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Review

Cancer Epigenomics: a review

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ABSTRACT: Epigenetic inactivation of genes that are crucial for the control of normal cell growth is a hallmark of cancer cells. Epigenetic modifications of the DNA do not alter the nucleotide sequence instead they involve the regulation of gene transcription and DNA methylation. Hypermethylation or histone deacetylation, which is within the promoter of a tumor suppressor gene, leads to the silencing as well as a deletion or a mutation of that gene. Cancer cells often show aberrant methylation and the frequency of aberrations increases is seen with the progression of disease. Hypermethylation events can occur early in tumorogenesis, involving the disruption of pathways that may predispose cells to malignant transformation. Epigenetic modification such as DNA methylation can be exploited for clinical purposes in cancer patients, first using hypermethylation as a molecular biomarker of cancer cells and second, epigenetic changes which are potentially reversible.

KEY WORDS: ***Cancer; Epigenomics; Methylation***

INTRODUCTION

Epigenetics refers to mitotically and/or meiotically heritable variations in gene expression that are not caused by changes in DNA sequence. Epigenetic mechanisms regulate all biological processes from conception to death, including genome reprogramming during early embryogenesis and gametogenesis, cell differentiation and maintenance of a committed lineage. Key epigenetic players are DNA methylation and histone post-translational modifications, which interplay with each other, with regulatory proteins and with non-coding RNAs, to remodel chromatin into domains such as euchromatin, constitutive or facultative heterochromatin and to achieve nuclear compartmentalization[1]. Epigenetics is one of the key areas of future research that can elucidate how genomes work. It combines genetics and the environment to address complex biological systems such as the plasticity of our genome. While all nucleated human cells carry the same genome, they express different genes at different times. Much of this is governed by epigenetic changes resulting in differential methylation of our genome/epigenomes[2,3]. Epigenetic mechanisms such as DNA methylation and modifications to histone proteins regulate high-order DNA structure and gene expression. Aberrant epigenetic mechanisms are involved in the development of many diseases, including cancer[4,5].

DNA METHYLATION

Epigenetic modifications of the DNA do not alter the sequence code instead they involve the regulation of gene transcription, DNA methylation[6]. In mammals, the major target for DNA methylation is a cytosine located next to a guanine (5'-CpG-3') found in CpG islands[7]. Methylation patterns are transmitted to the next generations during cell division. During embryonic development, currently undefined regulatory mechanisms allow rapid demethylation in very early stages followed by re-establishment of methylation patterns after implantation[8]. DNA methyltransferases (DNMTs) transfer the methyl group that is provided by S-adenosylmethionine to the 5'-carbon of a cytosine, there are only four types of DNMTs known of which three active DNA methyltransferases have been identified in mammals. They are named DNMT1, DNMT3A and DNMT3B. Fourth enzyme

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previously known as DNMT2 is not a DNA methyltransferase. However, DNMT3L is a protein that is closely related to DNMT3A and DNMT3B structurally and is critical for DNA methylation, but appears to be inactive.
The DNA methylation in the promoter regions of genes is correlated with gene silencing; however, methylation may, in some cases have a geneactivating effect[10,11,]. Twomain underlying mechanisms have been identified. First, binding of transcription factors or enhancer-blocking elements may be inhibited by DNA methylation and thus exert its effect on the transcription of downstream genes in the case of transcription factors[12,13]. Second and probably more common mechanism involves proteins that detect methylated DNA through methyl CpG-binding domains (MBDs)[14-17]. (**Figure 1**)

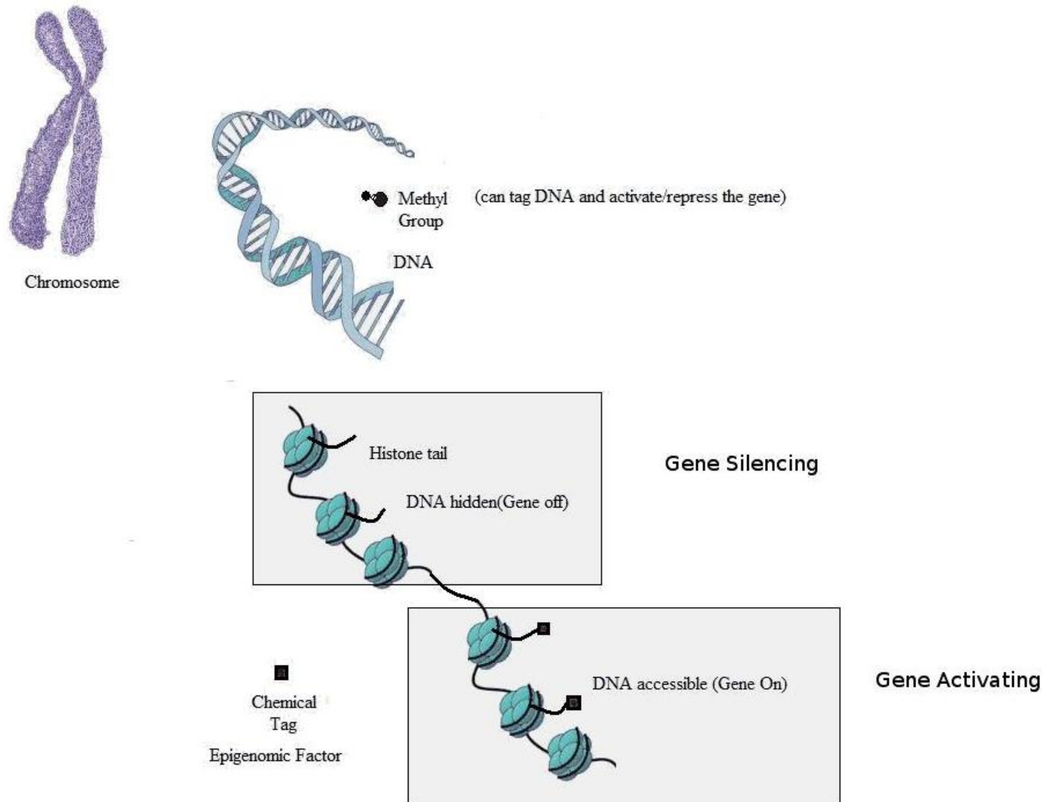

Figure 1: Epigenomics in tagging gene/diseases[17]

EPIGENOMICS AND CANCER

Studies by the Human Epigenome Project (HEP) studies now highlight the importance and complexity of cytosine DNA methylation in tissue-specific regulation of gene expression[18]. The cancer gene functions can be classified into six essential alterations in cell physiology, including self-sufficiency in growth signals, insensitivity to growth inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis[19]. In human cancer, the DNA methylation aberrations observed can be considered as falling into one of two categories: transcriptional silencing of tumor suppressor genes by CpG island promoter hypermethylation and a massive global genomic hypomethylation. Global DNA hypomethylation has been reported in almost every human malignancy[20]. This hypomethylation can be confirmed by HPLC by measurement of 5'-methyl cytosine level.the level was found to be decreased when as compared to normal tissue controls[21] Hypomethylation of the tumor genome could be verified by assays determining demethylation in specific sequences. Interestingly, the majority of hypomethylation events occur in repetitive elements localized in satellite sequences or centromeric regions[22]. While hypomethylation of repetitive elements is a common finding in human malignancies, gene-associated CpG islands are the targets of hypermethylation. Hypermethylation was initially discovered as a novel mechanism of tumor suppressor gene silencing in numerous genes that had been identified as targets for genetic alterations. Large-scale methylation studies on cancer genes became possible with the introduction of sodium bisulfite treatment of genomic DNA that results in a conversion of unmethylated cytosines to uracils but leaves methylcytosines unaltered/unmodified. [3].

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Several tumor suppressor genes have been identified today, solely based on silencing by promoter methylation. Ras associated domain family1, isoform1 (RASSF1A) was identified on chromosome 3p21.3, a region commonly deleted in lung cancer. No mutations have been found in RASSF1A; however, promoter methylation is associated with gene silencing in multiple human cancers, including lung[24]. Similarly, Suppressor of Cytokine Signalling1 (SOCS1) was found to be methylated in a Restricted Land Mark Genomic Scanning (RLGS) scan of hepatocellular carcinomas and is silenced by methylation. SOCS1 silencing results in constitutive activation of the JAK/STAT pathway and subsequent activation of target genes[25]. In another related tumor suppressor gene runt related transcription factors (RUNX3) expression is lost in more than 40% of gastric cancers. Recently, it was sobserved that loss of RUNX3 expression is due to loss of heterozygosity of LOH and promoter hypermethylation rather than mutations in the gene[26]. Epigenetic modifications are reversible, while genetic alterations areirreversible, this feature makes epigenetic modifications a perfect target for therapeutic interventions in cancer patients[27]. Epigenetic inactivation of genes that are crucial for the control of normal cell growth is a hallmark of cancer cells. These epigenetic mechanisms include crosstalk between DNA methylation, histone modification and other components of chromatin higher-order structure, and lead to the regulation of gene transcription. ?of genes epigenetically inactivated can result in the suppression of tumour growth or sensitization to other anticancer therapies. Small molecules that reverse epigenetic inactivation are now undergoing clinical trials in cancer patients. This, together with epigenomic analysis of chromatin alterations such as DNA methylation and histone acetylation, opens up the potential both to define epigenetic patterns of gene inactivation in tumours and to use drugs that target epigenetic silencing.. Cancer stem cells (CSCs) are thought to sustain cancer progression, metastasis and recurrence after therapy. There is *in vitro* and *in vivo* evidence supporting the idea that CSCs are highly chemoresistant. Epigenetic gene regulation is crucial for both stem cell biology and chemoresistance. CSC epigenomic profiling helps to dissect specific chemoresistance pathways, and have a significant clinical impact for patient stratification and rational design of therapeutic regimens[29]. The epigenome is little unusual since/for the fact that many changes may appear to be tissueor disease specific and perhaps less diverse and chaotic than those seen in cancer development. These epigenetic profiles, perhaps accessible through free DNA in body fluids, could be used as tools for diagnostics or as biomarkers once they have been mapped and catalogued.. An altered pattern of epigenetic modifications is central to many common human diseases, including cancer. Extensive studies have explored the mosaic patterns of DNA methylation and histone modification in cancer cells on a gene-by-gene basis.[30,31]. Epigenetic silencing in cancer cells is mediated by at least two distinct histone modifications. Polycomb-based histone H3lysine27 trimethylation (H3K27triM) and H3K9 dimethylation.suggests mechanism of tumor-suppressor gene silencing in cancer is potentially independent of promoter DNA methylation[32,33].

RECENT ADVANCEMENT AND FUTURE IMPLICATIONS

The discovery of 5methylcytosine, so called 5th base modification has immensely increased the filed of epigenomics /genetics[34,35]. Prior to the advent of the new sequencing technologies, the potential for epigenomics in medicine was already widely recognized[36,37]. Its role in cancer development, aging, gene regulation, embryogenesis and the modulation of genetic factors has been well described[36,38]. The most immediate impact of the new sequencing technologies has been on so-called ‘ChIP-seq’ experiments, where the locations of histone proteins can be mapped to the genome identifying epigenetic control of chromatin structure and gene expression[39]. These proteins leave a footprint on the DNA that protects it from shearing during sample preparation. This is a simpler experiment using the same genomic fragmentation and sequence remapping techniques used for mutation detection in sequencing experiments[40]. The significance of the identified regions can then be determined. This technique replaces an array-based method, ChIP on ChIP, and is generally considered hypothesis-free, more sensitive, and thus superior. Detecting the modification of cytosine, and its location on DNA from a given sample can currently be performed using any sequencing technique based on bi-sulfite treatment of DNA. Earlier embodiments required pulling down a subset of the genome to be analyzed using an antibody precipitation method known as ‘methylated DNA immunoprecipitation’ (MeDIP), often followed by an array based analysis[41]. Many other methylation site subsetting techniques have been described. Bi-sulfite treatment leaves 5-methylcytosine intact, but modifies cytosine (denoted as C) to a uracil analogue. During sequencing, by tghe use of technologies based on complementary synthesis or probe ligation, this is recognized as the base thymine (denoted T). A minor complication which might occur is remapping the resulting sequences to the genome in order to locate the site of the modifications. The other is the amount of DNA required for bi-sulfite

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treatment, and any biases or artefacts this treatment may introduce[36,42]. Nonetheless, genome-wide surveys of methylation have recently been performed using such techniques on second-generation sequencers. Stem cell chromatin control of gene expression, including relationships between histone modifications and DNA methylation, hold a key to understanding the origins of cancer epigenetic changes[30,42]. DNA methylation can be exploited for clinical purposes in cancer patients as a molecular biomarker of cancer cells. Since the presence of CpG island hypermethylation of the tumor suppressor genes described is specific to transformed cells. Example: Presence of hypermethylation of the glutathione *S*-transferase P1 (*GSTP1*) gene in prostate cancer[43]. Hypermethylation could also be used as tool for detecting cancer cells in multiple biological fluids or even for monitoring hypermethylated promoter loci in serum DNA from cancer patients[44]. Second, unlike genetic changes in cancer, epigenetic changes are potentially reversible. For years, in cultured cancer cell lines, we have been able to express genes that had been silenced by methylation by using DNA demethylating agents such as 5-aza-2-deoxycytidine, 5-azacitidine or zebularine[45,46]. These two factors makes epigenomics a potential area for cancer research, diagnosis and treatment.

REFERENCES

1. Delcuve GP, Rastegar M, Davie JR. Epigenetic control. *J Cell Physiol*. 2009 May;219(2):243-50.
2. Novik KL, Nimmrich I, Genc B, et al. Epigenomics: genome-wide study of methylation phenomena. *Curr Issues Mol Biol*. 2002 Oct;4(4):111-28.
3. Jones PA, Baylin SB. The epigenomics of cancer. *Cell*. 2007 Feb;128(4): 683-92.
4. Urdinguio RG, Sanchez-Mut JV, Esteller M. Epigenetic mechanisms in neurological diseases: genes, syndromes, and therapies. *Lancet Neurol*. 2009 Nov;8(11):1056-72.
5. Balch C, Fang F, Matei DE, et al. Minireview: Epigenetic changes in ovarian cancer. *Endocrinology*. 2009;150(9):4003-11.
6. Doerfler W. DNA methylation and gene activity. *Annu Rev Biochem*. 1983;52:93-124.
7. Riggs AD, Jones PA. 5-Methylcytosine, gene regulation, and cancer. *Adv Cancer Res*. 1983;40:1-30
8. Santos F, Hendrich B, Reik W, et al. Dynamic reprogramming of DNA methylation in the early mouse embryo. *Dev Biol*. 2002 Jan;241(1);172–182
9. Okano M, Bell DW, Haber DA, et al. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell*. 1999 Oct;99(3):247-57.
10. Plass C, Shibata H, Kalcheva I, et al. Identification of Grf1 on mouse chromosome 9 as an imprinted gene by RLGS-M. *Nat Genet*. 1996 Sep;14(1):106-9.
11. Hark AT, Schoenherr CJ, Katz DJ, et al. CTCF mediates methylation-sensitive enhancer-blocking activity at the H19/Igf2 locus. *Nature*. 2000 May;405(6785):486-9.
12. Iguchi-Ariga SM, Schaffner W. CpG methylation of the cAMP-responsive enhancer/promoter sequence TGACGTCA abolishes specific factor binding as well as transcriptional activation. *Genes Dev*. 1989 May;3(5):612-9.
13. Tate PH, Bird AP. Effects of DNA methylation on DNA-binding proteins and gene expression. *Curr Opin Genet Dev*. 1993 Apr;3(2):226-31.
14. Bell AC, Felsenfeld G. Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene. *Nature*. 2000 May;405(6785):482-5.
15. Boyes J, Bird A. DNA methylation inhibits transcription indirectly via a methyl-CpG binding protein. *Cell*. 1991 Mar;64(6):1123-34.
16. Hendrich B, Bird A. Identification and characterization of a family of mammalian methyl-CpG binding proteins. *Mol Cell Biol*. 1998 Nov;18(11):6538-47.
17. Epigenomics. Retrieved from: http://www.genome.gov/27532724
18. Brena RM, Huang TH, Plass C. Toward a human epigenome. *Nat Genet*. 2006 Dec;38(12):1359-60.
19. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000 Jan;100(1):57-70.
20. Feinberg AP, Vogelstein B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature*. 1983 Jan;301(5895):89-92.
21. Gama-Sosa MA, Slagel VA, Trewyn RW, et al. The 5-methylcytosine content of DNA from human tumors. *Nucleic Acids Res*. 1983 Oct;11(19):6883-94.
22. Ji W, Hernandez R, Zhang XY, et al. DNA demethylation and pericentromeric rearrangements of chromosome 1. *Mutat Res*. 1997 Sep;379(1):33-41.
23. Clark SJ, Harrison J, Paul CL, et al. High sensitivity mapping of methylated cytosines. *Nucleic Acids Res*. 1994 Aug;22(15):2990-7.
24. Dammann R, Li C, Yoon JH, et al. Epigenetic inactivation of a RAS association domain family protein from the lung tumour suppressor locus 3p21.3. *Nat Genet*. 2000 Jul;25(3):315-9.
25. Yoshikawa H, Matsubara K., Qian GS, et al. SOCS-1, a negative regulator of the

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JAK/STAT pathway, is silenced by methylation in human hepatocellular carcinoma and shows growth-suppression activity. *Nat Genet*. 2001 May;28(1):29-35.

26. Li QL, Ito K, Sakakura C, et al. Causal relationship between the loss of RUNX3 expression and gastric cancer. *Cell*. 2002 Apr;109:113-24.
27. Plass C. Cancer Epigenomics. *Hum Mol Gen*. 2002 Oct;11(20):2479-88.
28. Brown R, Strathdee G. Epigenomics and epigenetic therapy of cancer. *Trends Mol Med*. 2002;8(4 Suppl):S43-S8.
29. Crea F, Danesi R, Farrar WL. Cancer stem cell epigenetics and chemoresistance. *Epigenomics*. 2009;1(1):63-79.
30. Brown CG. The DNA sequencing renaissance and its implications for epigenomics. *Epigenomics*. 2009;1(1):5-8.
31. Esteller M. Cancer epiginomics: DNA methylomes and histone-modification maps. *Nat Rev Genet*. 2007;8(4):286-98.
32. Fraga MF, Ballestar E, Villar-Garea A, et al. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. *Nat Genet*. 2005 Apr;37(4):391-400.
33. Kondo Y, Shen L, Cheng AS, et al. Gene silencing in cancer by histone H3 lysine 27 trimethylation independent of promoter DNA methylation. *Nat Genet.* 2008 Jan;40(6):741-50.
34. Ehrlich M, Wang RY. 5-Methylcytosine in eukaryotic DNA. *Science*. 1981 Jan;212(4501):1350-7.
35. Kriaucions S, Heintz N. The nuclear DNA base 5-hydroxymethylacytosine is present in purkinje neurons and the brain. *Science*. 2009 May;324(5929):929-30.
36. Beck S, Olek A. The Epigenome. Wiley-VCH, Weinheim, Germany. 2003.
37. Egger G, Liang G, Aparicio A, et al. Epigenetics in human disease and prospects for epigenetic therapy. *Nature*. 2004 May;429(6990):457-63.
38. Bernstein BE, Meissner A, Lander ES. The mammalian epigenome *Cell*. 2007 Feb;128(4):669-81.
39. Johnson DS, Mortazavi A, Myers RM, et al. Genome-wide mapping of in vivo protein–DNA interactions. *Science*. 2007 Jan;316(5830):1497-502.
40. Li H, Ruan J, Durbin R. Mapping short DNA sequencing reads and calling variants using mapping quality scores. *Genome Res*. 2008 Nov;18(11):1851-8.
41. Frommer M, McDonald LE, Millar DS, et al. A genomic sequencing protocol that yields a positive display of 5-methylcytosine residues in individual DNA strands. *Proc Natl Acad Sci U S A*. 1992 Mar;89(5):1827-31.
42. Ting AH, McGarvey KM, Baylin SB. The cancer epigenome--components and functional correlates. *Genes Dev*. 2006 Dec;20(23):3215-31.
43. Hoque MO, Topaloglu O, Begum S, et al. Quantitative methylation-specific polymerase chain reaction gene patterns in urine sediment distinguish prostate cancer patients from control subjects. *J Clin Oncol*. 2005 Sep;23(27):6569-75.
44. Esteller M, Sanchez-Cespedes M, Rosell R, et al. Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients. *Cancer Res*. 1999 Jan;59(1):67-70.
45. Esteller M. DNA methylation and cancer therapy: new developments and expectations. Curr Opin Oncol 2005 Jan;17(1):55-60.
46. Esteller M. The necessity of a human epigenome project. *Carcinogenesis*. 2006 Jan;27(6):1121-1125.

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