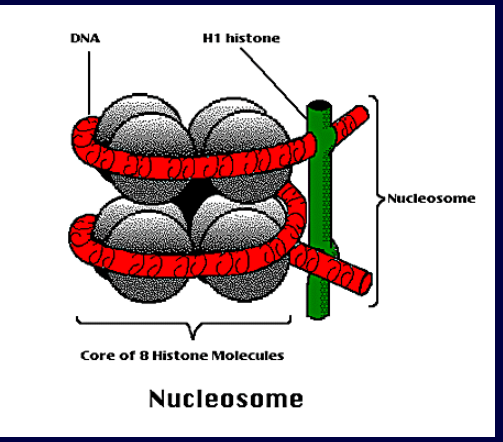
Opportunities in Epigenetic

NATIONAL CANCER INSTITUTE

Division of Cancer Control & Population Sciences

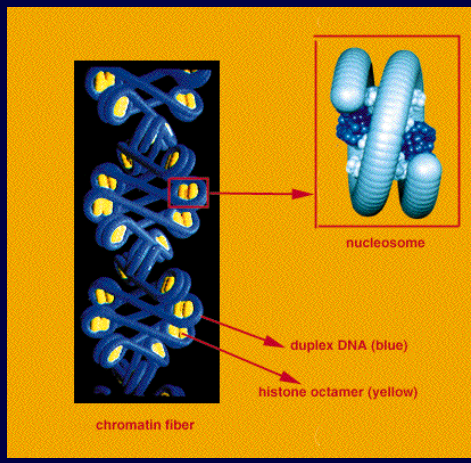


Mukesh Verma, PhD

Program Director

Analytic Epidemiology Research Branch

Epidemiology and Genetics Research Program



Chicago, September 20, 2004

Goal

To stimulate population based research on application of epigenetic markers in cancer epidemiology

Markers in Epidemiology Research

Currently Used:
Genetic markers
Biochemical markers

Unexplored:
Epigenetic markers
Proteomic markers

In epidemiology, biomarkers are used to follow disease prevalence by determining their level in cohort studies with potential of identifying the high risk population

Characteristics of Population Studies

In population based studies, questions of

- reliability,
- sensitivity,
- specificity,
- reproducibility, and
- scalable capacity for automation

are central in selecting assays to determine factors associated with carcinogenesis.

Epigenetics: Background

Epigenetics is the study of
- mitotically heritable changes
- not caused by DNA sequence alterations

Epigenetic controls
- essential for normal development
- misdirected in cancer cells

Background

Misdirected epigenetic controls can cause
- silencing of tumor suppressor genes
- activation of oncogenes

Epigenetic events vs. genetic events
- higher frequency
- can be reversed

Epigenetic Targets in Cancer Detection and Risk Assessment

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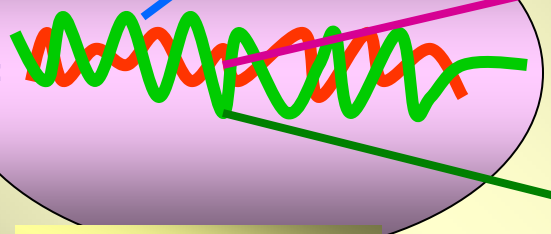
ces

Epigenetics regulates:
- Cell Cycle Control
- DNA Damage
- Apoptosis
- Invasion
- X-Chromosome Inactivation
- Imprinting
- Aging

Factors

Environmental
Hormonal
Genetic



Deacetylation Inhibitor:
Trichostatin
Methylation Inhibitors:
5-aza-2' deoxycytidine
Zebularine

DNA Methyl-transferases
DNMT1
DNMT3A
DNMT3B

Methionine Supplement:
S-Adenosylmethione

Hypermethylation

APC
BRCA1
ER
hMLH1
GSTP1
TIMP3
DAPK1

Hypomethylation

Raf
C-myc
C-fos
C-Ha-ras
C-K-ras

Acetylation/Deacetylation

Histone Acetyltransferase
Histone Decetylase
(Dynamic Equilibrium)

Verma and Srivastava (2003) Lancet Oncology. 3, 755-763

MV

Epigenetics and Gene Regulation

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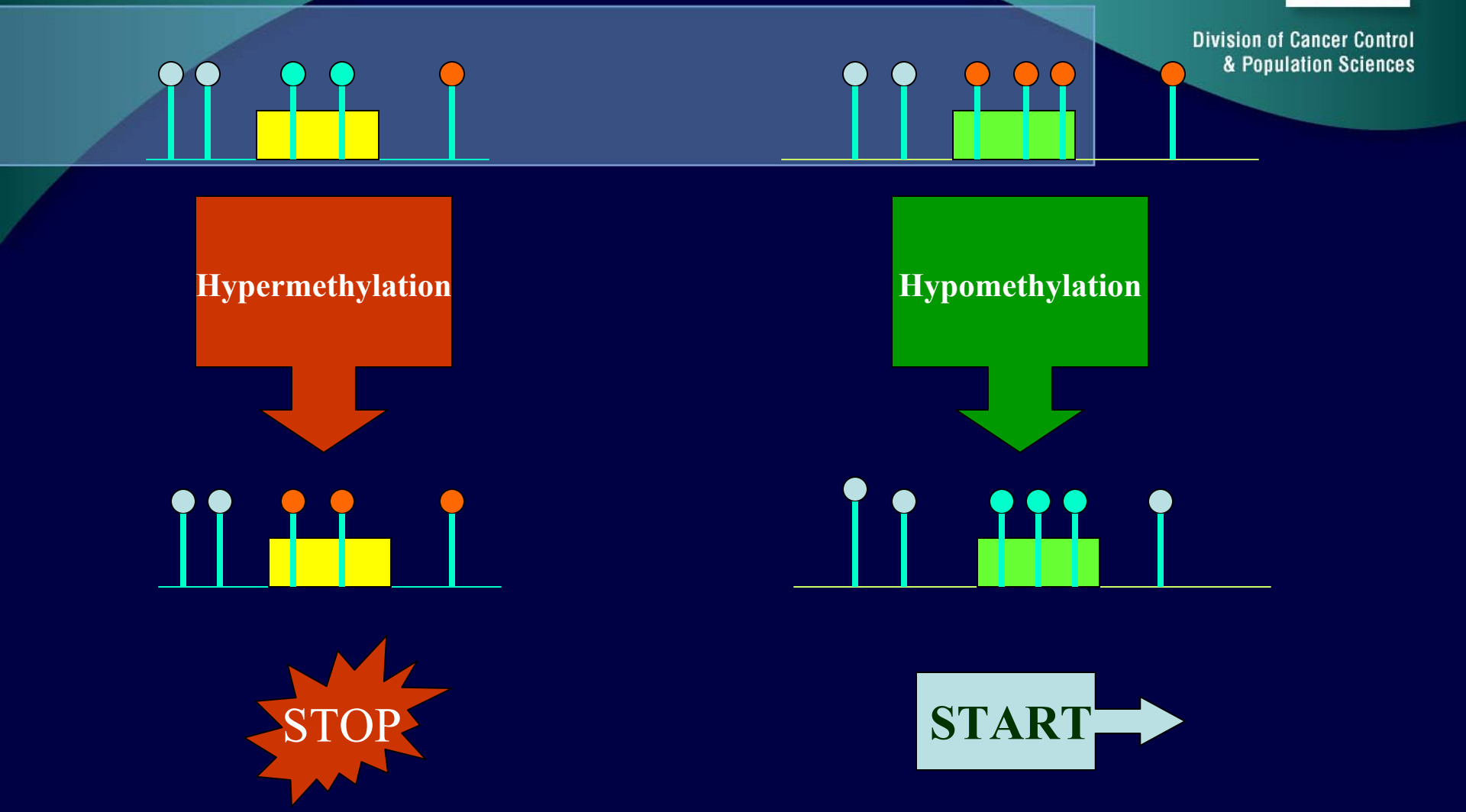
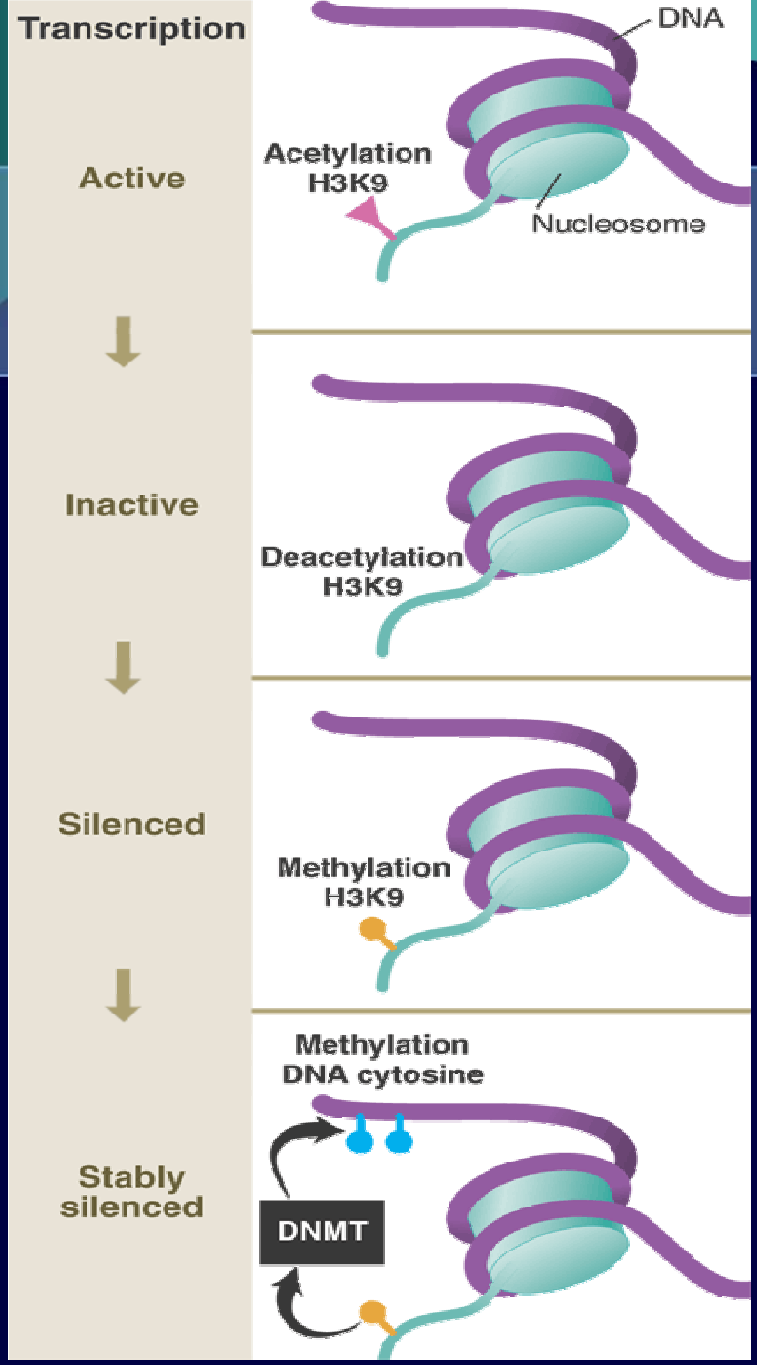
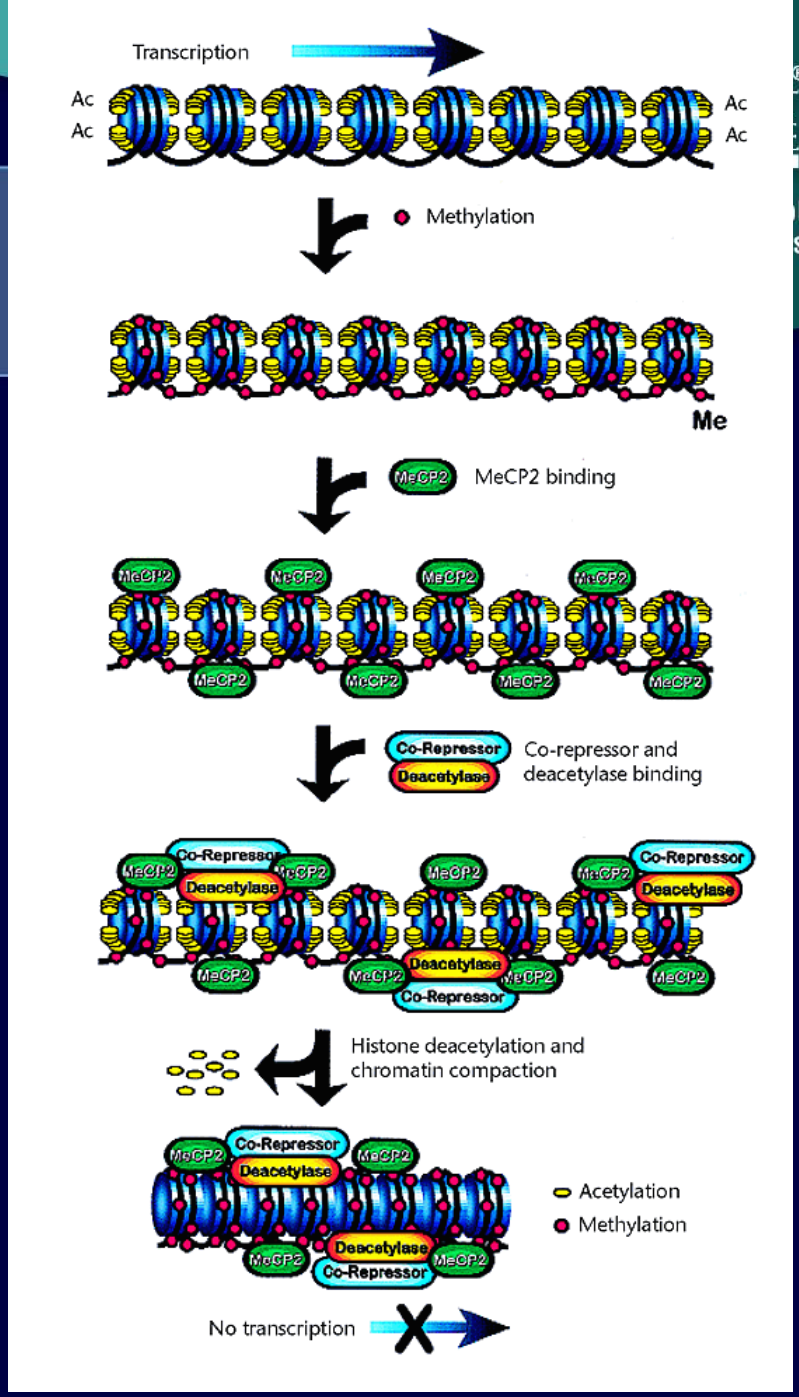


Silencing of Tumor Suppressor Gene

Activation of Proto-oncogene

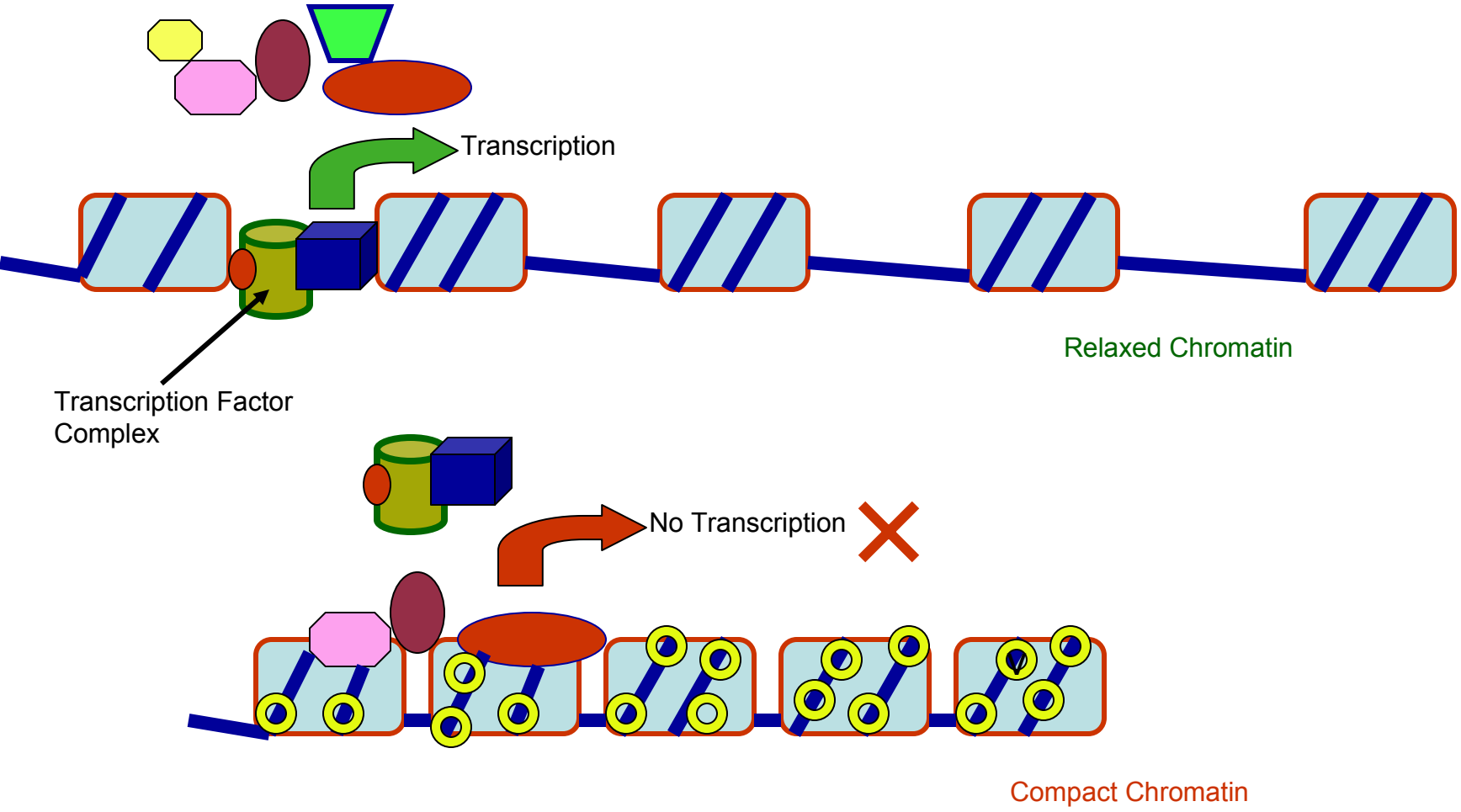
Verma and Srivastava (2003) Lancet Oncology. 3, 755-763





Jones and Laird (1999) Nat Rev Gen 21: 163

Inhibitors of DNA Methyltransferase (5-AZA-C) and Histone Deacetylase (TSA) can Restore Gene Activation



Verma and Srivastava (2003)
Lancet Oncology. 3, 755-763.

Epigenetics in Epidemiology

Molecular Targets

Tumor suppressor Genes

APC, p15, p16, p73, ARF/INK4A, VHL, ER, RARbeta, AR, HIC1, Rb

Invasive/Metastasis suppressor Genes

E-cadherin, TIMP-3, mts-1, CD-44

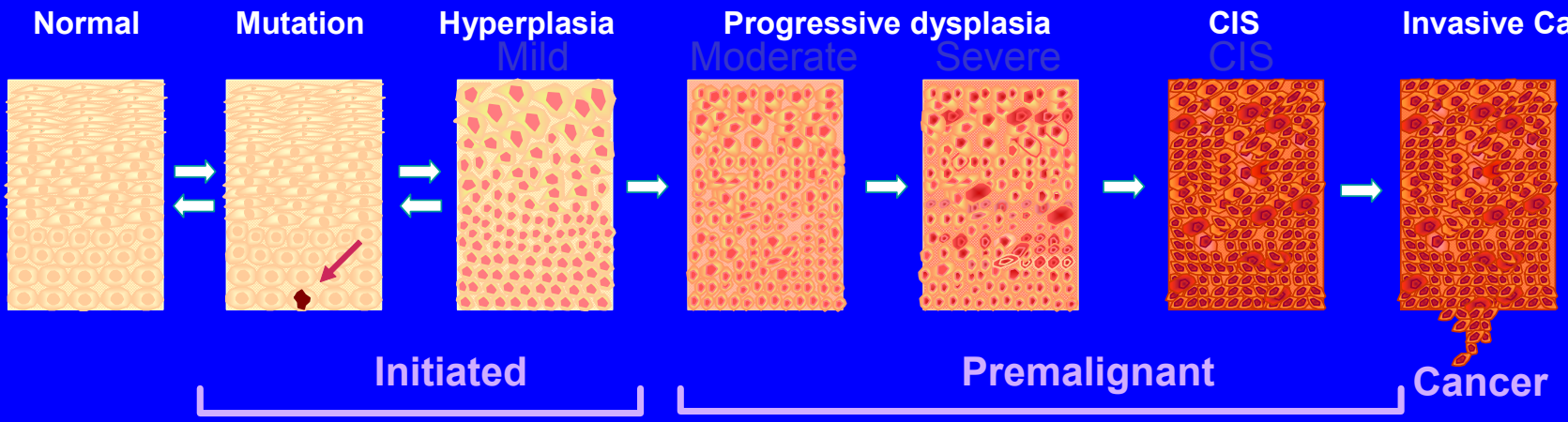
DNA Repair Genes

Methylguanine methyl transferase, hMLH1, BRCA1, GST

Angiogenesis

Thrombospondin-1 (TSP-1), TIMP-3

Cancer Development is Lengthy



Breast	14-18 yrs	6-10 yrs
Cervix	9-13 yrs	10-20 yrs
Colon	5-20 yrs	5-15 yrs
Prostate	20 yrs	3-15 yrs
Lung	? yrs	Many yrs

Epigenetic Markers in Lung Cancer

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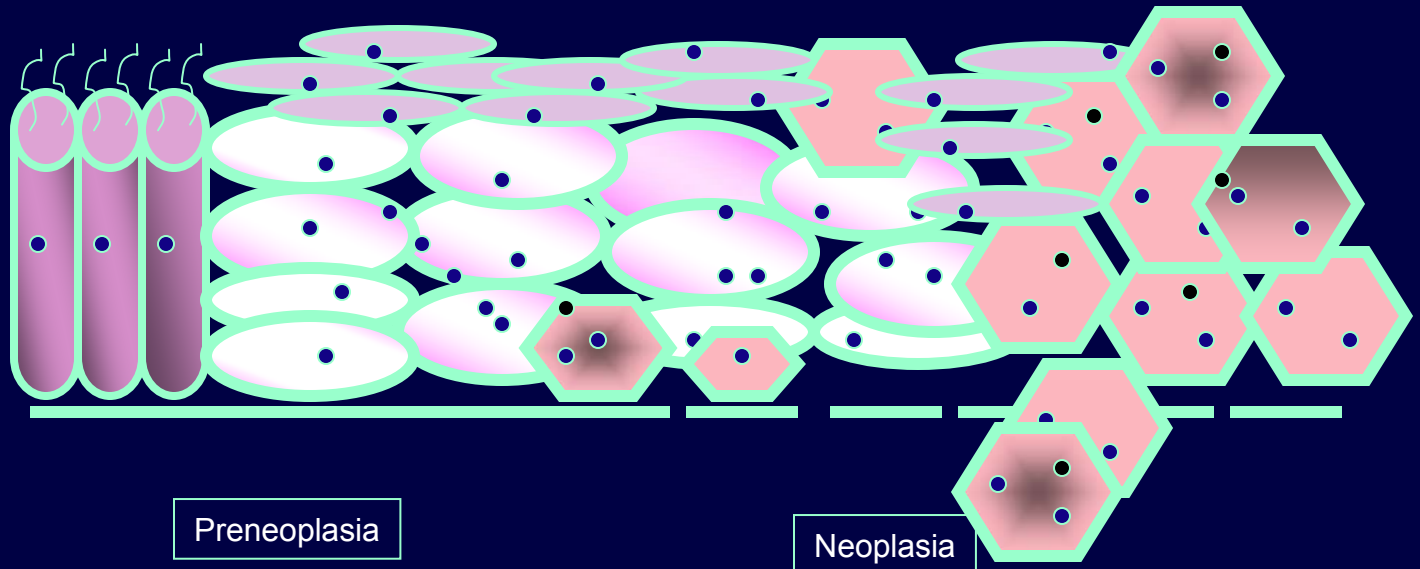
Annexin I and II

PIK3CA

p63

Methylation of RARbeta, DAPK, GSTP1, FHIT RASSF1, MGMT

Markers located on 3q



Preneoplasia

Neoplasia

Progression of Lung Cancer

Potential Epidemiological Markers in Colon Cancer

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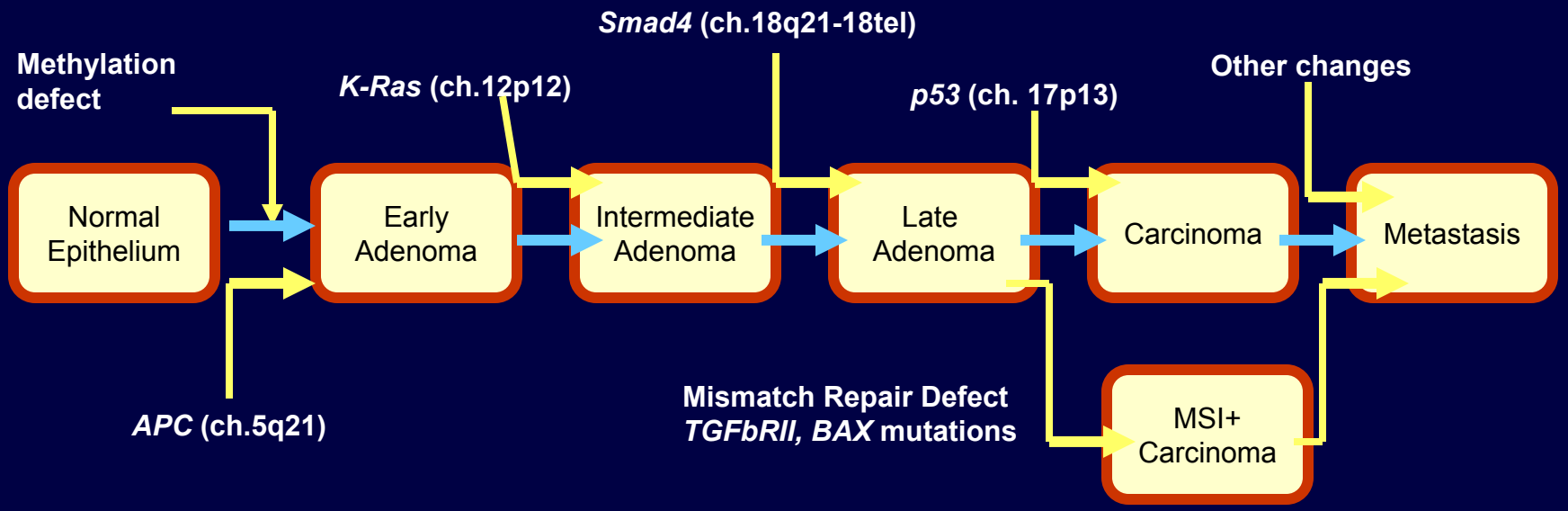

A Model for Colorectal Tumorigenesis

Modified from Jubb et al 2001. J Path. 195: 111.

Pancreatic Cancer: Methylation of p14ARF and p16INK4a

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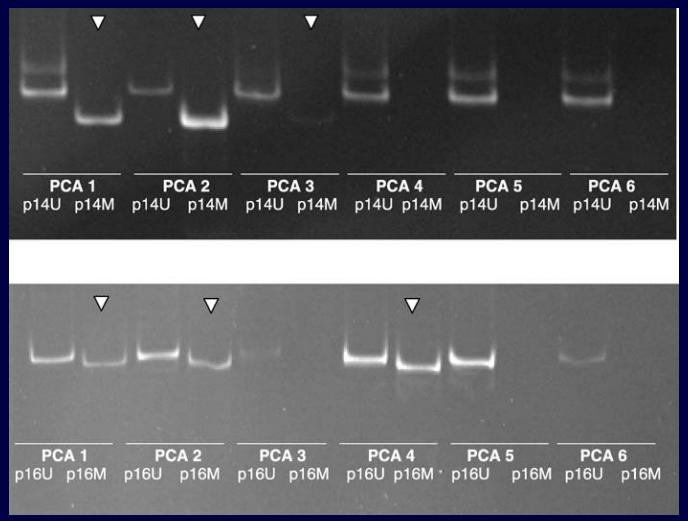
Pancreatic Carcinoma (PCA) : 39	**19/39**	**p16INK4a**
Chronic Pancreatitis (CP) : 16	**0/16**	**p16INK4a**
Normal Pancreatogram (NAD) : 6	**0/6**	**p16INK4a**



Sample: Pancreatic Fluid

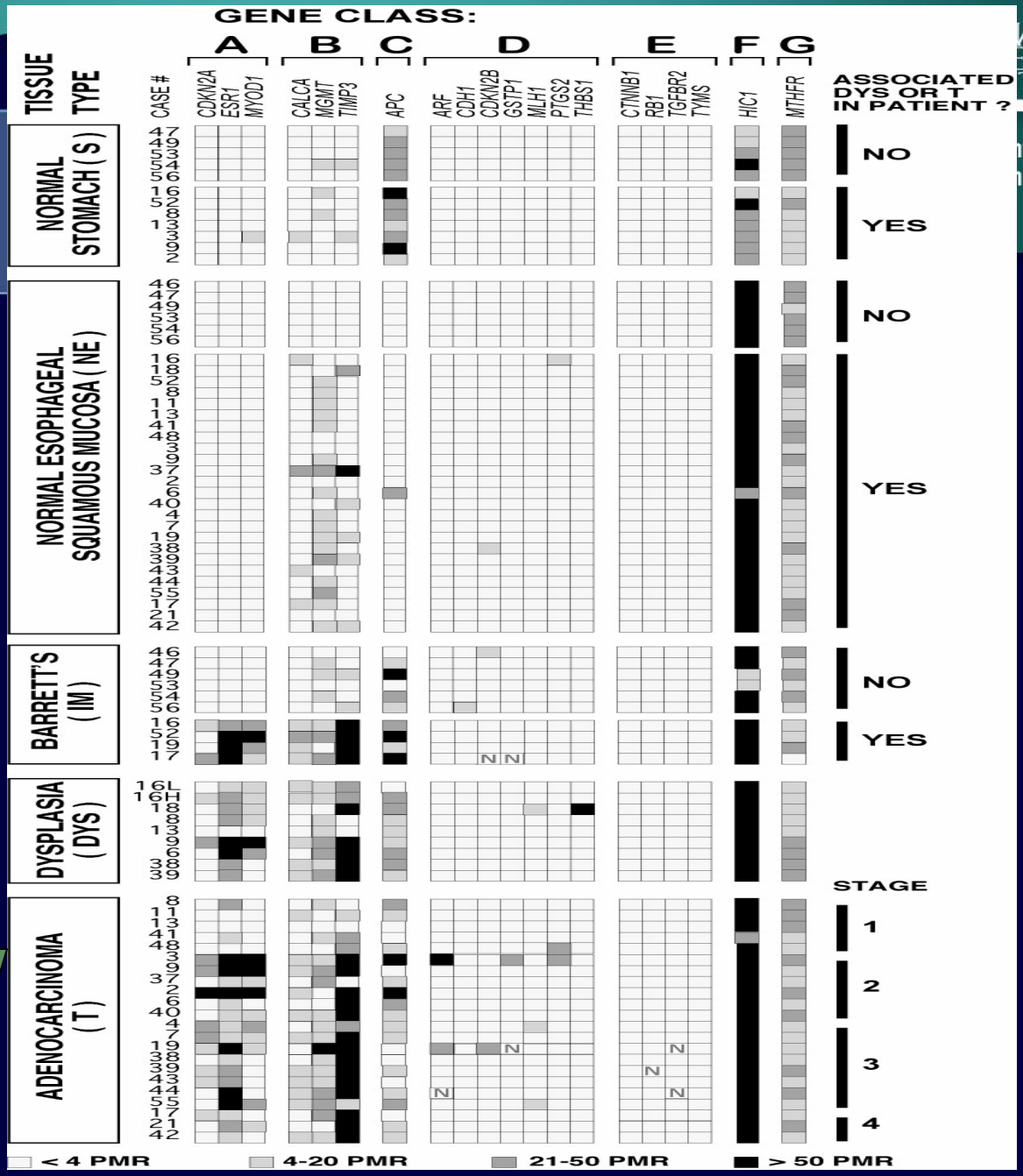
(Klump et al. Mol Cell Path 88: 217, 2003)

Epigenetic Patterns in the Progression of Esophageal Adenocarcinoma

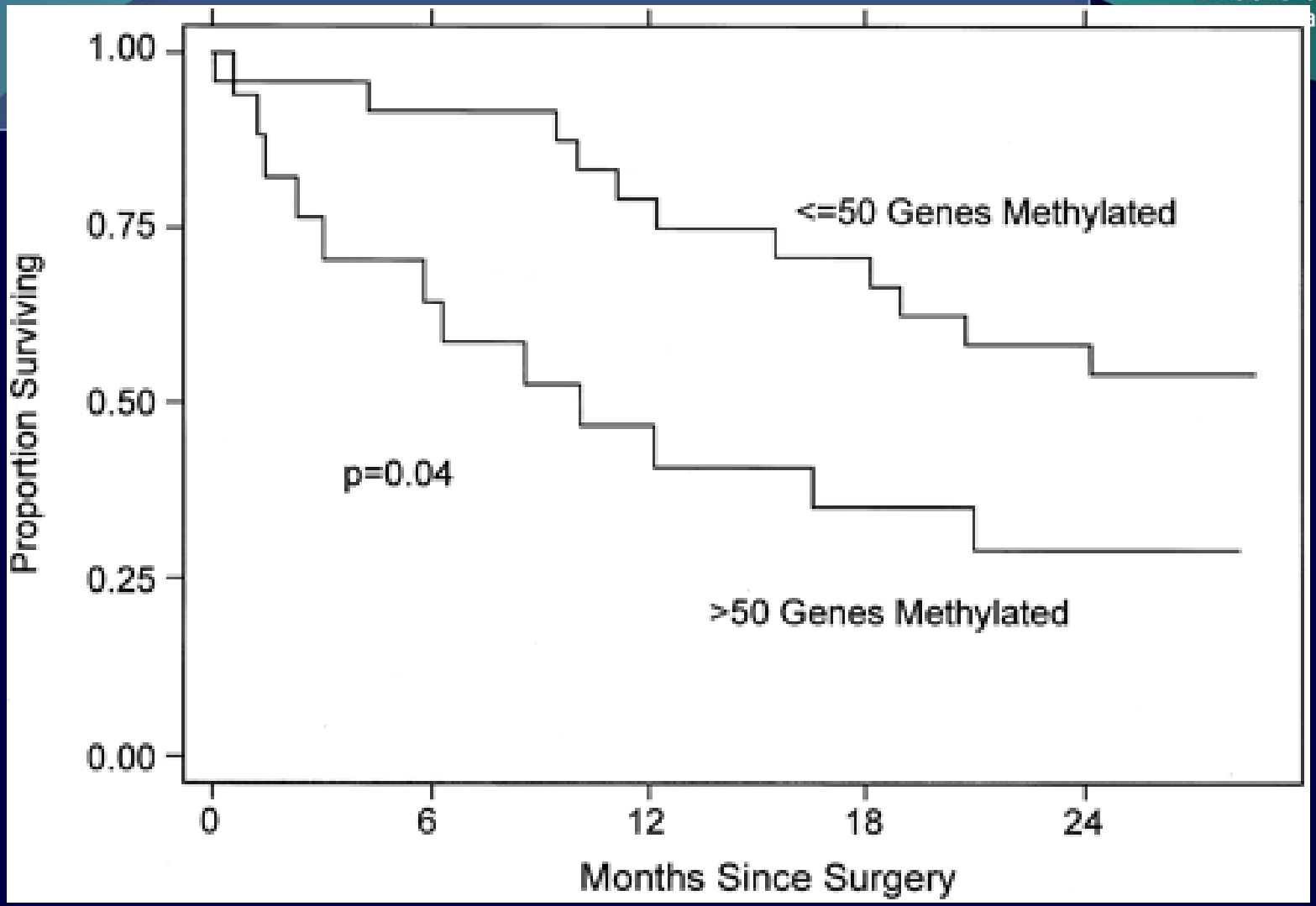
Cancer Research

61:3410

Cancer Progression



Esophageal Cancer: Probability of Survival



Brock et al. Clinical Cancer Research. 9: 2912 (2003)

Esophageal Cancer and Methylation

PATIENT #	TUMOR SAMPLES							NORMAL TISSUE						
	p16	MGMT	DAP-K	TIMP-3	E-CAD	ER	APC	p16	MGMT	DAP-K	TIMP-3	E-CAD	ER	APC
1	U	U	M	M	M	M	M	U	M	U	U	U	M	U
2	M	U	M	U	M	M	M	U	M	U	U	U	M	U
3	M	M	U	U	M	M	M	M	M	U	U	U	M	M
4	U	U	U	U	U	U	M	M	U	U	U	U	U	U
5	U	M	U	U	U	U	U	U	U	U	U	U	U	U
6	M	U	U	M	M	M	M	U	U	U	U	U	U	U
7	U	M	U	U	M	U	M	U	U	U	U	U	U	U
8	U	U	M	U	M	U	M	U	U	U	U	U	U	U
9	M	M	U	U	M	M	M	M	M	U	U	M	U	U
10	U	U	U	U	U	U	U	U	U	U	U	U	U	U
11	M	M	M	U	M	M	M	M	M	U	U	U	U	U
12	U	U	M	M	M	M	U	M	U	U	U	M	M	U
13	M	M	U	U	M	M	M	U	M	U	U	M	U	U
14	U	M	U	U	M	U	U	U	M	U	U	U	U	U
15	U	M	U	U	M	U	M	U	U	U	U	U	U	U
16	U	M	M	U	M	U	U	M	U	M	U	M	U	U
17	U	U	U	U	M	M	M	M	U	U	U	U	U	U
18	U	U	U	U	M	M	M	M	U	U	U	U	U	U
19	M	M	U	M	U	U	M	U	U	U	U	U	U	U
20	M	M	U	U	M	M	M	M	U	U	U	U	U	U
21	U	U	U	U	U	U	M	U	U	U	U	U	U	U
22	U	M	U	M	U	U	M	U	U	U	U	U	U	M
23	U	U	U	U	M	U	U	U	U	U	U	U	U	U
24	M	M	U	M	U	M	M	U	M	U	U	M	M	U
25	M	M	U	U	M	M	M	U	U	U	U	U	U	U
26	U	U	U	U	M	U	U	U	U	U	U	U	U	U
27	U	U	U	U	U	U	M	U	U	U	U	U	U	U
28	U	M	M	U	U	U	U	U	U	U	U	U	U	U
29	U	M	M	U	M	M	M	U	U	U	U	U	U	U
30	M	M	U	U	M	U	U	U	U	U	U	U	U	U
31	M	M	U	M	M	M	M	M	M	U	U	U	U	U
32	U	U	U	M	U	M	M	U	M	U	U	U	U	U
33	U	M	U	U	U	M	M	U	U	U	U	U	U	U
34	M	M	U	U	M	M	M	U	U	U	U	U	U	U
35	M	M	U	U	U	M	M	U	U	U	U	U	U	M
36	U	M	U	U	U	U	U	U	U	U	U	U	U	U
37	U	U	U	U	M	M	M	U	U	U	U	U	U	U
38	M	M	U	U	M	U	U	U	U	U	U	U	U	U
39	U	U	U	U	M	U	U	U	U	U	U	U	U	U
40	M	M	U	U	M	M	M	U	U	U	U	U	U	U
41	U	U	U	U	U	U	U	U	U	M	U	U	U	U

Brock et al. Clinical Cancer Research. 9: 2912 (2003)

Bladder Cancer: Methylation of LAMC2 in Exfoliated Cells

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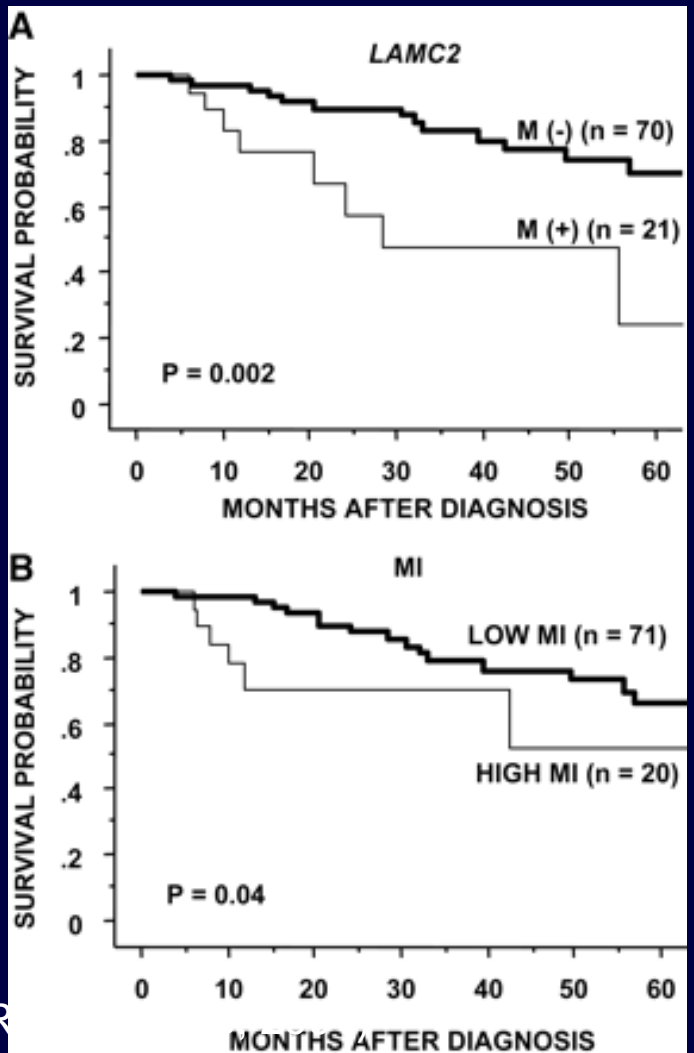


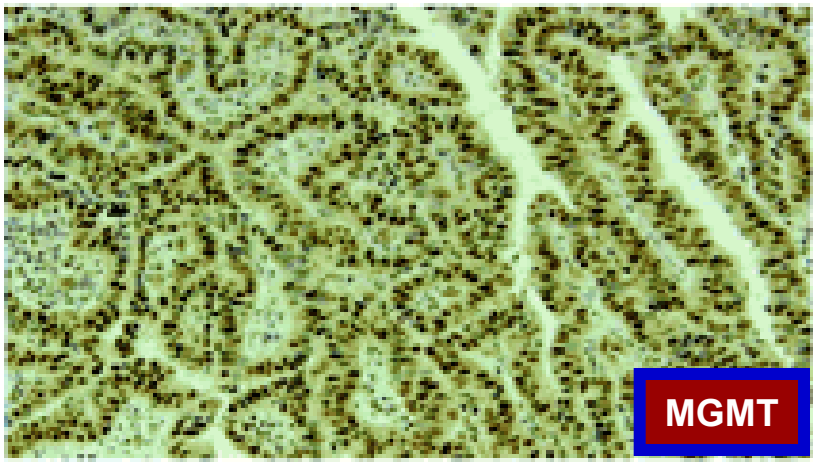
Sample: Urine

(Sathyanarayana et al. Can R

Esophageal Cancer: Immuno-histochemistry

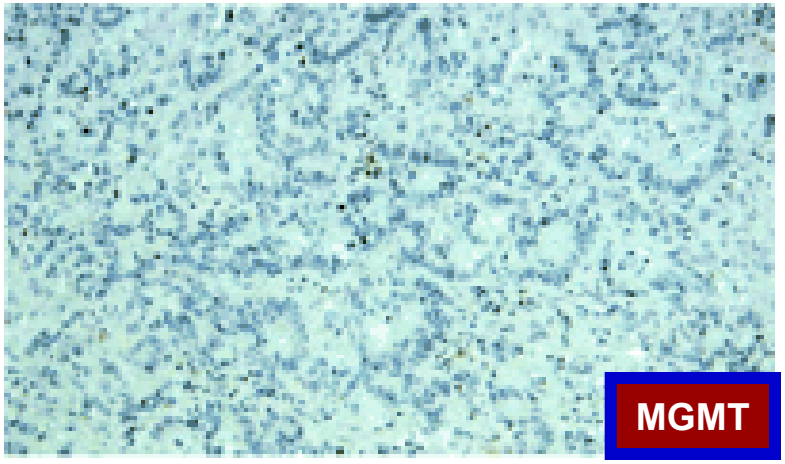
Unmethylated

A

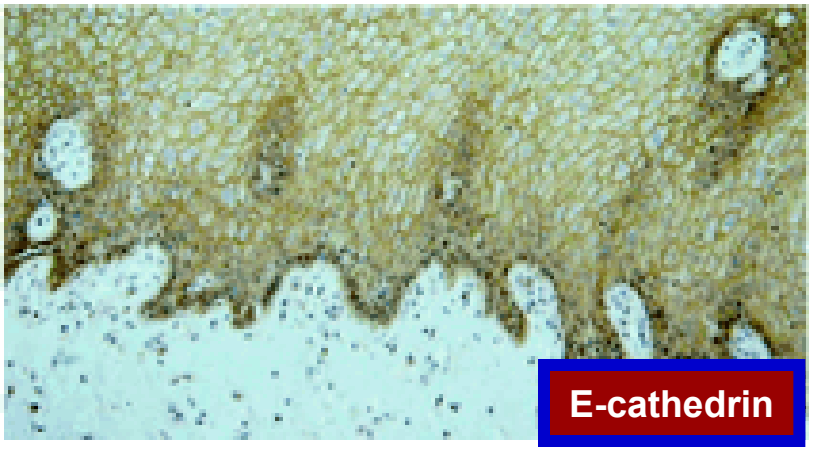


Methylated

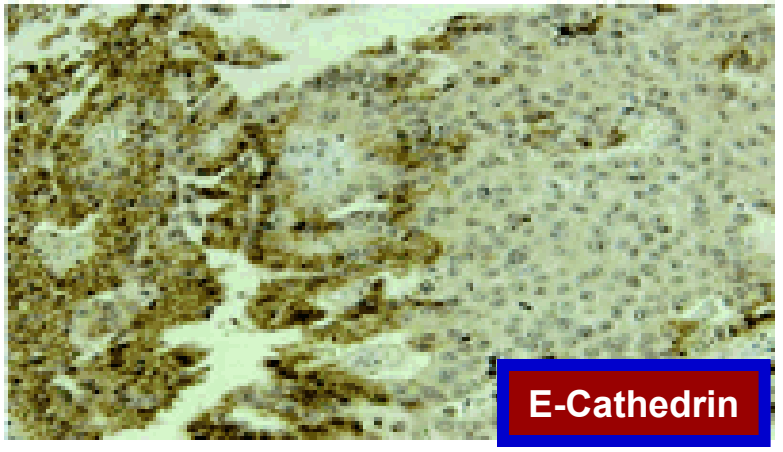
B



C

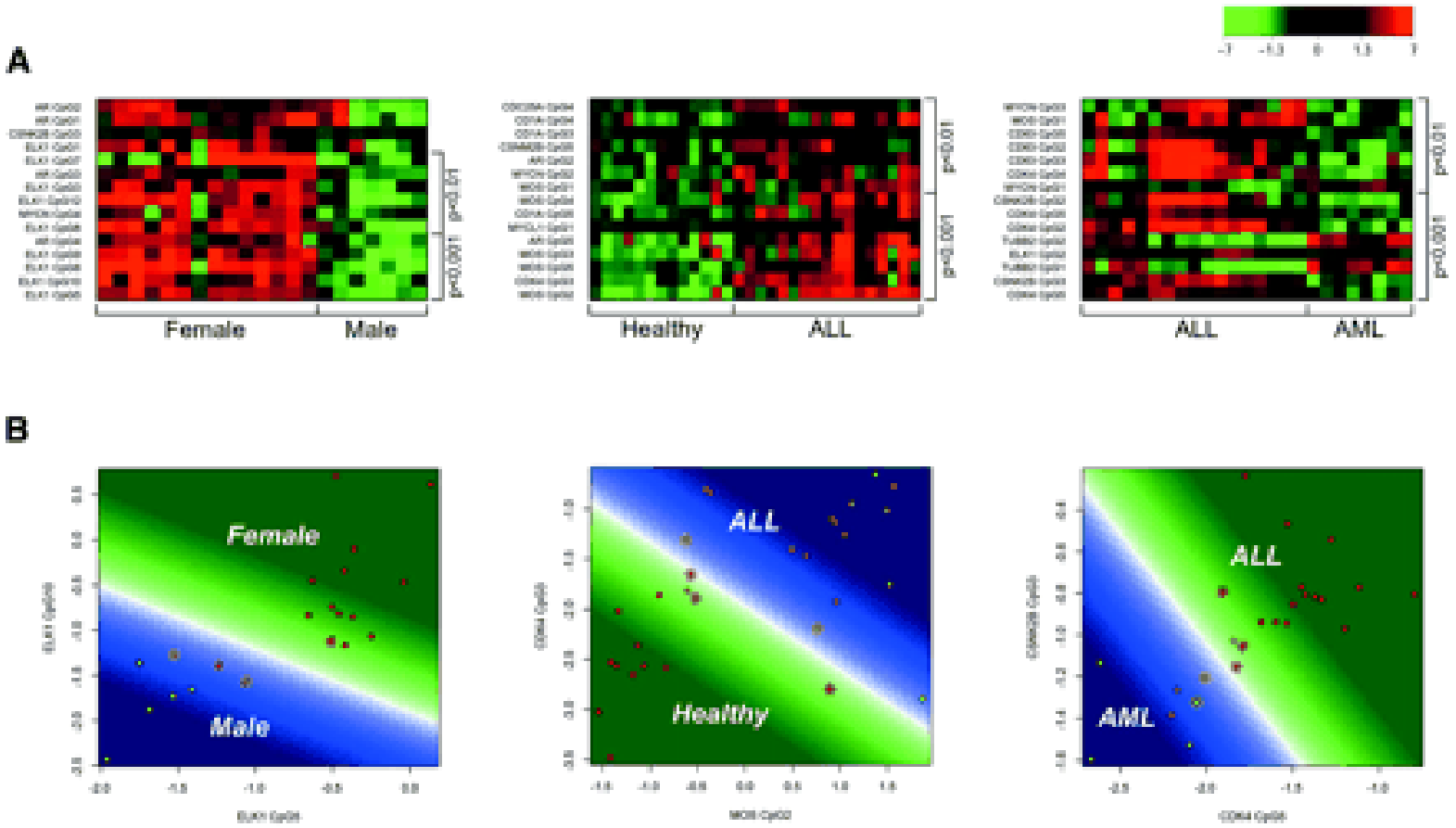


D



Brock et al. Clinical Cancer Research. 9: 2912 (2003)

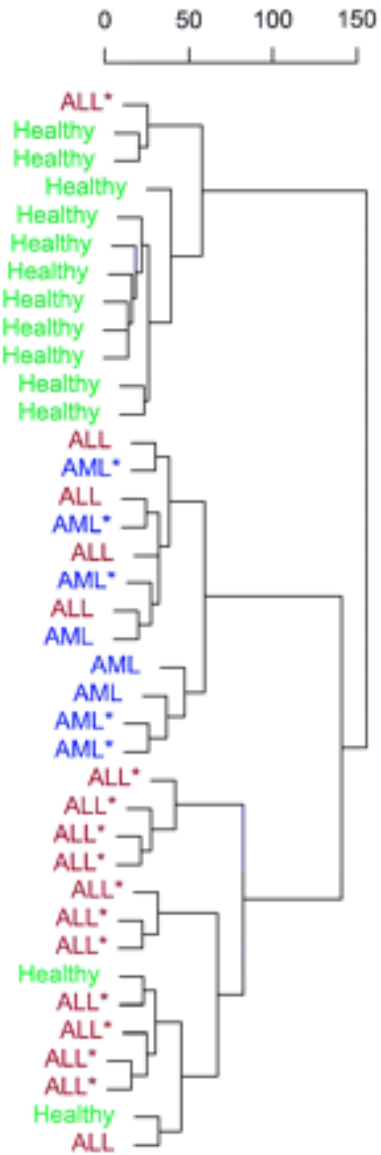
Tumor Class Prediction by Methylation
AML and ALL



AML:Acute Myeloid Leukemia
ALL: Acute Lymphoblastic Leukemia

Adorjan et al (2002) Nuc Ac Res. 30: e21

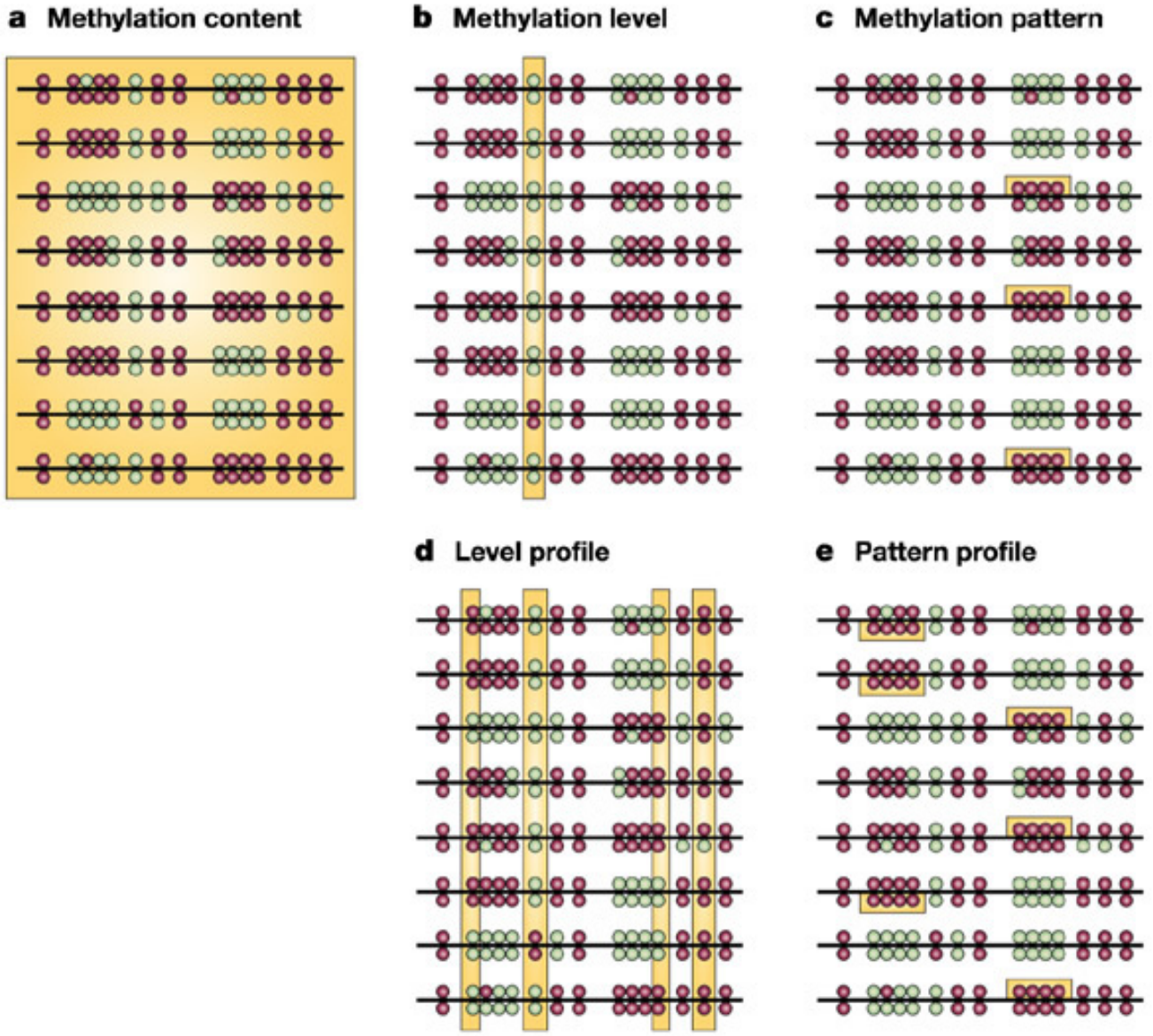
Tumor Class Prediction by Methylation
AML and ALL



AML:Acute Myeloid Leukemia
ALL: Acute Lymphoblastic Leukemia

Adorjan et al (2002) Nuc Ac Res. 30: e21

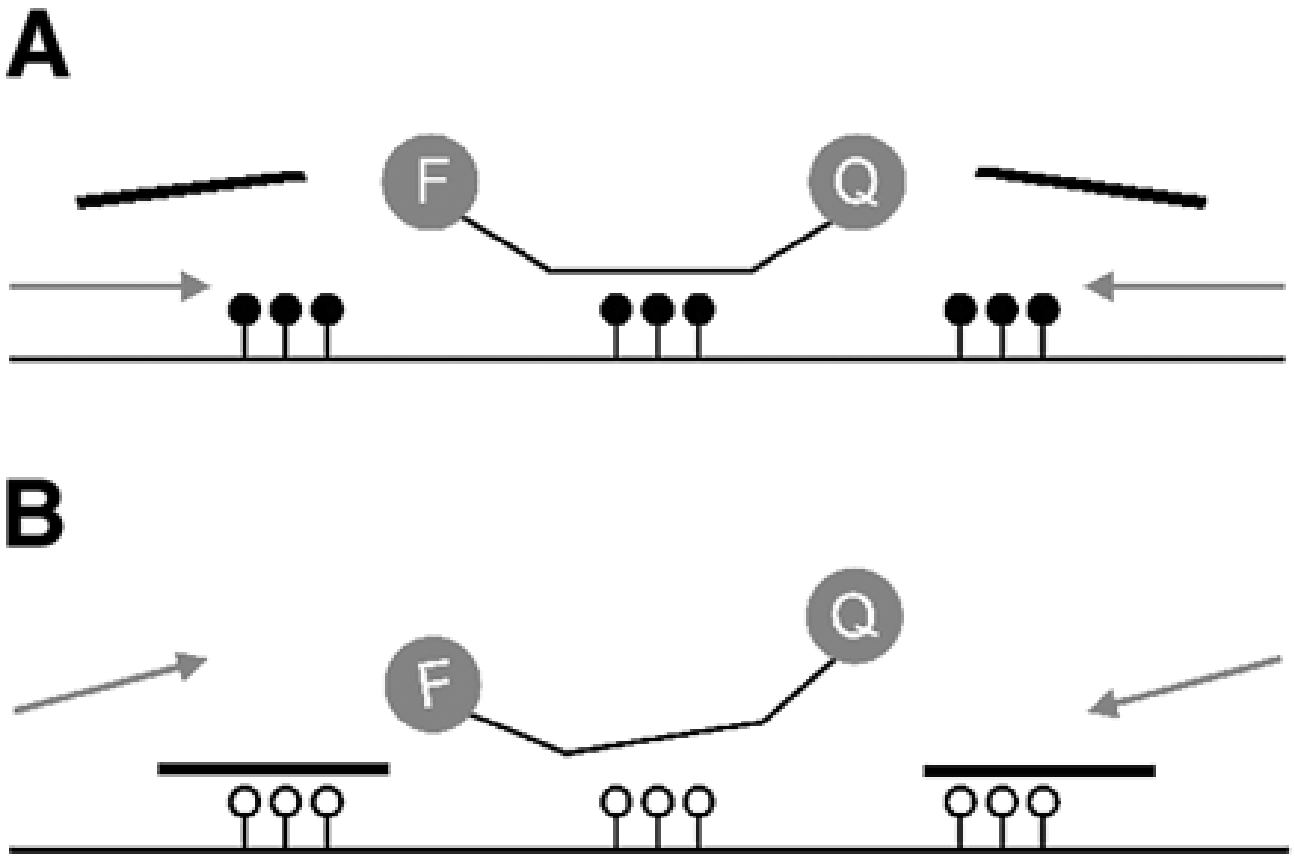
Principle of Methylation



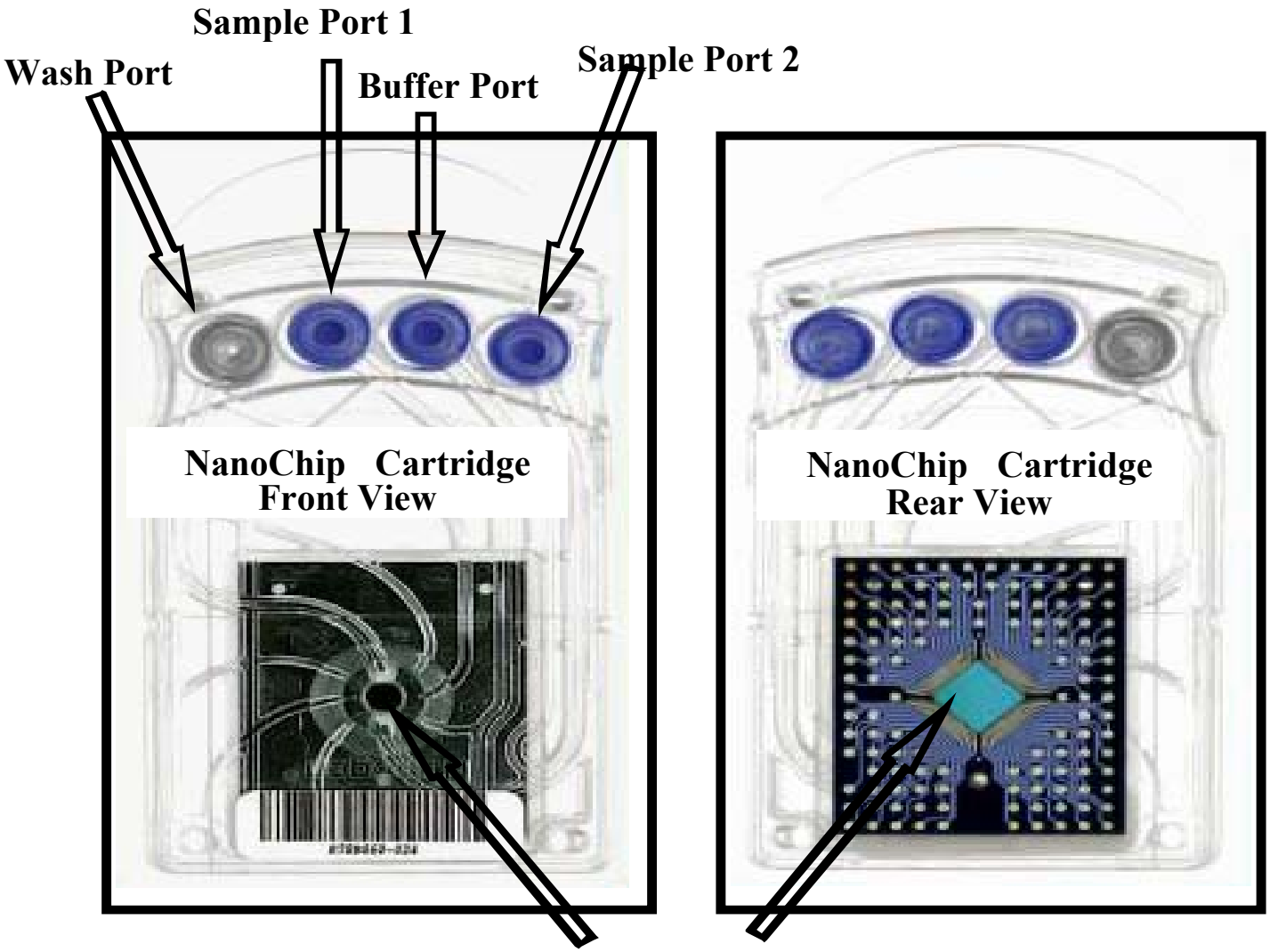
Laird (2003) Nat Rev Cancer 3:253

Circulating DNA to Detect Methylation

Real time PCR HeavyMethyl Method (Cottrell et al NAR 32: e10, 2004)



Nanochip for Methylation



Why Epigenetic Markers

- Multiple markers are better than single marker
- Complementary to biochemical and genetic markers
- Epigenetic events occur early in cancer development
- Easy to assay in small sample size (MS-PCR based assay)
- Source of markers: biofluids, exfoliated cells
- Automation possible (nanochips)

National Cancer Institute

Division of Cancer Control & Population Sciences

Funding Opportunities

R03 (Small Grant): PAR-03-010

R21 and R33 (IMAT Program)

R01

R41/42 and R43/44

Epigenetics Interest Group

SIGS: Epigenetics Interest Group - Microsoft Internet Explorer

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Address http://tango01.cit.nih.gov/sig/home.taf?_function=list&SIGInfo_SIGID=134&_UserReference=FB80AD74F29CEE52412BB485

Go Links

Epigenetics Interest Group

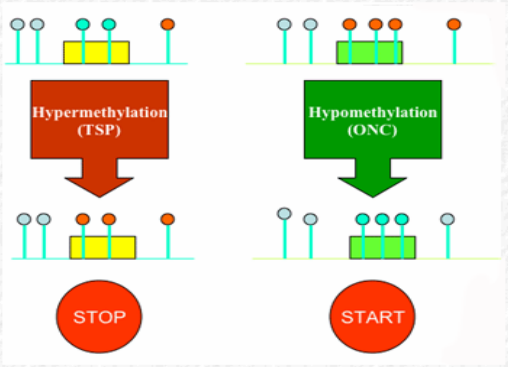
Mtgs/Seminars | Members | Join the SIG | Special Items | Archives | Related Links

CONTENTS

- Meetings & Seminars
  Schedule of upcoming meetings & seminars
- Members
  A listing of current members
- Join the Group
  Register as a member, join the mailing list
- Special Items
  No items listed at this time
- Archives
  Listserv archives, past meetings & seminars
- Related Links
  Other related sites of interest

EPGN

Epigenetics represents a new frontier in cancer research. Information in the genome exists in at least two forms, genetic and epigenetic. The genetic information provides the blue print for the manufacture of all the proteins necessary to create a living organism, whereas the epigenetic information provides additional instructions on how, where, and when the genetic information will be used. The focus of the Epigenetics Interest group is on applications of epigenetic approaches in disease detection, risk assessment and treatment. The group meets on last Thursday of every month from 3- 4 pm.



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[Main SIGs Page]

http://www.nih.gov/sigs/epgn

Internet

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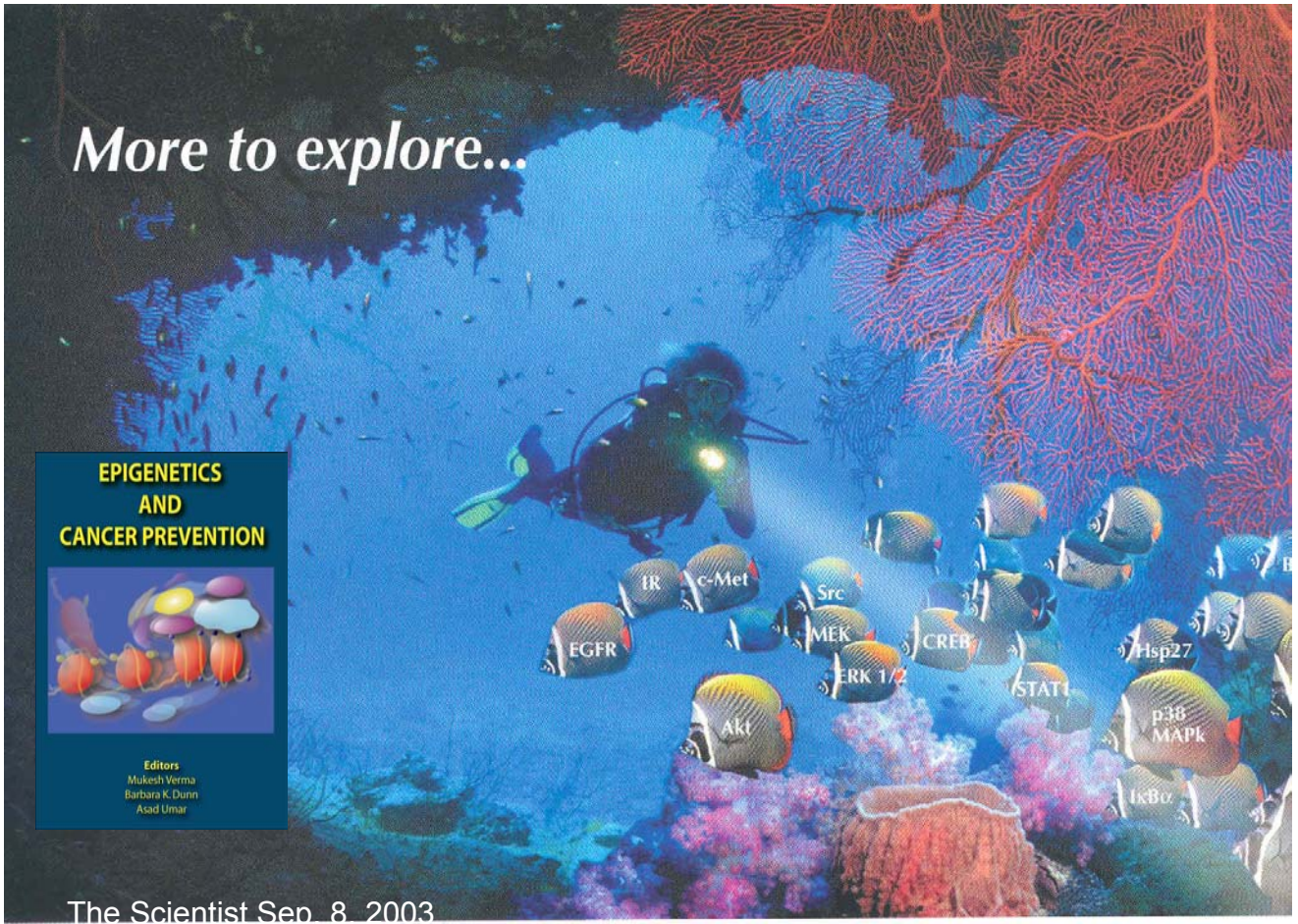
SUMMARY

Epigenetic markers can be used for cancer detection and risk assessment to identify populations at high risk of developing cancer