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. 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. 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.

DNA METHYLATION

Epigenetic modifications of the DNA do not alter the sequence code instead they involve the regulation of gene transcription, DNA methylation. In mammals, the major target for DNA methylation is a cytosine located next to a guanine (5'-CpG-3') found in CpG islands. 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. 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
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 gene-activating effect\textsuperscript{10,11}. Two main 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\textsuperscript{12,13}. Second and probably more common mechanism involves proteins that detect methylated DNA through methyl CpG-binding domains (MBDs)\textsuperscript{14-17}. (Figure 1)
Several tumor suppressor genes have been identified today, solely based on silencing by promoter methylation. Ras associated domain family 1, isoform 1 (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. Similarly, Suppress of Cytokine Signalling 1 (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. In another related tumor suppressor gene runt related transcription factors (RUNX3) expression is lost in more than 40% of gastric cancers. Recently, it was observed that loss of RUNX3 expression is due to loss of heterozygosity of LOH and promoter hypermethylation rather than mutations in the gene. Epigenetic modifications are reversible, while genetic alterations are irreversible, this feature makes epigenetic modifications a perfect target for therapeutic interventions in cancer patients. 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. The epigenome is little unusual since for the fact that many changes may appear to be tissue 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. Epigenetic silencing in cancer cells is mediated by at least two distinct histone modifications. Polycomb-based histone H3lysine27 trimethylation (H3K27triM) and H3K9 dimethylation suggest mechanism of tumor suppressor gene silencing in cancer is potentially independent of promoter DNA methylation.

RECENT ADVANCEMENT AND FUTURE IMPLICATIONS

The discovery of 5 methylocytosine, so called 5th base modification has immensely increased the filed of epigenomics /genetics. Prior to the advent of the new sequencing technologies, the potential for epigenomics in medicine was already widely recognized. Its role in cancer development, aging, gene regulation, embryogenesis and the modulation of genetic factors has been well described. 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. 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. 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. 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 the 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
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.39

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.43,44 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 make epigenomics a potential area for cancer research, diagnosis and treatment.

REFERENCES

25. Yoshikawa H, Matsubara K., Qian GS, et al. SOCS-1, a negative regulator of the


