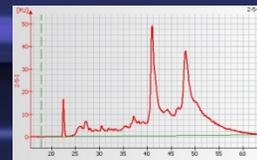
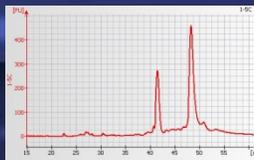
t = 60 mins



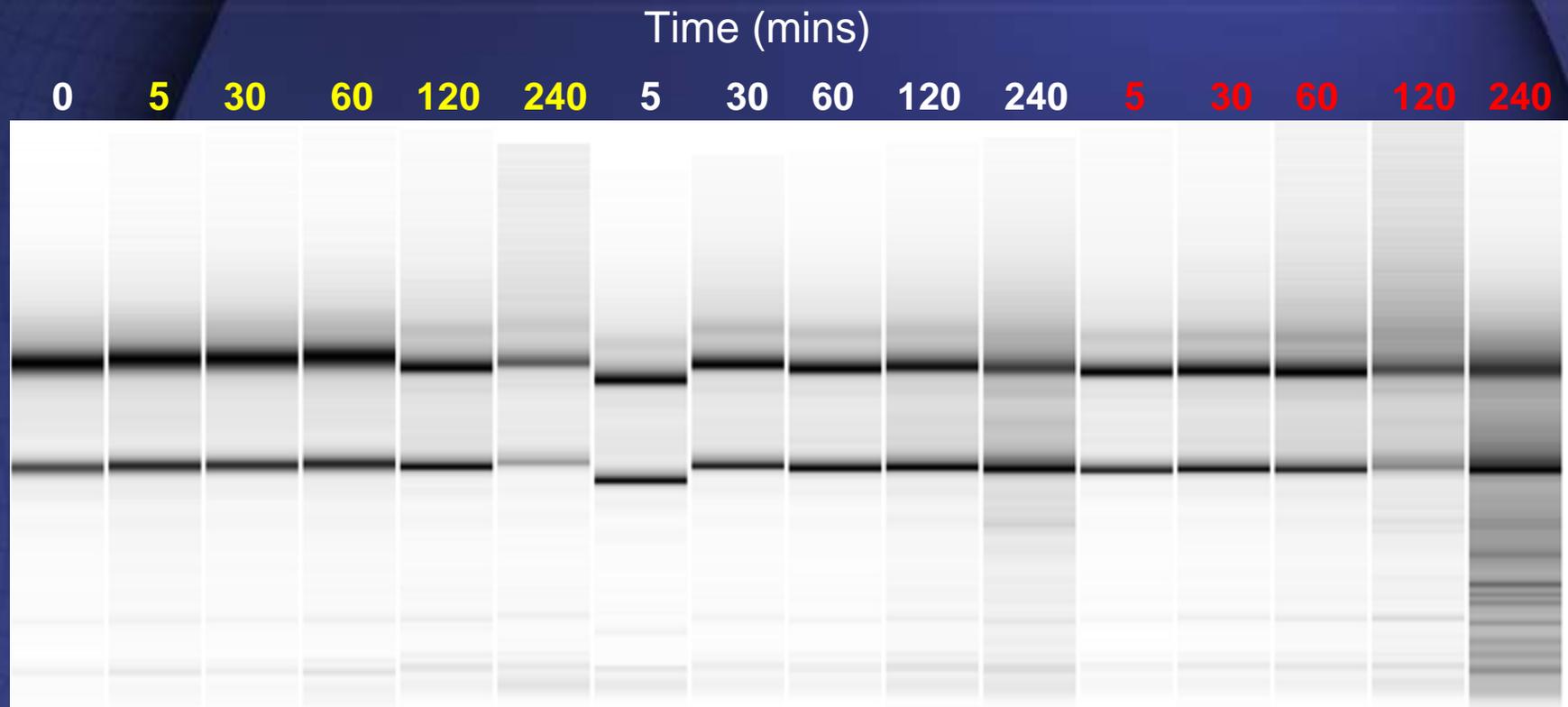
t = 120 mins



t = 240 mins



Gel Electrophoresis of Tumor RNA



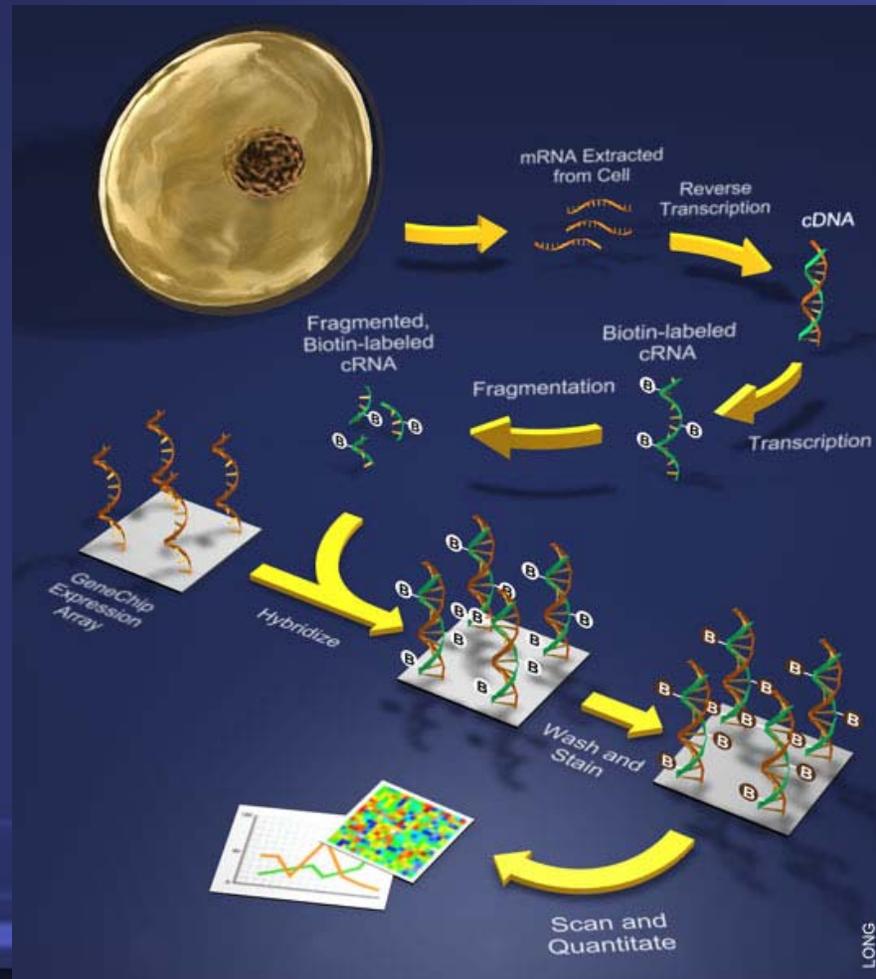
4°C

22°C

37°C

Temperature (°C)

Gene Expression of Renal Cell Carcinoma – cRNA Microarray Analysis

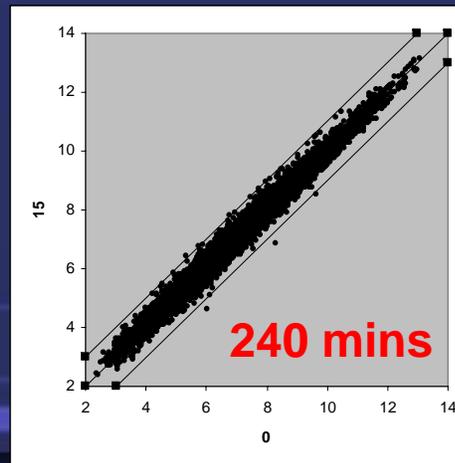
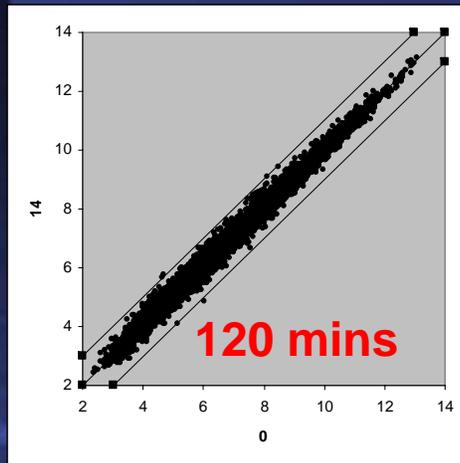
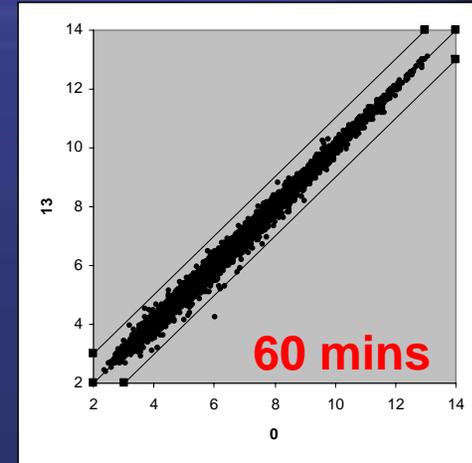
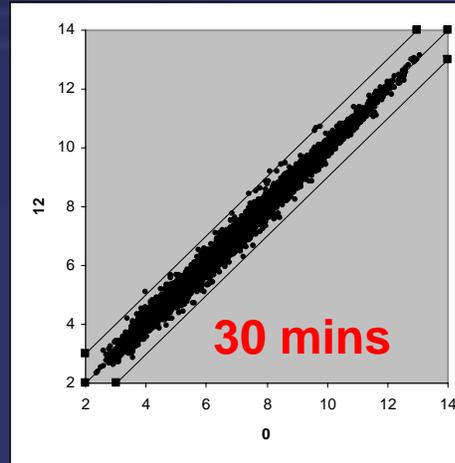
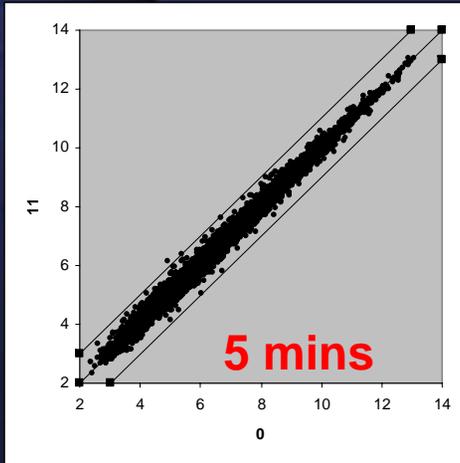


>24,000 genes represented by >50,000 probe sets

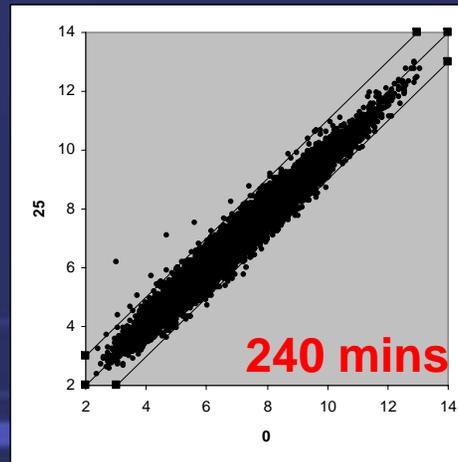
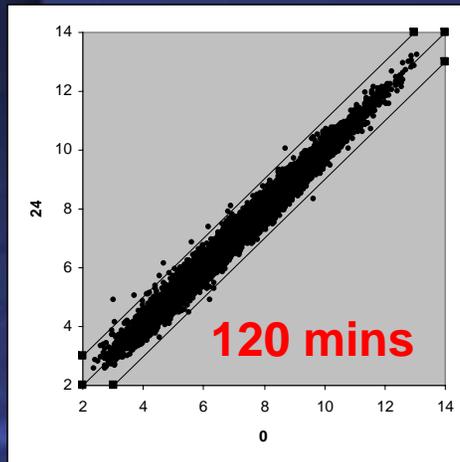
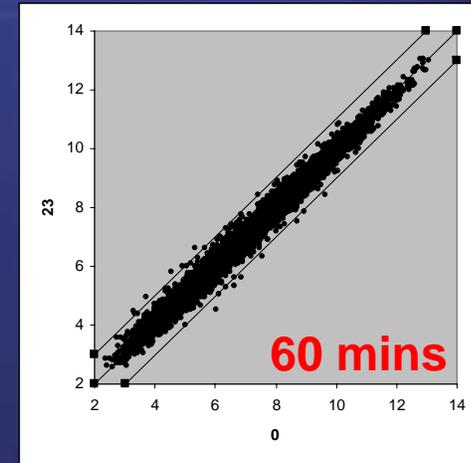
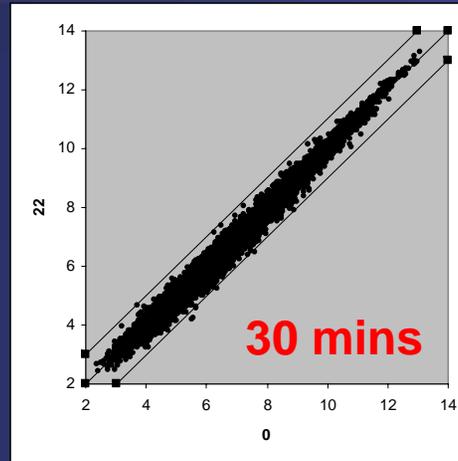
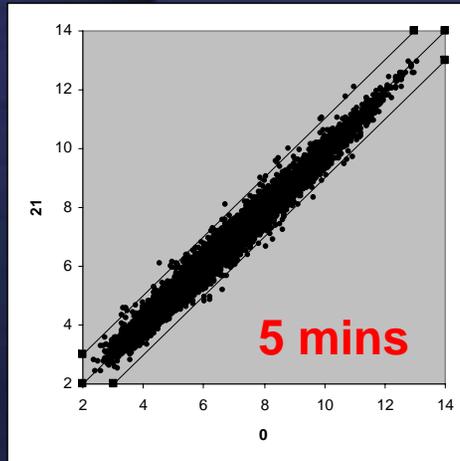
Gene Expression of Renal Cell Carcinoma – cRNA Microarray Analysis

- One hundred and twenty-six gene expression microarrays were performed

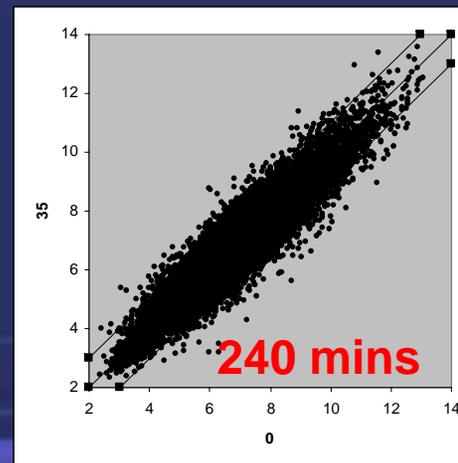
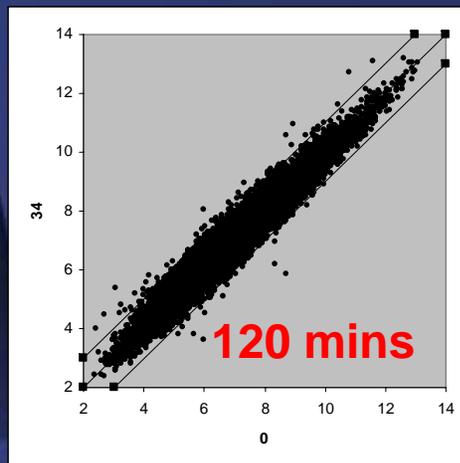
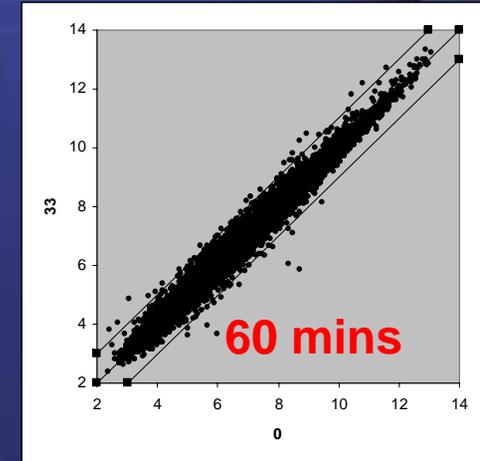
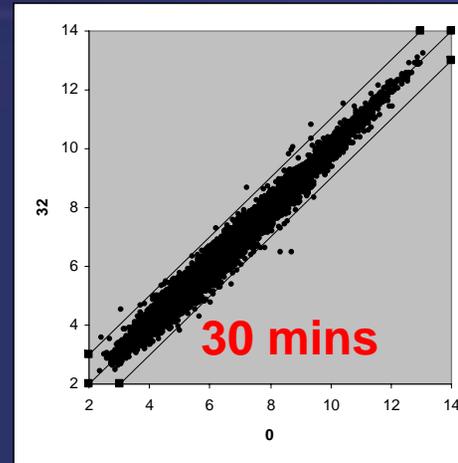
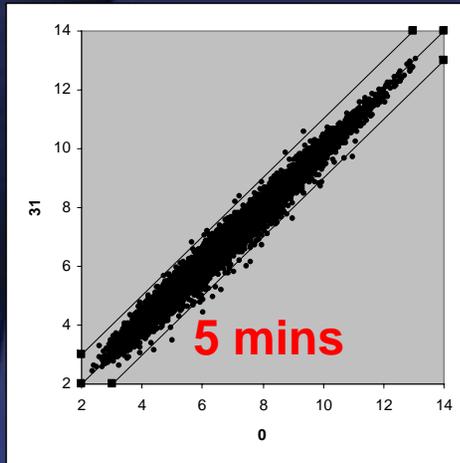
Global Gene Expression: 4C – Ice



Global Gene Expression – 22C



Global Gene Expression – 37C



Differential Gene Expression

Temperature	Time	Number of Genes	P-value
4C	5	33	<0.001
	30	8	<0.001
	60	29	<0.001
	120	29	<0.001
	240	616	<0.001
22C	5	8	<0.001
	30	75	<0.001
	60	269	<0.001
	120	289	<0.001
37C	5	298	<0.001
	30	246	<0.001
	60	700	<0.001
	120	3835	<0.001

Differentially Expressed Genes – 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

Review article

Molecular markers for predicting prognosis of renal cell carcinoma

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Table 2
Molecular markers predictive of survival in patients with renal cell carcinoma

	Year	n	H	A		Year	n	H	A
Apoptosis					Cell cycle regulation				
BAX	2004 [20]	138	mi	U	CAV1 (caveolin-1)	2004 [152]	67	c	U
BCL2	2004 [153]	101	mi	M		2003 [154]	114	mi	M
	2004 [20]	138	mi	U	CDKN1B (p27, Kip1)	2004 [61]	129	c	M
BIRC5 (survivin)	2006 [102]	312	c	M		2002 [64]	67	c	M
CLU (clusterin)	2005 [155]	131	mi	U		2001 [65]	104	c	M
CTSD (cathepsin D)	2005 [79]	150	c	U	EGFR	2005 [156]	149	c	M
DIABLO	2005 [104]	78	mi	U	GMNN (geminin)	2005 [27]	176	mi	U
IGF1R	2003 [157]	280	c	U	MCM2	2005 [27]	176	mi	U
VEGFA	2004 [21]	48	c	M	MKI67 (Ki-67)	2005 [27]	176	mi	M
	2004 [158]	179	c	U		2004 [25]	224	c	M
Degradation of extracellular matrix						2004 [46]	73	c	M
MMP2	2001 [112]	153	c	U		2004 [20]	138	mi	U
	2003 [159]	131	mi	U		2003 [158]	131	mi	U
MMP7	2006 [113]	156	c	M		1999 [23]	118	un	M
MMP9	2001 [112]	153	c	U		1997 [19]	50	c	U
PLAU (uPA)	2005 [122]	106	mi	U		1997 [22]	87	mi	M
PLAUR	2005 [122]	106	mi	U	NME1 (nm23)	1998 [160]	95	mi	M
SERPINE1 (PAI)	2005 [122]	106	mi	M	PCNA	1998 [161]	109	mi	M
Hypoxia inducible factors						1997 [22]	87	mi	U
CA9	2004 [25]	224	c	M	RB1 (pRb)	2001 [65]	104	c	M
	2004 [26]	318	c	M	PRDX2	2006 [162]	138	mi	U
	2003 [142]	321	c	M	SAT1 (spermine)	2004 [46]	73	c	M
ENG (endoglin)	2006 [163]	168	c	U	SKP2	2004 [61]	129	c	M
HIF1A	2006 [145]	176	c	U	TIMP1	2001 [112]	153	c	M
	2005 [144]	66	c	M	TIMP2	2001 [112]	153	c	U
	2005 [143]	56	c	M	TP53 (p53)	2005 [55]	193	c	M
Immune regulation						2004 [54]	134	c	M
CCL4 (MIP-1 β)	2006 [164]	67	ns	U		2004 [26]	318	c	M
CCL5 (RANTES)	2006 [39]	67	ns	U		2001 [65]	104	c	U
CD274 (B7-H1)	2006 [134]	196	c	M		2000 [24]	73	c	U
CXCL9 (MIG)	2006 [164]	67	c	M		1997 [19]	50	c	M
CXCL11 (I-TAC)	2006 [164]	67	c	U		1997 [51]	72	mi	M
CXCR3 (IP10)	2006 [164]	67	c	U	Miscellaneous				
SPP1 (osteopontin)	2006 [165]	171	c	U	AQP1	1998 [166]	58	c	M
VTCN1 (B7-H4)	2006 [135]	259	c	U	ADFP	2005 [167]	103	c	M
Cell adhesion					DPYD (DPD)	2003 [168]	65	mi	U
CDH6 (cadherin-6)	2004 [169]	216	mi	M	EBAG9	2005 [170]	78	c	M
CD44	2004 [171]	173	c	U	ECCGF1(TP)	2003 [168]	65	mi	M
	2001 [172]	73	c	M	FHIT	2002 [173]	149	c	M
	2001 [174]	92	c	U	IMP3	2006 [175]	371	mi	M
	2000 [176]	52	c	U	KLK6 (HK6)	2006 [177]	70	mi	U
CTNNA1	2004 [178]	124	mi	U	MME (CD10)	2004 [179]	131	c	U
	1997 [180]	90	mi	M	NUDT6 (bFGF)	2005 [181]	259	ns	M
CTNNB1	2004 [178]	124	mi	M	PTGS2 (COX2)	2003 [159]	131	mi	U
	1997 [180]	90	mi	U	VHL	2002 [141]	187	c	M
CTNNG	1997 [180]	90	mi	U		2005 [143]	56	c	U
MUC1 (EMA)	2004 [179]	131	c	U	VIM (vimentin)	2004 [26]	318	c	M
	2003 [182]	92	ns	M					
	2002 [183]	60	c	U					
MUC16 (CA 125)	2003 [182]	92	ns	M					
L1CAM	2005 [78]	72	c	M					
TACSTD1 (Ep-Cam)	2005 [184]	96	c	M					
	2004 [97]	417	mi	M					
VCAM1	2006 [99]	485	mi	M					

n = number of patients; H = tumor histology; mi = mixed; c = clear cell; ns = not specified; A = analysis; U = univariate; M = multivariate.

Ischemia Sensitive Prognostic Genes

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

Over Expressed Genes

**VIM, TYMP, JUN, CCL5,
CD44, DPYD, HIF1A, RB1,
SPP1, TIMP2**

Under Expressed Genes

**TIMP1, PRDX2, PTEN,
CTSD, CDH6, CA9,
DIABLO, CLU, NUDT6,
CTNNA1, NME1, IMP3,
AQP1, BAX**

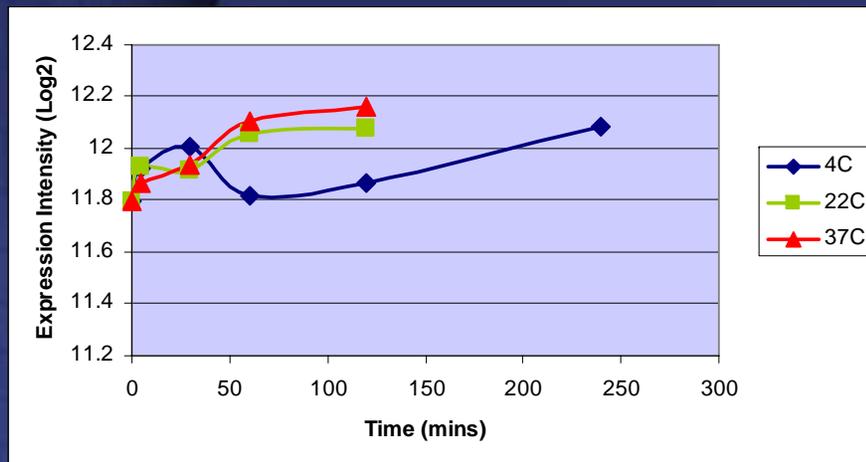


RT-PCR

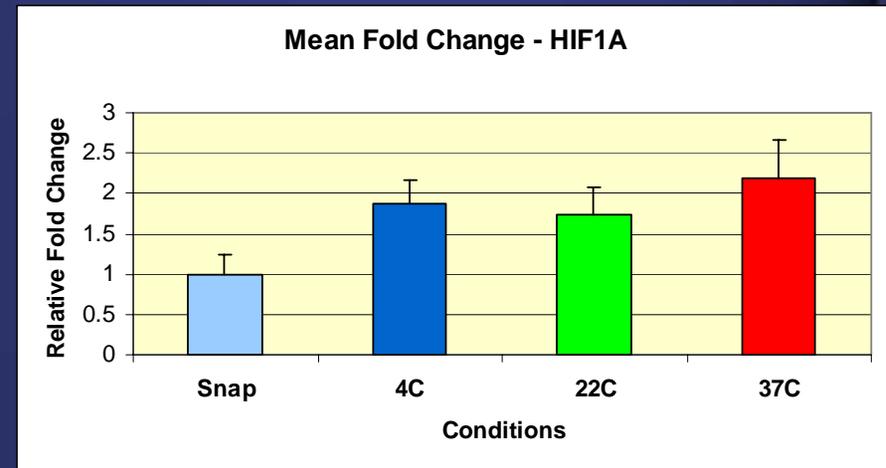
HIF1A, VIM, JUN

HIF-1A

Microarray

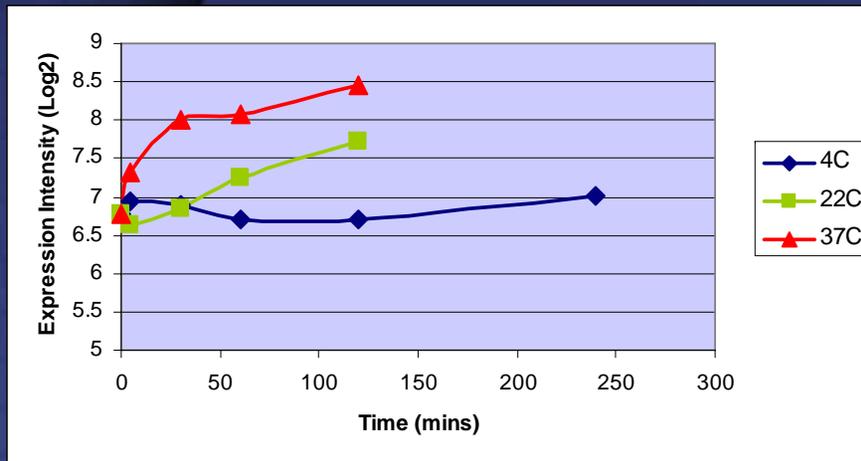


RT-PCR

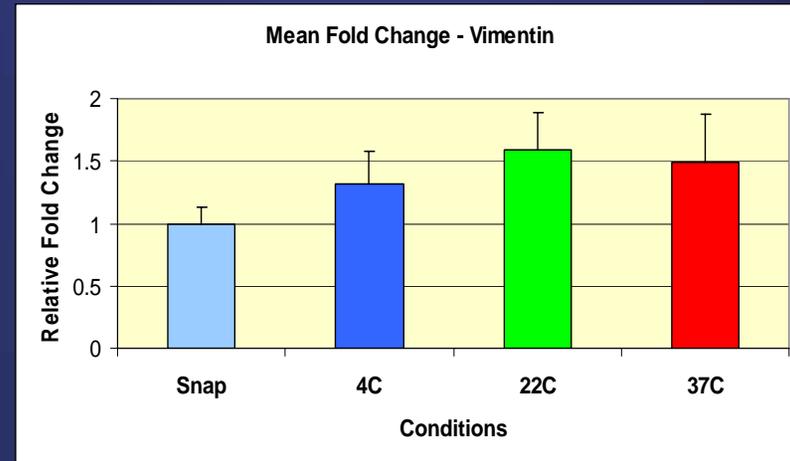


Vimentin

Microarray

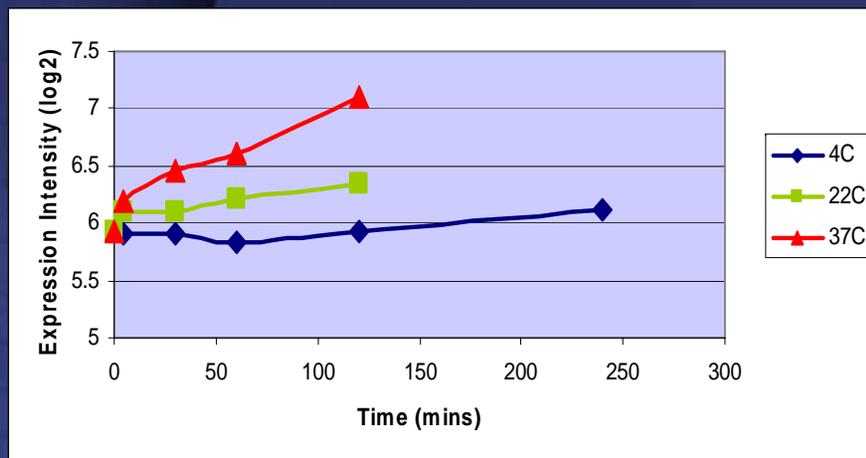


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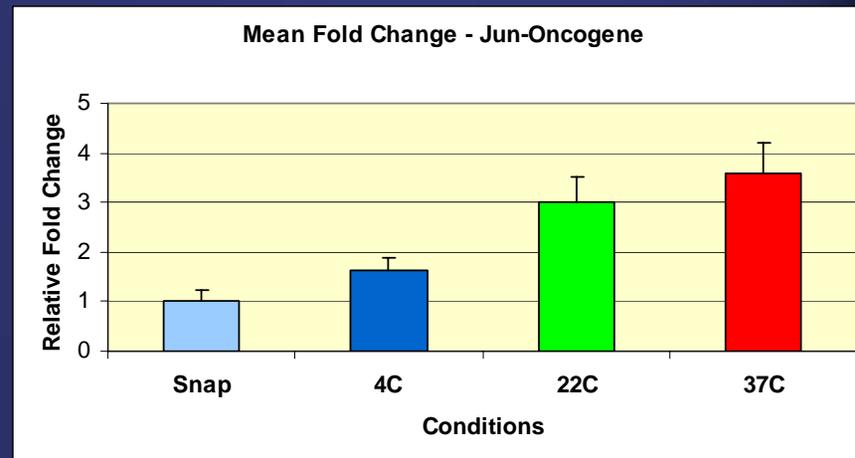


JUN

Microarray



RT-PCR



Conclusion

- Despite excellent RNA quality in most samples, there are significant changes in gene expression
- RNA from kidney cancer remains intact for up to 4 hours post surgical resection
- Gene expression is best preserved when specimens are stored on ice
- Warm procurement conditions are associated with greatest changes in gene expression profiles
- It is important to account for ischemia sensitive genes when evaluating renal biomarkers for prognostication and therapeutics

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- Dr. Peter Pinto
- Dr. W. Marston Linehan

