Genetic variants influencing global DNA methylation and TGCT risk

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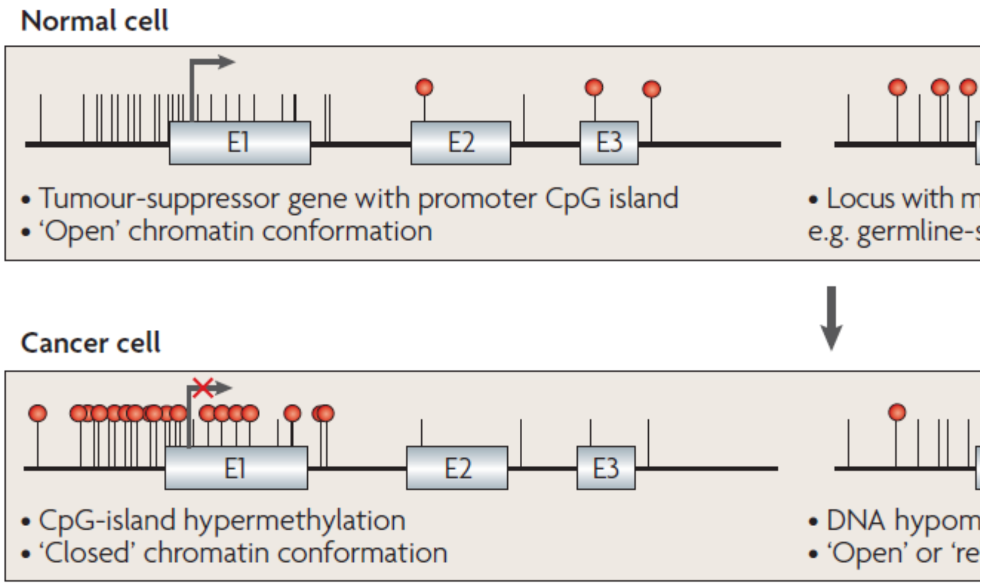
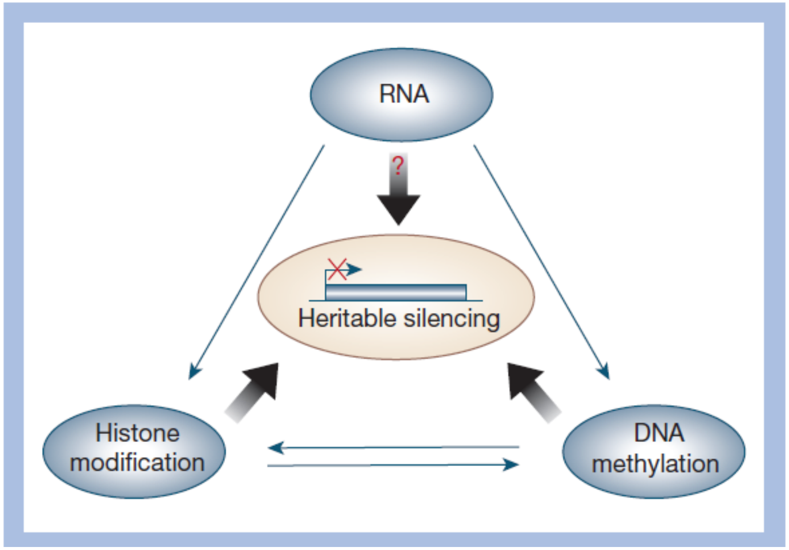
Background

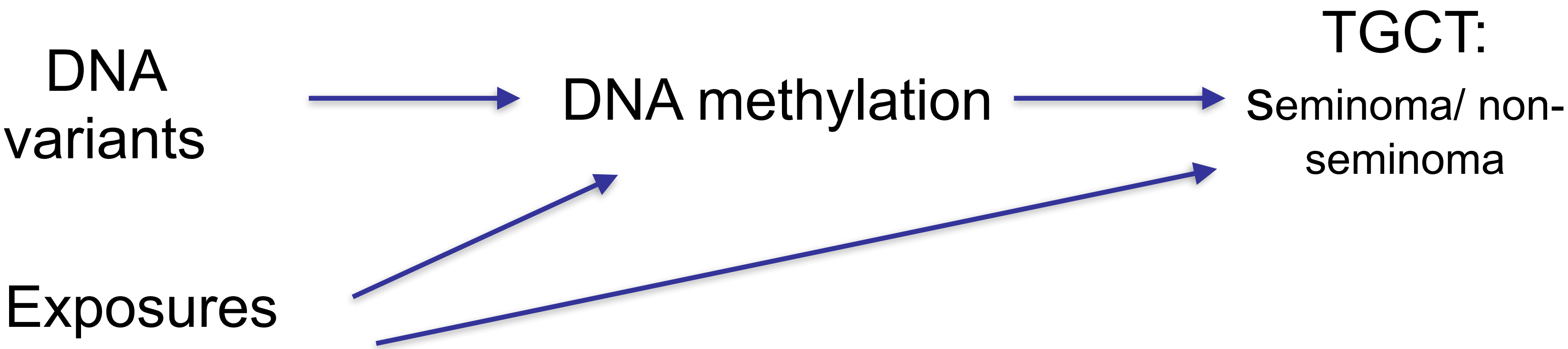
- Epigenetic programming is crucial for germ cell development
- Epigenetic alterations, including aberrant DNA-methylation, is frequently found in tumors including TGCT



Egger et al. Nature 2004



Model



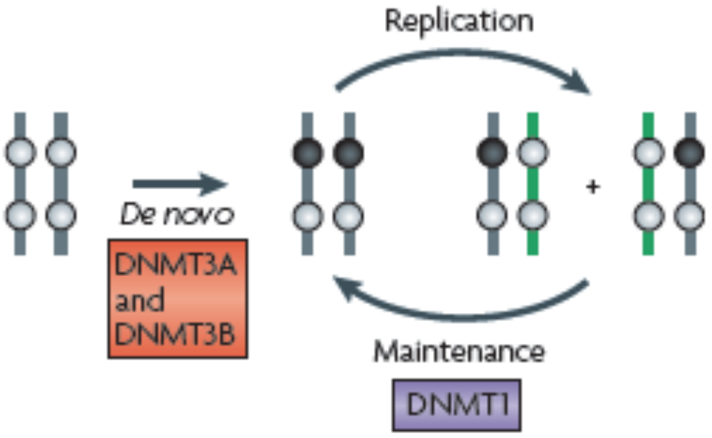
DNA variants

DNA methylation

TGCT:

Seminoma/ non-seminoma

Exposures



Jones, Nat Rev Genet 2009

DNA methylation process requires a large number of factors, involved in different mechanisms:

1) DNA methylation machinery
- Writers of the methylation marks (DNA methyltransferases): enzymes that catalyze the transfer of a methyl group typically to the cytosine of a CpG site of DNA

- Erasers of the methylation marks (DNA demethylases): enzymes that remove methyl groups from nucleic acids

- Readers of the methylation marks (methyl-CpG-binding proteins): proteins that bind to DNA that contains one or more symmetrically methylated CpGs; they directly interpret the DNA methylation marks on a gene

2) Folate-dependent one-carbon pathway
Metabolic pathway that is necessary for the synthesis of S-adenosylmethionine (SAM), the major methyl donor for all cellular methylation reactions

Selection of candidate genes involved in DNA methylation process

-BioCarta (http://www.biocarta.com/genes/index.asp)
→ **Mechanisms of transcriptional repression by DNA methylation**

- Reactome (http://www.reactome.org)
→ **Metabolism of folate and pterines (Homo sapiens)**
→ **Epigenetic regulation of gene expression**
    → **TET1,2,3 and TDG demethylate DNA**
    → **DNA methylation**

- KEGG pathway (http://www.genome.jp/kegg/kegg2.html)
→ **One carbon pool by folate**

- NCI-PID (http://pid.nci.nih.gov/index.shtml)
→ **Mechanisms of transcriptional repression by DNA methylation**
→ **Metabolism of folate and pterines**

42 candidate genes

Selection of candidate genes involved in DNA methylation process

Check of the function of each of the 42 candidates to select only those definitely involved in the DNA methylation process

- UniProtKB (http://www.uniprot.org/help/uniprotkb)

28 candidate genes selected for genotyping analyses

Selection of the TagSNPs using Haploview 4.2

[minor allele frequency (MAF) of >5%, r^2 value of >0.8, expansion of the 5′- and 3′-untranslated flanking regions (+/-10 kb)]

273 SNPs

Selected tagSNPs (1)

Pathway	Gene name	TagSNP number
DNA methylation machinery-WRITER	DNMT1	9
DNA methylation machinery-WRITER	DNMT3A	18
DNA methylation machinery-WRITER	DNMT3B	9
DNA methylation machinery-WRITER	DNMT3L	16
DNA methylation machinery-READER	MBD1	10
DNA methylation machinery-READER	MBD2	7
DNA methylation machinery-READER	MBD3	4
DNA methylation machinery-READER	MBD3L1	5
DNA methylation machinery-READER	MBD4	5
DNA methylation machinery-READER	MECP2	4
DNA methylation machinery-READER	UHRF1	13
DNA methylation machinery-READER	ZBTB38	4
DNA methylation machinery-READER	ZBTB4	7
DNA methylation machinery-ERASER	TET1	8
DNA methylation machinery-ERASER	TET2	9
DNA methylation machinery-ERASER	TET3	11
DNA methylation machinery-ERASER	GADD45B	8

Selected tagSNPs (2)

Pathway	Gene name	TagSNP number
One-carbon metabolism	MTR	8
One-carbon metabolism	MTHFR	16
One-carbon metabolism	CBS	17
One-carbon metabolism	AHCY	4
One-carbon metabolism	MTRR	26
One-carbon metabolism	BHMT	14
One-carbon metabolism	CTCF	3
One-carbon metabolism	MAT1A	14
One-carbon metabolism	MAT2A	5
One-carbon metabolism	MAT2B	11
One-carbon metabolism	SHMT1	8

Statistical analysis

- Single SNP analysis (Q-Q plot, adjustment for multiple comparisons)
- gene-based approach —> adaptive rank truncated product (ARTP): (i) gene-level statistics, (ii) pathway
- adjustment for matching variables, study, cryptorchidism (and restriction to subjects who has normal descent), population stratification
- Comparison of seminomas vs. non-seminomas, and, whenever possible, nonseminoma subtypes
- Analysis of heterogeneity across studies: US vs. Europe and high incidence countries vs. medium-low incidence countries

Function

- involvement in development and progression of other cancer types
- data on the role of identified SNPs in DNA methylation and expression
- (other)