Renal Cancer Epigenetics

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Renal Cell Carcinoma

~2% of all human cancers

Pathologically and genetically heterogeneous

Metastatic disease has very poor prognosis

Understand the molecular basis of RCC to:
- guide novel therapeutic approaches
- develop biomarkers for diagnosis and prognosis

Identifying the molecular basis of renal cancers

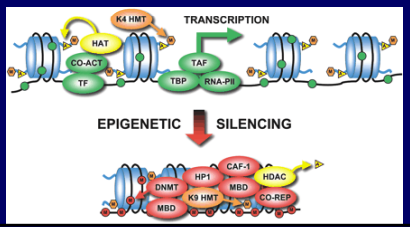
Somatic cancer genetics:
- candidate gene sequencing
- epigenetic studies
- exome/genome sequencing

Germline cancer genetics:
- candidate gene sequencing
- Translocation mapping
- genetic linkage studies and candidate positional analysis

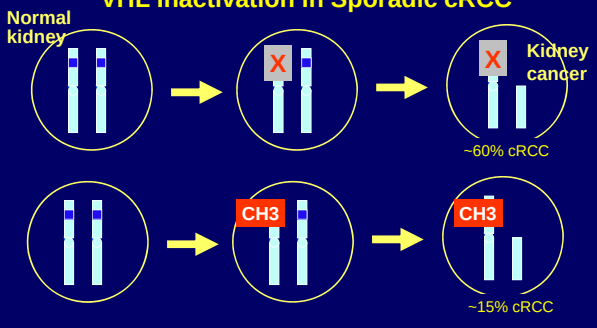
DNA methylation and cancer

Hypomethylation and hypermethylation may occur

Hypermethylation of tumour suppressor genes promoters causes epigenetic silencing

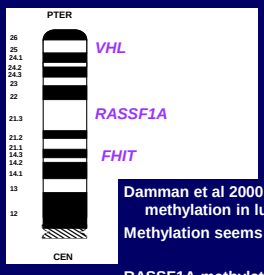


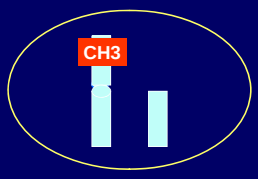
VHL Inactivation in Sporadic cRCC



Foster et al 1994, Gnarra et al 1994, Herman et al 1994, Clifford et al 1997

Epigenetic silencing of RASSF1A Tumour suppressor gene





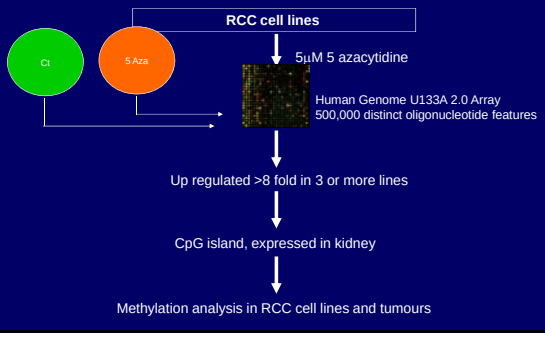
Damman et al 2000 found a high incidence of RASSF1A methylation in lung cancer.

Methylation seems to be the main route of inactivation

RASSF1A methylated in ~40% of sporadic RCC

Morrissey et al 2001

Functional Epigenetics

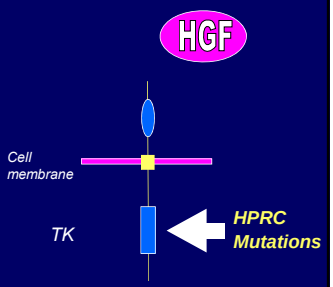


Hereditary Papillary RCC and MET Proto-oncogene

Zbar et al J Urol 1995
10 families with Type 1 papillary RCC
Dominantly inherited
Multiple tumours, incomplete penetrance

Rare cause of familial renal cell carcinoma (in UK)

Activating mutations in *MET* protooncogene (Schmidt et al 1997)

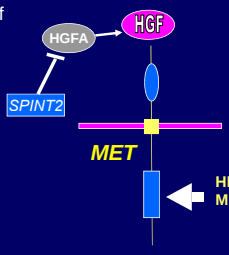


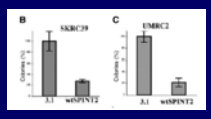
Identification of Candidate TSGs by Functional Epigenetic Analysis

Tumour specific methylation for 7 of 60 genes analysed

SPINT2 methylated in 30% cRCC and 40% pRCC

Re-expression of *SPINT2* suppressed RCC cell growth





Identification of Candidate TSGs in Sporadic RCC by Functional Epigenetic Analysis

Gene	% RCC Methylated	Tumour suppression
SPINT2	33%	Y
CST6	47%	Y
SFRP1	52%	Y
BNC1	45%	Y
GREM	23%	
COL15A1	46%	
RPRM	44%	Y

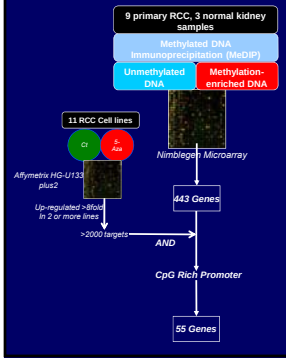
Morris MR et al 2005; Morris MR et al 2008; Morris et al 2010

Identification of Candidate TSGs in Sporadic RCC by Functional Epigenetic Analysis

Effective but inefficient strategy for detecting RCC TSGs (normal tissue methylation/unmethylated genes)
So combine with Methods to directly detect DNA methylation status?

- MeDIP, MeDIP-seq, MIRA
- Illumina Methylation assays (Goldengate, Infinium 27k, Infinium 450k)
- Whole genome bisulphite sequencing

MeDIP Results



9/56 genes showed frequent promoter region methylation in primary RCC:

KLHL35 (39%), QPCT (19%), SCUBE3 (19%), ZSCAN18 (32%), CCDC8 (35%), FBN2 (34%), ATP5G2 (36%), PCDH8 (58%) and CORO6 (22%)

RNAi knockdown for KLHL35, QPCT, SCUBE3, ZSCAN18, CCDC8 and FBN2 resulted in an anchorage-independent growth advantage

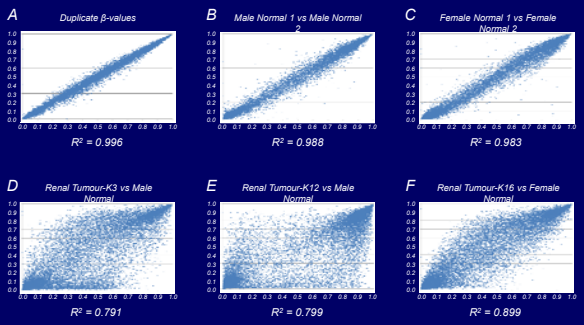
Morris MR, Ricketts CJ et al 2011

Identification of Candidate RCC TSGs with Infinium Human Methylation Array

- CpG methylation analysis for ~27,500 CpGs and >14,000 genes
- 38 sporadic RCC analysed
- Imprinted, X-linked genes etc excluded
- Results validated by bisulphite sequencing and expression analysis for selected genes (good correlation)
- Data analysed for methylation only and methylation plus functional data (total 205 genes)
- Methylated and reduced expression genes: OVOL1 (methylated in 40%), DLEC1 (20%), TMPRSS2 (26%), SST (32%), BMP4 (35%)

Ricketts CJ et al (in press)

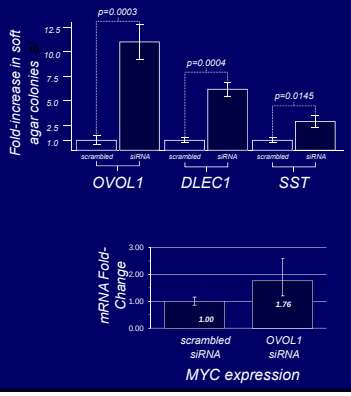
Infinium Human Methylation27 Array β-values



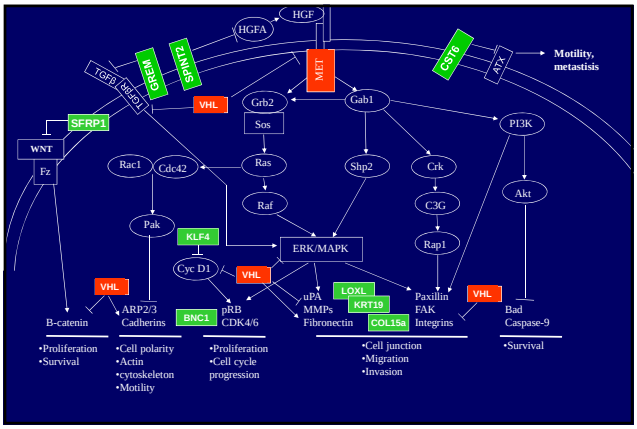
OVOL1 methylated in 40% RCC
- ovo-like 1 (fly);
?genitourinary development;
?regulates Myc

DLEC1 methylated in 20%
– TSG methylated in RCC

SST
– Somatostatin (also methylated in breast cancer)

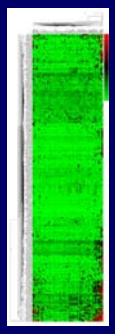


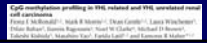
Ricketts CJ et al (in press)



Methylation Profiling in VHL and non-VHL RCC

- High-throughput analysis using Illumina Goldengate methylation assay (1505 CpGs from 807 genes)
- 29 VHL RCC, 20 non-VHL sporadic cRCC and 13 sporadic pRCC
- More CpG methylation in pRCC than cRCC
- Mean of 45.4 tumour-specific methylated CpGs per tumour in pRCC, 29.9 in non-VHL cRCC and 20.9 in VHL RCC
- Less methylation in VHL RCC as earlier stage?
- 14 genes significantly more CH3 in pRCC than cRCC




CpG methylation profiling in VHL related and VHL unrelated renal cell carcinoma

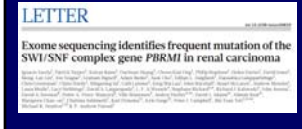
Genomic Analysis of Sporadic RCC


LETTERS

Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes

Sequenced 3500 genes in ~100 clear cell RCC

Identified inactivating mutations in genes encoding enzymes involved in histone modification (SETD2/JARID1C/UTX) (2-5% each)


LETTER

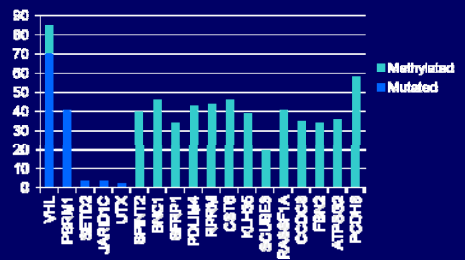
Exome sequencing identifies frequent mutation of the SWI/SNF complex gene *PBRM1* in renal carcinoma

Identification of the SWI/SNF chromatin remodelling complex gene PBRM1 as a second major clear cell RCC cancer gene

Truncating mutations in 41% (92/227) of cases.

Complementary approaches:
Epigenetic analysis
Understand the molecular basis of familial RCC

TSG inactivation in RCC



PBRM1 is a SWI/SNF chromatin remodelling complex gene
Mutations in histone modifying genes (SETD2, UTX and JARID1C) in ~3% of cases

Dalgliesh et al 2010, Varela et al 2011

Translational Epigenetics: Molecular Biomarkers and RCC

Mutation analysis:

- No clear association with VHL mutations
- Other mutations (except PBRM1) infrequent

Epigenetic analysis:

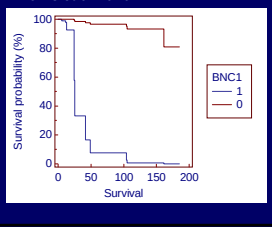
- Frequent TSG promoter methylation in RCC (~60 genes)
- Less heterogeneous than mutation analysis – easier detection
- Methylated DNA from tumours detectable in plasma or urine
  - potential for non-invasive diagnosis

Epigenetic analysis of tumours might guide prognostic predictions and therapeutic choices?

Epigenetic prognostic biomarkers?

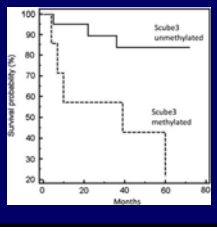
Methylation of BNC1 or COL14A1 was associated with a poorer prognosis independent of tumour size, stage or grade.

Morris et al 2010



SCUBE3 methylation associated with higher risk of death (P<0.009) and cancer death/relapse (P<0.0046)

Morris et al 2011



Translational Epigenetics

Epigenetic biomarkers

- prognosis
- tumour detection and monitoring (methylated DNA detectable in blood and urine)
- treatment (e.g. SPINT2 CH3 and MET inhibitors)

But

Many "methylated TSGs" analysed in <100 RCC and in a single study (Morris and Maher 2010)

Prognostic biomarkers require validation

Epigenetic therapies not yet investigated in RCC

Genomic Analysis of Sporadic RCC

The Cancer Genome Atlas Data Portal — Understanding genomics to improve cancer care

Comprehensive analysis (copy number analysis, DNA methylation, exome sequencing and gene expression) for 500 clear cell RCC and 75 papillary RCC

Cancer Genomics of Kidney Cancer

Comprehensive analysis (copy number analysis, DNA methylation, genome sequencing and gene expression, protein expression) in up to 2000 cases focussing on clear cell RCC

Molecular Advances in RCC

Advances in knowledge of the genetics AND epigenetics of RCC necessary to understand molecular pathology of RCC

Cancer genome projects are accelerating sporadic RCC gene discovery

Increasing candidate molecular markers for prognosis, stratified therapy etc

Major challenges ahead to identify those molecular biomarkers relevant to clinical practice

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CANCER RESEARCH UK

AICR
CANCER KNOWS NO BOUNDARIES
FORTUNATELY, NEITHER DO WE

8 of 14 genes more methylated in pRCC than cRCC linked to TGFβ and ERK/AKT nodes