ROLE AND SIGNIFICANCE OF DNA METHYLATION IN NORMAL AND ABNORMAL HEMATOPOIESIS

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Abstract

Data suggested that cancer appears to be a process that is fuelled both by genetic alterations and by epigenetic mechanisms. Epigenetics refer to the study of changes in gene expression that can be mitotically inherited, but is not associated with the changes in the coding sequence of the affected genes. The DNA methylation in the promoter regions is a powerful epigenetic mechanism for the suppression of gene activity. Hypomethylation and hypermethylation are the two kinds of methylation defect that is observed in a wide variety of malignancies. Hypomethylation is common in solid tumors such as metastatic hepatocellular cancer, in cervical cancer, prostate tumors, and also in hematological malignancies such as B-cell chronic lymphocytic leukemia. A large number of genes involving fundamental cellular pathways may be affected by aberrant CpG island methylation in association with transcriptional silencing in virtually all tumor types.

Key words: DNA methylation, CpG islands, Epigenetics in hematological malignancies, Hypomethylation, Hypermethylation

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List of abbreviations

- AICAR ---------- 5-aminoimidazole-4-carboxamide ribonucleotide
- AICARFT ---------- 5-aminoimidazole-4-carboxamide ribonucleotide transformylase
- AIF ----------Apoptosis inducing factor
- APC ---------- Adenomatous polyposis coli
- ARJP ---------- Autosomal recessive juvenile parkinsonism
- BRCA1 ----------Breast cancer 1
- CBS ---------- Cystathionine beta-synthase
- CDKN2A/p16 ---------- Cyclin-dependent kinase 2A
- CHL ---------- Classical Hodgkin lymphoma
- CH_3 ---------- Methyl group
- DAPK1 ---------- Death-associated protein kinase 1
- DHF ---------- Dihydrofolate
- DHFR ---------- Dihydrofolate reductase
- DISC ---------- Death-inducing signal complex
- DLBCL ---------- Diffuse large B-cell lymphoma
- DNA-MTase - *DNMT*----- DNA methyltransferases
- dUMP ---------- deoxyuridine monophosphate
- dTMP ---------- deoxythymidine monophosphate
- ER ---------- Estrogen receptor
- FasL ---------- Fas ligand
- FCL ---------- Follicular center lymphoma
- FADD ---------- Fas-associated death domain protein
- 5-FU ---------- 5-fluorouracil
- GAR ---------- Glycinamide ribonucleotide
- GSTP1 ---------- Glutathione S-transferase Pi 1
- H3 ---------- Histone
- HDAC ---------- Histon deacetylase
- hFR ---------- human Folate Receptor
- hMLH1 ---------- human Mut L Homologue 1
- Igf2 ---------- Insulin-like growth factor
- LOH ---------- Loss of heterozygosity
- MBD ---------- Methyl CpG binding domain
- MGMT, O-6 ---------- Methylguanine-DNA methyltransferase.
- MMR^+/MET^- ---------- Methylation proficiency
- MRD ---------- Minimal residual disease
- MS ---------- Methionine synthase
- MSP ---------- Methylation-specific PCR)
- MTHFR ---------- 5,10-Methylenetetrahydrofolate reductase
- NHL ---------- Non-Hodgkin's lymphoma.

- PCNA ---------- Proliferating cell nuclear antigen
- PEL ---------- primary effusion lymphoma
- PML-RAR ---------- Promoting promyelocytic leukemia retinoic acid receptor
- pRb ---------- Protein retinoblastoma
- RASSF1A ---------- Ras association domain family member 1
- Rb ---------- Retinoblastoma
- RFC ---------- Reduced folate carrier
- RLGS ---------- Restriction Landmark Genomic Scanning
- ROS ---------- Reactive oxygen species
- SAH ---------- S-adenosylhomocysteine
- SAM ---------- S-adensyl methionine
- TDG ---------- Thymine DNA glycosylase
- THF ---------- Tetrahydrofolate
- TKO ---------- Technical knock-out
- TRD ---------- Transcriptional repressor domain
- TS ---------- Thymidylate synthase
- TSA ---------- Trichostatin
- UDG ---------- Uracil DNA glycosylase
- VHL ---------- Von Hippel-Lindau transformylase
- X ---------- A variety of substrates for methylation

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Normal cell biology – An overview

(1) Normal DNA

Historical view

The modern era of molecular biology began in 1953, when James Watson and Francis Crick described the double-helical structure of DNA based on the analysis of X-ray diffraction patterns coupled with careful model building. A closer look at the "thread of life", as the DNA molecule is sometimes called shows why the discovery of its basic structure suggests its function ***(Lodish et al., 1997).***

The native state of DNA

DNA as described by ***Harvey et al (1997) and Micklos et al (2003)*** consists of two associated polynucleotide strands that wind together through space in a helical fashion which is often described as a double helix. The two sugar-phosphate backbones are on the outside of the double helix. And the bases project into the interior. The adjoining bases in each strand stack on top of each other in parallel planes (Fig. 1).

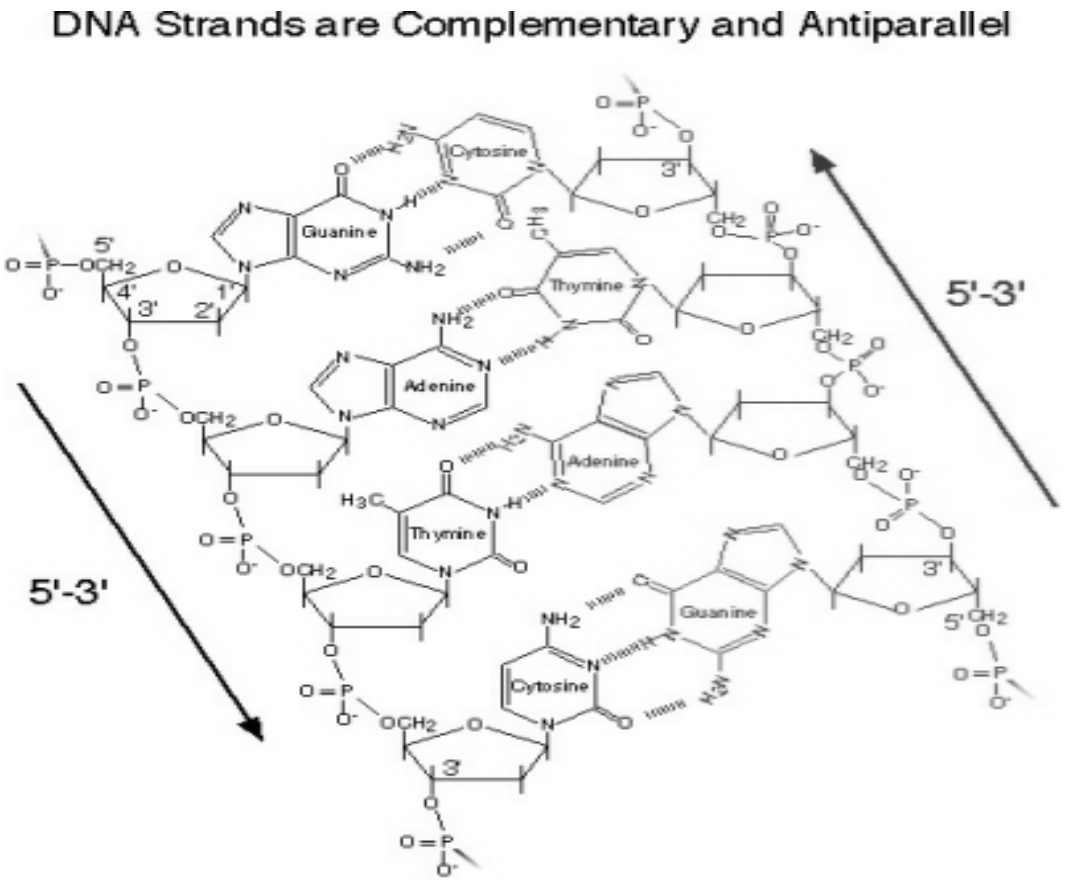

Fig. 1: Normal structure of DNA. ***(Harvey et al., 1997)***

The orientation of the two strands is antiparallel (their 5' ---- 3' directions are opposite); they are held together by the cooperative energy of many hydrogen bonds in addition to hydrophobic interactions. The opposite strands are held in precise register by a regular base pairing between the two strands: A is paired with T by two hydrogen bonds; G is paired with C by three hydrogen bonds. This base-pair complementarity is a consequences of the size, shape and chemical composition of the bases. Hydrophobic and Van der-Waals interactions between the stacked adjacent base pair also contribute significantly to the overall stability of the double helix. To maintain the geometry of the double helix, a larger purin (A or G) must pair with a smaller pyrimidine (C or T) in natural DNA it is almost always A with T and G with C. However, in theory and

in synthetic DNAs other interactions can occur. For example, a guanine (a purine) could theoretically form hydrogen bonds with a thymine (a pyrimidine), causing only a minor distortion in the helix. ***Harvey et al (1997)*** mentioned that the space available in the helix also would allow pairing between the two pyrimidines cytosines and thymine. While neither G-T nor C-T base are normally found in DNA, G-U base pairs in helical regions of RNA are quite common. Two polynucleotide strands can, in principle, form either a right-handed or a left-handed helix, but the geometry of the sugar-phosphate backbone is more compatible with the former, and therefore natural DNA is right-handed.

Gene Structure

Historically, a gene is a heritable unit of phenotypic variation. However, from a molecular stand point, a gene is the linear collection of DNA sequences required to produce a functional RNA molecule, or a single transcriptional unit ***(Emery*** and ***Riomin, 1992).***

Each gene (Fig. 2) is composed of:

a. **Exons** are the functional portions of gene sequences that code for portions.

b. **Introns** that are non-coding DNA sequences of unknown function that interrupt most mammalian genes. The number and size of intron vary in different genes.

c. The boundaries between exons and interons are not random base sequences. In most instances the first two bases at the 5 end of each interon are GT and the last two bases of each interon are AG.

d. The open reading frame is the sequence binding with the triplet ATG-the universal translation initiation codon, which specifiy the intiation of protein synthesis. ATG resides at the 5 ends of genes.

e. Promotos, a promotor is the region of DNA, immediately upstream from the transcription intiation site. It binds RNA polymerase. The presence of promotor is an absolute requirement for initiation of transcription. In general strong promotors (associated with a high level of transcription) are those that bind RNA polymerase with a high affinity. Two regions defined as promotors on a functional basis (i.e. their presence is required for normal initiation of transcription) are found at the consensus sequences 5′-TATA-3 and 5′-CCAT-3. ***(Friedman et al., 1992).***

f. Termination codon is the end of transcription and signified by a TAA,TAGor TGA ***(Emery*** and ***Riomin, 1992).***

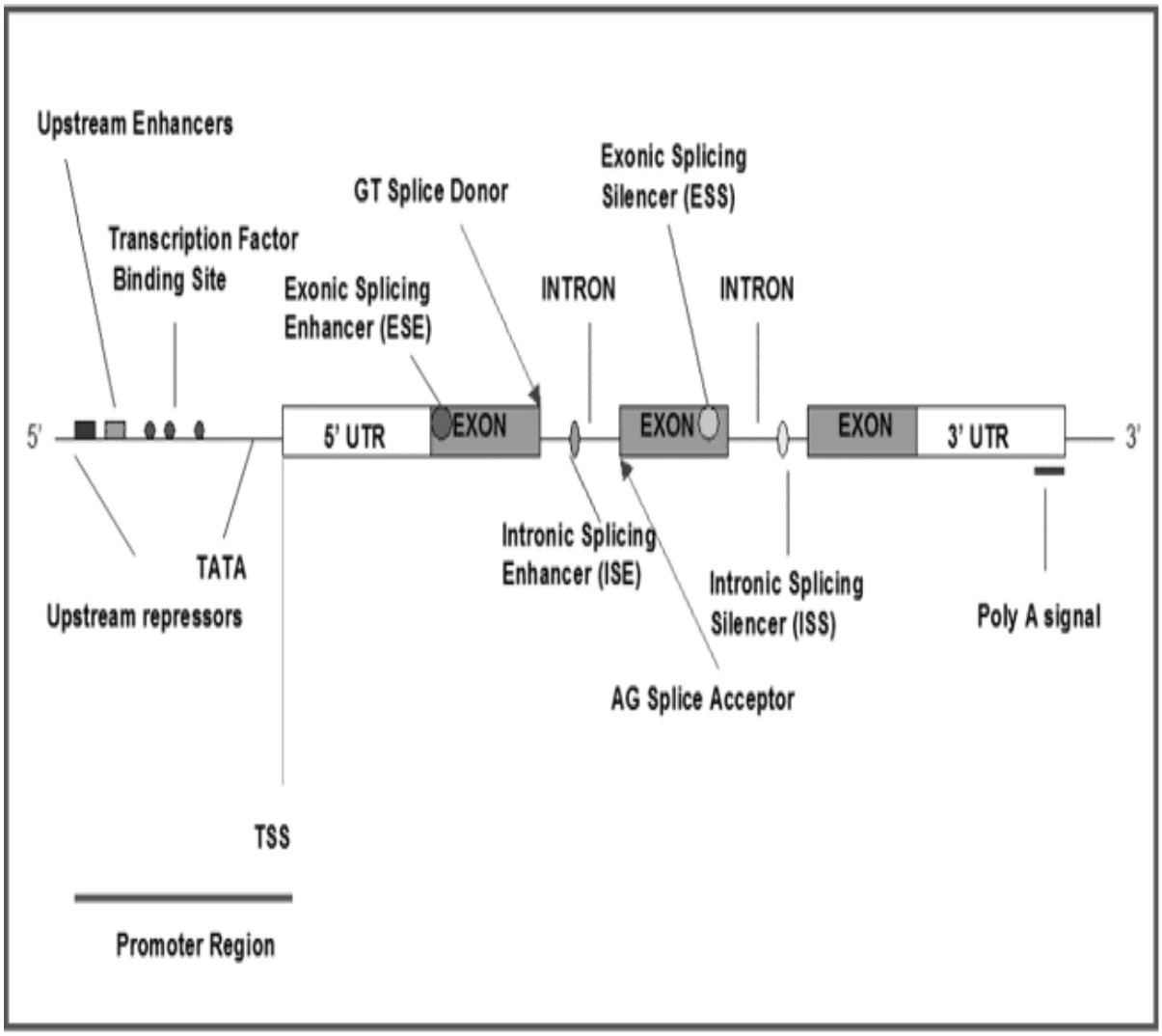

Fig. 2: Simplified structure of the gene. ***(Emery and Riomin, 1992)***

(2) RNA structure

Micklos et al (2003) stated that RNA is a structural component of the cell and an intermediate between DNA and protein. There are a variety of RNAs present in the eukaryotic cell:

1- Messenger RNA (mRNA).

2- Transfer RNA (tRNA).

3- Ribosomal RNA (rRNA).