



Methylation patterns in cancer patients

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<http://zbo.ipipan.waw.pl>

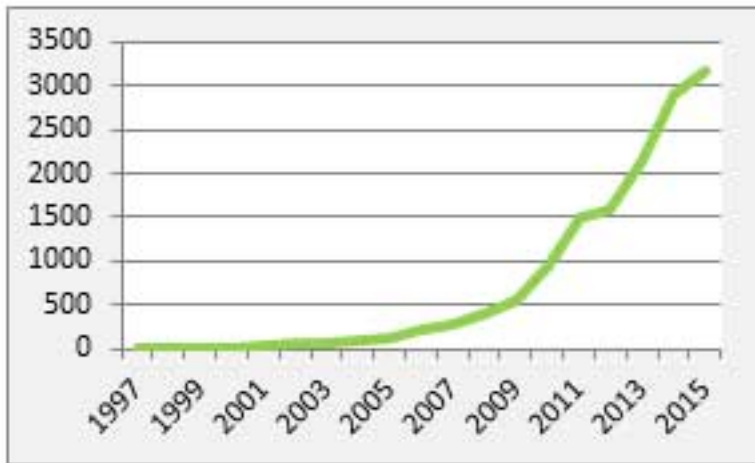
- DNA methylation
 - Definition of the process
 - Targets in DNA
 - Function of DNA methylation
 - CpG islands
 - Repression of CpG-dense promoters
 - Other associations
 - Detection methods
- Role of DNA methylation in cancer development
- Glioma tumor classification
- Glioma patients prognosis

What is DNA methylation?

It is an epigenetic change

Modification that changes gene expression without affecting genetic sequence

Are these modifications important?



Epigenetics-related publications in PubMed

What is DNA methylation?

Purines

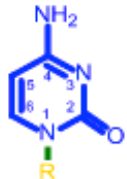


Adenine

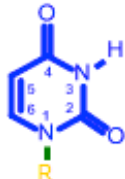


Guanine

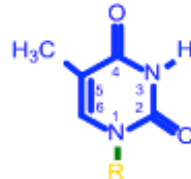
Pyrimidines



Cytosine

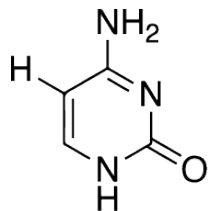


Uracil

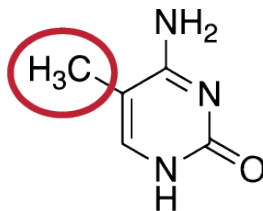


Thymine

- Addition of a methyl group to DNA
- 5-carbon of the cytosine ring
- Methyl groups project into the major groove of DNA
- Inhibit transcription

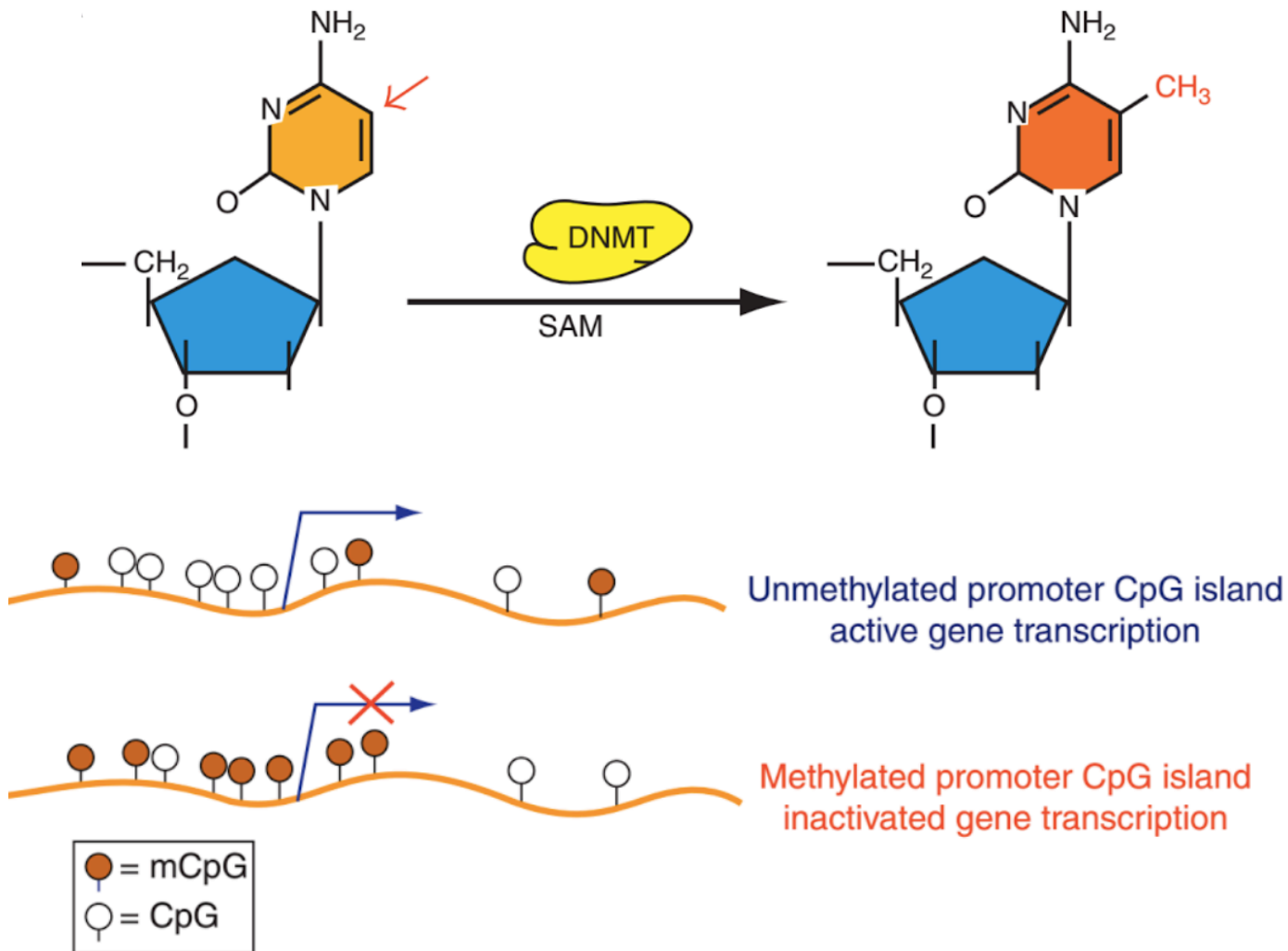


Cytosine

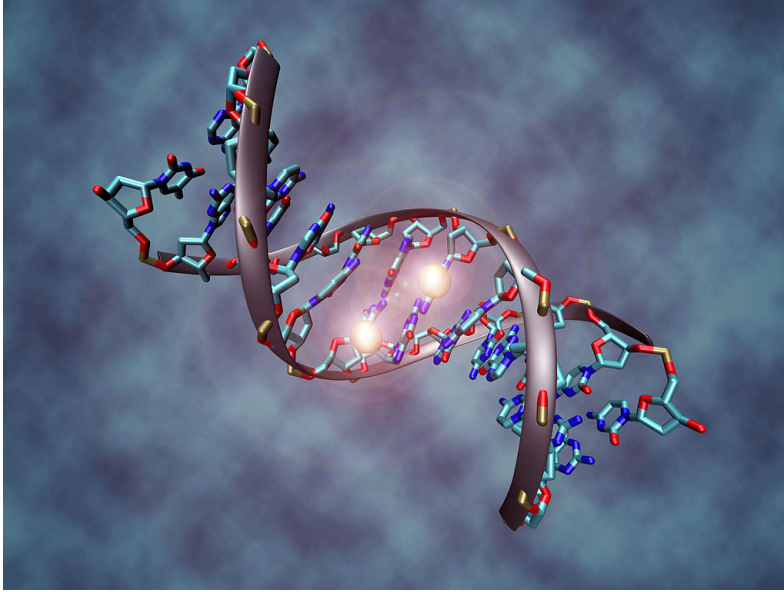


methylated Cytosine

DNA methyltransferase



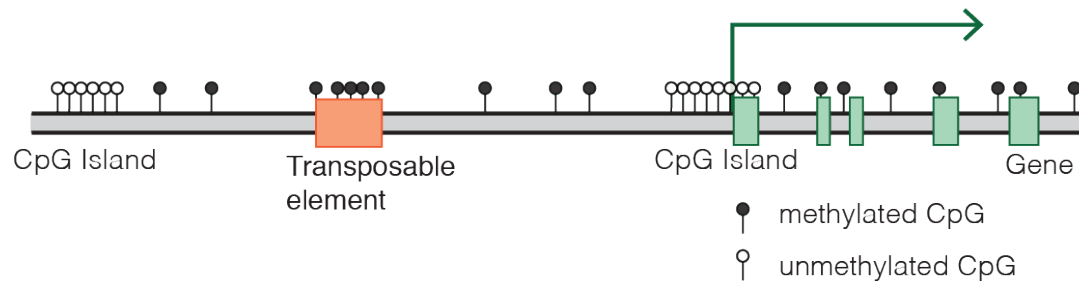
DNA methylation targets



- Vertebrates – 60-80% of CpG are methylated in somatic cells
- DNA methylation appears as default state
- Methylated C residues spontaneously deaminate to form T residues
- CpG to TpG

DNA methylation targets

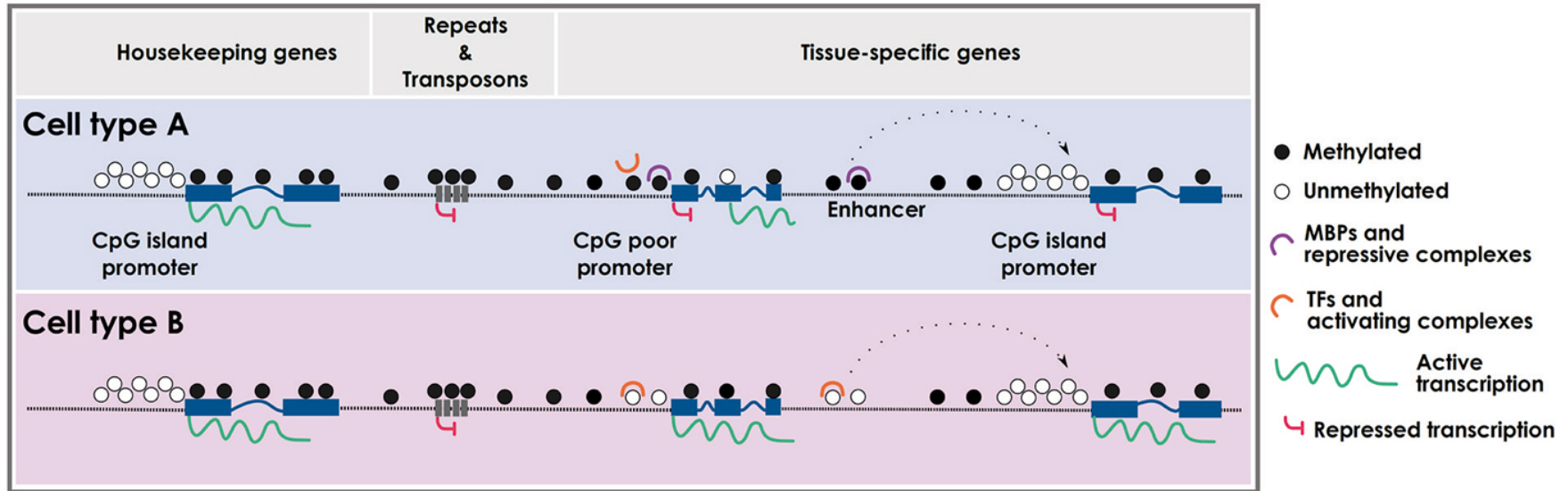
Typical mammalian DNA methylation landscape



CpG islands:

- Length greater than 200bp
- G+C content above 50%
- ~ 25,000 of CpG islands
- ~75% being less than 850bp long
- ~50% being located in promoter regions
- ~25% being located within gene bodies
- 60% to 70% of genes have an island in the promoter
- Unmethylated and enriched in chromatin modification H3K4

Repression of CpG-dense promoters



- CpG-dense promoters of actively transcribed genes are never methylated
- CpG-poor promoters – DNA methylation function is unclear
- Physical disturbance (TF-DNA)
- Bound by proteins known as methyl-CpG-binding domain proteins (MBDs)

Methyl-CpG-binding domain



Crystal structure of methyl CpG Binding Domain of MBD4 in complex with the 5mCG/TG sequence

- Effects of DNA methylation are mediated
- Specific domain of ~70 residues
- Linked to other domains that recruit:
 - histone deacetylase complexes
 - chromatin remodeling factors

Journal List > Nucleic Acids Res > v.21(21); 1993 Oct 25 > PMC311401

Nucleic Acids Research

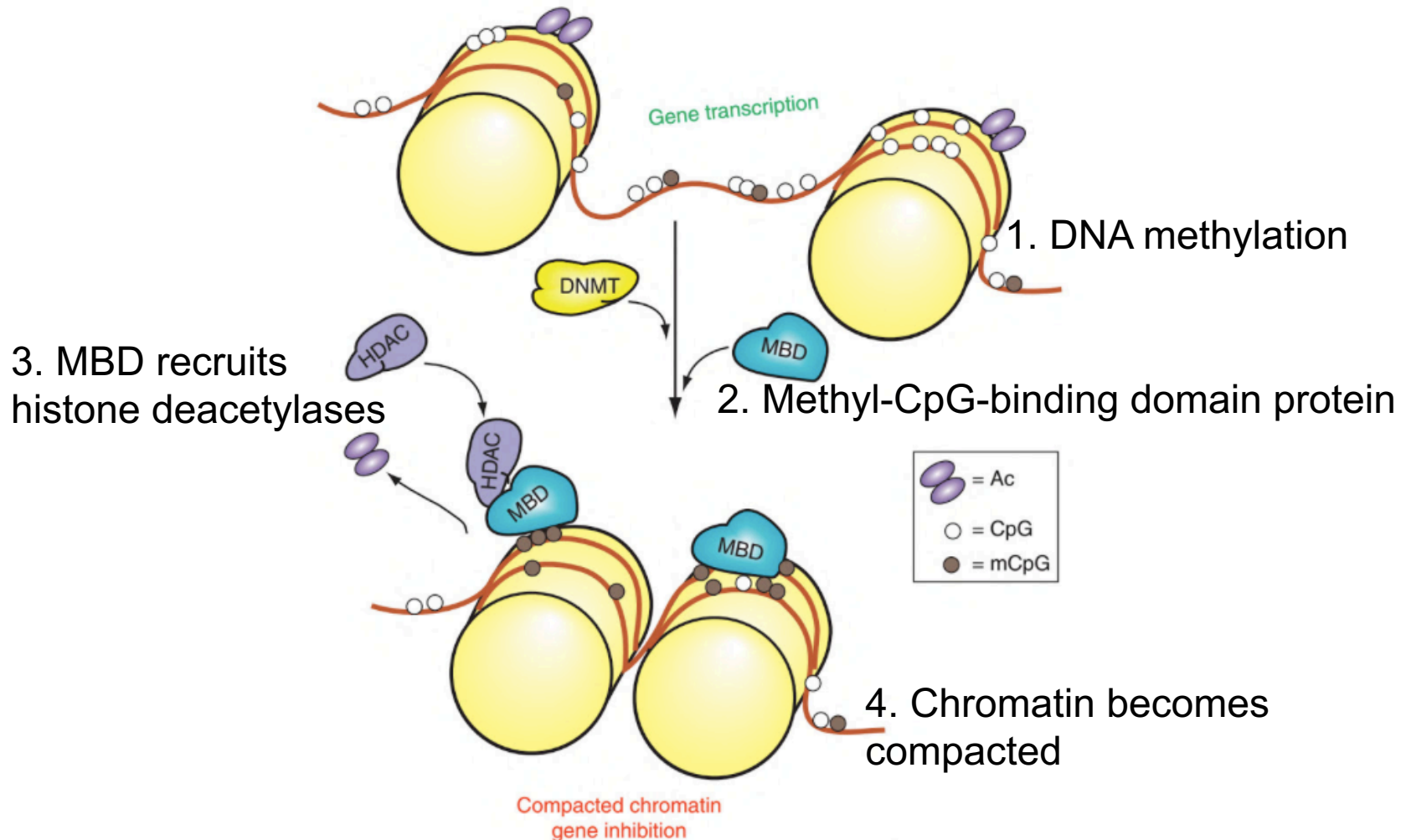
[Nucleic Acids Res.](#) 1993 Oct 25; 21(21): 4886-4892.

PMCID: PMC311401










Dissection of the methyl-CpG binding domain from the chromosomal protein MeCP2.

[X Nan](#), [R R Meehan](#), and [A Bird](#)

Methyl-CpG-binding domain



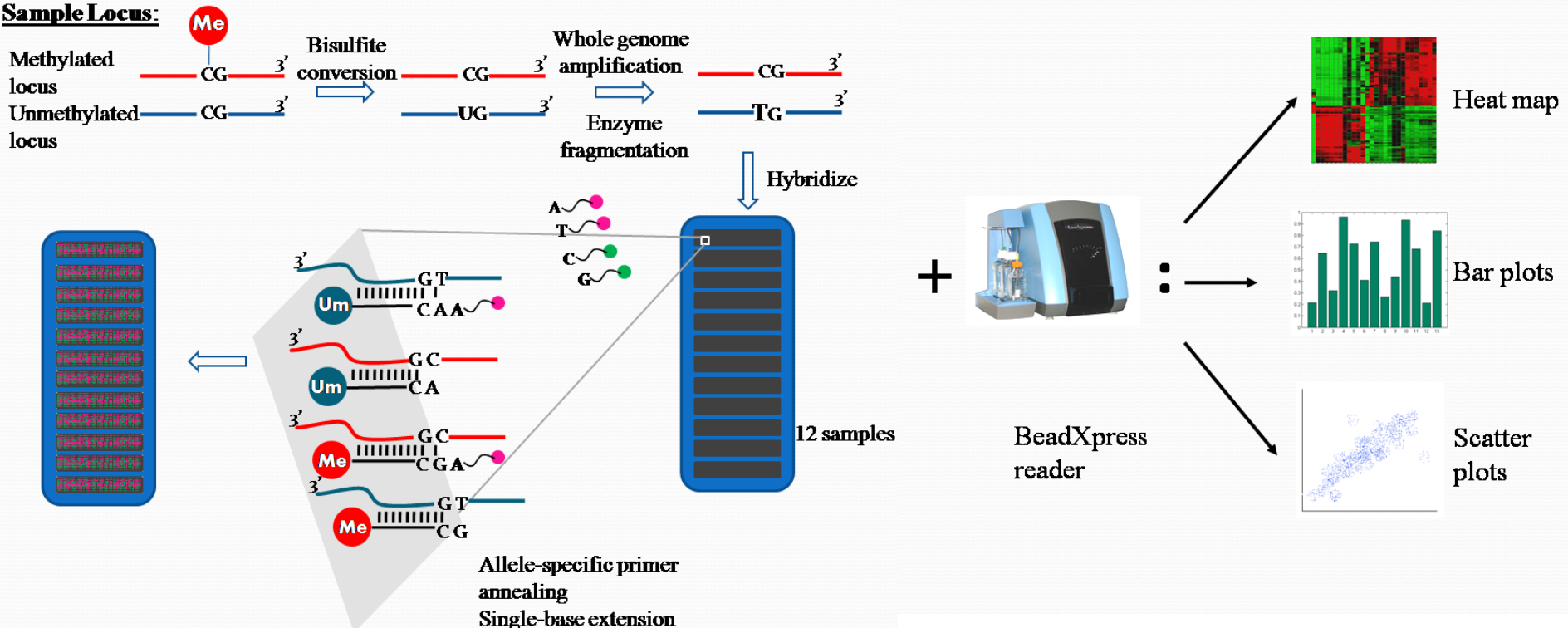
Kaiso-like family of proteins binds by zinc-finger motifs to methylated DNA

-  Repress gene transcription
-  Essential for normal development
-  Associated with a number of key processes:
 -  genomic imprinting,
 -  chromatin structure,
 -  X-chromosome inactivation,
 -  repression of transposable elements, repression of CpG-dense promoters, methylation of the gene body of highly transcribed genes
 -  aging,
 -  carcinogenesis.

Methods

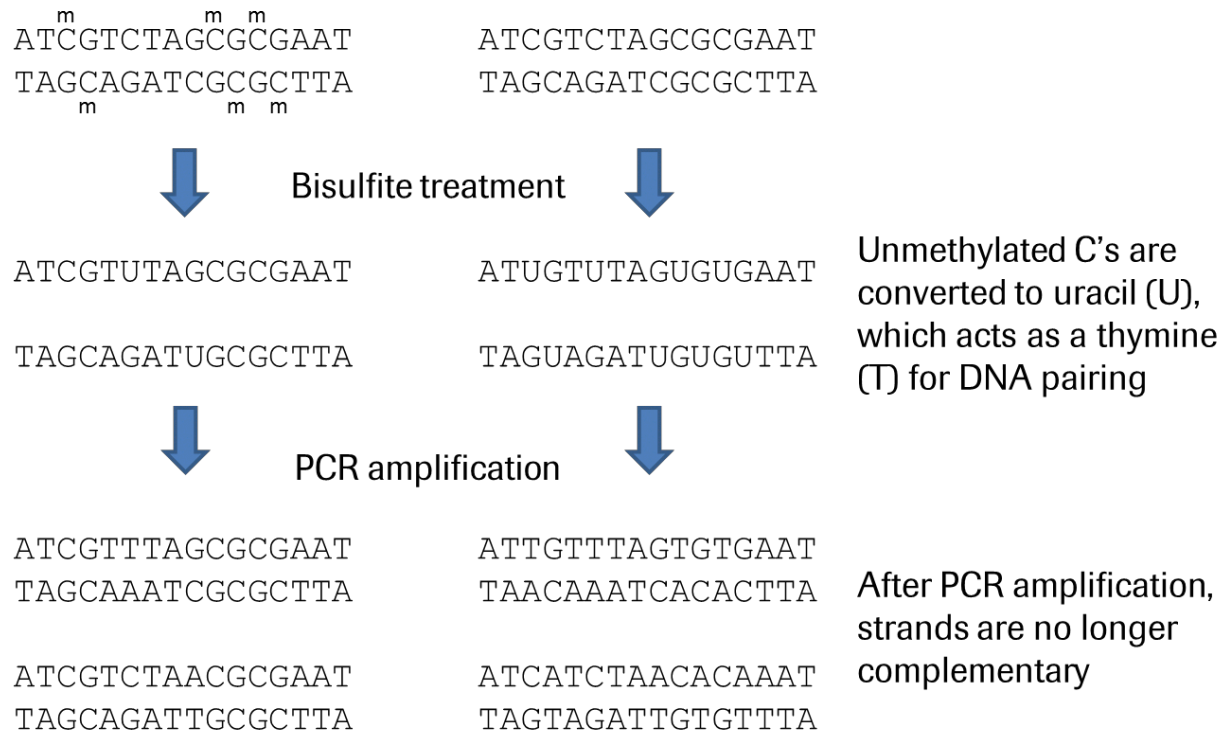
- The Illumina Methylation Assay using the Infinium I platform uses 'BeadChip' technology. The chip contains 27,578 individual CpG sites, spread across 14,495 genes.
- Illumina Infinium 450k Human Methylation Assay. Covers more than 485,000 methylation sites per sample

Sample Locus:



Methods

- NimbleGen SeqCap Epi target enrichment data
 - Over 5.5 million methylation sites per sample
 - Capture size of 80.5 Mb



DNA methylation in cancer

Normal/healthy regulation of gene expression to disease pattern

CpG island methylation is inherited from one cell division to the next through the DNA methylation maintenance machinery.

Colorectal cancers typically have:

- 3 to 6 driver mutations

- 33 to 66 passenger mutations

- 600-800 genes are transcriptionally silenced by CpG island methylation

- MGMT* repair gene

- PMS2* needs *MLH1* for stabilization

Promoters close to TSS (70%),

Distal promoters i.e. DNA repair gene *ERCC1* (5,400bp)

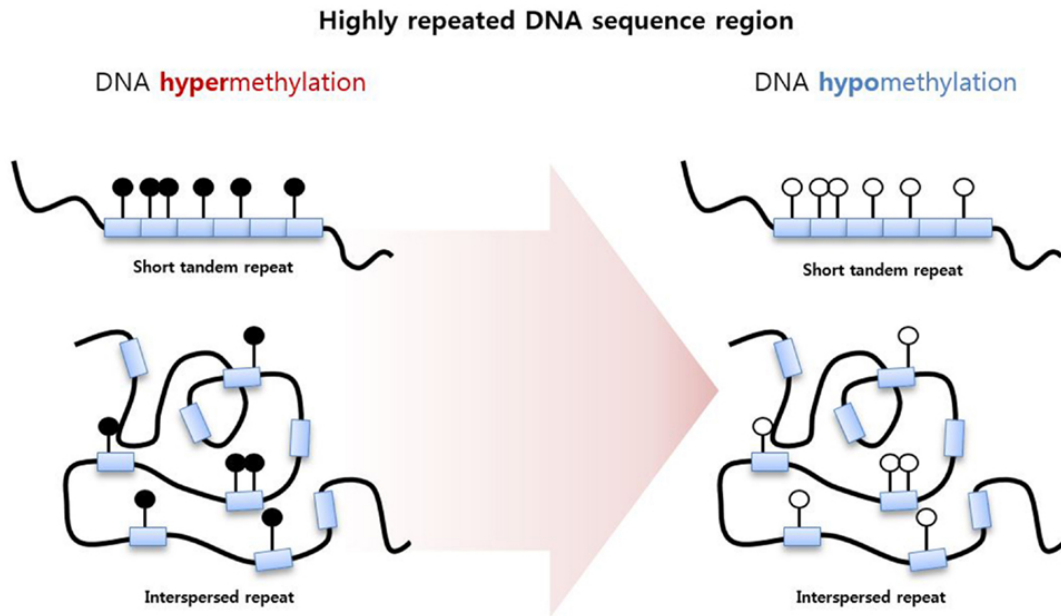
CpG frequently present in noncoding functional RNAs

DNA repair genes

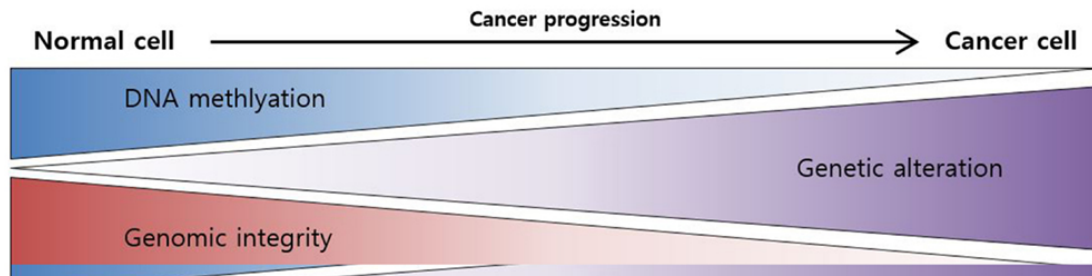
DNA methylation in cancer

Methylome changes:
Hypo- and hypermethylation, both observed in cancer

Suppressor
of tumorigenesis



A global
decrease in
Methylated
CpG is observed



Hypermethylation

Table 2.1. Examples of Genes Silenced by CpG Island Promoter Hypermethylation in Most Frequently Reported Cancer Types

Gene	Function	Breast cancer	Lung cancer	Prostate cancer	Leukemia/ lymphomas	Colon cancer
APC	Antagonist of the Wnt signaling pathway involved in cell migration and adhesion	X	X	X	–	X
BMAL1	Core component of the circadian clock	–	–	–	X	–
BRCA1	DNA repair double-stranded breaks, transcription	X	–	–	–	–
CDH1	E-cadherin, cell adhesion	X	X	X	X	X
CDH13	H-cadherin, cell adhesion	X	X	–	X	X
CDKN2A (<i>p16^{INK4a}</i>)	CDK4 inhibitor, control of cell-cycle G1 progression	X	X	X	X ^a	X
CDKN2B (<i>p15^{INK4b}</i>)	CDK4 and CDK6 inhibitor, control of cell-cycle G1 progression	–	–	–	X ^b	–
<i>p14^{ARF}</i>	Control of cell-cycle G1 progression, stabilizer of the tumor-suppressor protein p53	–	–	–	–	X
COX2	Cyclooxygenase, prostaglandin biosynthesis	–	–	–	–	X
CRBP1	Transport of retinol necessary for growth or differentiation of epithelial tissues	–	–	–	X	X
DAPK1	Positive mediator of γ -interferon induced programmed cell death	–	X	–	X	X
ESR1	Estrogen receptor, regulation of gene expression	X	X	X	X	–
GATA4	GATA family of zinc-finger transcription factors	–	–	–	–	X
GATA5	GATA family of zinc-finger transcription factors	–	–	–	–	X
GSTP1	Metabolism, detoxification, and elimination of genotoxic foreign compounds	–	X	X	–	–
HIC1	Transcription factor	X	X	–	X	X
IGFBP3	Insulin-like growth factor-binding protein	–	X	–	–	–
MGMT	DNA repair	–	X	–	X ^a	X
MLH1	DNA mismatch repair, DNA damage signaling	–	–	–	–	X
NORE1A	Ras effector homolog	–	X	–	–	–
PYCARD	TMS1/ASC, apoptotic signaling pathways	X	X	–	–	–
RARB2	Retinoic acid receptor, limits growth of many cell types by regulating gene expression	–	X	–	X	X
RASSF1A	Inhibit the accumulation of cyclin D1, involved in cell cycle arrest at G1/S phase transition and DNA repair	X	X	X	X	X
TLE1	Groucho homolog	–	–	–	X	–
TP73	p53 family of transcription factors, apoptotic response to DNA damage	–	–	–	X	–

X, reported frequent hypermethylation.

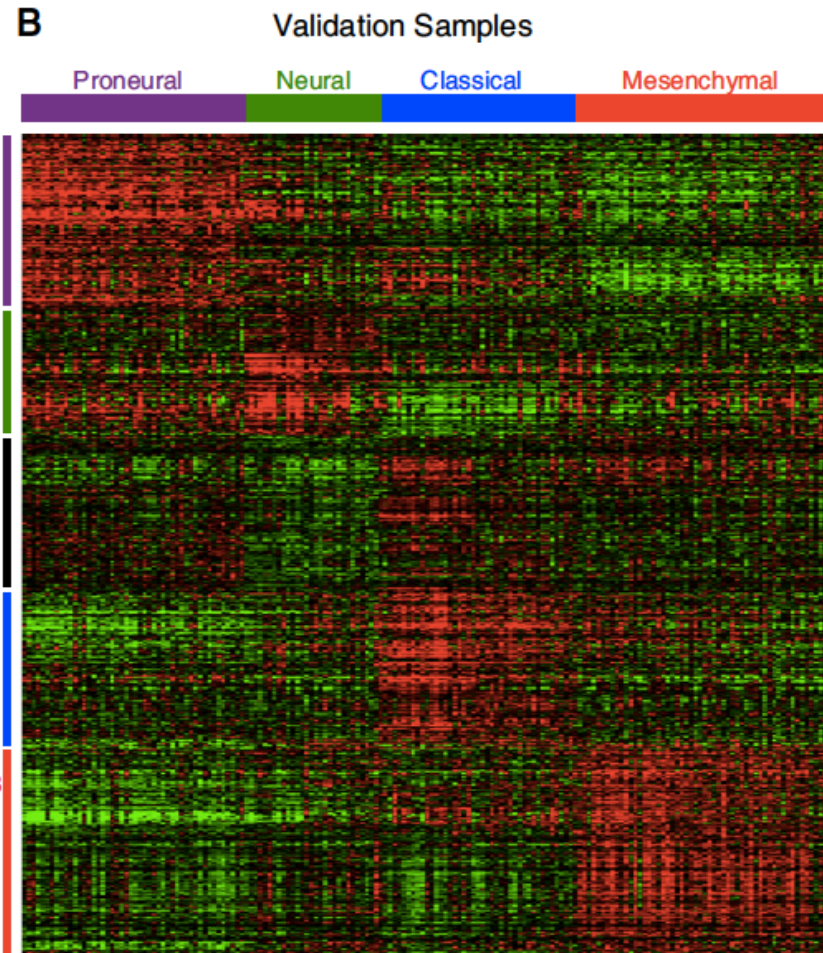
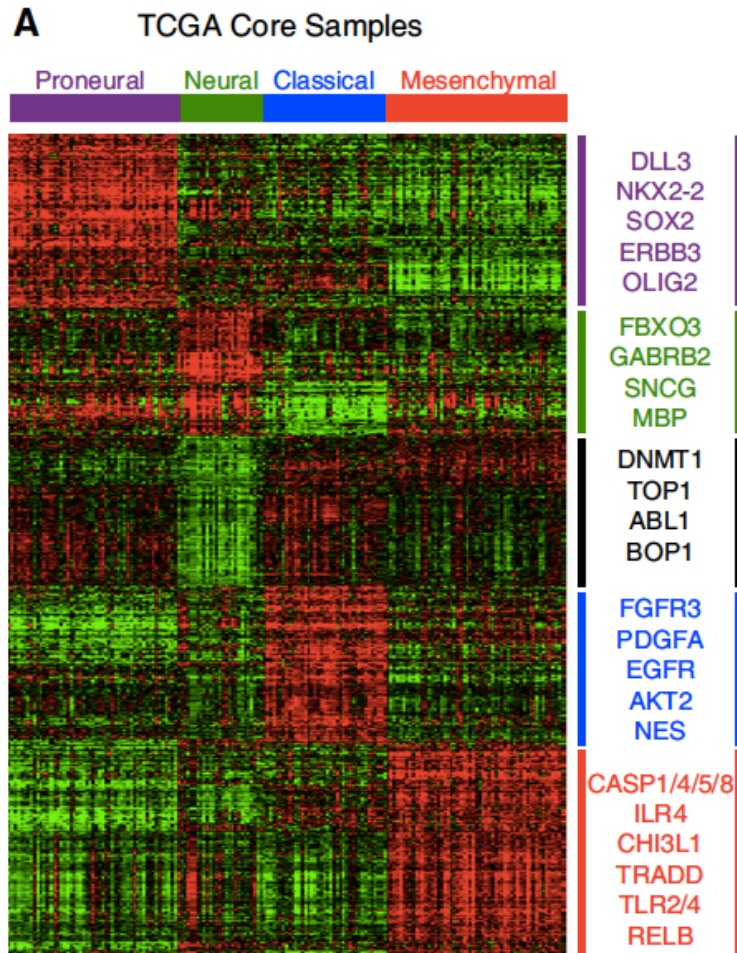
^aDNA methylation only in lymphomas.

^bDNA methylation only in leukemia.

Molecular patterns in human gliomas

Integrated Genomic Analysis Identifies Clinically Relevant Subtypes of Glioblastoma Characterized by Abnormalities in *PDGFRA*, *IDH1*, *EGFR*, and *NF1*

Roel G.W. Verhaak,^{1,2,17} Katherine A. Hoadley,^{3,4,17} Elizabeth Purdom,⁷ Victoria Wang,⁸ Yuan Qi,^{4,5} Matthew D. Wilkerson,^{4,6} C. Ryan Miller,^{4,6} Li Ding,⁹ Todd Golub,^{1,10} Jill P. Mesirov,¹ Gabriele Alexe,¹ Michael Lawrence,^{1,2} Michael O'Kelly,^{1,2} Pablo Tamayo,¹ Barbara A. Weir,^{1,2} Stacey Gabriel,¹ Wendy Winckler,^{1,2} Supriya Gupta,¹ Lakshmi Jakkula,¹¹ Heidi S. Feller,¹¹ J. Graeme Hodgson,¹² C. David James,¹² Jann N. Sarkaria,¹³ Cameron Brennan,¹⁴ Ari Kahn,¹⁵ Paul T. Spellman,¹¹ Richard K. Wilson,⁹ Terence P. Speed,^{1,16} Joe W. Gray,¹¹ Matthew Meyerson,^{1,2} Gad Getz,¹ Charles M. Perou,^{3,4,8} D. Neil Hayes,^{4,5,*} and The Cancer Genome Atlas Research Network



nature

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Article

Nature **462**, 739–744 (10 December 2009) | doi:10.1038/nature08617; Received 15 July 2009; Accepted 29 October 2009; Published online 22 November 2009

There is an Addendum (17 June 2010) associated with this document.

Cancer-associated IDH1 mutations produce 2-hydroxyglutarate

ARTICLE LINKS

- ▶ [Figures and tables](#)
- ▶ [Supplementary info](#)

Cancer Cell

Previews

IDH1 Mutations in Gliomas: When an Enzyme Loses Its Grip

Christian Frezza,¹ Daniel A. Tennant,¹ and Eyal Gottlieb^{1,*}

¹Cancer Research UK, The Beatson Institute for Cancer Research, Glasgow G61 1BD, UK

*Correspondence: e.gottlieb@beatson.gla.ac.uk

DOI 10.1016/j.ccr.2009.12.031

The most frequent mutations involve R132 (IDH1) and R172 (IDH2)

Significance of complete 1p/19q co-deletion, *IDH1* mutation and *MGMT* promoter methylation in gliomas: use with caution

Sandra HE Boots-Sprenger^{1,2}, Angelique Sijben^{3,4}, Jos Rijntjes¹, Bastiaan BJ Tops¹, Albert J Idema⁵, Andreana L Rivera^{6,7}, Fonnet E Bleeker⁸, Anja M Gijtenbeek², Kristin Diefes^{6,7}, Lindsey Heathcock⁷, Kenneth D Aldape⁶, Judith WM Jeuken^{1,9} and Pieter Wesseling^{1,10,11}

Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 8681–8686, July 1999
Medical Sciences

CpG island methylator phenotype in colorectal cancer

MINORU TOYOTA, NITA AHUJA, MUTSUMI OHE-TOYOTA, JAMES G. HERMAN, STEPHEN B. BAYLIN,
AND JEAN-PIERRE J. ISSA*

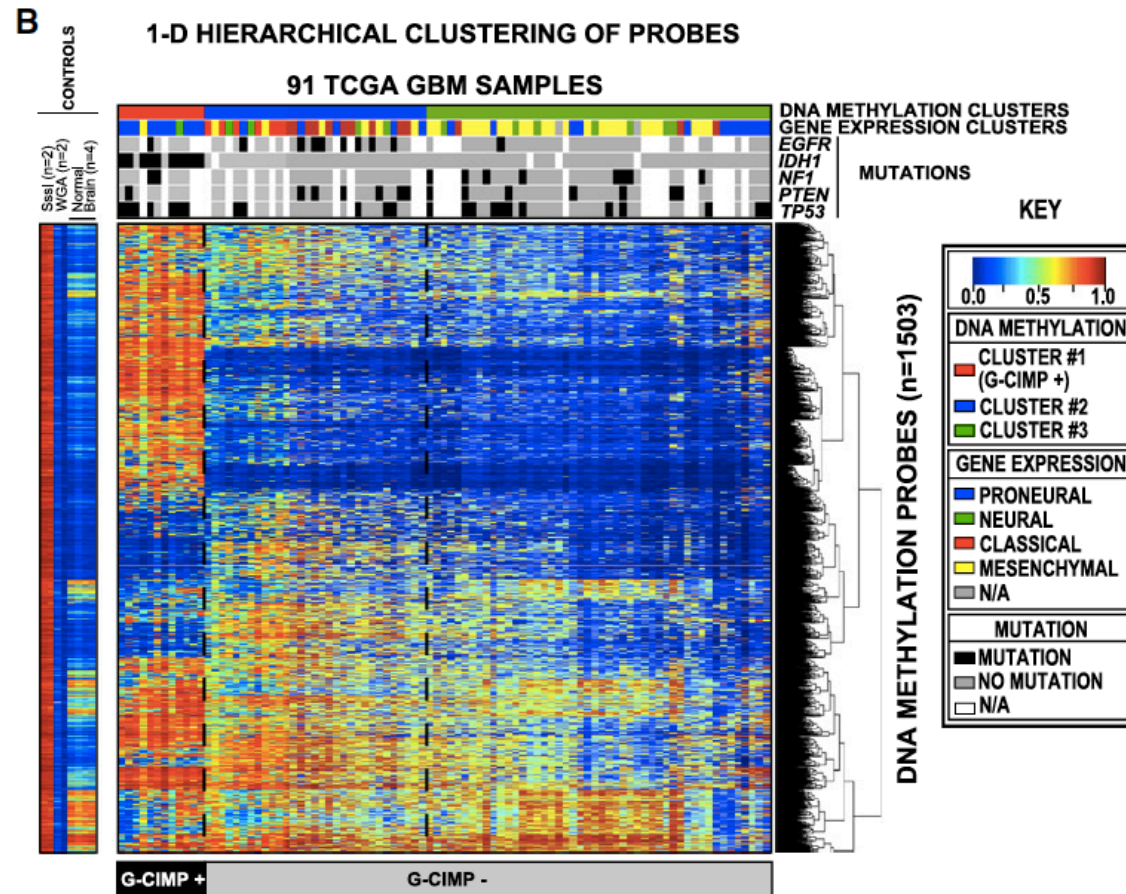
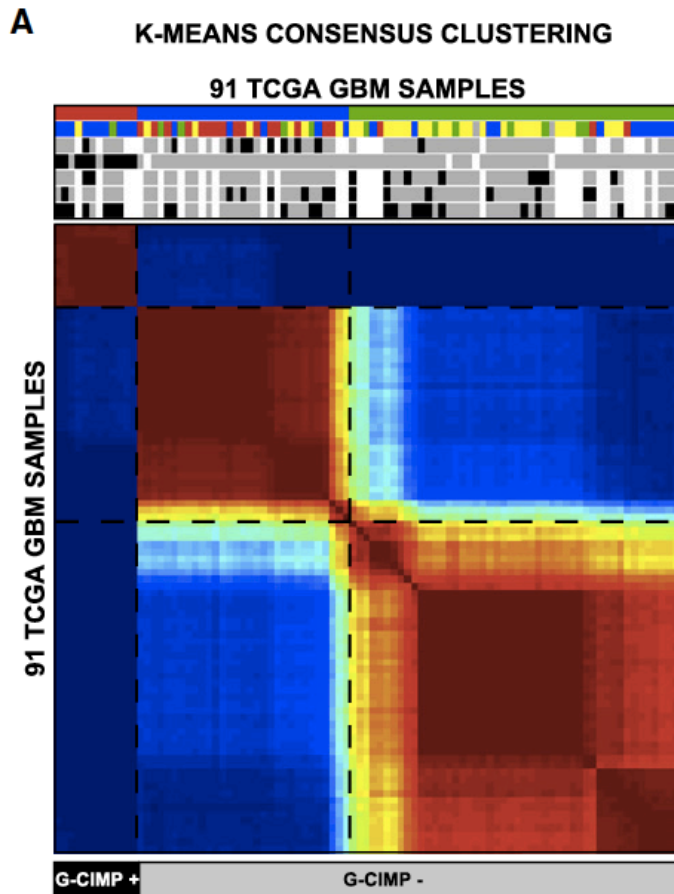
The Johns Hopkins Oncology Center, 424 North Bond Street, Baltimore, MD 21231

normal colon as an age-related phenomenon; and (ii) virtually all the other methylation events occur in a distinct subset of CRCs and adenomas which appear therefore to have a new phenotype, which we termed CpG island methylator phenotype (CIMP). These data shed additional light on the global patterns of CGI methylation in human cancer and delineate a distinct pathway involving tumor-suppressor gene hypermethylation in the evolution of CRC.

Glioma-CpG island methylator phenotype

Identification of a CpG Island Methylator Phenotype that Defines a Distinct Subgroup of Glioma

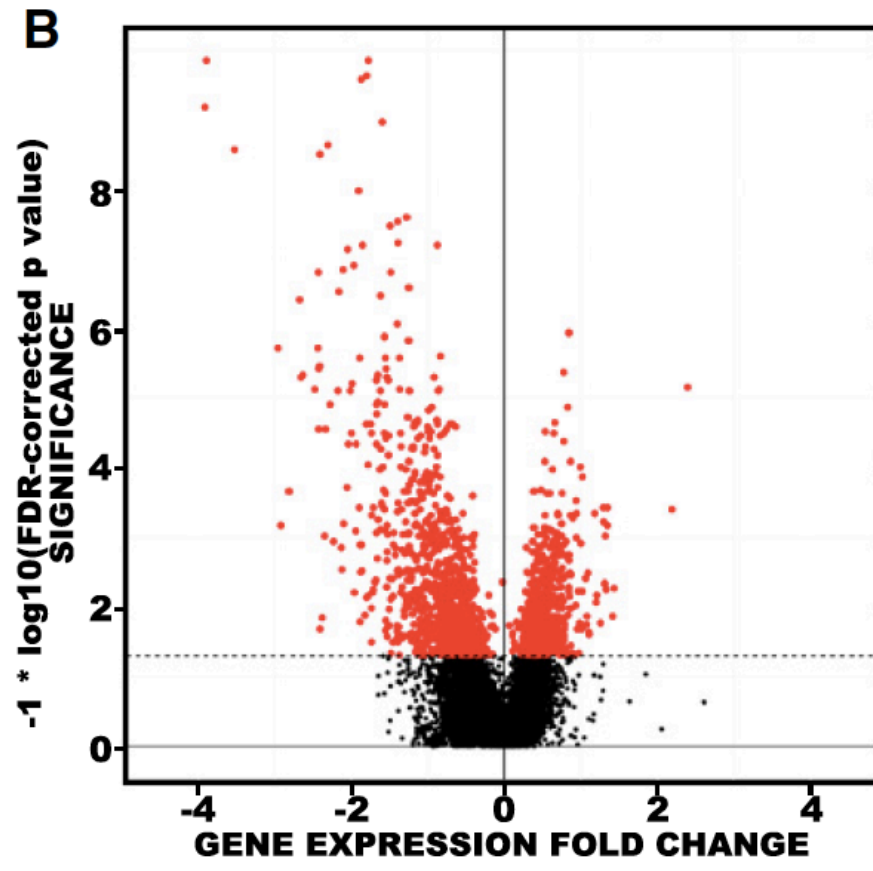
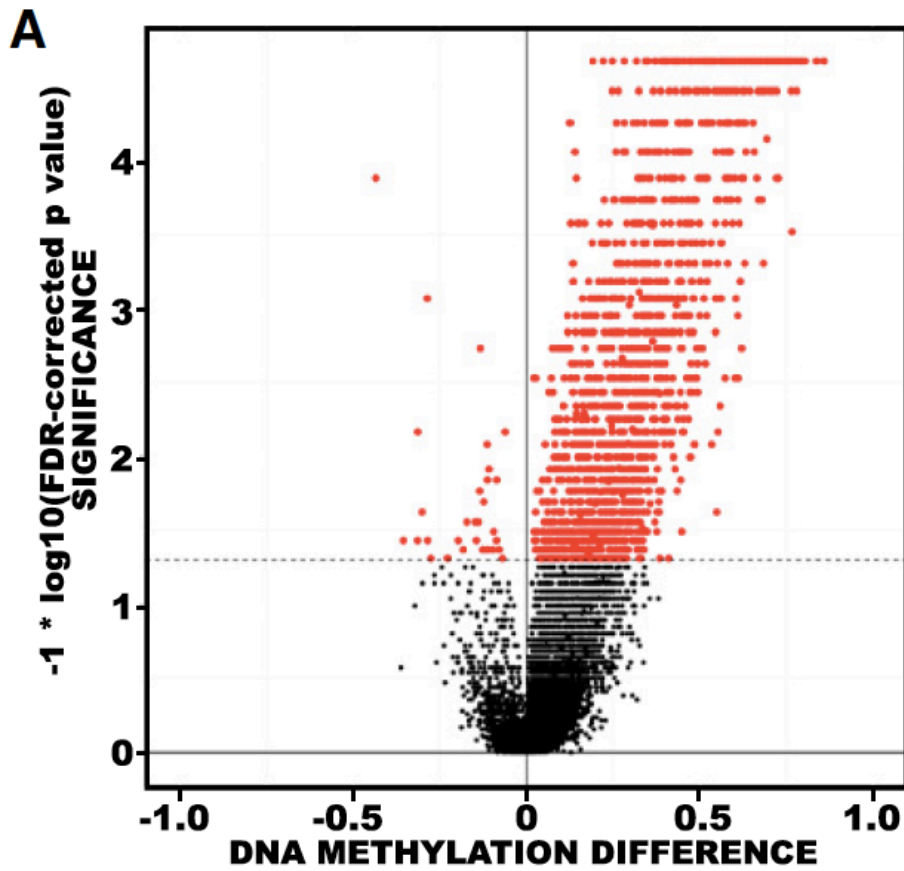
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Glioma-CpG island methylator phenotype

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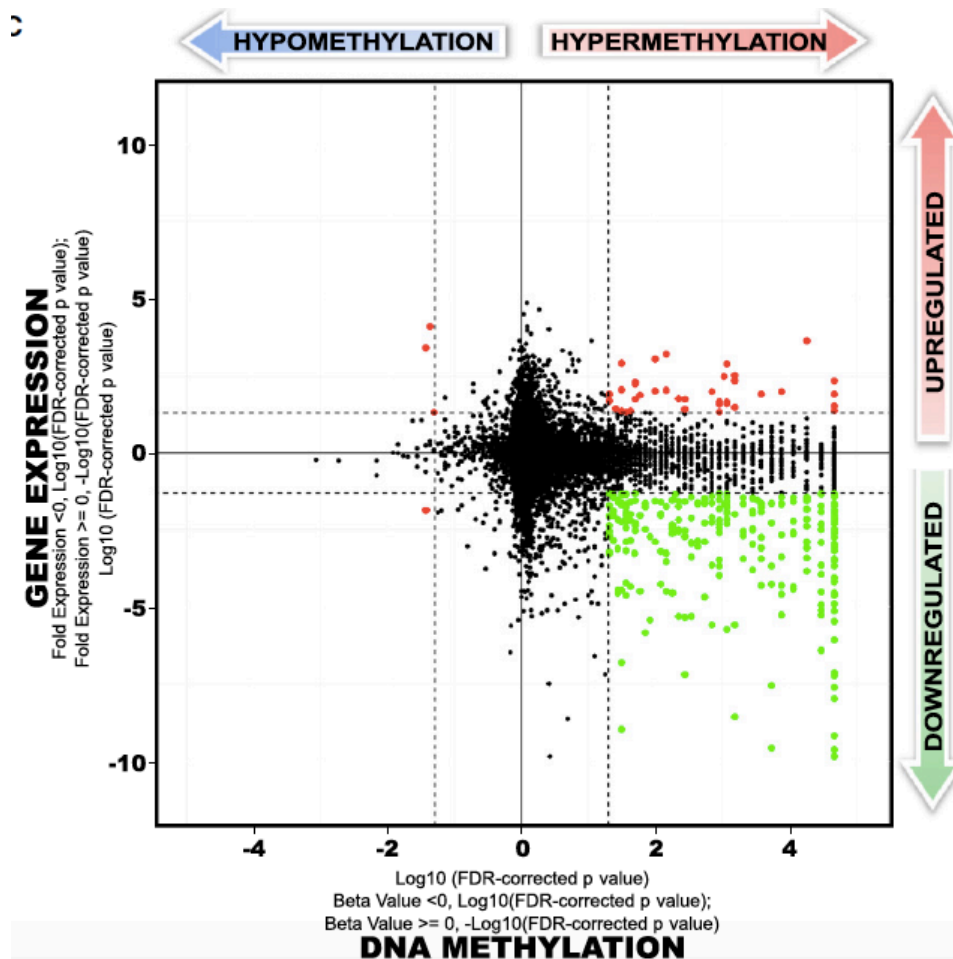
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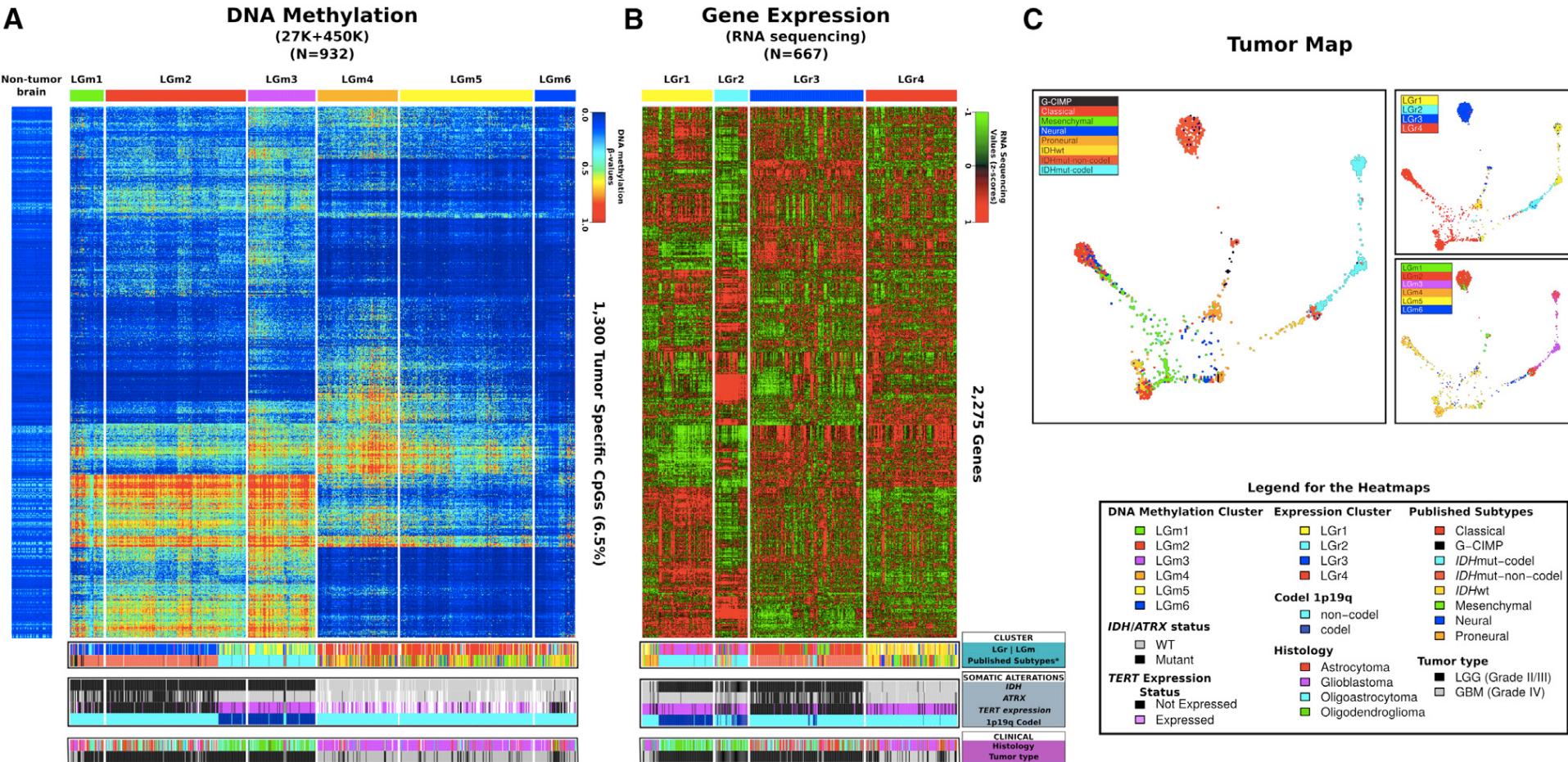
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Molecular patterns in human gliomas

Molecular Profiling Reveals Biologically Discrete Subsets and Pathways of Progression in Diffuse Glioma

Michele Ceccarelli,^{1,2,24} Floris P. Barthel,^{3,4,24} Tathiane M. Malta,^{5,6,24} Thais S. Sabedot,^{5,6,24} Sofie R. Salama,⁷ Bradley A. Murray,⁸ Olena Morozova,⁷ Yulia Newton,⁷ Amie Radenbaugh,⁷ Stefano M. Pagnotta,^{2,9} Samreen Anjum,¹ Jiguang Wang,¹⁰ Ganiraju Manyam,³ Pietro Zoppoli,¹⁰ Shiyun Ling,³ Arjun A. Rao,⁷ Mia Grifford,⁷ Andrew D. Cherniack,⁸ Hailei Zhang,⁸ Laia Poisson,¹¹ Carlos Gilberto Carloti, Jr.,^{5,6} Daniela Pretti da Cunha Tirapelli,^{5,6} Arvind Rao,³ Tom Mikkelsen,¹¹ Ching C. Lau,^{12,13} W.K. Alfred Yung,³ Raul Rabadan,¹⁰ Jason Huse,¹⁴ Daniel J. Brat,¹⁵ Norman L. Lehman,¹⁶ Jill S. Barnholtz-Sloan,¹⁷ Siyuan Zheng,³ Kenneth Hess,³ Ganesh Rao,³ Matthew Meyerson,^{5,18} Rameen Beroukhi,^{5,19,19} Lee Cooper,¹⁹ Rehan Akbani,³ Margaret Wrensch,²⁰ David Haussler,⁷ Kenneth D. Aldape,²¹ Peter W. Laird,²² David H. Gutmann,²³ TCGA Research Network, Houtan Noushmehr,^{5,6,25,*} Antonio Iavarone,^{10,25,*} and Roel G.W. Verhaak^{3,25,*}



Glioma subtypes

DNA Methylation level
(cartoon representation)

Survival
months

Grade

Age
years

Histology

AS: Astrocytoma
GBM: Glioblastoma
OA: Oligoastrocytoma
OD: Oligodendroglioma

LGG/GBM subtypes

CL: Classical
MES: Mesenchymal
NEU: Neural
PN: Proneural

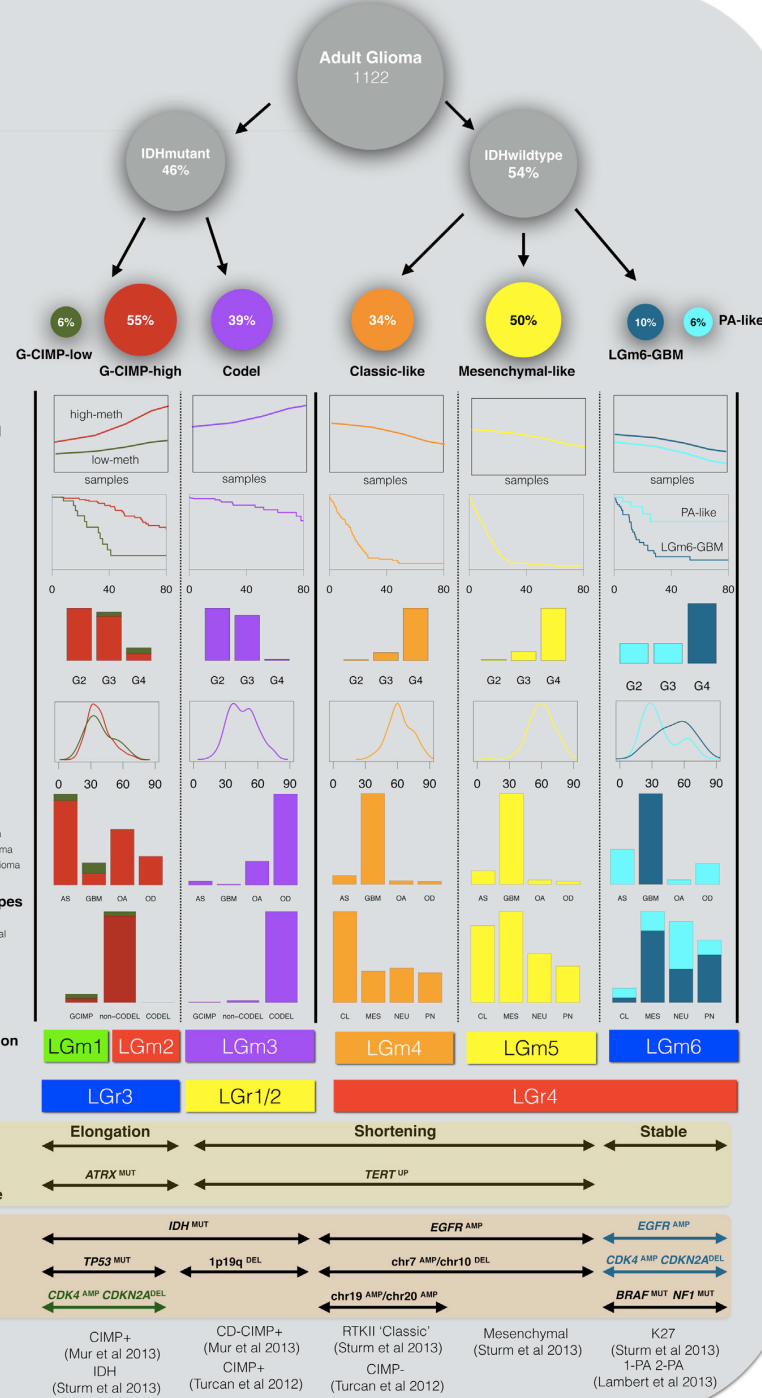
DNA Methylation Cluster

RNA Cluster

Telomere Length
Telomere Maintenance

Biomarkers

Validation datasets



DNA Methylation Signatures Identify Biologically Distinct Subtypes in Acute Myeloid Leukemia

Maria E. Figueroa,¹ Sanne Lugthart,⁵ Yushan Li,¹ Claudia Erpelinck-Verschueren,⁵ Xutao Deng,² Paul J. Christos,³ Elizabeth Schifano,⁷ James Booth,⁷ Wim van Putten,⁶ Lucy Skrabanek,^{2,4} Fabien Campagne,^{2,4} Madhu Mazumdar,³ John M. Greally,⁸ Peter J.M. Valk,⁵ Bob Löwenberg,⁵ Ruud Delwel,^{5,*} and Ari Melnick^{1,*}

Szyf *Genome Medicine* 2012, 4:26
<http://genomemedicine.com/content/4/3/26>



REVIEW

DNA methylation signatures for breast cancer classification and prognosis

Moshe Szyf*

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Establishment of a Strong Link Between Smoking and Cancer Pathogenesis through DNA Methylation Analysis

Received: 28 September 2016
Accepted: 3 April 2017
Published online: 12 May 2017

Yunlong Ma^{1,2} & Ming D. Li^{1,2,3}

Review

DNA Methylation in Cancer and Aging

Michael Klutstein, Deborah Nejman, Razi Greenfield, and Howard Cedar

Cancer
Research