Molecular Pathogenesis of Bladder Cancer

American Society of Cytopathology Annual Meeting

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Epidemiologic Features

- **Genetic Factors:**
  - ~8% have a family history
  - *GSTM1* null Phenotype status (Hazard Ratio ~1.4)
  - Muir-Torre Syndrome: type of HNPCC, young age, multifocal, bilateral upper tract disease
- **Cigarette Smoking Exposure (poly cyclic aromatic hydrocarbons [PAH], arylamines):**
  - estimates as high as 66% of male and 30% of female TCC
  - Hazard Ratio: 2-6
  - Cessation for 15 y lead to equality of risk

Epidemiologic Features

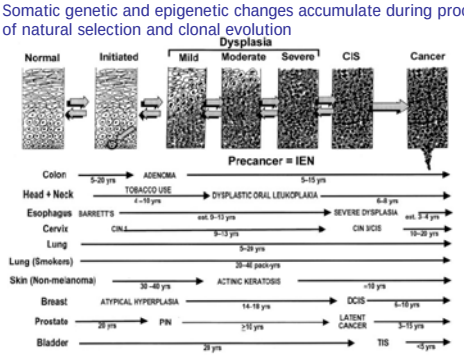
- **Occupational Exposures:**
  - **Estimates as high as 25% of cases**
  - **Arylamines: e.g. Benzidine, naphtylamines, aniline dye (workers in rubber, petroleum, paint, textile dye industries)**
- **Iatrogenic Exposures: e.g, Phenacetin, X-ray Rx and Cyclophosphamide**
- **Drinking Water Exposure: Arsenic in water: Taiwan, Argentina, Chile**
- ***Infectious/Inflammatory Exposures: Schistosoma haematobium*: Egypt, Sudan, Iraq, Zambia, Malawi, and Zimbabwe**

Genotype/Exposure Joint Effects:

- **Slow acetylators (*NAT2*) have higher arylamine and smoking related risk**
- ***GSTM1*, *GSTT1* null genotypes have higher risk of smoking related cancers**

Multi-Step Epithelial Carcinogenesis –

Somatic genetic and epigenetic changes accumulate during process of natural selection and clonal evolution



Clinical Cancer Research Vol. 8, 314-346, February 2002

Two Main Pathways to Bladder Cancer

TABLE II. Distribution of DNA Values in 277 Untreated Bladder Tumors†

Distribution by Grade

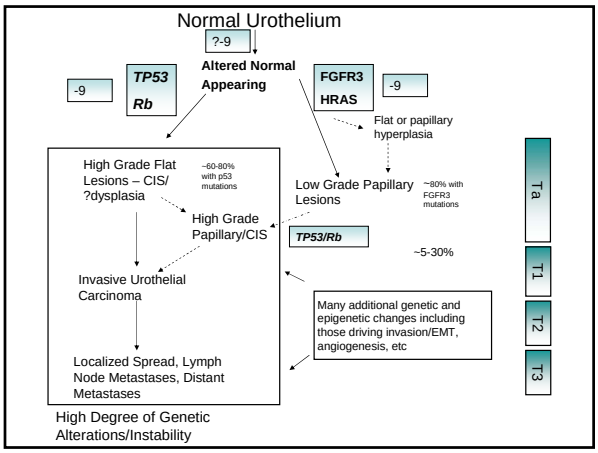
Grade	No. in Group	Diploid	Aneuploid*
0	2	2	0
I	30 (100%)	24 (80%)	6 (20%)
II	107 (100%)	56 (52%)	51 (48%)
III	130 (100%)	6 (5%)	124 (95%)
Adenocarcinoma	8	1	7
Total	277	89	188

Distribution by Stage

Stage	No. in Group	Diploid	Aneuploid
T0	42 (100%)	32 (76%)	10 (24%)
T1	118 (100%)	50 (42%)	68 (58%)
T2, T3, T4	93 (100%)	7 (7.5%)	86 (92.5%)
TIS	24 (100%)	0	24 (100%)
Total	277	89	188

† Modified from Tribukait [19].
* Includes tetraploid-aneuploid tumors.

Koss, J Cell Biochem, Supp 161:23-29 (1992)



Chromosome 9 Alterations in Urothelial Carcinoma

- LOH of Chromosome 9 is an early event shared by both pathways
- Potential tumor suppressor genes:
  - 9p21: *CDKN2A* (encodes p16 and p14); homozygous deletion in up to 14% of superficial carcinomas, associated with progression
  - 9q34: *TSC1* (up to 50% of all tumors have LOH)
  - 9q32-33: *DBC1*
  - *9q32-33: DBCCR1 (often methylated in carcinomas)*

CIS

- Zieger et al, *Int. J. Cancer, 125:2095-, 2009:* used 10k SNP Chips to evaluate allelic loss/gains from LCM samples
  - Low grade dysplasia – n = 4
    - No changes seen
  - CIS
    - overall 8 or 12 had chromosomal instability
    - FGFR3 – 0/16
    - 5p gain – 8/12
      - Lesions with 5p gain had many of the of other alterations listed here, but those without generally did not
    - 5q loss – 6/12
    - 9p loss - 6/12
    - 9q loss – 5/16
    - 17 p loss – 4/12
    - 17q gain – 5/12
    - 13 q loss – 6/12
    - 18q loss – 4/12
    - 20q gain – 5/12

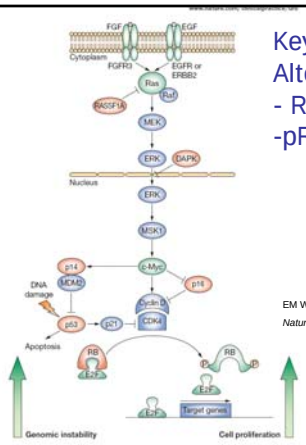
T1 Urothelial Carcinoma Lesions

- Zeiger et al., n = 48
- *FGFR3* mutations in 17/48, wild-type in 31/48
- *FGFR3* mutations and gains of chromosome 5p were mutually exclusive
- Loss of chromosome 9 was common in both FGFR3 mutated and CIS-type tumors
- Summary – of 48 T1 lesions
  - N = 22 CIS type tumors – lack of *FGFR3*, gains of 5p, 6p and 10p, and loss of 5q
  - N= 12 Papillary type tumors with *FGFR3* mutations
  - N = 11 tumors with no characteristic changes ("undetermined")
  - N= 5 had both CIS related chromosomal changes and *FGFR3* mutations ("equivocal") – none of these had 5p gain
  - Lesions with CIS type molecular changes were highly associated with concomitant CIS at some point in the disease

No FGFR3 Mutations in Adjacent Normal Urothelium in Patients with LG Bladder Tumors Containing FGFR3 Mutations

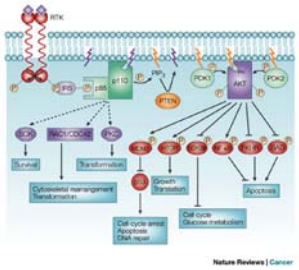
- Otto et al. Int. J. Cancer, 125:2205-, 2009.
- LCM captured normal epithelium from:
  - 38 patients with FGFR3 mutated bladder tumors
  - 15 patients without any urothelial tumors
- Results: no mutations in normal urothelium in vicinity of urothelial carcinoma harboring tumors with FGFR3 mutations.
- Conclusions:
  - FGFR3 mutations are not the earliest somatic events leading to low grade bladder neoplasia
  - Chromosome 9 loss and methylation changes appear to occur first

Key Pathways Altered – I - Ras-ERK -pRB/p16/p14



EM Wolff, G Liang, and PA Jones
Nature Clin. Prac. Urol, 2, 502-, 2005

PI3-Kinase\PTEN Pathway



PIK3CA mutations: PUNLUMP (26%); pTa (16%); T1 (20%); muscle invasive (0%); associated with *FGFR3* mutations

PTEN:LOH and/or mutation is fairly uncommon, but protein loss is more common in high grade invasive tumors

Combined knockout of *Pten* and *Tp53* in mice in the bladder results in invasive carcinomas (Puzio Juter et al., Genes Dev, 23:675-, 2009

Bladder Urothelial Carcinoma Chromosomal Aberrations

Additional LOH:

- LOH 8p in up to 83% of high grade and invasive UrCa
- LOH 15q in 40% of UrCa; ? RAD51 DNA repair

Chromosomal Gains:

- 17q gains: HER2 amplification; TOP2A
- 3q,7p,17q gains

Diagnostic Application:

- Numerical Ch 3, 7, 17 are exploited in UroVysion FISH assay (in addition to loss of p16 @ 9p21)

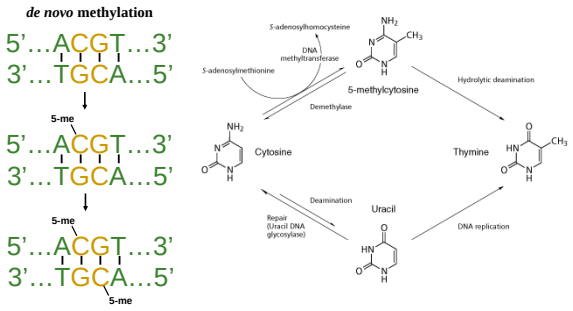
Slide from G. Netto MD

Bladder Urothelial Carcinoma Epigenetic Alterations

- Cytosine methylation in CpG islands of promoter regions of tumor suppressor genes is a common mechanism of silencing their transcription activity
- Methylation in *RASSF1a* and *CDH1* (E Cadherin) has been most frequently shown
- Variable rates regarding other TSGs: *CDKN1B* (p16, p14), *DAPK*

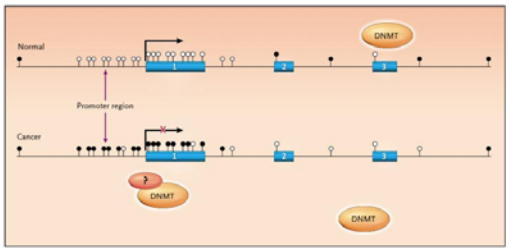
Slide from G. Netto MD

DNA methylation occurs at CpG dinucleotides in mammalian genomes

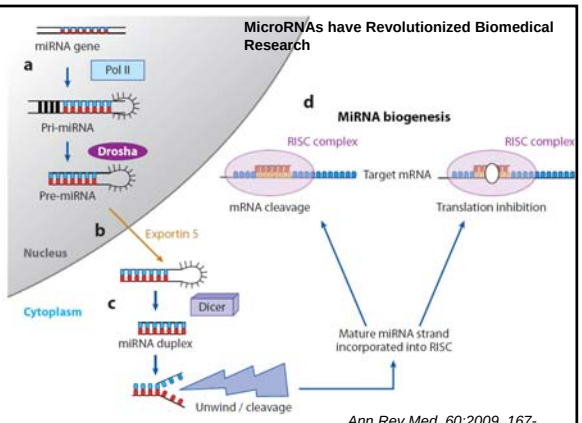


Slide from S. Yegnasubramanian

Proposed methylation patterns in normal and cancer genomes



Herman and Baylin, NEJM, 2003



Ann Rev Med, 60:2009, 167-

Research Article

The Putative Tumor Suppressor microRNA-101 Modulates the Cancer Epigenome by Repressing the Polycomb Group Protein EZH2

Jeffrey M. Friedman,[1] Gangning Liang,[1] Chun-Chi Liu,[2] Erika M. Wolff,[1] Yvonne C. Tsai,[1] Wei Ye,[1] Xianghong Zhou,[2] and Peter A. Jones[1]

[1]Department of Urology, Biochemistry and Molecular Biology, Norris Comprehensive Cancer Center, Keck School of Medicine, and [2]Department of Molecular and Computational Biology, University of Southern California, Los Angeles, California

Abstract

The Polycomb Repressive Complex 2 (PRC2) mediates epigenetic gene silencing by trimethylating histone H3 lysine 27 (H3K27me3) and is known to aberrantly silence tumor suppressor genes in cancer. EZH2, the catalytic subunit of PRC2, enhances tumorigenesis and is commonly overexpressed in several types of cancer. Our microRNA profiling of bladder transitional cell carcinoma (TCC) patient samples

illustrates a strong association between the function of PRC2 in cancer and stem cells, which represent dedifferentiated and proliferative cell states. Therefore, EZH2 overexpression might cause a normal cell to dedifferentiate back to a stem cell–like state by epigenetically repressing cell fate–regulating genes and tumor-suppressor genes leading to tumor development (1, 9, 11).

MicroRNAs (miRNA) are ~22 nucleotide noncoding RNA molecules that usually function as endogenous repressors of

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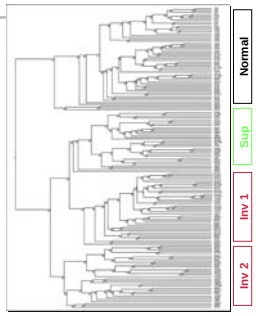
Defining Molecular Profiles of Poor Outcome in Patients With Invasive Bladder Cancer Using Oligonucleotide Microarrays

Marta Sanchez-Carbayo, Nicholas D. Socci, Juanjo Lozano, Fabien Saint, and Carlos Cordon-Cardo

Sanchez-Carbayo et al JCO 2006

- 106 pts, (33 superficial,72 muscle inv UrCa & 52 normals)
- Affymetrix arrays
- Hierarchical clustering classified normal vs Sup vs inv with 89% accuracy
- 82% accurate prediction of OS (90% in invasive)
- 174 probes marked LN positive and poor survival



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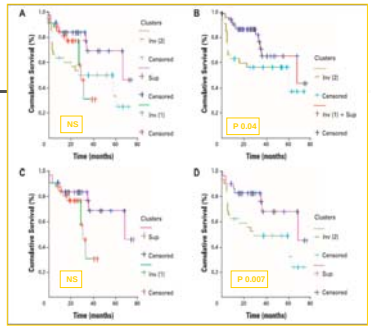


Sanchez-Carbayo et al JCO 2006

"The Cancer Biomarker Problem"

"Genomic technologies .. are being used to characterize tumors at the molecular level, and several clinical successes have shown that such information can guide the design of drugs targeted to a relevant molecule.."

Nature. 2008 Apr 3;452(7187):548-52. CL Sawyers

Biological Markers in Clinical Settings

- "there is an urgent need to develop and consistently apply more systematic and effective approaches to the development of cancer biomarkers"

Translational Research Working Group: Clinical Cancer Research 2008; 14 (18) September 15, 2008

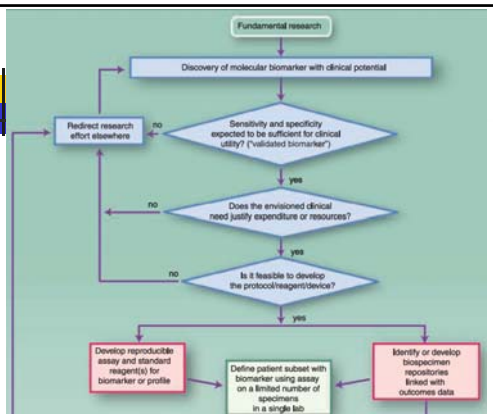
Steps in Biomarker Development

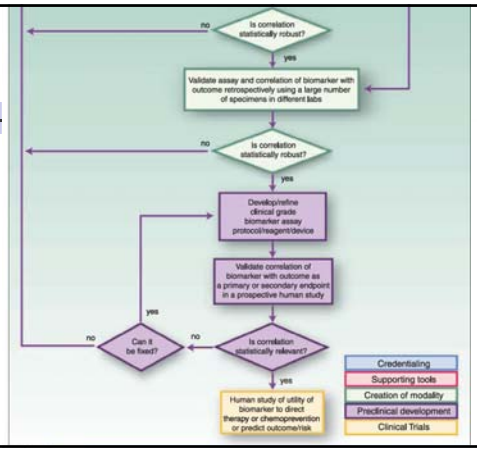
- Translational Research Working Group
  - Posed a developmental pathway that lays out a biomarker translational research process describing it in terms of key activities and decision points along the path from concept of assay development to clinical testing

Translational Research Working Group Developmental Pathway for Biospecimen-Based Assessment Modalities

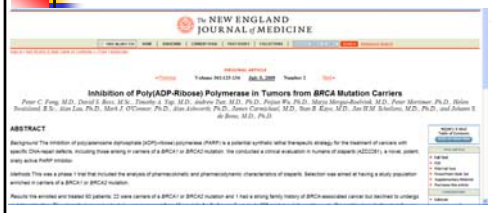
Sudhir Srivastava,[1] Joe W. Gray,[2] Brian J. Reid,[3] Oren Grad,[4] Addison Greenwood,[5] and Ernest T. Hawk[6] for the Translational Research Working Group

Clin Cancer Res 2008;14(18) September 15, 2008





New Approaches to Rx: Concept of Synthetic Lethal

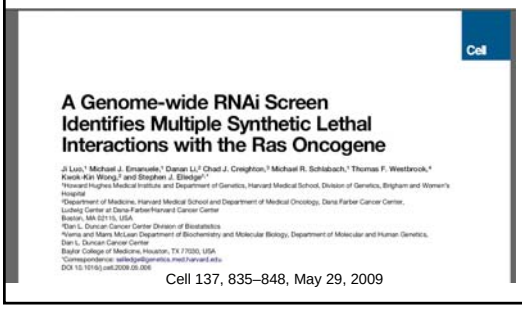


PARP facilitates DNA repair by binding to DNA breaks and attracting DNA repair proteins to the site of damage.

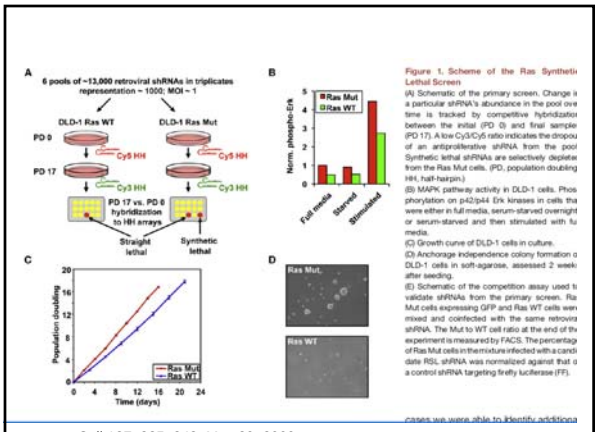
PARP1 activity is essential in homologous recombination-deficient BRCA1/BRCA2 mutant cells.

Inhibiting PARP in BRCA1/2 deficient cells is lethel => SYNTHETIC LETHAL

Synthetic Lethal Screens will Lead to Many New Drug Targets that Will Need to be Measured!



Cell 137, 835–848, May 29, 2009



Cell 137, 835–848, May 29, 2009

Cancer cells with mutant Ras experience elevated mitotic stress and are more dependent on key mitotic proteins such as PLK1, the APC/C complex, the COP9 signalosome, and the proteasome for proper mitotic progression

Cell 137, 835–848, May 29, 2009

Trends in DNA sequencing will change the face of cancer research and medicine

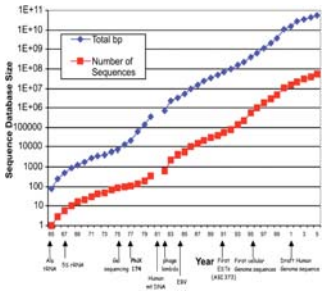
Service RF, Science, 2006

2007: 10^{-4}/bp

2008: 10^{-5}/bp

2009: 10^{-6}/bp

… still going …



Hutchison CA, Nucl Acids Res, 2007

Next Generation Sequencing technologies harness innovations in imaging, fluidics, molecular biology and engineering to allow sequencing of millions of targets simultaneously...

Slide from S. Yegnasubramanian

Next Generation Sequencing opens doors to take on questions at extraordinary scales...

The diploid genome sequence of an Asian individual

Nature, 2008

Dynamic Regulation of Nucleosome Positioning in the Human Genome

Cell, 2008

Shotgun bisulphite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning

Nature, 2008

The Antisense Transcriptomes of Human Cells

Science, 2008

Stem cell transcriptome profiling via massive-scale mRNA sequencing

Nat Methods, 2008

Paired-End Mapping Reveals Extensive Structural Variation in the Human Genome

Science, 2007

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Multiple currently commercialized platforms...

SOLiD System
Applied Biosystems

Genome Analyzer
From Illumina

Genome Sequencer FLX System
From 454 LifeSciences/Roche

Heliscope
From Helicos Biosciences

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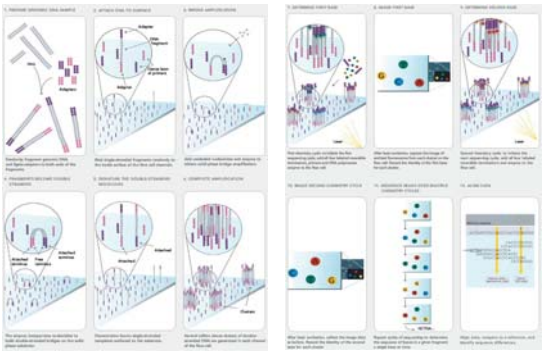
Illumina Solexa Platform



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Thank You!