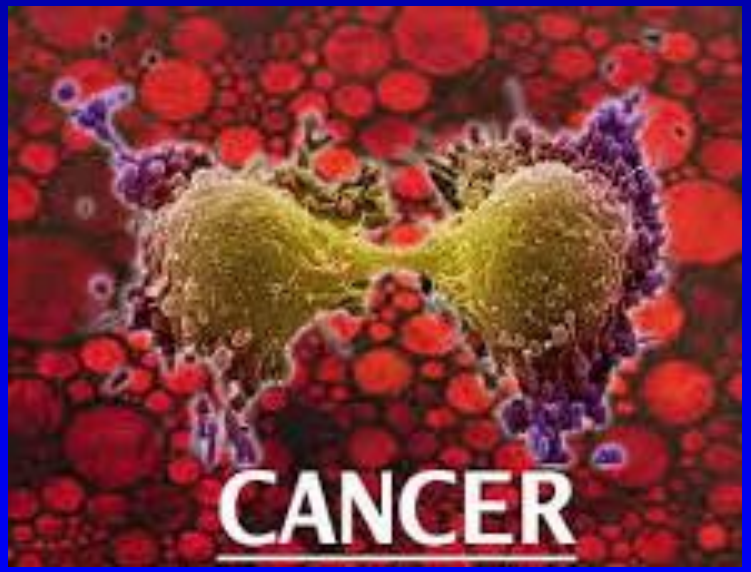
Epigenetic targets

Qian Tao

Professor, Cancer Epigenetics Laboratory
Department of Clinical Oncology, State Key Laboratory of Oncology in South China
The Chinese University of Hong Kong

Cancer medicine

molecular targets



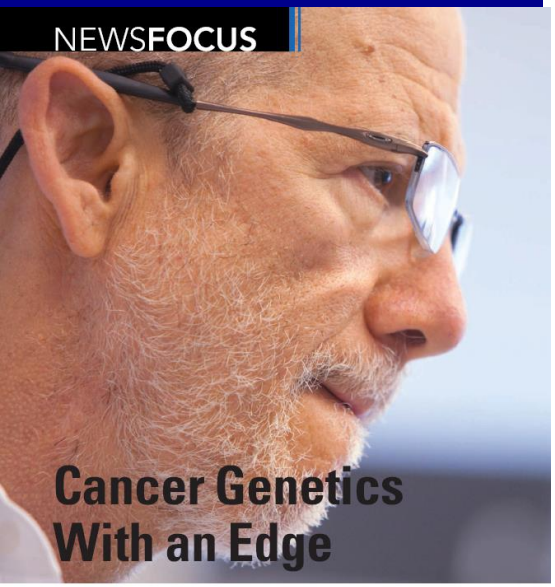
Genetic angle (sequencing):

- WESeq
- WGSeq
- Single-cell seq

GWAS

…

Cancer genome sequencing



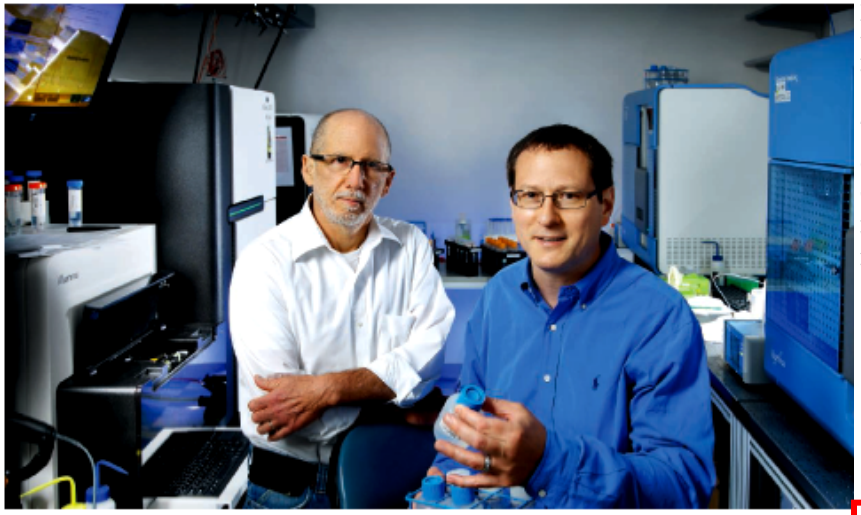
Their lab helped reveal how faulty genes cause cancer, but Bert Vogelstein and Kenneth Kinzler sometimes irk colleagues with their "reality check" comments on genomic medicine

The gamble paid off: In 2006, Vogelstein's group published the first rough exomes for breast and colorectal cancers in *Science* (13 October 2006, p. 268). Data revealed both known and new cancer genes in varying frequencies—the authors called them mountains and hills—as well as daunting variations in patterns from one tumor to the next. Some researchers were critical: In Technical Comments in *Science*, TCGA leader and genome researcher Eric Lander of the Broad Institute in Cambridge, Massachusetts, among others, argued that the Johns Hopkins team had used faulty statistical methods and tested too few tumors to yield meaningful results.

Vogelstein and Kinzler moved on to glioblastoma exomes in 2008 and found an important new oncogene, *IDH1*. This was a slight embarrassment for the NCI collaboration, whose glioma project sequenced only a set of candidate cancer genes in a large number of tumors and missed *IDH1*.

Reviewing work by his group and others in his talks, Vogelstein seems ambivalent about the value of tumor DNA scans. More than 1000 of these surveys have added only a few dozen new cancer-driver genes to the 80 or so that were previously known, Vogelstein says. This includes some intriguing one family. (Researchers compared the genomes of affected and nonaffected family members to pinpoint a responsible gene, *PALB2*.) Like screening for the *BRCA* breast cancer genes, identifying individuals with rare but high-risk pancreatic cancer genes might help some avoid death from the disease. This work led the group to wonder what might be gained if everyone's entire genome were sequenced, Vogelstein says. If a major fraction of the population carried high-risk genes, then sequencing everyone might make sense.

Because homozygous twins have identical genomes, the researchers could learn about their inherited disease risks without DNA sequencing, by comparing health records. Data on nearly 54,000 twin pairs, most in Europe, allowed the Johns Hopkins group to model how risks were distributed across the population. They found that genome testing had potential value. With a positive result defined as an overall disease risk of 10%, the average person would likely test positive for at least one disease, such as heart disease or diabetes. But genome scans would not help much with identifying risks for common cancers, they reported in *Science Translational Medicine* and at the meeting of the American Association for Cancer Research (AACR) in Chicago in April. For most people, the risks associated with inherited DNA are small or nonexistent, they found—and trivial compared to risks from random mutations and factors such as smoking and obesity.



Reality check. Vogelstein and Kenneth Kinzler have identified flaws in some plans to use cancer genomics for treatment and risk prediction. They aim to develop a blood screening test to find early cancers.

Twins and cancer

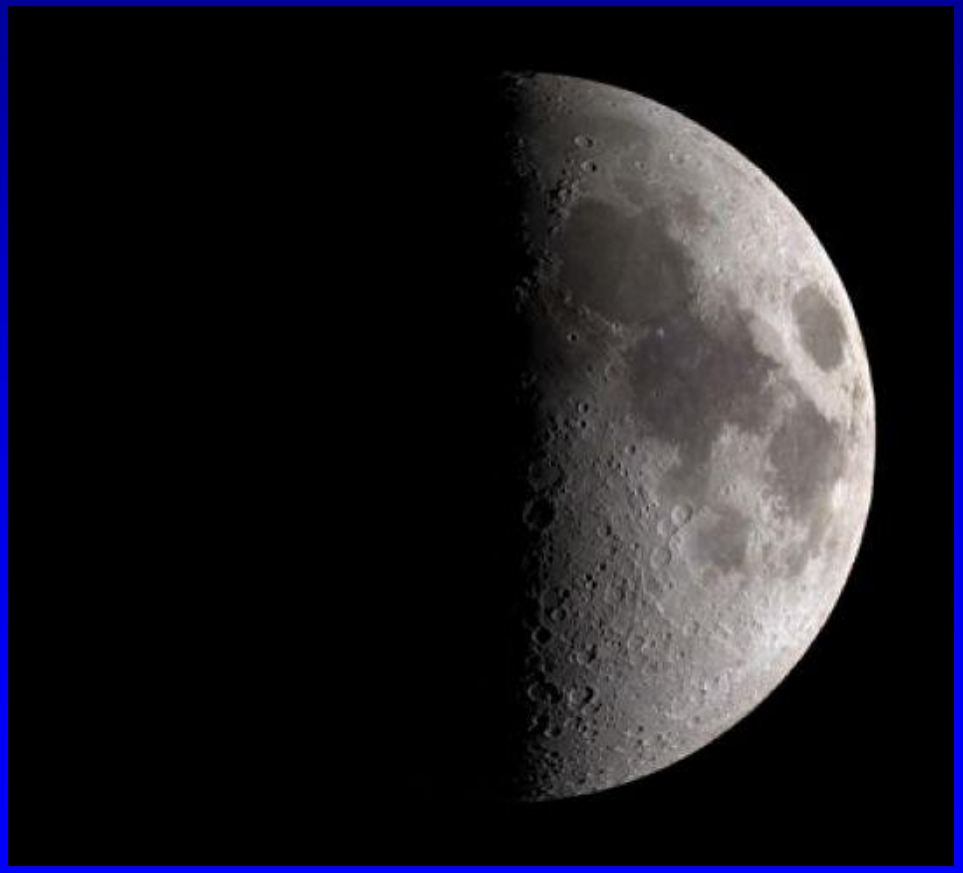
One question that drew the Johns Hopkins group was: What fraction of the population would benefit from whole-genome sequencing? They had already used exome sequencing in 2009 to look for genes linked to pancreatic cancer common

Cancer medicine

molecular targets

Epigenetic angle:

- **CpG methylation**
- *Histone marks*
- *miR, lncRNA …*



Genetic angle (sequencing):

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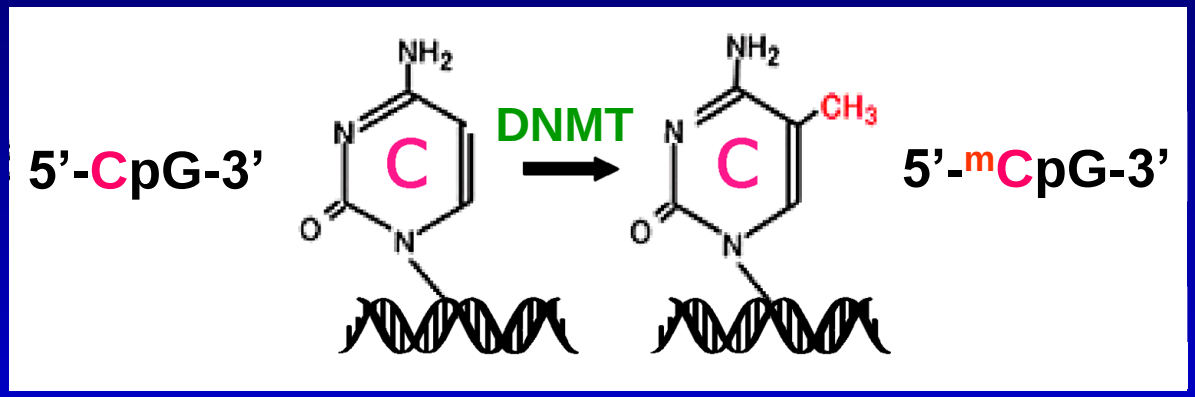
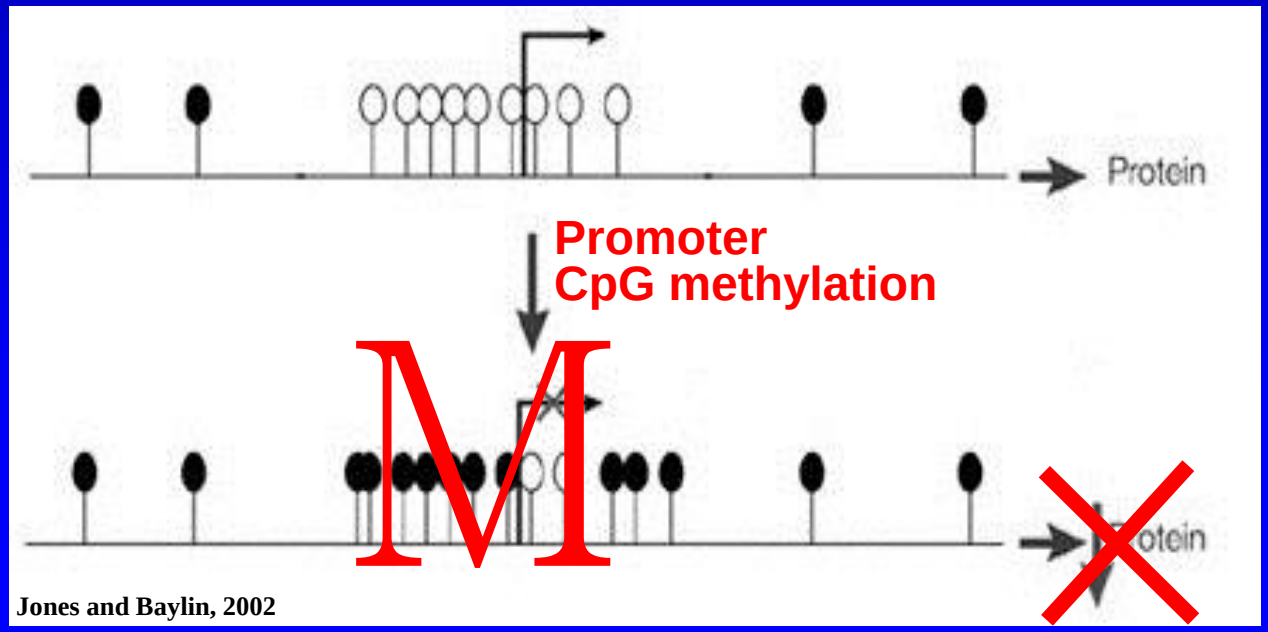
GWAS

…

CpG methylation





Jones and Baylin, 2002

Cancer genes (CAN-gene)

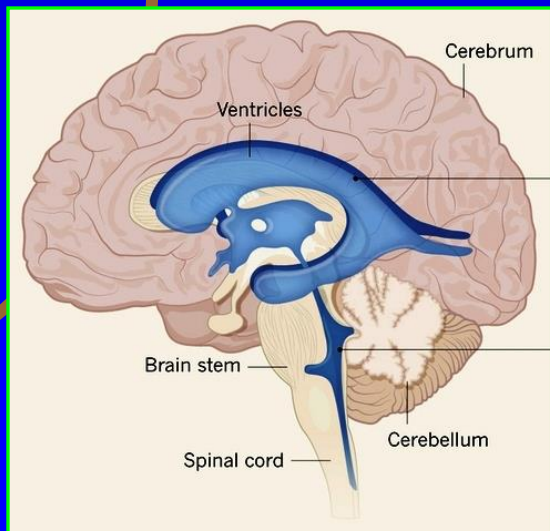
Genetic mutation

TP53
BRCA2
ATM
PTEN
Oncog
…

Rb
p16
CHD5
FHIT
…

Epi-mutation (methylation)

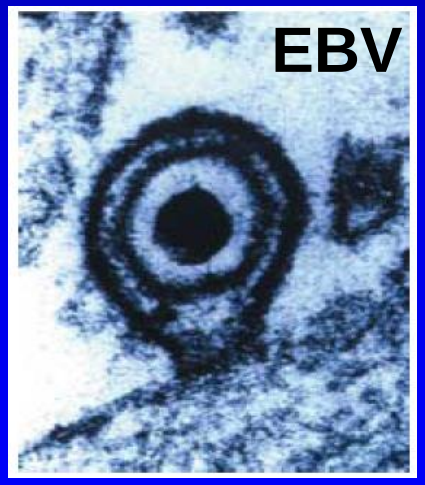
RUNX3
p14
CHD5
HIC1
RASSF1A
TP73
GSTP1
CDH1
RASAL1
…
…
…



Virus-associated tumors:

Nasopharyngeal carcinoma (NPC)
Some gastric cancers

Strong
epigenetic driver!



EBV infection in
normal epithelial cells
↓
Viral proteins
↓
DNMT1, 3A, 3B upregulation
↓
CpG methylation
TSG silencing
↓
Aberrant signaling
Abnormal growth regulation
Cancer

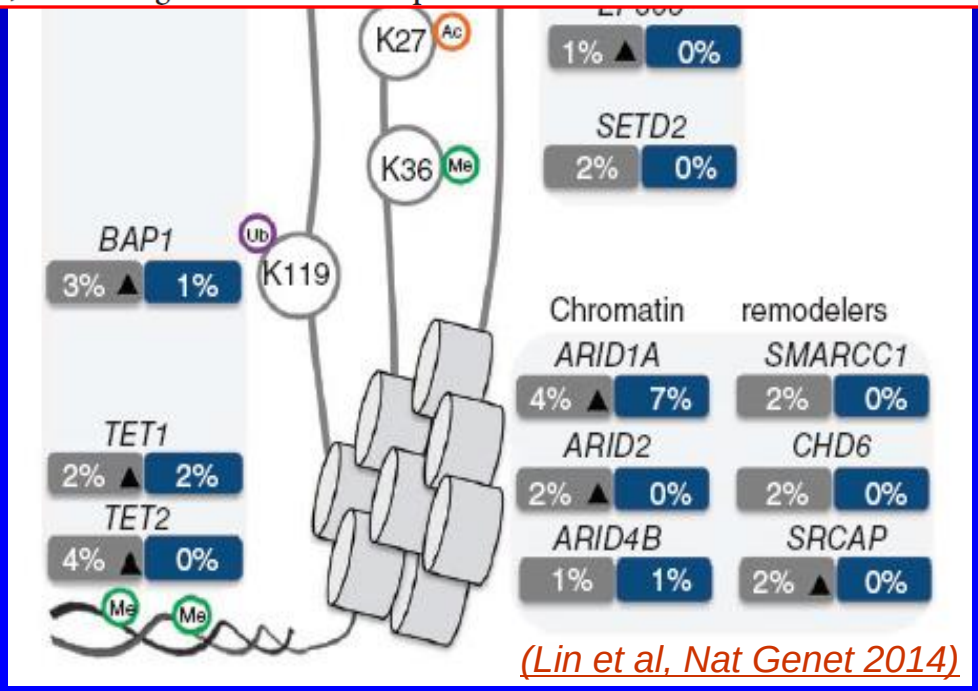
EBV induces more CpG methylation in tumor cells

NPC exome seqing

nature genetics

The genomic landscape of nasopharyngeal carcinoma

De-Chen Lin[1,2,12], Xuan Meng[1,3,12], Masaharu Hazawa[1], Yasunobu Nagata[4,5], Ana Maria Varela[1], Liang Xu[1], Yusuke Sato[4,5], Li-Zhen Liu[1], Ling-Wen Ding[1], Arjun Sharma[1], Boon Cher Goh[1,6], Soo Chin Lee[1,6], Bengt Fredrik Petersson[7], Feng Gang Yu[8], Paul Macary[9], Min Zin Oo[9], Chan Soh Ha[10], Henry Yang[1,13], Seishi Ogawa[4,5,13], Kwok Seng Loh[8,13] & H Phillip Koeffler[1,2,11,13]



(Lin et al, Nat Genet 2014)

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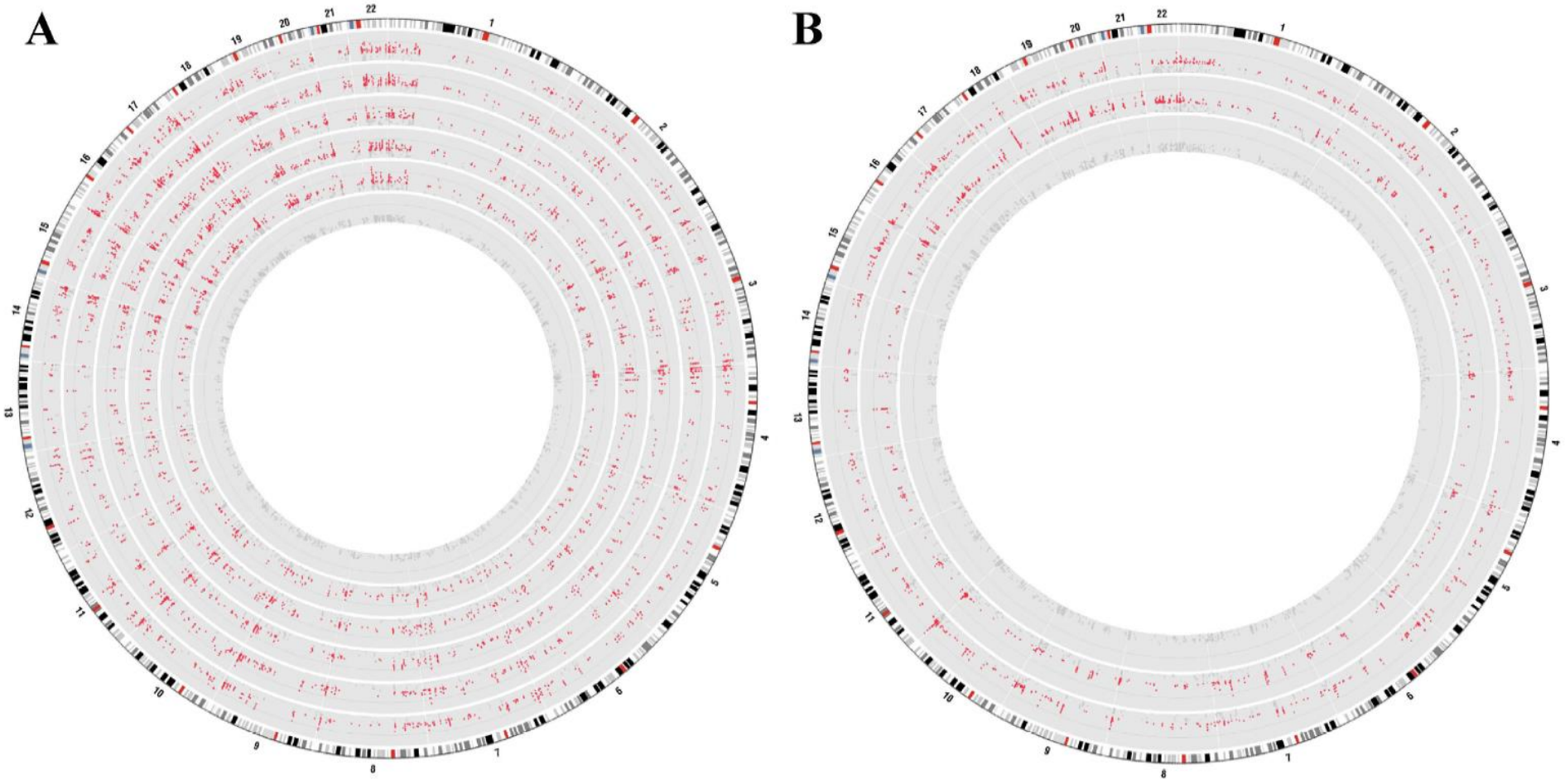
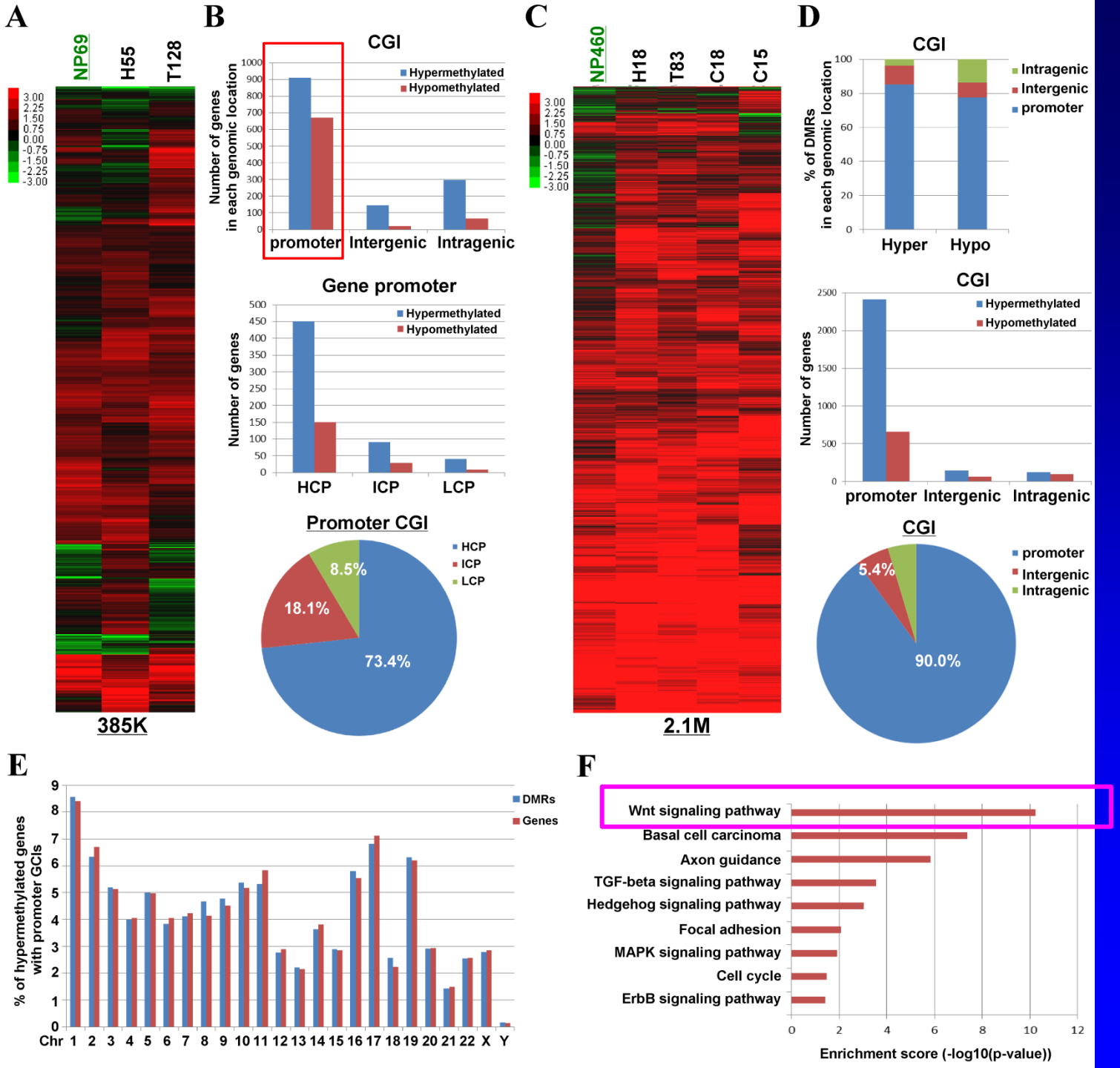
The Chinese University of Hong Kong

NPC methylomes



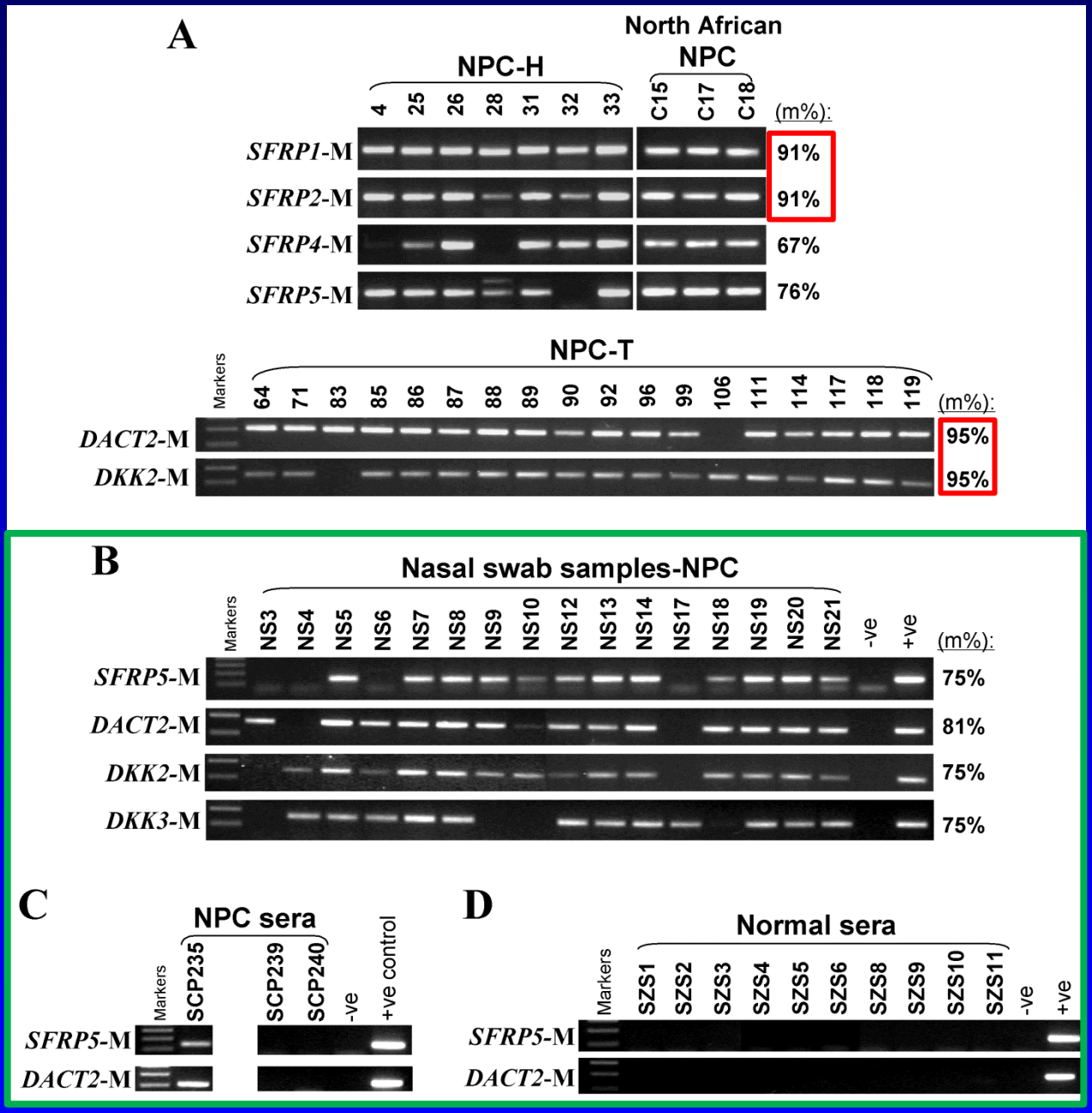
(~1,000-2,000 methylated genes/sample)


A
NP69
H55
T128
3.00
2.25
1.50
0.75
0.00
-0.75
-1.50
-2.25
-3.00
385K
B
CGI
Number of genes in each genomic location
Hypermethylated
Hypomethylated
promoter
Intergenic
Intragenic
Gene promoter
Number of genes
HCP
ICP
LCP
Promoter CGI
8.5%
18.1%
73.4%
C
NP460
H18
T83
C18
C15
2.1M
D
CGI
% of DMRs in each genomic location
Intragenic
Intergenic
promoter
Hyper
Hypo
CGI
Number of genes
5.4%
90.0%
E
% of hypermethylated genes with promoter GCIs
DMRs
Genes
Chr 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
F
Wnt signaling pathway
Basal cell carcinoma
Axon guidance
TGF-beta signaling pathway
Hedgehog signaling pathway
Focal adhesion
MAPK signaling pathway
Cell cycle
ErbB signaling pathway
Enrichment score (-log10(p-value))

Validation of methylome data

TSGs	Methylation identified by MeDIP-chip	Methylation reported previously	Refs
ADAMTS8	m	m	Mol Cancer Res 2014; 12: 228-38.
ADAMTS9	m	m	Int J Cancer 2008; 123: 401-8.
ADAMTS18	m	m	Oncogene 2007; 26: 7490-8.
CACNA2D3	m	m	Int J Cancer 2013; 133: 2284-95.
CADM1	m	m	Int J Cancer 2004; 112: 628-35; Mol Cancer 2011; 10: 48.
CCNA1	m	m	Oral Oncol 2008; 44: 400-6.
CDH4	m	m	Cancer Lett 2011; 309: 54-61.
CDH11	m	m	Oncogene 2012; 31: 3901-12.
CHFR	m	m	Mol Carcinog 2005; 43: 237-45. Mol Cancer 2011; 10: 48.
CMTM3	m	m	Cancer Res 2009; 69: 5194-201.
CMTM7	m	m	Oncogene 2014; 33: 3109-18.
DACT1	m	**verified in this study**	
DKK3	m	**verified in this study**	
DLC1	m	m	Oncogene 2007; 26: 934-44; Oncogene 2011; 30: 1923-35. Mol Cancer 2011; 10: 48; Mol Carcinog 2013.
DUSP6	m	m	Int J Cancer 2012; 130: 83-95.
ESR1	m	m	Int J Cancer 2011; 128: 1393-403.
FEZF2	m	m	Carcinogenesis 2013; 34: 1984-93.
KIF1A	m	m	Int J Cancer 2011; 128: 1393-403.
MGMT	m	m	Clin Cancer Res 2002; 8: 131-7; Int J Oncol 2003; 22: 869-74. Clin Chim Acta 2012; 413: 795-802.
PAX5	m	**verified in this study**	
PCDH8	m	m	Eur J Cancer Prev 2012; 21: 569-75.
PTEN	m	m	Oncol Rep 2014; 31: 2206-12.
PTPRG	m	m	Cancer Res 2008; 68: 8137-45.
RASSF1	m	m	Cancer Res 2001; 61: 3877-81; Clin Cancer Res 2002; 8: 131-7; Int J Oncol 2003; 22: 869-74; Neoplasia 2005; 7: 809-15; Mol Cancer 2011; 10: 48.
RRAD	m	m	Cancer Lett 2012; 323: 147-54.
SCGB3A1	m	m	Clin Cancer Res 2003; 9: 3042-6.
SFRP1	m	**verified in this study**	
SFRP2	m	**verified in this study**	
SFRP5	m	**verified in this study**	
SLIT2	m	**verified in this study**	
SOX11	m	m	Cancer Cell Int 2013; 13: 109.
THBS1	m	m	Int J Oncol 2003; 22: 869-74.
TP73	m	m	Int J Oncol 2003; 22: 869-74.
UCHL1	m	m	Clin Cancer Res 2010; 16: 2949-58.

TSG methylation as candidate biomarkers



PCDH10 methylation - biomarker

Oncogene (2006) 25, 1070–1080

www.nature.com/onc

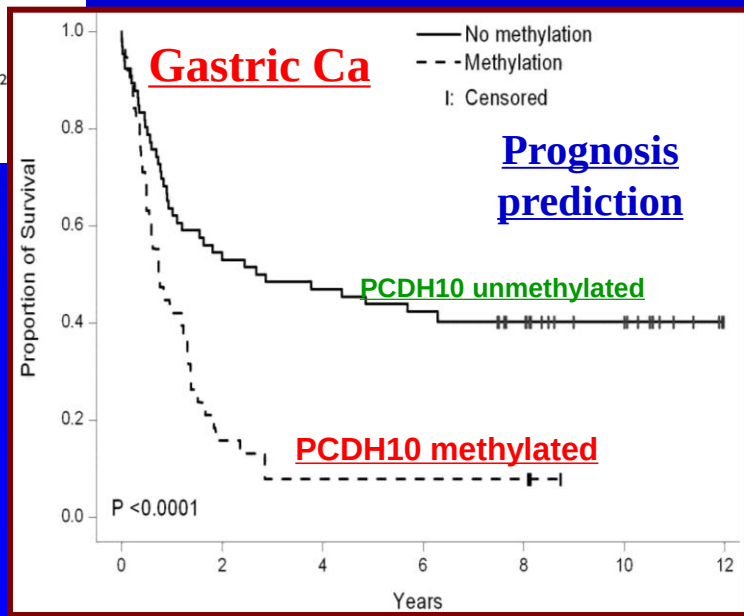
ORIGINAL ARTICLE

Functional epigenetics identifies a protocadherin *PCDH10* as a candidate tumor suppressor for nasopharyngeal, esophageal and multiple other carcinomas with frequent methylation

J Ying[1], H Li[1], TJ Seng[2], C Langford[3], G Srivastava[4], SW Tsao[5], T Putti[6], P Murray[7], ATC Chan[1] and Q Tao[1,2]

Protocadherin PCDH10, Involved in Tumor Progression, Is a Frequent and Early Target of Promoter Hypermethylation in Cervical Cancer

Gopeshwar Narayan,[1†] Luigi Scotto,[1] Vijayalakshmi Neelakantan,[1] Sherine H. Kottoor,[1] Ada Ho Yan Wong,[2] Shee-Loong Loke,[3] Mahesh Mansukhani,[1] Bhavana Pothuri,[4] Jason D. Wright,[4] Andreas M. Kaufmann,[5] Achim Schneider,[5] Hugo Arias-Pulido,[6] Qian Tao,[2] and Vundavalli V. Murty[1,7*]



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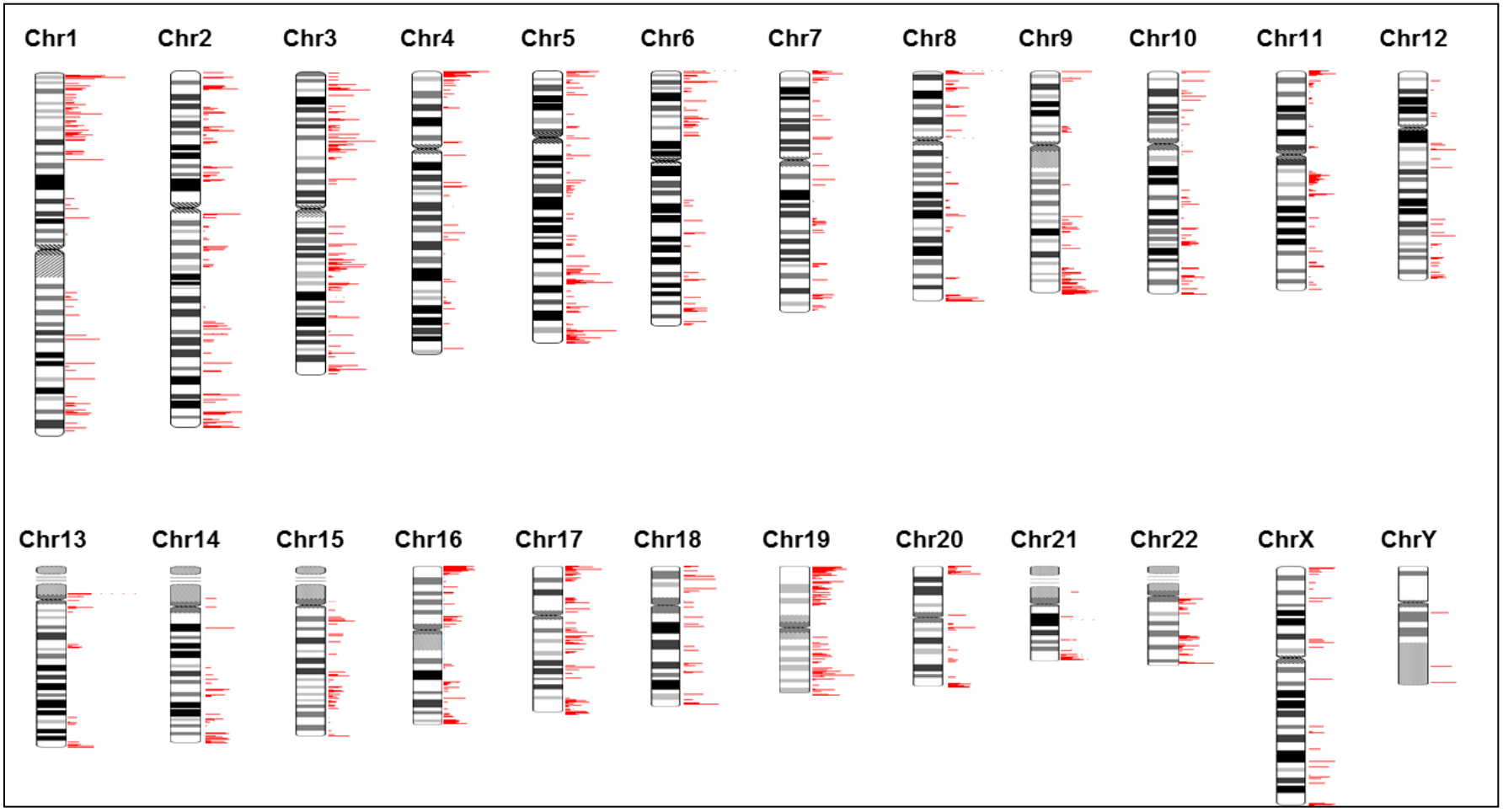
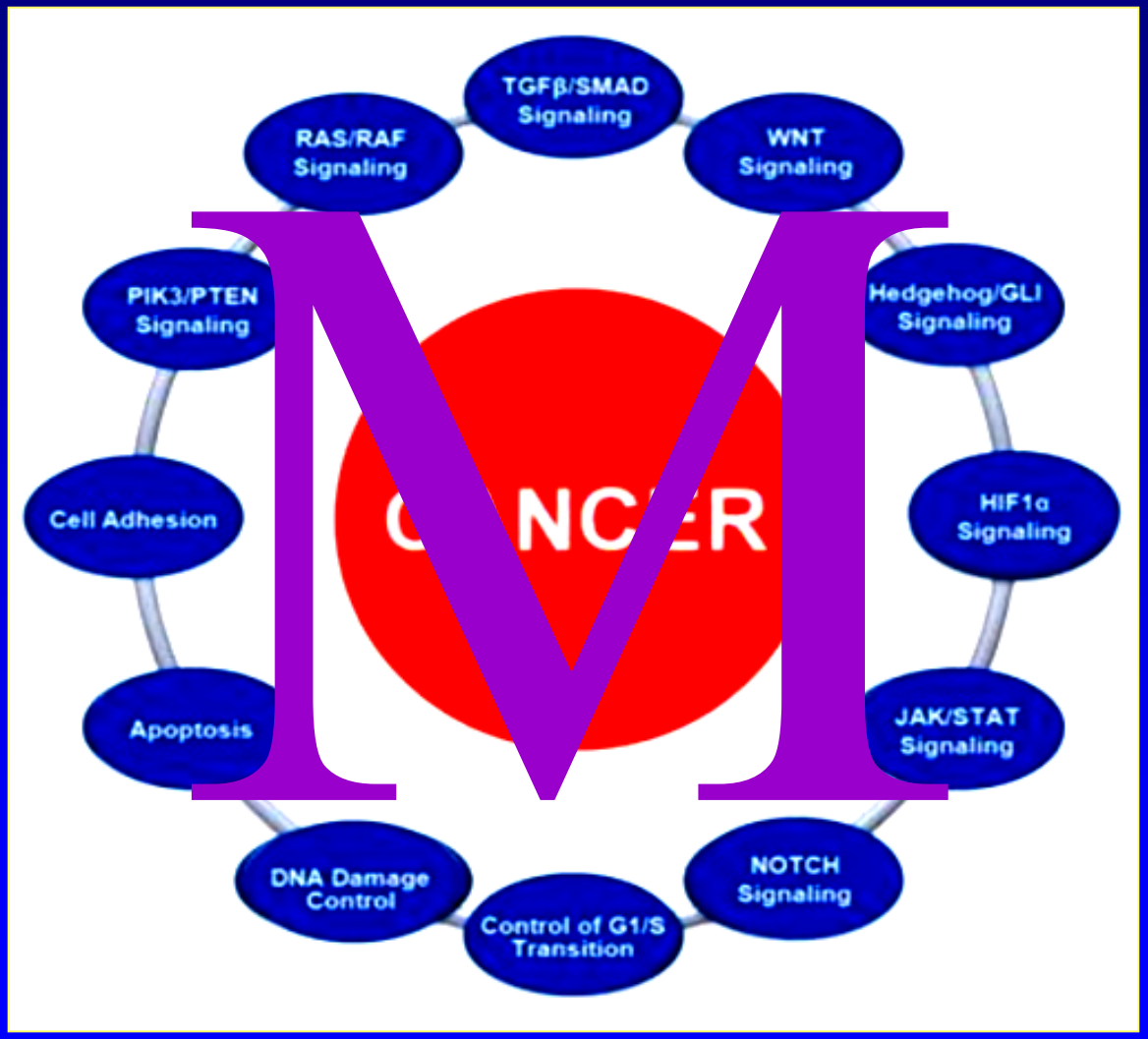
Tumor methylomes

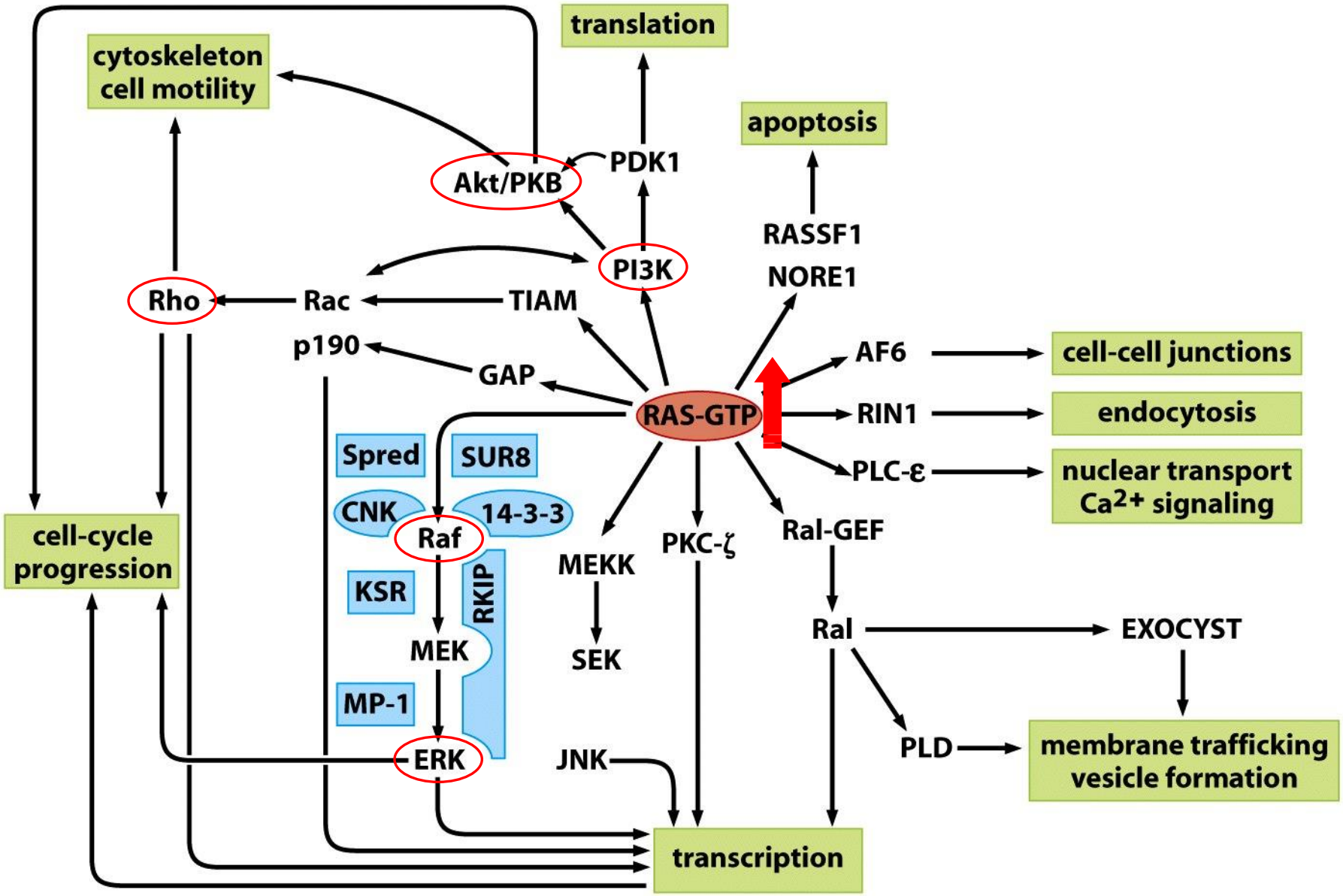
(ESCC, lung Ca, HCC, RCC, …)



Epigenetic disruption of cell signaling in tumors

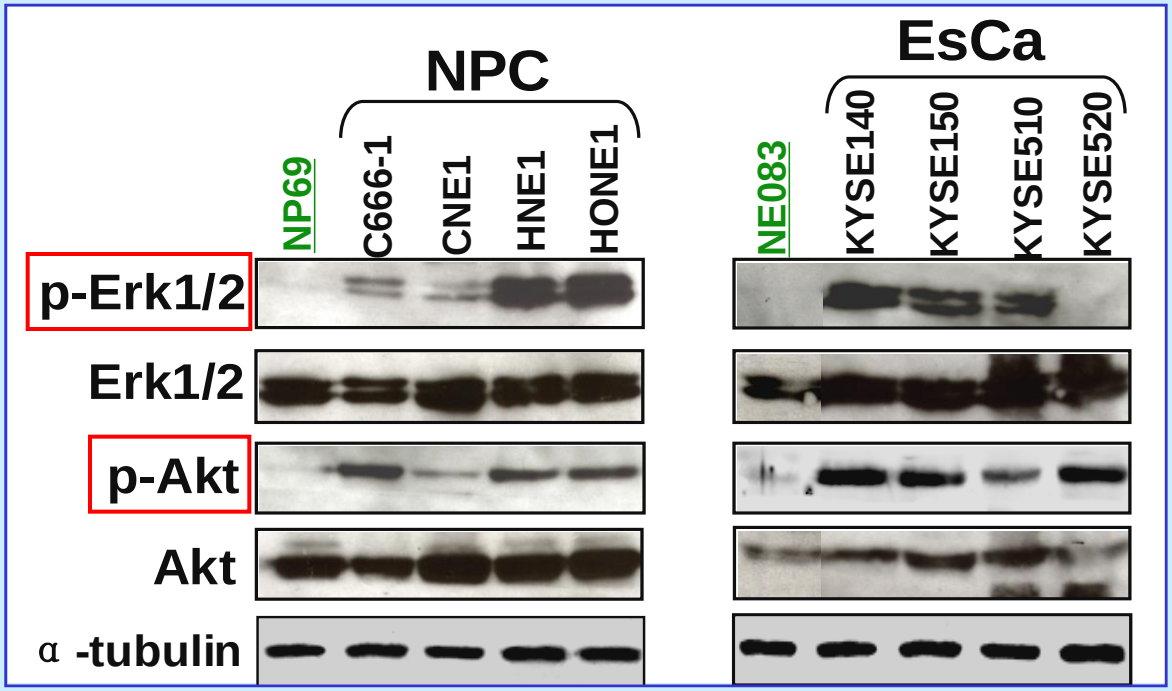


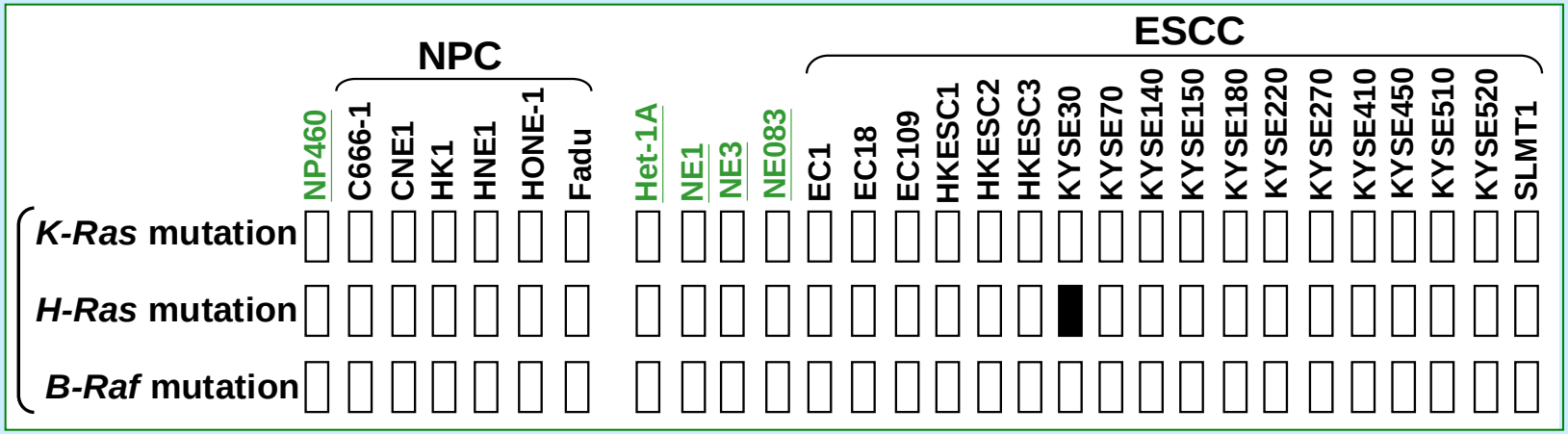
Ras-signaling



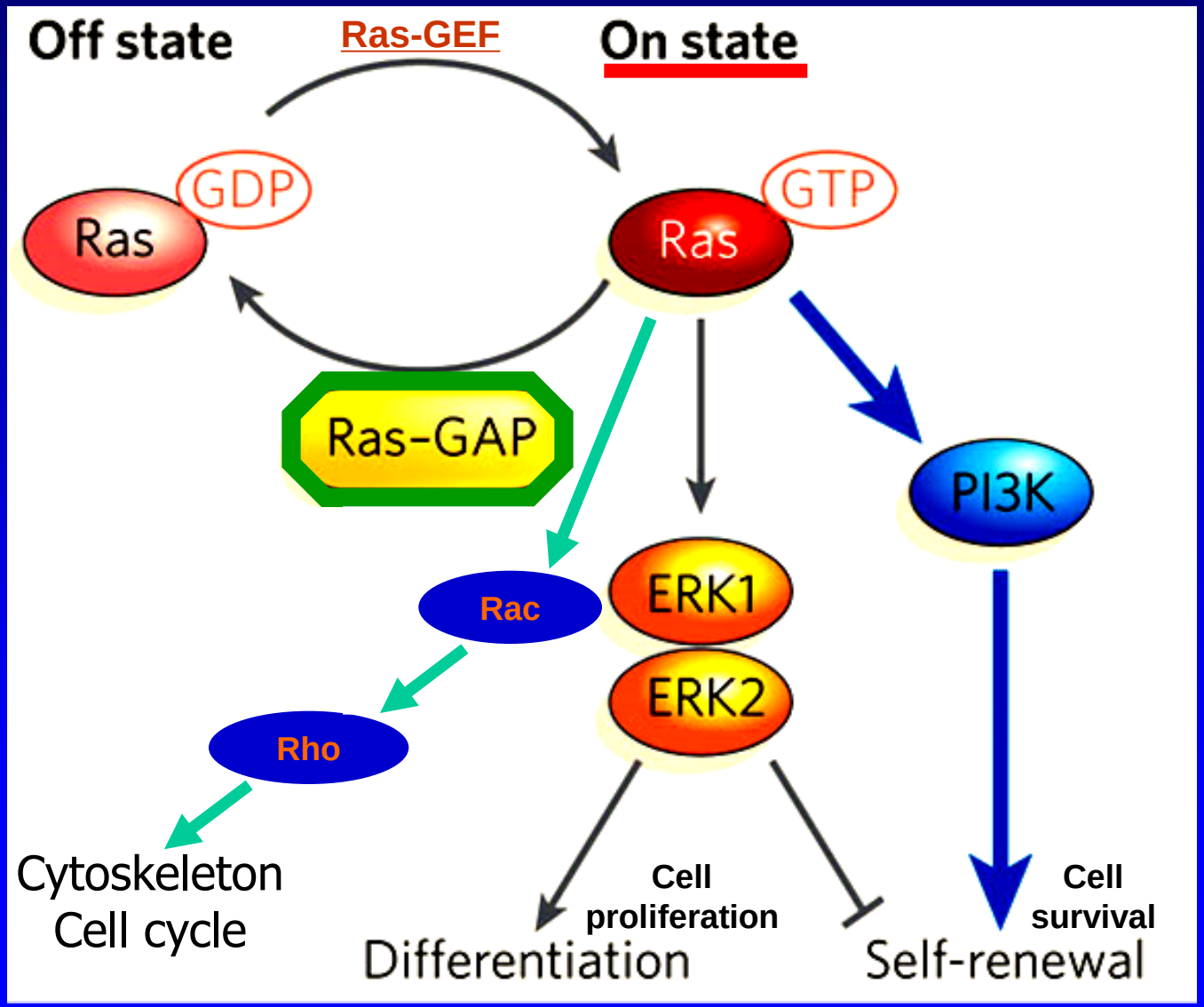
The Biology of Cancer (© Garland Science 2007)

Aberrant activation of Ras/Akt-signaling in cancers





Ras-signaling

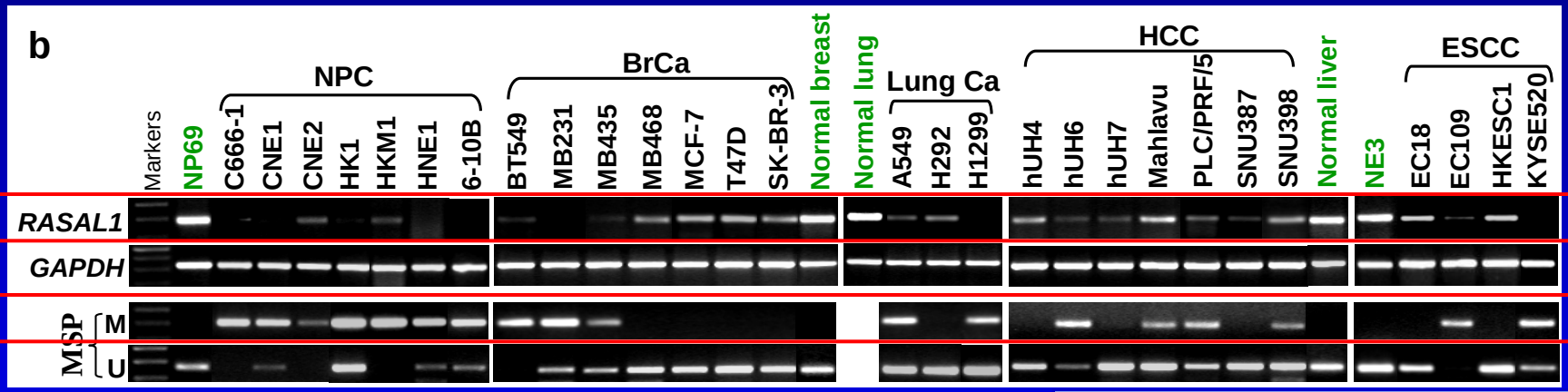


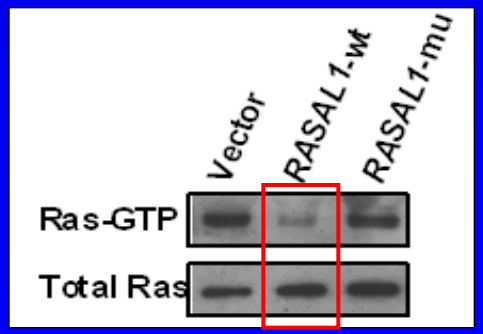
Novel epigenetic targets

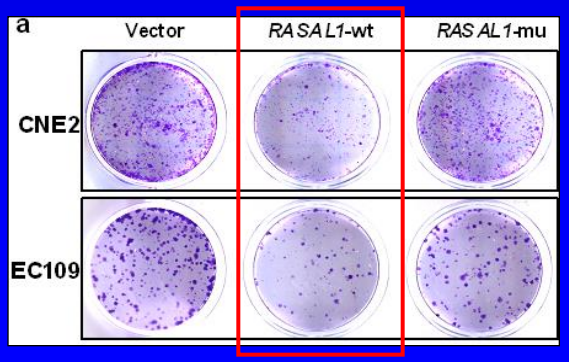
PNAS

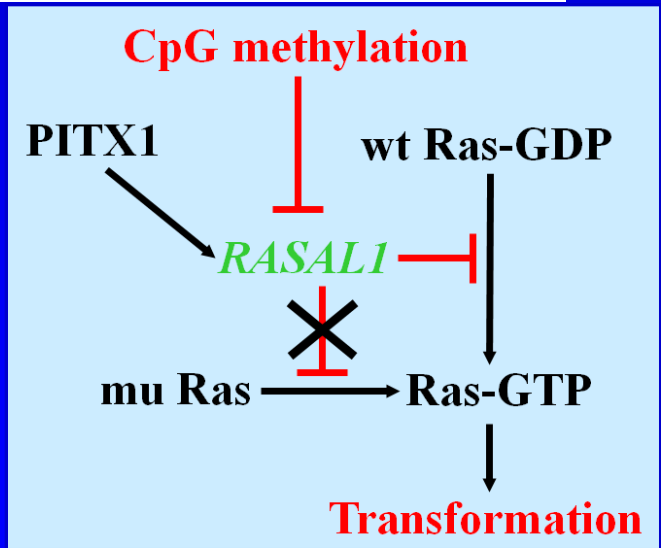
Epigenetic silencing of a Ca^{2+}-regulated Ras GTPase-activating protein RASAL defines a new mechanism of Ras activation in human cancers

Hongchuan Jin*, Xian Wang*, Jianming Ying*, Ada H. Y. Wong*, Yan Cui*, Gopesh Srivastava†, Zhong-Ying Shen
En-Min Li‡, Qian Zhang§, Jie Jin§, Sabine Kupzig¶, Anthony T. C. Chan*, Peter J. Cullen¶‖, and Qian Tao*‡‖









Ras mutation *vs* *RASAL* methylation

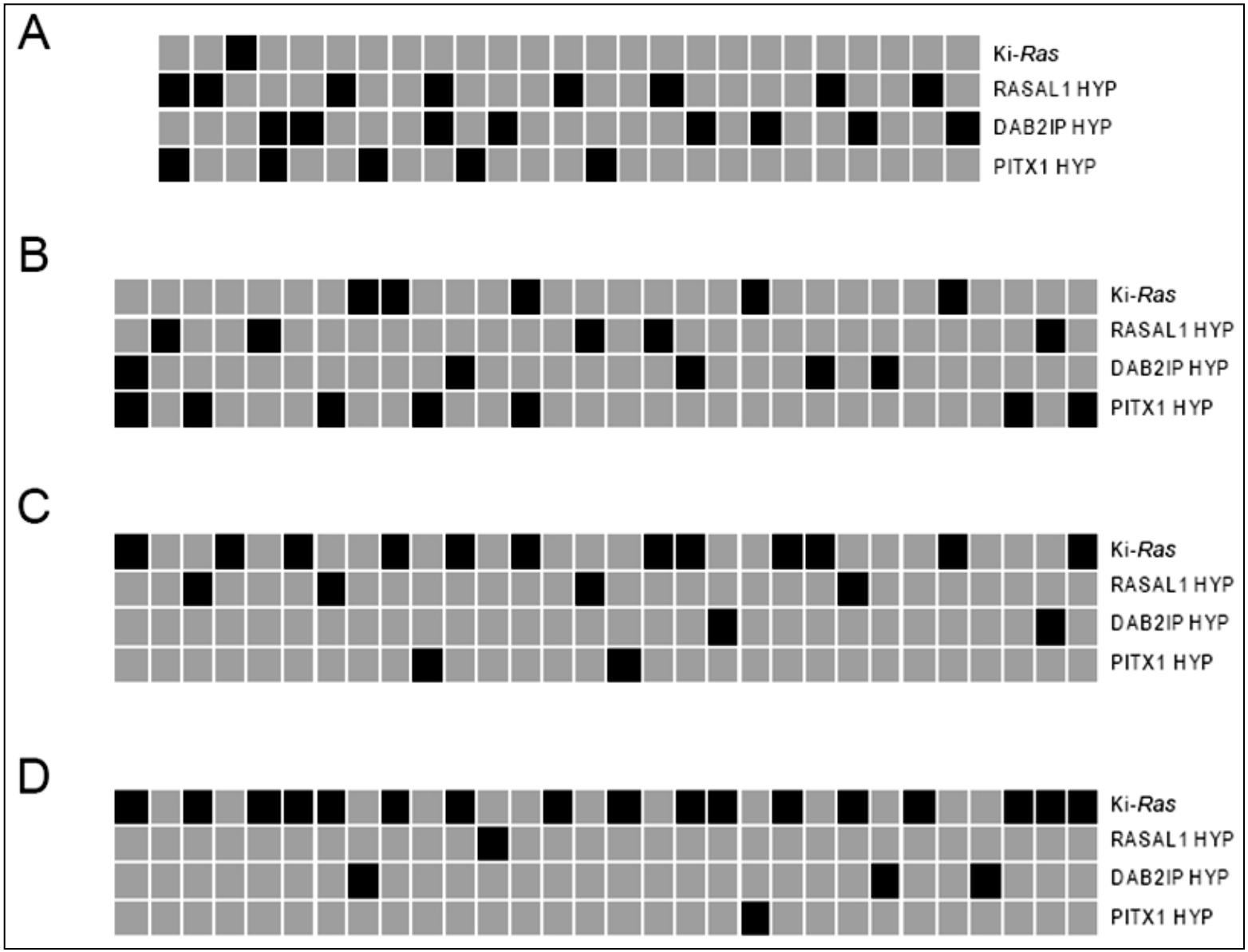
– mutaually exclusive!

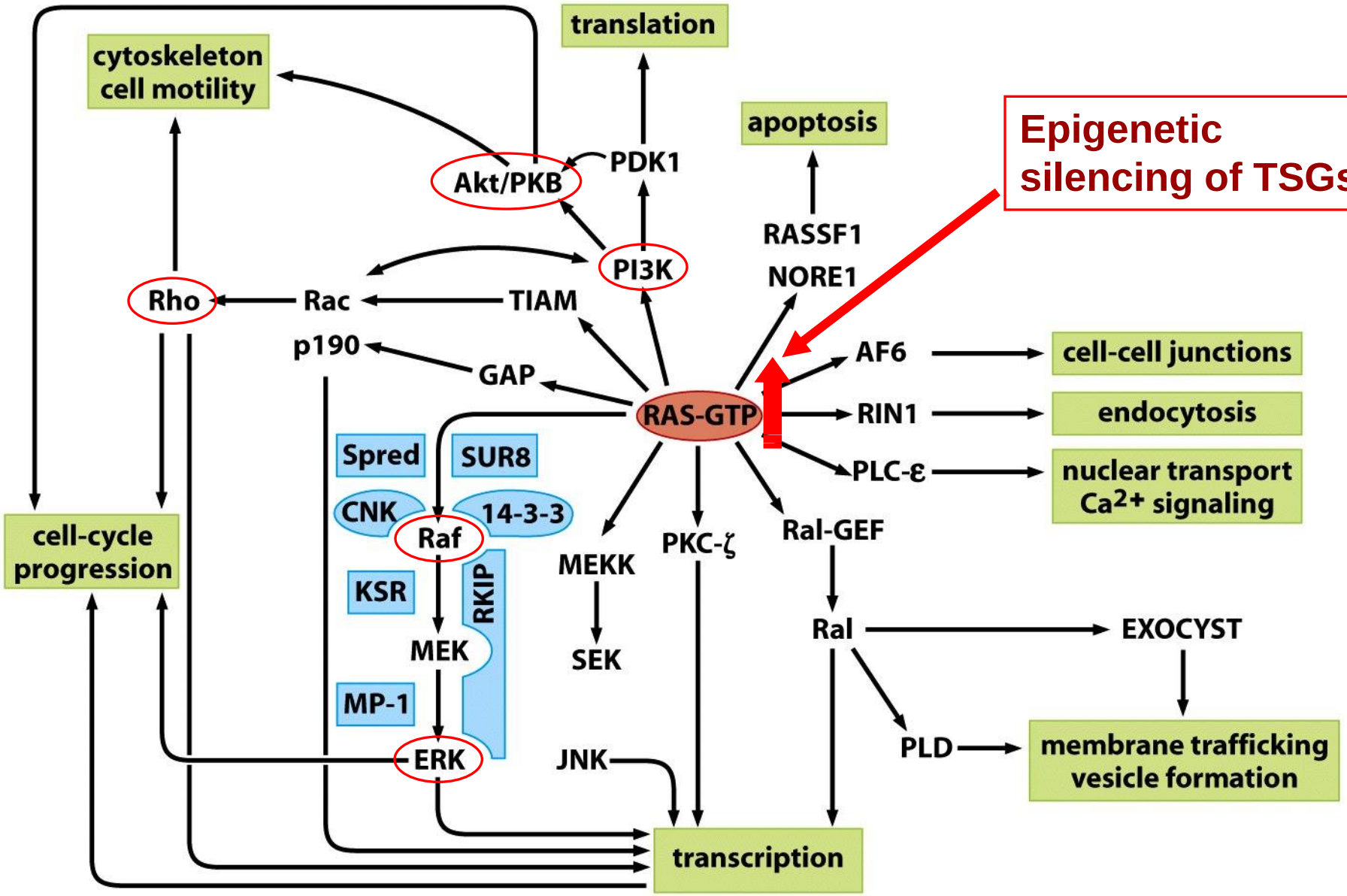
Breast Ca

Lung Ca

Colon Ca

Pancreatic Ca

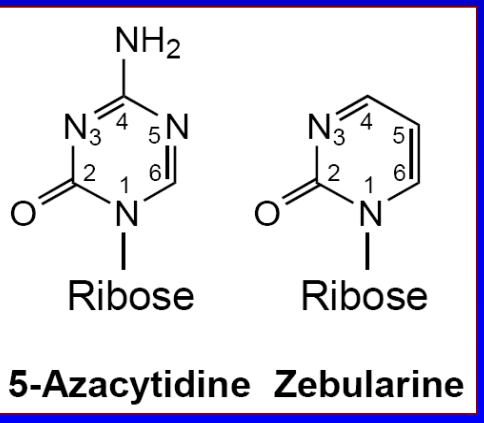




The Biology of Cancer (© Garland Science 2007)

Epigenetic therapy

DNMTi: Aza, Zebularine, RG108 …
HDACi: SAHA, FK228, LBH589, MS275 …



5-Azacytidine Zebularine

Epigenetic drugs:

Drug target	Generic or trade name	Development name	Chemical class	Pharmaceutical sponsor	Status
DNMT inhibitor	Azacitidine Vidaza	5-azacytidine	Nucleoside analog	Celgene (Summit, NJ)	FDA approved, 2004 EMEA approved, 2008
DNMT inhibitor	Decitabine Dacogen	2′-deoxy-5-azacytidine	Nucleoside analog	Eisai Co., Ltd. (Tokyo, Japan)	FDA approved, 2006
DNMT inhibitor	Zebularine		Nucleoside analog		
DNMT inhibitor		RG108	Active site inhibitor		
HDAC inhibitor	Sodium phenylbutyrate Buphenyl Ammonaps		Small chain fatty acid	Ucyclyd Pharma (Scottsdale, AZ) Orphan International (Stockholm, Sweden)	FDA approved EMEA approved (urea cycle disorders)
HDAC inhibitor	Valproic acid Depakote, (others)	VPA	Small chain fatty acid	Abbott Laboratories (Abbott Park, IL)	FDA approved (seizure disorders
HDAC inhibitor	Vorinostat Zolinza	SAHA	Hydroxamic acid	Merck & Co.	FDA approved, 2006
HDAC inhibitor	Panobinostat	LBH589	Hydroxamic acid	Novartis Pharmaceuticals (East Hanover, NJ)	
HDAC inhibitor	Belinostat	PXD101	Hydroxamic acid	TopoTarget (Rockaway, NJ)	
HDAC inhibitor	Romidepsin	Depsipeptide, FK228	Cyclic peptide	Gloucester Pharmaceuticals (Cambridge, MA)	New drug application filed with FDA
HDAC inhibitor	Entinostat	MS275	Benzamide	Syndax Pharmaceuticals (Waltham, MA)	

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JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

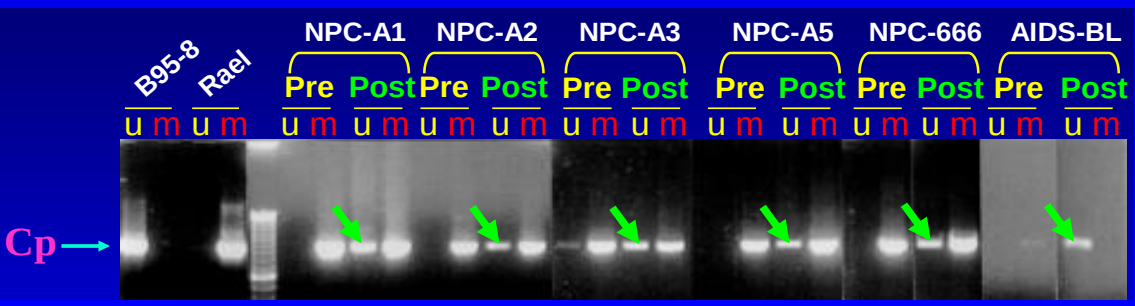
Azacitidine Induces Demethylation of the Epstein-Barr Virus Genome in Tumors

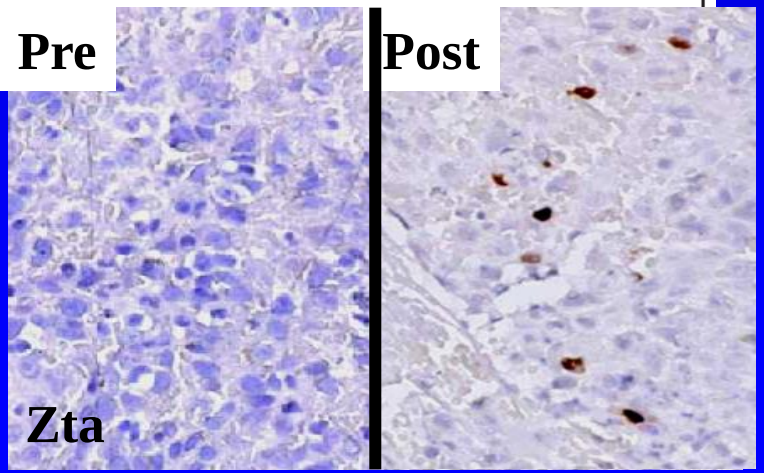
Anthony T.C. Chan, Qian Tao, Keith D. Robertson, Ian W. Flinn, Risa B. Mann, Barbara Klencke, Wing Hong Kwan, Thomas Wai-Tong Leung, Philip J. Johnson, and Richard F. Ambinder

Table 2. Changes of Percentage of Methylated CpG Sites in EBV Promoters After Azacitidine Treatment*

	Wp		LMP1/2Bp		Zp		Rp	
Cases	Pre	Post	Pre	Post	Pre	Post	Pre	Post
NPC1	83	27	97	17	69	60	100	57
NPC2	87	88	9	5	—	—	65	50
NPC3	—	—	98	23	53	15	79	26
NPC5	80	85	10	5	—	—	—	—
AIDS-L	92	55	2	0	—	—	89	7

Abbreviations: EBV, Epstein-Barr virus; Wp, W promoter; LMP, latency membrane protein; Zp, Z promoter; Rp, R promoter; NPC, nasopharyngeal carcinoma; AIDS-L, AIDS lymphoma.
*Percentage refers to methylated CpG sites/all CpG sites.

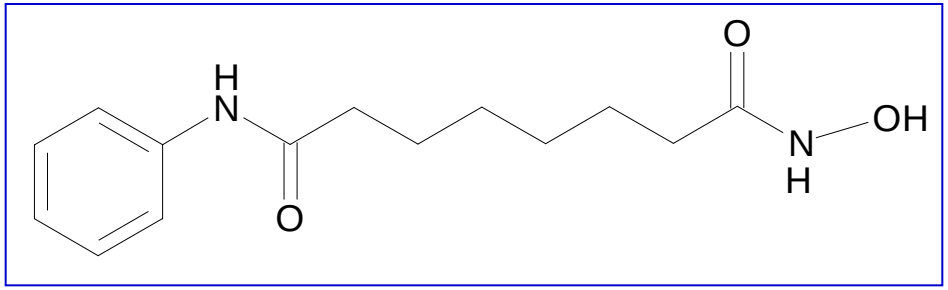




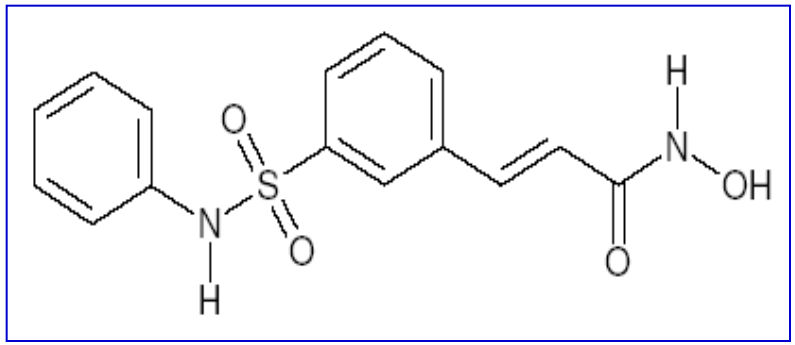
Epigenetic therapy to NPC

Aza + HDACi for NPC

(NCI-supported, in vitro and Phase I / II trials)

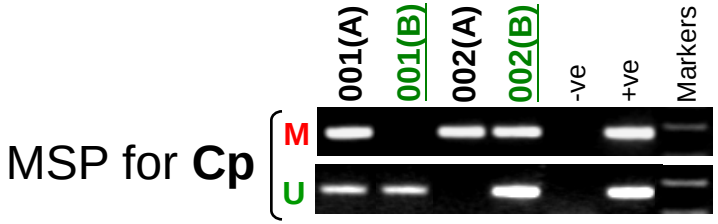


SAHA (Suberoylanilide hydroxamic acid)



PXD101 (N-hydroxy-3-[phenylsulphamoylphenyl] acryl amide)

Aza+SAHA treatment for NPC patients



(Hsieh, Tao, Chan…Ambinder, unpublished)

Epigenetic therapy

RESEARCH ARTICLE

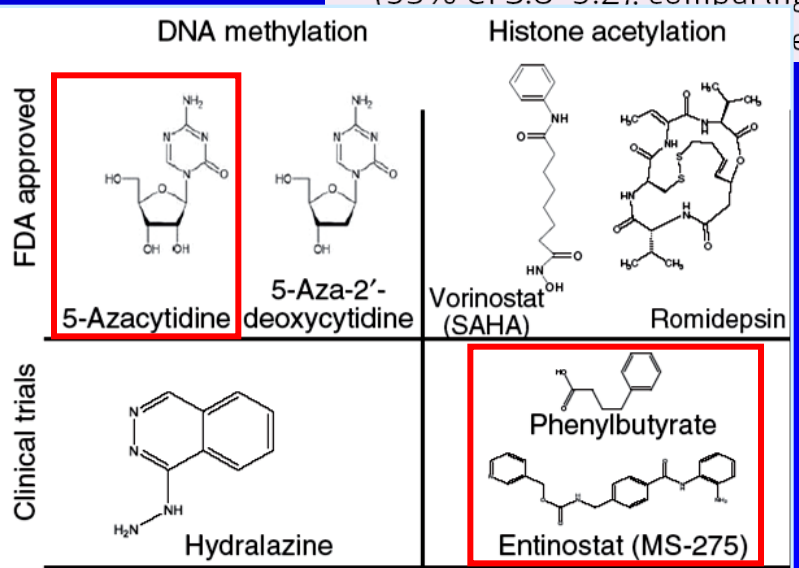
Combination Epigenetic Therapy Has Efficacy in Patients with Refractory Advanced Non-Small Cell Lung Cancer

Rosalyn A. Juergens[1], John Wrangle[1], Frank P. Vendetti[3], Sara C. Murphy[1], Ming Zhao[1], Barbara Coleman[1], Rosa Sebree[1], Kristen Rodgers[2], Craig M. Hooker[1], Noreli Franco[1], Beverly Lee[1], Salina Tsai[4], Igor Espinoza Delgado[5], Michelle A. Rudek[1], Steven A. Belinsky[6], James G. Herman[1], Stephen B. Baylin[1], Malcolm V. Brock[2], and Charles M. Rudin[1]

Cancer Discov. 2011 Dec;1(7):598-607.

ABSTRACT

Epigenetic alterations are strongly associated with the development of cancer. We conducted a phase I/II trial of combined epigenetic therapy with azacitidine and entinostat, inhibitors of DNA methylation and histone deacetylation, respectively, in extensively pretreated patients with recurrent metastatic non-small cell lung cancer. This therapy is well tolerated, and objective responses were observed, including a complete response and a partial response in a patient who remains alive and without disease progression approximately 2 years after completing protocol therapy. Median survival in the entire cohort was 6.4 months (95% CI 3.8–9.2), comparing favorably with existing therapeutic options. Demethylation of a set of genes known to be associated with lung cancer was detectable in serial



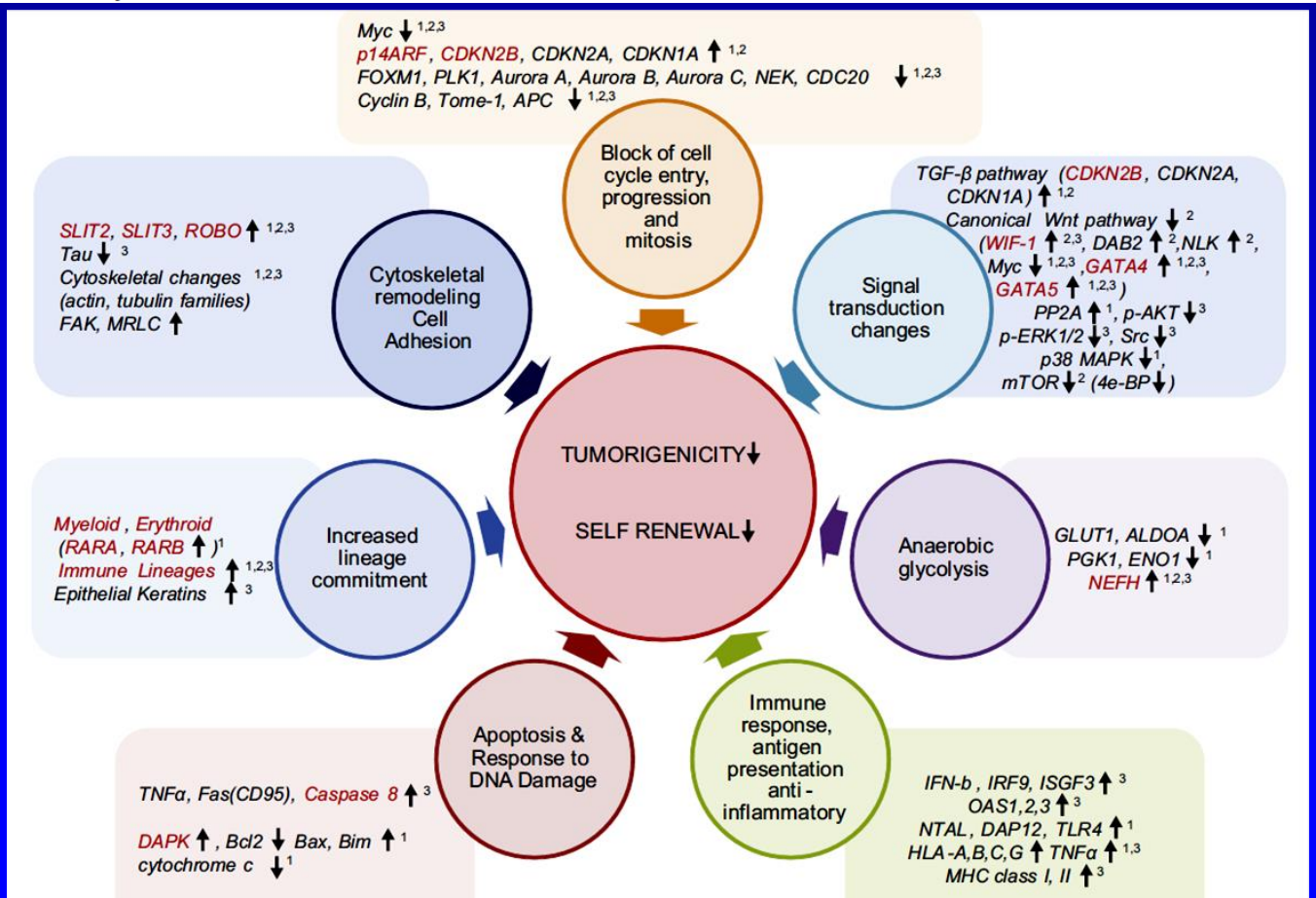
Cell PRESS

Cancer Cell
Article

Epigenetic therapy using low dosage of Aza

Transient Low Doses of DNA-Demethylating Agents Exert Durable Antitumor Effects on Hematological and Epithelial Tumor Cells

Hsing-Chen Tsai,[1,2,10] Huili Li,[2,10] Leander Van Neste,[3,10] Yi Cai,[2] Carine Robert,[4] Feyruz V. Rassool,[4] James J. Shin,[2,5] Kirsten M. Harbom,[2] Robert Beaty,[2] Emmanouil Pappou,[2,5] James Harris,[2,5] Ray-Whay Chiu Yen,[2] Nita Ahuja,[2,5] Malcolm V. Brock,[2,5] Vered Stearns,[2,6] David Feller-Kopman,[7] Lonny B. Yarmus,[7] Yi-Chun Lin,[8] Alana L. Welm,[8] Jean-Pierre Issa,[9] Il Minn,[2] William Matsui,[1,2] Yoon-Young Jang,[2] Saul J. Sharkis,[1,2] Stephen B. Baylin,[1,2,*] and Cynthia A. Zahnow[2,6,*]



Demethylation triggers a cellular antiviral program

Cell

Volume 162, Issue 5, 27 August 2015, Pages 974–986

Article

Inhibiting DNA Methylation Causes an Interferon Response in Cancer via dsRNA Including Endogenous Retroviruses

Katherine B. Chiappinelli[1,7], Pamela L. Strissel[2,7], Alexis Desrichard[3,7], Huili Li[1], Christine Henke[2], Benjamin Akman[1], Alexander Hein[2], Neal S. Rote[4], Leslie M. Cope[1], Alexandra Snyder[3,5], Vladimir Makarov[3], Sadna Buhu[5], Dennis J. Slamon[6], Jedd D. Wolchok[5], Drew M. Pardoll[1], Matthias W. Beckmann[2], Cynthia A. Zahnow[1], Taha Mergoub[5,8], Timothy A. Chan[3,5,8], Stephen B. Baylin[1,8], Reiner Strick[2,8],

Cell

Volume 162, Issue 5, 27 August 2015, Pages 961–973

Article

DNA-Demethylating Agents Target Colorectal Cancer Cells by Inducing Viral Mimicry by Endogenous Transcripts

David Roulois[1], Helen Loo Yau[1,2], Rajat Singhania[1], Yadong Wang[1], Arnavaz Danesh[1], Shu Yi Shen[1], Han Han[4], Gangning Liang[4], Peter A. Jones[4,6], Trevor J. Pugh[1,2], Catherine O'Brien[1,3,5], Daniel D. De Carvalho[1,2],

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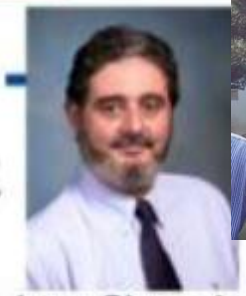
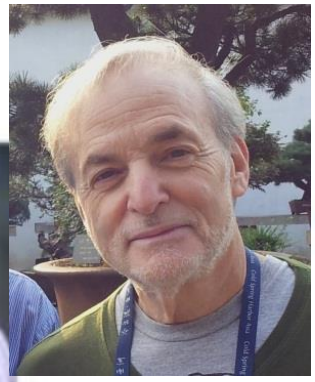


Jean-Pierre Issa



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Casey O'Connell
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Leukemia

Breast, Lung, & Colon

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