Developing In Vivo Models Of Human Renal Cell Carcinoma: Relevant Biology That Approaches The Human Condition

MD Anderson Cancer Center
Making Cancer History®

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None Of This Would Be Possible Without

- Tapasi Maity
- Xiu-Ying Zhang (Tina)
- Shih-Chi Su
- Vitaly Margulis
- Steven Culp
- Jose Karam
- Scott Delacroix
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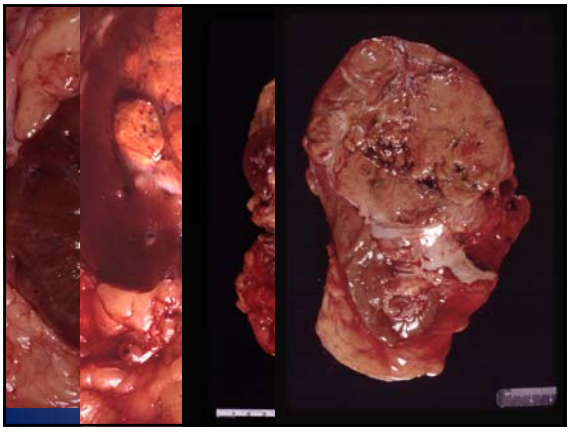
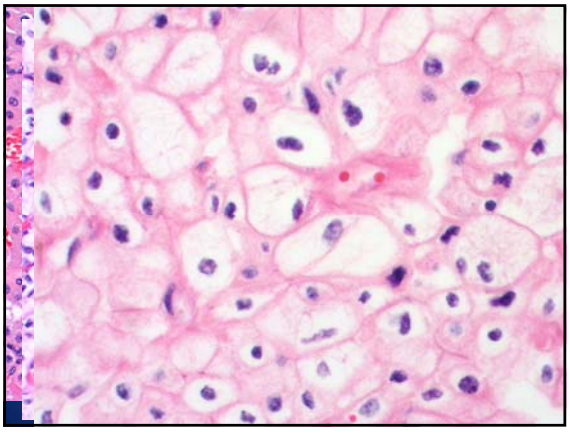
- Patrick Kenney
- Pheroze Tamboli
- Bruce Luxom
- David Engler

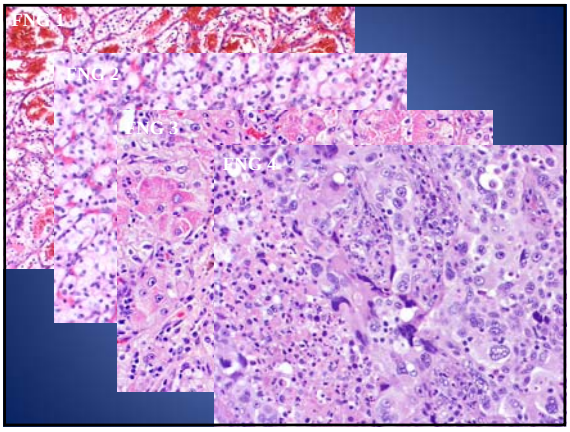
Why Do We Need Models Of Renal Cell Carcinoma?

- Study tumor biology under controlled conditions
  - Identify factors that mitigate local and metastatic progression
  - Models need to mimic clinical behavior to achieve meaningful insight into molecular mechanisms
- Assess differences in tumor behavior based on:
  - Histology
  - Clinical variables (grade, stage, site of origin)
- Preclinical therapy development
- Biomarker identification/validation

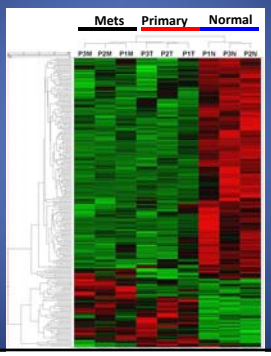
What Constitutes A Good Model Of Human Renal Cell Carcinoma?



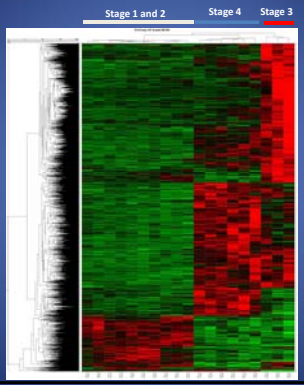




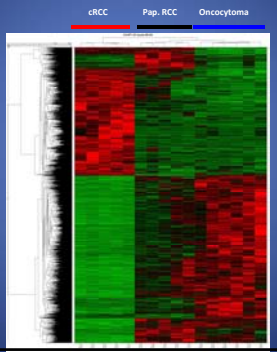
Altered genes in cRCC patients with metastatic disease



Genes altered in Stage I - IV cRCC



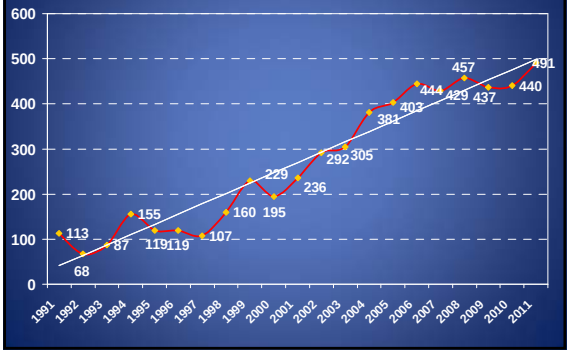
Differential Gene Expression in RCC Based On Histology



Renal Cell Carcinoma Models

- RENCA (murine in origin)
- 786-0 (ccRCC, VHL mutation, ?stage)
- ACHN (unknown histology, pleural effusion)
- Caki-1 (ccRCC, skin metastasis)
- Caki-2 (ccRCC, ?papillary morphology/genotype)
- A-498 (kidney “carcinoma”)
- 769-P (ccRCC, ?stage)
- Ecker Rat (Tuberous Sclerosis)

Nephrectomies At MDACC



Renal Cell Carcinoma Program at The University of Texas M. D. Anderson Cancer Center

RCC Surgical Specimens (Primary and Metastasis)*

Pathologic Analysis

Clinical Database Entry

RFS, OS, Therapy, Demographics

Tissue samples (normal/tumor) collected for:

1. RNA
2. Snap Frozen (DNA/Protein)
3. Paraffin embedded (IHC/TMA)
4. OCT (IHC)
5. Primary Culture
6. Ex vivo implant into athymic animals

*2004- Pre- and Post-operative serum and urine collected

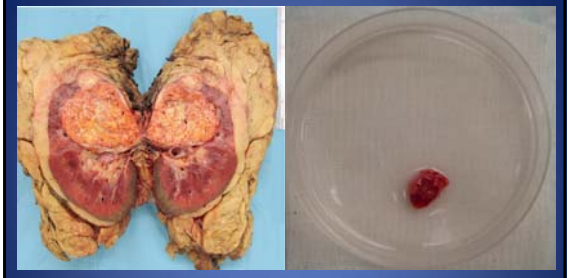
Primary Cultures

subtype	number (total 261)	
Renal Cell Carcinoma		
Conventional RCC	185	
Papillary RCC		
	type 1	13
	type 2	10
	unidentified 1 or 2	9
Chromphobe RCC	16	
Unclassified RCC	4	
Translocation RCC (Xp11.2)	3	
Mucinous tubular and spindle cell type RCC	1	
Renal medullary RCC	1	
Tubulocystic type RCC	1	
Benign Tumors		
renal oncocytoma	14	
angiomyolipoma	2	
mixed epithelial and stromal tumor	1	
Hyperplastic polyp	1	

Xenografts

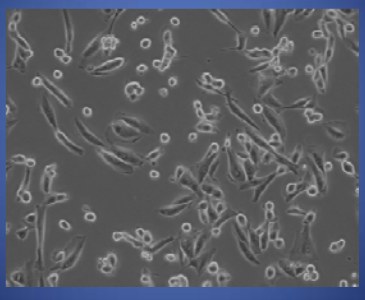
subtype	number (total 11)	
Conventional RCC	pure	3
	with sarcomatoid dedifferentiation	2
Papillary RCC	type 1	1
	type 2	2
Renal medullary RCC	1	
Unclassified RCC	1	
RCC associated with Xp11.2 translocation	1	

Immunodeficient Mice

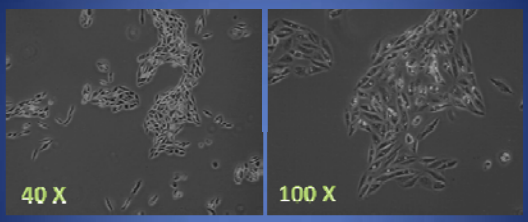


Tumor Specimens from OR

Established Tumor Cell Lines

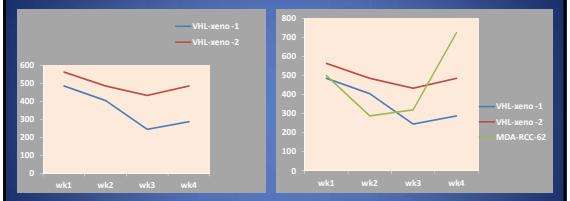


hRC(MSC21) – D6



Primary culture from VHL patient's ccRCC tumor

VHL MSC21 growth curve



Xenograft from VHL patient's ccRCC tumor

RCC Xenograft Model

Subcutaneous RCC model

orthotopic RCC model

Left Kidney Exposed

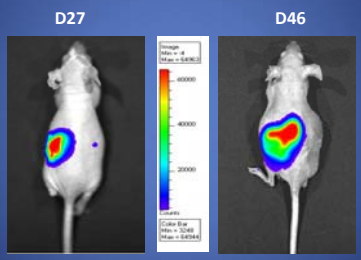
9 weeks Post-Injection

Normal vs. Tumor

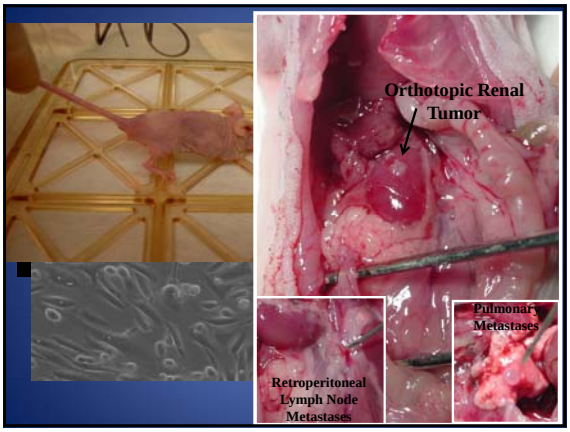
Real-time in vivo orthotopic RCC model

Stable transfected with firefly Luciferase gene

Real-time in vivo orthotopic RCC model



MDA-RCC-55 (papillary type II)

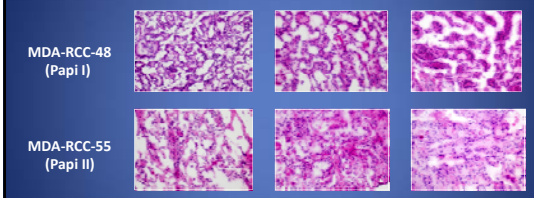


RCC Xenograft Model Validation

- original patient
- early passage xenografts (F2-F3)
- late passage xenografts (F5-F15)

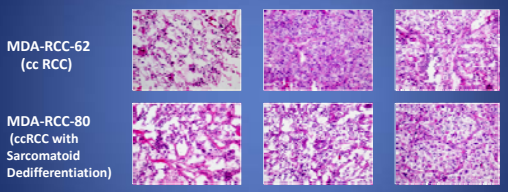
Stable Histological Characteristics

Microscopic analysis of patients VS xenografts



Karam JA & Wood CG, European Urology 59 (2011): 619-628

Microscopic analysis of patients VS xenografts



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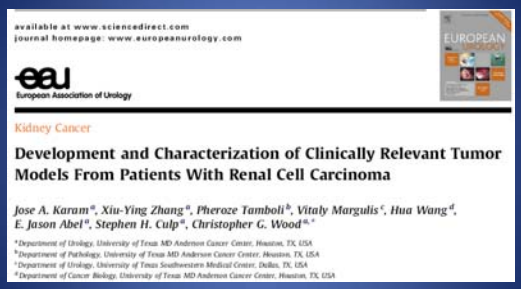
Stable Molecular Characteristics



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Kidney Cancer

Development and Characterization of Clinically Relevant Tumor Models From Patients With Renal Cell Carcinoma

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Patient characteristics of established RCC models

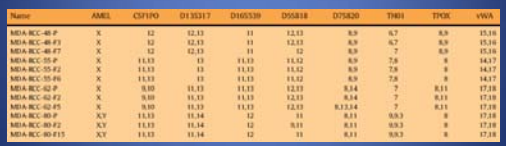
Name	Age, yr	Gender	Ethnicity	Histology	AJCC 2010 stage	Grade	Status
MDA-RCC-48	65	Female	Black	Papillary RCC type I	pT3aN1M0	3	Alive, NED
MDA-RCC-55	80	Male	White	Papillary RCC type II	pT3aN0M0	3	DOD
MDA-RCC-62	71	Female	Asian	Clear cell RCC	pT3cN0M1	4	DOD
MDA-RCC-80	35	Male	White	Clear cell RCC with sarcomatoid features	pT3aN1M1	4	DOD

AJCC = American Joint Committee on Cancer; RCC = renal cell carcinoma; NED = no evidence of disease; DOD = dead of disease.

Karam JA & Wood CG, European Urology 59 (2011): 619-628

Stable Molecular Characterictics

Short tandem repeat fingerprinting



Name	AMEL	CSF1PO	D13S317	D16S539	D5S818	D7S820	TH01	TPOX	vWA
MDA-RCC-48-P	X	12	12,13	11	12,13	8,9	6,7	8,9	15,16
MDA-RCC-48-F3	X	12	12,13	11	12,13	8,9	6,7	8,9	15,16
MDA-RCC-48-F7	X	12	12,13	11	12	8,9	7	8,9	15,16
MDA-RCC-55-P	X	11,13	13	11,13	11,12	8,9	7,8	8	14,17
MDA-RCC-55-F2	X	11,13	13	11,13	11,12	8,9	7,8	8	14,17
MDA-RCC-55-F6	X	11,13	13	11,13	11,12	8,9	7,8	8	14,17
MDA-RCC-62-P	X	9,10	11,13	11,13	12,13	8,14	7	8,11	17,18
MDA-RCC-62-F2	X	9,10	11,13	11,13	12,13	8,14	7	8,11	17,18
MDA-RCC-62-F5	X	9,10	11,13	11,13	12,13	8,13,14	7	8,11	17,18
MDA-RCC-80-P	XY	11,13	11,14	12	11	8,11	9,9.3	8	17,18
MDA-RCC-80-F2	XY	11,13	11,14	12	9,11	8,11	9,9.3	8	17,18
MDA-RCC-80-F15	XY	11,13	11,14	12	11	8,11	9,9.3	8	17,18

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Stable Molecular Characterictics

Single nucleotide polymorphism analysis

Name	Assay	Call	Zygosity
MDA-RCC-48-P	PIK3R1_M326I_G978	A,A	Homozygous
MDA-RCC-48-F3	PIK3R1_M326I_G978	A,A	Homozygous
MDA-RCC-48-F7	PIK3R1_M326I_G978	A,A	Homozygous
MDA-RCC-80-P	MET_N375S	AG,AG	Heterozygous
MDA-RCC-80-F2	MET_N375S	AG,AG	Heterozygous
MDA-RCC-80-F15	MET_N375S	AG,AG	Heterozygous

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Stable Molecular Characterictics

Von Hippel-Lindau sequencing

Name	Base change	Exon	Position	Type	Effect	Amino acid change
MDA-RCC-62-P	245G>C	1	245	Substitution	Missense	R82P
MDA-RCC-62-F2	245G>C	1	245	Substitution	Missense	R82P
MDA-RCC-62-F6	245G>C	1	245	Substitution	Missense	R82P
MDA-RCC-62-P	246C>A	1	246	Substitution	Missense	R82P
MDA-RCC-62-F2	246C>A	1	246	Substitution	Missense	R82P
MDA-RCC-62-F6	246C>A	1	246	Substitution	Missense	R82P
MDA-RCC-80-P	30delA	2	30	Deletion	Frameshift deletion	–
MDA-RCC-80-F2	30delA	2	30	Deletion	Frameshift deletion	–
MDA-RCC-80-F15	30delA	2	30	Deletion	Frameshift deletion	–

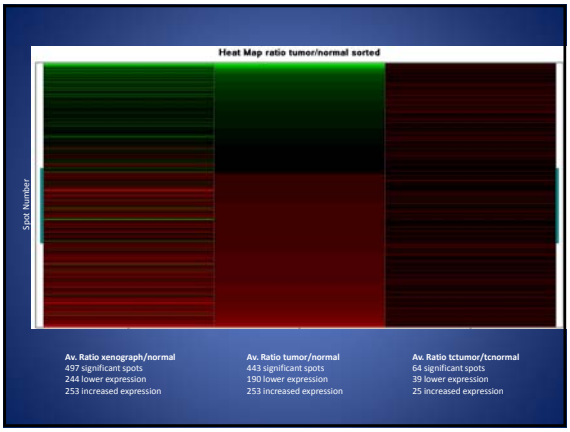
Karam JA & Wood CG, European Urology 59 (2011): 619-628

able 2. Results of VHL mutation analysis in original tumours and corresponding xenografts

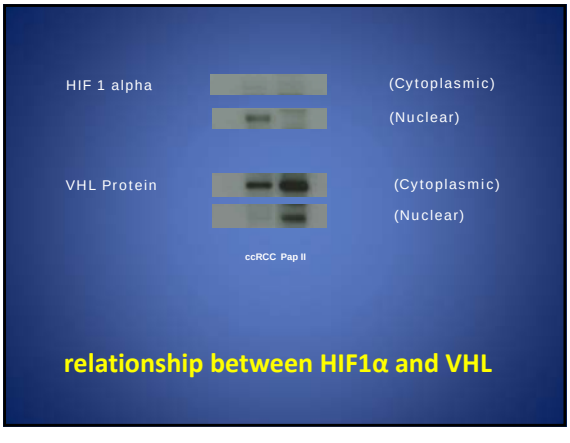
Case no.	Histological diagnosis	Tissue type	VHL mutation	Exon	Mutation type	Codon and predicted aa change*
3	cRCC	Original	Yes	1	FS	N67fsX158
		Xenograft	Yes	1	FS	N67fsX158
5	cRCC	Original	Yes	1	MS	V74G
		Xenograft	Yes	1	MS	V74G
6	cRCC	Original	Yes	1	NS and MS	S65X and R64S
		Xenograft	Yes	1	NS and MS	S65X and R64S
8	cRCC	Original	Yes	2	MS	L168R
		Xenograft	Yes	2	MS	L168R
9	cRCC	Original	Yes	3	FS	L152fsX188
		Xenograft	Yes	2	FS	L136fsX158
10	cRCC	Original	No	–	–	–
		Xenograft	No	–	–	–
11	pRCC	Original	No	–	–	–
		Xenograft	No	–	–	–
12	cRCC	Original	Yes	3	FS	L168fsX188
		Xenograft	Yes	3	FS	L152fsX188
13	cRCC	Original	Yes	2	FS	L136fsX158
		Xenograft	Yes	2	FS	L136fsX158
14	cRCC	Original	No	–	–	–
		Xenograft	No	–	–	–

aa, amino acids.

Chiara Grisanzio & Sabina Signoretti, J Pathol (2011); 225: 212-221

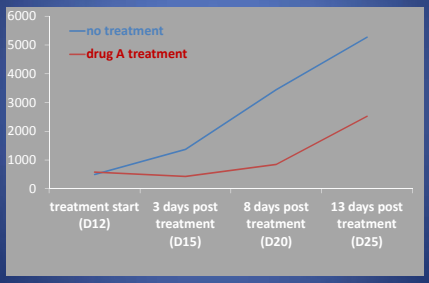


RCC Xenograft Model Application

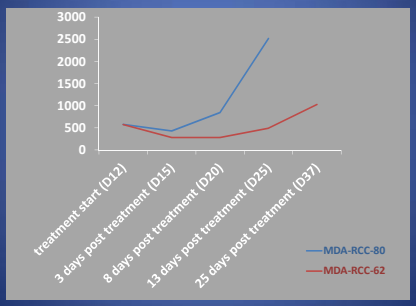


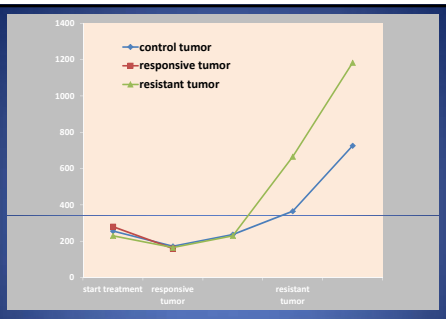
relationship between HIF1α and VHL

Effect of drug A on RCC xenografts models



response of different RCC xenografts to drug

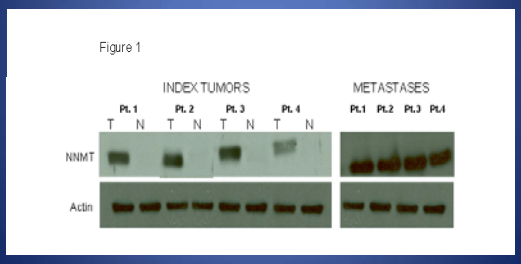




ccRCC in vivo responsive-resistance model

NNMT Expression In Renal Cell Carcinoma

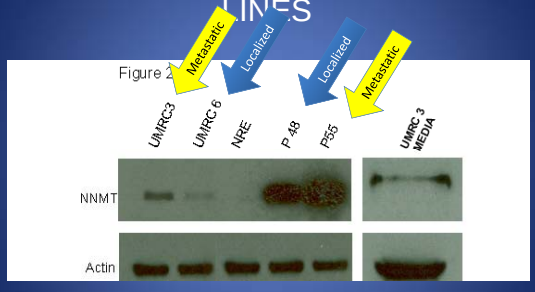
Patient Samples: Western Blot



What is NNMT?

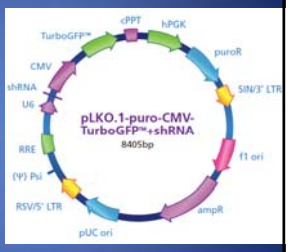
- Monomeric Enzyme (Methyl Transferase)
- Function:
  - N-methylation is one method by which drugs and xenobiotic compounds are metabolized (through conjugation).
  - Methyl Donor : SAM (SAM dependent methyl transferase)
  - Many other interactions/regulatory functions being explored

NNMT EXPRESSION IN CELL LINES

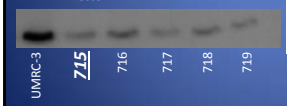


Lentivirus

- Commercially Available Lentivirus
- 5 Constructs for NNMT gene + Control
- PURO and GFP Selection

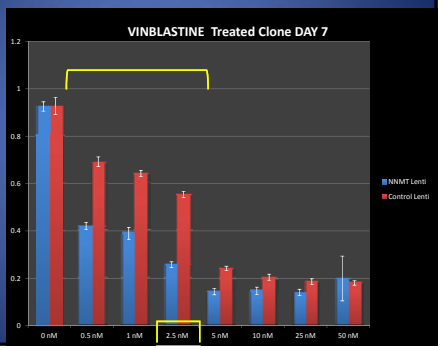


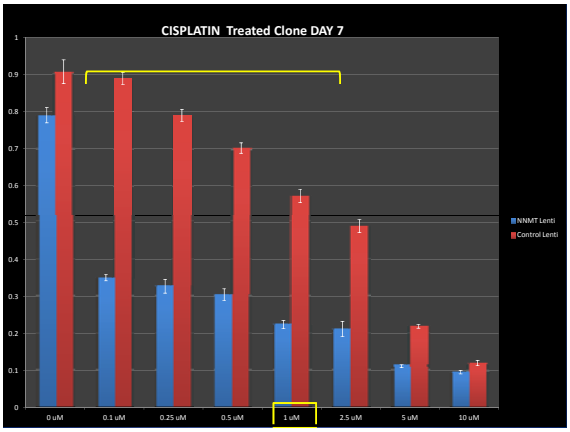
NNMT Western



NNMT Lentiviral Knockdown Increases Susceptibility to Cytotoxic Agents

Sulforhodamine B Tox
Y-Axis : Absorbance
Time 7 days





IN VIVO DATA

- Hypothesis: Tumors grown in the in vivo nude mouse model will be more susceptible to cytotoxic agents and possibly contemporary targeted agents (at physiologic doses).

CELL CULTURE

UMRC-3

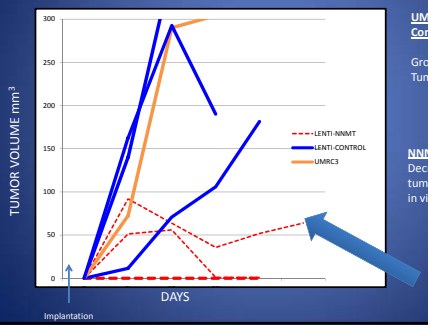
CONTROL LENTIVIRUS

NNMT LENTIVIRUS

5 Million cells / implant

SUBCUTANEOUS IMPLANTATION

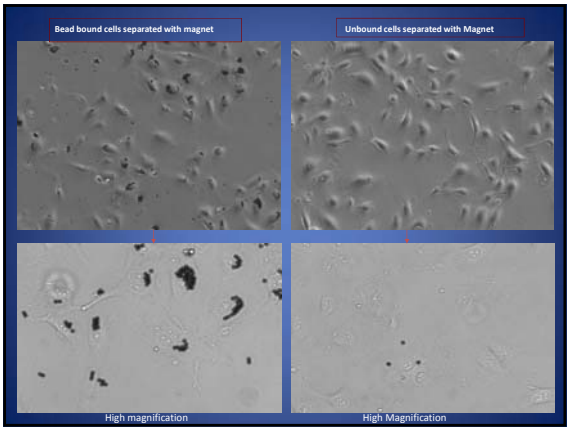
Tumor Formation Inhibited by NNMT Knockdown



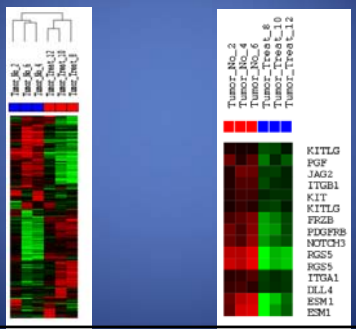
UMRC3 and Lenti Control:

Growth to Maximal Tumor Volume

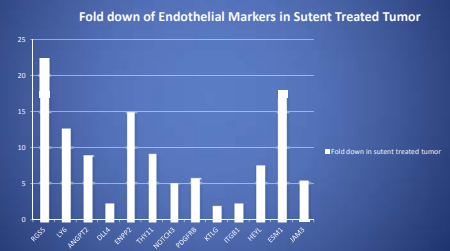
NNMT-LENTI:
Decreased tumorigenicity and in vivo growth



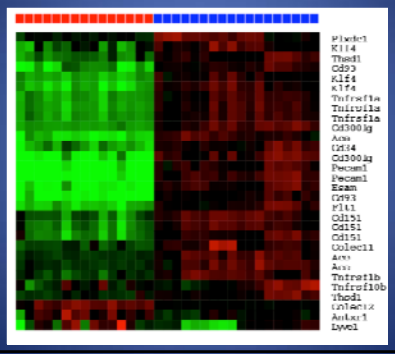
Gene Expression Alterations In Responsive and Resistant Endothelial Cells



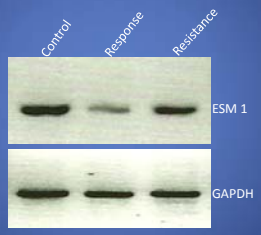
Micro Array data in Sunitinib Treated Endothelial Cell RNA from ccRCC patient.



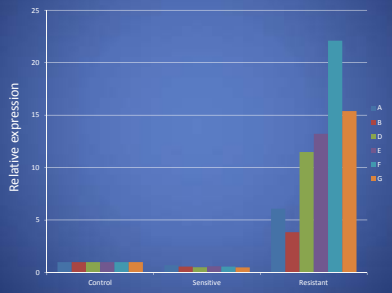
Heat Map of differentially expressed Endothelial markers in Sunitinib Treated ccRCC Xenograft.



Endocan (ESM1) expression in Sunitinib Treated ccRCC Xenograft

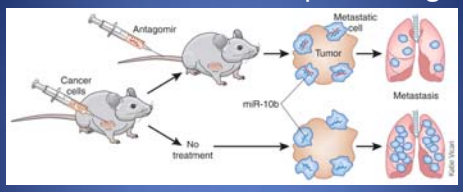


AngiomiRs upregulated in resistance



MDACC Wood Lab, Unpublished data

MicroRNAs: Viable therapeutic targets



- Drugs targeting microRNAs have shown promise in preclinical models of several cancers including metastatic melanoma, lung, glioma, liver and breast
- Mouse breast cancer model
  - Treatment with antagomir blocked miR-10b
  - Reduced number of pulmonary metastases
- First human trial of a microRNA therapy
  - Antagomir for miR-122 for hepatitis C infection
  - Currently enrolling

DePalma and Naldini, Nature Biotech, 2010; Wilmott et al, Pathology, 2011

Xenograft Utilization

- Tissue based and circulating biomarker identification and development
- Evaluate novel therapeutics
  - "Co-clinical trials concept"
- Study microenvironment interactions
  - Endothelial, stromal, tumor compartments can be separated or studied in aggregate
  - EMT
- Identify and target pathways of therapeutic resistance
- Biggest weakness: Lack of host immunity

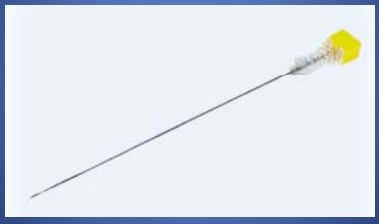
Future

- **Expand the panel of RCC xenograft modelS**
- **Develop biopsy xenografts model**

Expand the panel of RCC xenografts model

- **Clear cell RCC (√)**
- **Type I and II Papillary RCC xenografts (√)**
- **Xp11.2 translocation RCC xenografts (√)**
- **Renal medullary carcinoma (√)**
- **Sarcomatoid Dedifferentiation (√)**
- **Hybrid oncocytoma / chromophobe type xenografts)**
- **other RCC models**

Develop biopsy xenografts model



Develop biopsy xenografts model

Subcutaneous **orthotopic**

Thank You !