

## Epigenetic Profiles in Clear Cell Renal Cell Carcinoma

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## UNC Team...

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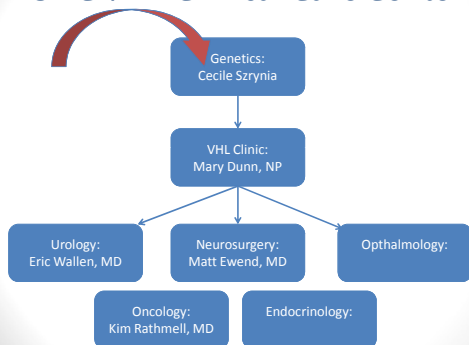
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Billy Kim  
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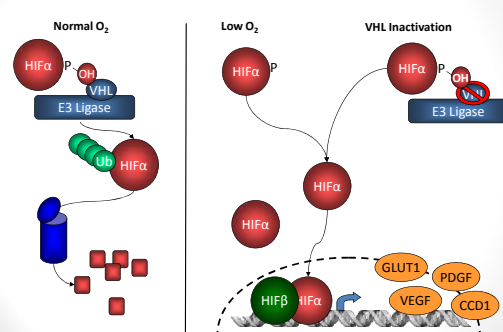
### Funding

R01 CA94031,  
ACS,  
AACR INNOVATOR,  
CALGB,  
UCRF

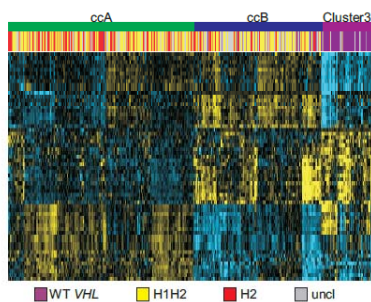
## UNC VHL Clinical Care Center



## VHL/HIF Regulatory Pathway

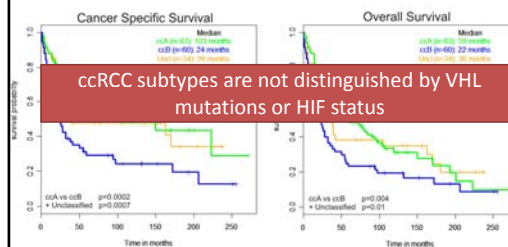


## Cluster3 tumors display gene signature indicative of wild type VHL



Brannon et al. Eur. Urology. 2011

## Marked survival differences between ccRCC subtypes



Brannon et al. Genes and Cancer. 2010

## Mutations in Epigenetic Modifying Genes and ccRCC

- Mutations in known cancer genes, including RAS, BRAF, TP53, RB, CDKN2A, PTEN, etc, **rarely** observed
- Enriched for mutations in histone modifying genes
- PBRM1 mutations

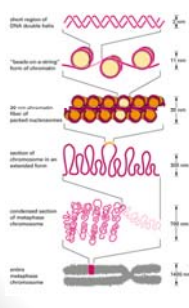
| Gene    | Mutation Frequency | Function                                    |
|---------|--------------------|---|
| SETD2   | 3% (13 of 407)     | H3K36 methyltransferase                     |
| JARID1C | 3% (13 of 407)     | H3K4 demethylase                            |
| UTX     | 3% (12 of 407)     | H3K4 methyltransferase<br>H3K27 demethylase |
| MLL2    | 4% (17 of 407)     | H3K4 methyltransferase                      |
| PBRM1   | 41% (92 of 227)    | SWI/SNF chromatin remodeling complex member |

Dakiles et al. Nature 2010

## Histone Modifications Modulate Gene Expression

| Histone Residue | Methylation Status | Genomic Location  | Transcriptional Effect                                      |
|-----------------|--------------------|---|---|
| H3K4            | me1<br>me2<br>me3  | Enhancers<br>Promoters, Enhancers, ORFs<br>Promoters, Enhancers | Poised state, Inhibits histone clipping<br>Active<br>Active |
| H3K27           | me3                | Promoters, ORFs   | Repressive  |
| H3K36           | me3                |   | Active  |
| H3K79           | me3                | ORFs  | Active  |
| H4K20           | me1<br>me3         | Promoter, ORFs  | Active<br>Repressive  |

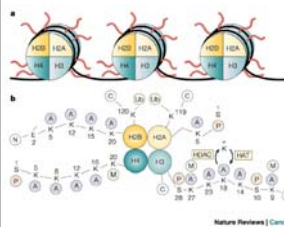
## Epigenetics and Chromatin Structure



- **Epigenetics**: Heritable changes in gene expression or phenotype NOT due to underlying changes in DNA
- Types of epigenetic changes affecting gene expression:
  - Chromatin Remodeling
  - DNA Methylation
  - Histone Modifications

<http://library.thinkquest.org/CD04535/chromatin.html>

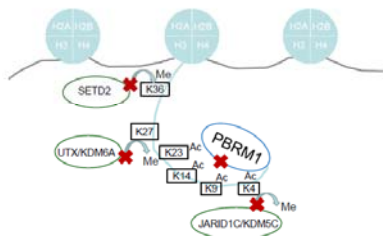
## Unit of Chromatin: Nucleosome



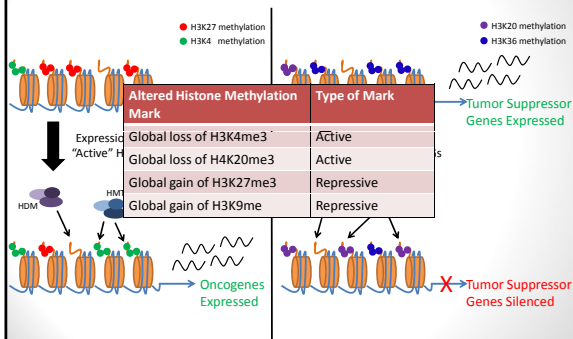
- Nucleosome = Octamer of histones around which DNA wraps
- Histone Tail Modifications
  - Acetylation
  - Phosphorylation
  - Methylation
  - Ubiquitination
  - Sumoylation

Marks et al. Nature Reviews Cancer 1, 194-207 (December 2001)

## Epigenetic modifiers mutated in RCC



## Altered Histone Modifications could promote tumorigenesis through transcriptional dysregulation



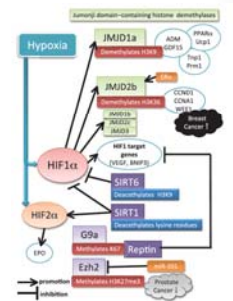
## Mutations in histone modifying genes are enriched for in ccA tumors

| ccA Tumors | ccB Tumors |
|------------|------------|
| 10% SETD2  | 3% SETD2   |
| 5% JARID1C |            |
| 2% UTX     | 5% UTX     |
| 2% MLL     | MLL4       |
| 2% MLL2    |            |
| JMJD1B     |            |
| NSD1       |            |
| SUV420H1   |            |

Dakiles et al. Nature 2010, Rose Brannon

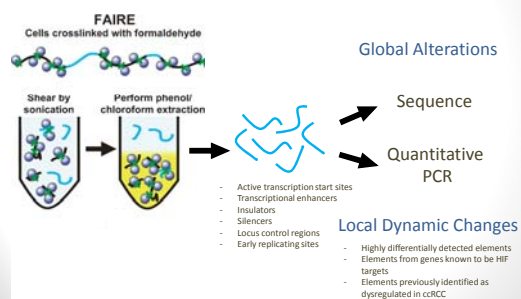
## Evidence for Altered Histone Methylation in ccRCC

- Mutations in epigenetic modifying genes
- Links to classical ccRCC biology



Miyawaki et al. J Pharmacol Sci 2010

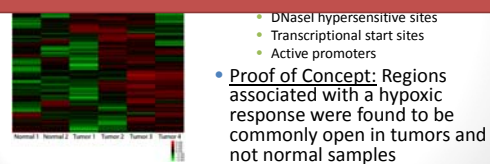
## Formaldehyde-Assisted Isolation of Regulatory Elements



Adapted from Groud et al., 2007

## FAIRE identifies altered chromatin organization in ccRCC

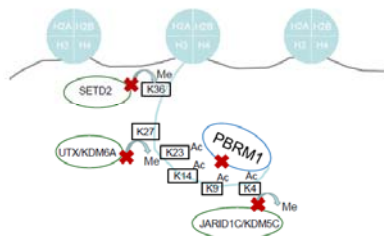
- Formaldehyde-Assisted Isolation of Regulatory Elements
- FAIRE data suggests that chromatin organization differs between and within normal kidney and ccRCC tumor samples.



- **Proof of Concept:** Regions associated with a hypoxic response were found to be commonly open in tumors and not normal samples

Jeremy Simon and Rose Brannon

## Exploring histone marks in RCC tumors



## Investigating ccRCC histone modification patterns

- Created tissue microarrays containing:
  - 71 tumors
  - 11 paired normals
- Each sample in triplicate
- Efficiently determine protein expression in ccRCC samples

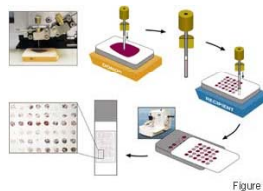
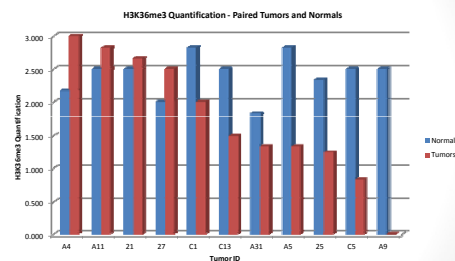


Figure 1

http://www.microarraystation.com/images/tissue-microarray-process.jpg

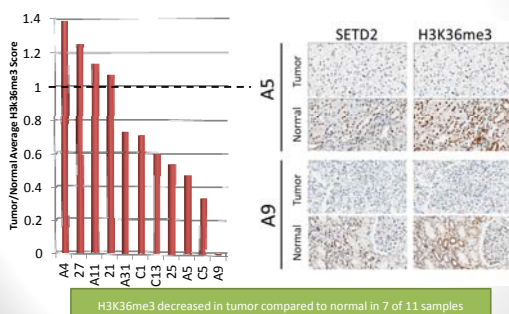
How do H3K36me3 patterns differ both between tumor and normal samples and within ccRCC tumor subsets?

## H3K36me3 - Paired Tumors and Normals



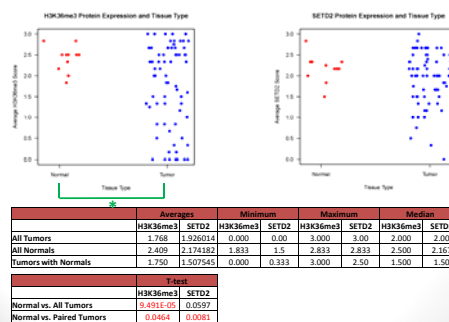
H3K36me3 decreased in tumor compared to normal in 7 of 11 samples

H3K36me3 is decreased in a subset of ccRCC tumors compared to paired normal kidneys



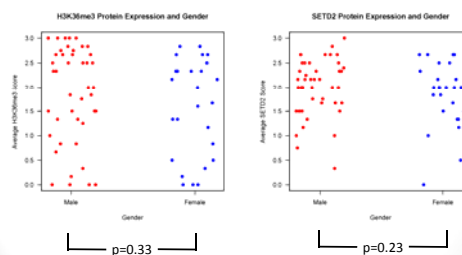
H3K36me3 decreased in tumor compared to normal in 7 of 11 samples

H3K36me3 and SETD2 expression in tumor compared to normal kidney



Are decreased H3K36me3 and SETD2 expression levels associated with tumor characteristics?

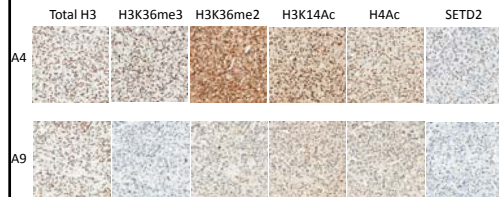
H3K36me3 and SETD2 Expression are not associated with gender



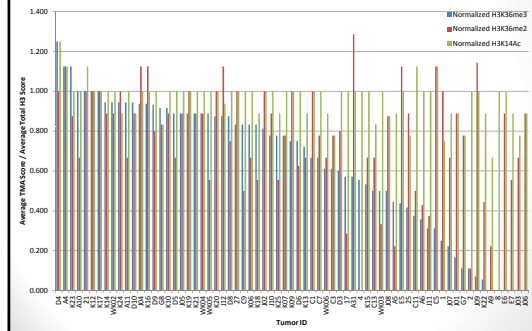




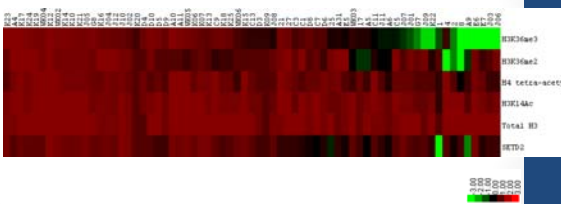
## Representative TMA Images



## H3K36me2 is not associated with H3K36me3 levels in ccRCC



## Of the marks tested, H3K36me3 displays the greatest loss in ccRCC



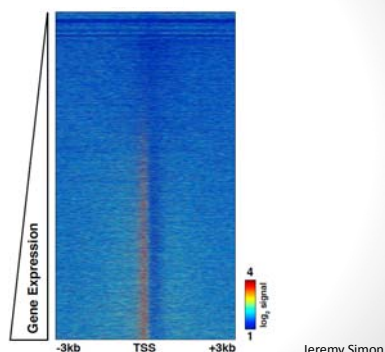
## Conclusions

- The effect of SETD2 mutations in VHL mutated ccRCC has not yet been fully explored.
- Preliminary evidence suggests that aberrations in SETD2 result in measurable alterations in chromatin organization, histone modification patterns, and transcriptional profiles.

## Future Directions

- Confirm tumor mutational status
- Define the relationship of gene expression and chromatin modification.
- Identify SETD2/H3K36me3 modulated genes
- Integrated/Mechanistic analysis

## Integration of ccRCC RNA-seq and FAIRE data



## Tumor Sample Progress to Date

| H3K36me3       | Low (17) |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    | Normal (10) |    |    |    |    |    |    |    |    |    | Unknown |    |    |    |
|----------------|----------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|-------------|----|----|----|----|----|----|----|----|----|---------|----|----|----|
| Sample         | A9       | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17          | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27      | 28 | 29 | 30 |
| RNA isolation  |          |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |             |    |    |    |    |    |    |    |    |    |         |    |    |    |
| FAIRE prep     |          |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |             |    |    |    |    |    |    |    |    |    |         |    |    |    |
| gDNA isolation |          |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |             |    |    |    |    |    |    |    |    |    |         |    |    |    |
| RNA Library    |          |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |             |    |    |    |    |    |    |    |    |    |         |    |    |    |
| FAIRE Library  |          |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |             |    |    |    |    |    |    |    |    |    |         |    |    |    |
| gDNA Capture   |          |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |             |    |    |    |    |    |    |    |    |    |         |    |    |    |
| RNA sequence   |          |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |             |    |    |    |    |    |    |    |    |    |         |    |    |    |
| FAIRE sequence |          |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |             |    |    |    |    |    |    |    |    |    |         |    |    |    |
| gDNA sequence  |          |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |             |    |    |    |    |    |    |    |    |    |         |    |    |    |
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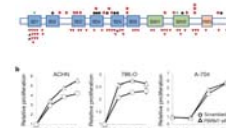
Not Attempted  
Completed  
Assessed

| Sample    | Gene  | cDNA Annotation                        | Protein Annotation | Domain |
|-----------|-------|--|--------------------|--------|
| A489      | SETD2 | c.6098_6099delTG (hom)                 | p.V20336*9         |        |
| PD3338a   | SETD2 | c.92_122delCTAATGAACGGGATCCGAGAGGGTATC | p.P3115*35         |        |
| PD3408a   | SETD2 | c.1850_1853delTAGC                     | p.R1791*33         |        |
| PD3437a   | SETD2 | c.2965C>T                              | p.R989*            |        |
| PD3528a   | SETD2 | c.3152T>G                              | p.V1051G           |        |
| PD3379a   | SETD2 | c.4333A>T                              | p.K1445*           |        |
| PD3431a   | SETD2 | c.4561delC                             | p.S1614fs*30       | SET    |
| PD3363a   | SETD2 | c.5341C>T                              | p.Q1781*           |        |
| PD3419a   | SETD2 | c.5540_5555delCAGCAGTGACTACAAT         | p.A1847fs*17       |        |
| PD2205a   | SETD2 | c.2659delT                             | p.S887fs*4         |        |
| PD2214a   | SETD2 | c.3392_3393delAT                       | p.N1131fs*2        |        |
| PD1754a   | SETD2 | c.5218delC                             | p.Q1740fs*16       |        |
| PD2186a   | SETD2 | c.5619_5626delGGATCTGCC                | p.L1873fs*50       |        |
| PD2153a   | SETD2 | c.3545G>T                              | p.C1182F           |        |
| PD2146a   | SETD2 | c.3383G>A                              | p.C1128Y           |        |
| LB996-RCC | SETD2 | c.3421G>T (hom)                        | p.G1141*           |        |

Dalglish et al. 2010

## PBRM1 Mutations in ccRCC

- SWI/SNF complex modifier (chromatin remodeling)
- Mutated in 41% of human ccRCC tumors (92/227)
- Silencing results in increased proliferation in 4/5 ccRCC cell lines (remaining cell line already has PBRM1 inactivation)
- Increased migration with PBRM1 knock-down
- Unanswered:** How does modulation of PBRM1 affect chromatin patterns in ccRCC?



Vignati et al. 2011

## Causes of Gene Expression Patterns

- Chromosomal Alterations
  - Loss of: 3p, 6q, 8p, 9p, 14q
  - Gain of: 5q (good prognosis), 20

- Epigenetics

We hypothesize that epigenetic alterations play a role in ccRCC tumorigenesis and progression. Furthermore, they may result in distinct gene expression patterns and survival differences.