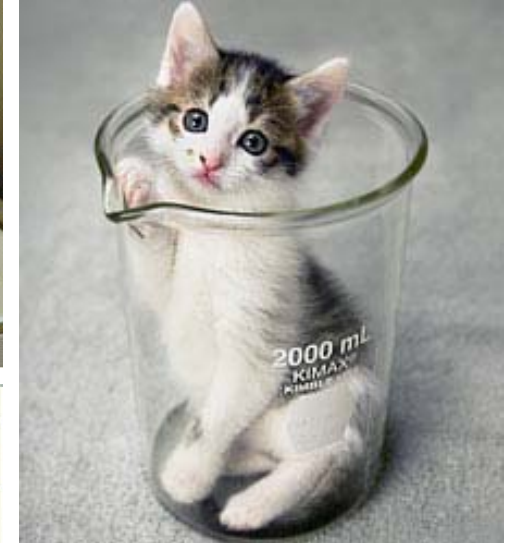


Epigenetic Inactivation of *SFRP1* and *SFRP2* genes as  
Biomarkers of Invasive Bladder Cancer

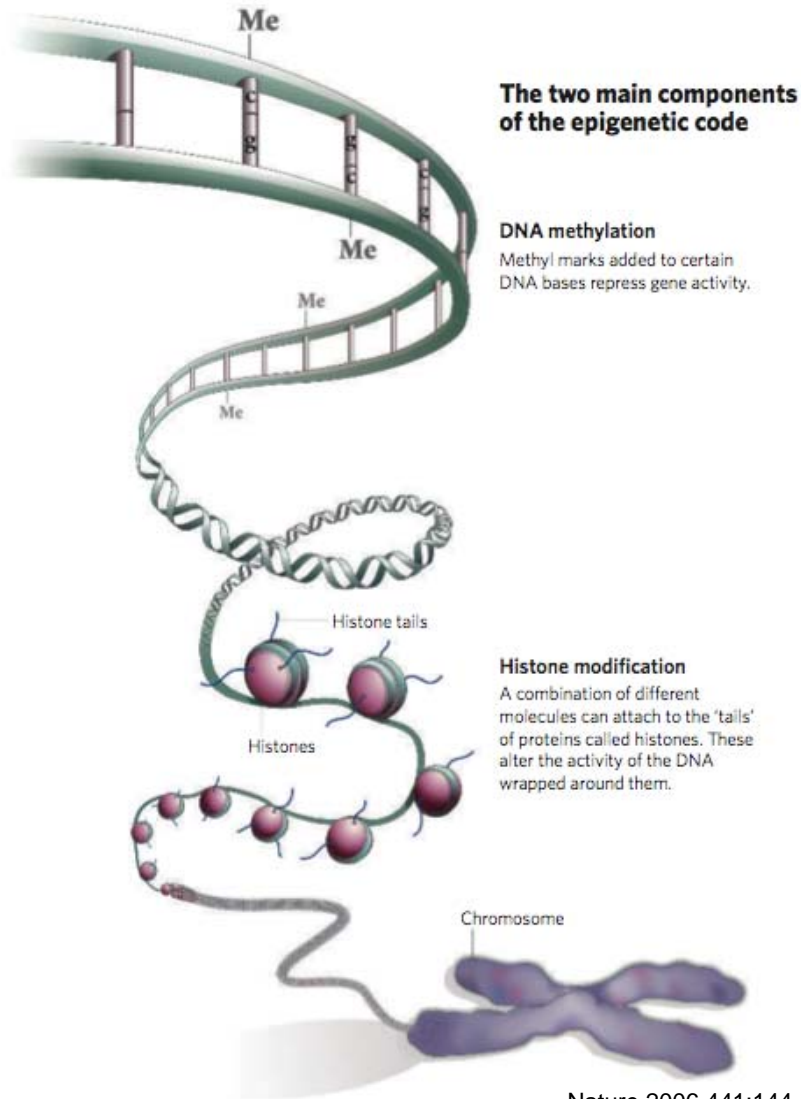
*Thesis Presentation*  
*Shuying Glenda Tan, 2009*

# Epigenomics

- ‘Carbon copy’ does not look like ‘Rainbow’ despite being genetically identical
- Colors on a Calico cat are determined epigenetically
- X-inactivation



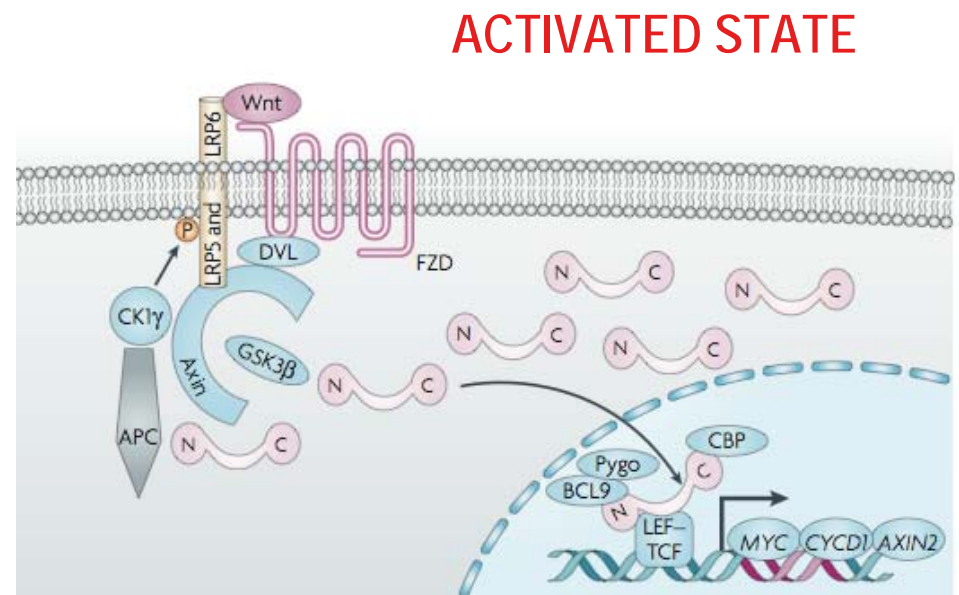
# So how does it 'work'?



- Methylation of the gene promoter silences the gene
  - Form of cellular control and occurs in normal cells
  - Can become a problem in cancer

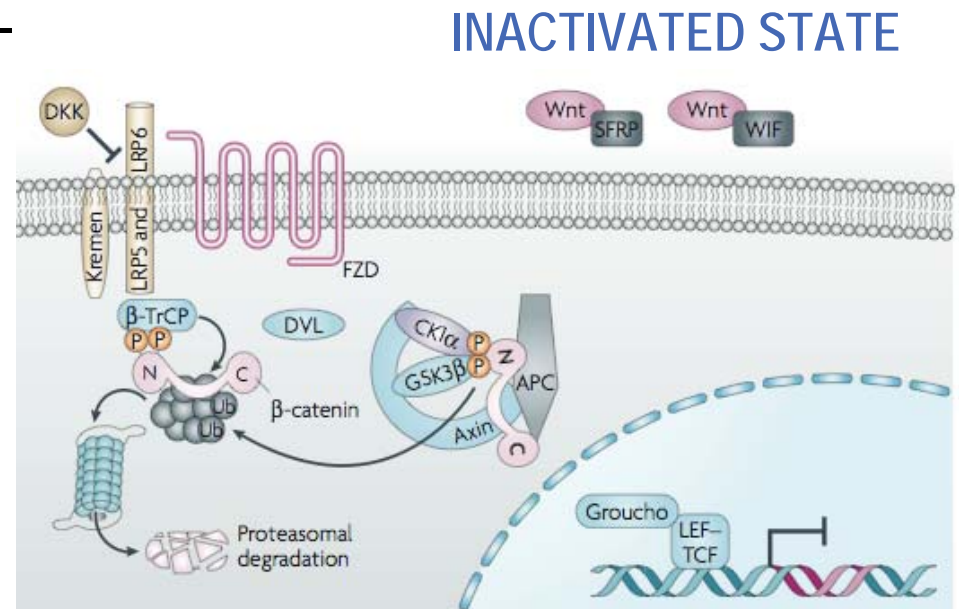
# The Wnt pathway

- Important in embryonic development
- Regulates cell proliferation, survival, differentiation through maintenance of  $\beta$ -catenin levels
- Pathway activation
  - Transcription of target genes
  - In cancer: cells develop, multiply and form tumors



# SFRPs in the Wnt pathway

- SFRPs: Secreted Frizzled-Related Proteins
  - Soluble antagonists that bind directly to Wnt proteins
- *SFRP* gene regulation an alternative way to (in)activate or stabilize this pathway



# *TP53* and cancer

---

- *TP53* is the most frequently altered gene in human cancer
- Tumor suppressor function
  - Alteration associated with more aggressive invasive bladder cancer
- Commonly used in determining patient prognosis and treatment
  - Alone, test may not be effective
  - Looking for additional biomarkers to be used in conjunction

# Hypotheses

---

1. *SFRP* methylation is associated with invasive bladder cancer
2. Smoking is associated with *SFRP* methylation
3. *SFRP* methylation and *TP53* mutations act jointly as markers of invasive bladder cancer

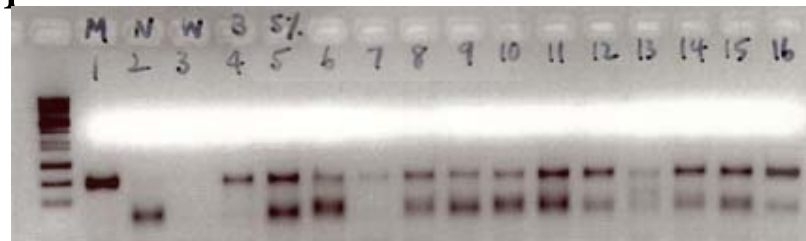
# Patients & Methods

- Patients: sub-sample of the NE Bladder Cancer Study
- DNA extraction
- Sodium Bisulfite Modification



[http://epigenetics-noe.net/images/protocolfigs/id34\\_fig1.gif](http://epigenetics-noe.net/images/protocolfigs/id34_fig1.gif)

- Methylation specific PCR



MS-PCR analysis of sFRP1 gene

- Statistical analyses

# Results

**Table 1.** Bladder tumor SFRP gene methylation by patient demographics

Characteristic	SFRP1 Methylation			SFRP2 Methylation			Any SFRP Methylation			Totals
	Negative n (%)	Positive n (%)	Adjusted OR* (95% CI)	Negative n (%)	Positive n (%)	Adjusted OR* (95% CI)	Negative n (%)	Positive n (%)	Adjusted OR* (95% CI)	
Overall Prevalence	122 (60)	82 (40)		123 (60)	81 (40)		85 (42)	119 (58)		204
Gender										
Female	35 (63)	21 (37)	1.0 (reference)	39 (70)	17 (30)	1.0 (reference)	26 (46)	30 (54)	1.0 (reference)	56
Male	87 (59)	61 (41)	1.1 (0.6-2.2)	84 (57)	64 (43)	1.8 (0.9-3.5)	59 (40)	89 (60)	1.3 (0.7-2.4)	148
Age										
<65	43 (56)	34 (44)	1.0 (reference)	47 (61)	30 (39)	1.0 (reference)	34 (44)	43 (56)	1.0 (reference)	77
≥65	79 (62)	48 (38)	0.7 (0.4-1.3)	76 (60)	51 (40)	1.1 (0.6-2.1)	51 (40)	76 (60)	1.2 (0.7-2.2)	127
Tumor Stage										
Non-invasive	91 (65)	50 (35)	1.0 (reference)	98 (70)	43 (30)	1.0 (reference)	69 (49)	72 (51)	1.0 (reference)	141
Invasive	31 (49)	32 (51)	1.9 (1.0-3.8)	25 (40)	38 (60)	3.4 (1.7-6.9)	16 (40)	47 (60)	3.1 (1.5-6.4)	63
TP53 Staining										
Score <3	90 (61)	58 (39)	1.0 (reference)	96 (65)	52 (35)	1.0 (reference)	65 (44)	83 (56)	1.0 (reference)	148
Score 3+	32 (55)	24 (45)	0.9 (0.5-1.9)	27 (48)	29 (52)	1.1 (0.5-2.3)	20 (36)	36 (64)	0.8 (0.4-1.7)	56
Smoking Status <sup>^</sup>										
Never	22 (71)	9 (29)	1.0 (reference)	21 (68)	10 (32)	1.0 (reference)	16 (51)	15 (49)	1.0 (reference)	31
Former	57 (53)	49 (47)	2.2 (0.9-5.3)	62 (58)	44 (42)	1.3 (0.5-3.2)	40 (38)	66 (62)	1.6 (0.7-3.8)	106
Current	41 (64)	23 (36)	1.3 (0.5-3.4)	39 (61)	25 (39)	1.3 (0.5-3.4)	28 (44)	36 (56)	1.4 (0.6-3.4)	64

\* OR adjusted for all other variables in the table and limited to subjects with complete data for all variables (n= 201).

<sup>^</sup> Three tumors were missing smoking status classification.

# Results

**Table 2.** Methylation of multiple SFRP genes and altered TP53 status are independently associated with invasive bladder cancer

Covariate	n (no. of invasive disease)	Invasive bladder cancer * OR (95% CI)^	P
No. of SFRP genes methylated			
0	85 (16)	1.0 (reference)	
1	75 (24)	2.2 (1.0-4.9)	0.0519
2	44 (23)	4.6 (1.9-11.3)	0.0008
TP53 alteration (staining intensity)			
<3	148 (28)	1.0 (reference)	
3+	56 (35)	7.1 (3.5-14.6)	<0.0001

\*Model predicts invasive compared with non-invasive

^Model controls for all covariates, limited to subjects with complete data (n=201)

- Increased odds of having invasive bladder cancer with increasing number of *SFRP* genes methylated
- *TP53* alteration correlates with invasive disease

# Results

**Table 3.** Stratified analysis of the association between TP53 alteration and invasive bladder cancer by methylation of any SFRP gene

	n (no. invasive disease)	Invasive bladder cancer OR (95% CI)*	P
No SFRP methylation			
TP53 WT	69 (7)	1.0 (reference)	0.002
TP53 altered	16 (9)	<u>6.7 (2.0-22.6)</u>	
Positive SFRP methylation			
TP53 WT	83 (21)	1.0 (reference)	0.00001
TP53 altered	36 (26)	<u>7.7 (3.2-18.8)</u>	

\*Models controlled for age and sex, limited to subjects with complete data for all variables (n=201)

WT = wild type

- *SFRP* methylation status enhanced association between p53 staining intensity and invasiveness of disease

# Discussion: Consistency

---

- Consistent with hypothesis:
  - *SFRP* methylation is an important biomarker for invasive bladder cancer
- Highlight importance of SFRPs as the likely candidates employed in bladder cancer to control Wnt signaling
- Only able to look at 2 *SFRP* genes
  - *SFRP1* & *SFRP2* may be the main actors that participate in Wnt pathway inhibition

# Discussion: Consistency

---

- Consistent with hypothesis:
  - Strong joint relationship of *SFRP* methylation and *TP53* alteration on invasive disease
- Relationship though significant, not as strong as previously suggested
  - OR of 7.7 compared to >30
  - Reflects range of odds in population of bladder cancer patients

# Discussion: Importance

---

- Prognosis:
  - Joint classification of tumors based on *TP53* and *SFRP* identifying patients who need more aggressive treatment
  - *SFRP* methylation first of multiple ‘hits’?
- Therapy:
  - Therapeutic drugs to mimic SFRPs, easy target since it is soluble and works in the intercellular matrix
- Research:
  - Focus on key genes in subsequent research

# Discussion: Smoking

---

- Hypothesis rejected?
  - Inconclusive relationship between smoking and *SFRP* gene methylation
- Original position:
  - Exposure to tobacco smoke carcinogens can select for epigenetic silencing of these *SFRP* genes
- Is this no longer true? Why do we no longer see a clear relationship?
  - Maybe to do with this particular sub-sample
  - Change in risk factors from the 1990s to 2000s
  - Should we be looking for other exposures?

# Future Directions

---

- Cancer Statistics (2008) shows elevated levels of bladder cancer in northeastern USA
  - RI has the highest incidence rate of bladder cancer
  - Differential exposure, or chance?
- More research on smoking and alternative exposures
- More research on therapeutic drugs targeting the Wnt pathway
- Epigenetic silencing as alternative mechanism of cancer progression

# Thanks

---

- Karl T. Kelsey
- Carmen J. Marsit
- Silvia Plaza
- Kelsey Lab: Rondi Butler, Brock Christensen, Gretchen Gee, Lauren Ouellet, Graham Poage
- Mary Hixon
- Dov Sax