Review Article

Role of Epigenetics In Tumor-formation: A Review

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Abstract

Objective: Epigenetics is a non-genetic regulation of heritable traits including gene expressions, which are non-hereditary in nature. It is an external modification of DNA of the cell that turn genes 'on' and 'off' affecting the way genes are read (or expressed) but without altering the DNA sequence. Epigenetic regulatory factors are related to 3 aspects: DNA, histones and chromatin remodelling. This physiological process gets disturbed in many human diseases such as neoplasm, diabetes, and other neurodegenerative disorders. So, the main objective of this review is, as epigenetic changes are reversible, which allow the functional recovery of the affected genes so to find out that, targeting epigenetic changes provides great potential in current therapeutic development in the field of cancer.

Study Design: Literature search of the PubMed database taken from the last decade and half, studies based on epigenetic mechanism and its relationship over tumorigenesis were selected for this review.

Conclusion: In this review, we summarize the recent updates of epigenetic regulatory mechanisms in tumerogenesis with the strong emphasis on histone modifications, enhancer reprogramming, chromosome accessibility, and transcription regulation; and discuss the significance of epigenetic aberrations in cancer-cure. From this way, emergence of epigenetics is fueling new hope in the field of cure from cancer, in cancer research and mainstream oncology.

Key Words: Epigenetics, gene expression, DNA sequence, histones and chromatin remodelling, tumor

Introduction

Cell is the unit of life. At present, to explore the undergoing changes in the cell, in various diseases, Genomics (study of complete genome of the organism) and proteomics (molecular biology that studies the set of proteins expressed by the genome of the organism) are centre of the researches. Both exerts a great impact on the epigenetics. “Epigenetics” are defined as non-genetic regulation of heritable traits including gene expression.[1] In normal physiological condition, epigenetics is a regular and natural phenomenon, but it may get dysregulated in multiple human diseases. In this review, we shed a light on comprehensive understanding of the epigenetic mechanisms in normal physiologic condition in mammalian cells and their comparative aberrations that results in carcinogenesis.[2] We also discuss the idea of the prospective role of epigenetics in the field of cancer detection and also proposed its role in designing efficient strategies for cancer treatment.

Methods

The present review was based on a literature search of the PubMed database taken from the last decade and half, with a strong emphasis on reviews published over the last five-year period. Studies based on epigenetic mechanism and its relationship over tumorigenesis were selected to access the interested readers to attain further in-depth knowledge. We acknowledge that although search not exhaustive, but we try our best to present a concise update on the epigenetic alteration which results in tumor formation. We express our regret to those authors whose work on this field could not be cited due to limitation of space and resources.

Epigenetics-Definition

Epigenetics is defined as an external alteration of expression of DNA of the cell that turn genes 'on' and 'off' affecting the way of actions of genes.[3] It is the term coined by C. H. Waddington. The given definition of epigenetics is 'the study of heritable changes in the expression of genes that occurs, irrespective of changes in the DNA sequence'.[1] Hence, it is the study of stable, long-term alteration in the transcriptional potential of a cell-expression. Thus, in the contrary of genetics, which is based on changes to the DNA sequence (the genotype), in epigenetics, the changes occurs in gene expression (or cellular phenotype). hence, epigenetics is referred to as those changes in the genes functions which are transmitted through both mitosis and meiosis without causing any alterations in the DNA sequence.[4] Epigenetics controls gene expression by silencing it (making it dormant) or turning it on (becoming active), modify the DNA transcription and translation steps.[3] It is the role of

epigenetics to make each individual phenotypically unique." The epigenetic aberrations described in current literature generally comprise histone variants, posttranslational modifications of amino acids on the amino-terminal tail of histones, and covalent modifications of DNA bases.[3]

Basically, all these modifications cumulatively referred as epigenome.

Epigenetic mechanisms in normal cellsEpigenetic mechanisms in normal cells:

In general, physiologic condition, epigenetic regulates factors over 3 aspects: DNA, histones and chromatin remodelling. These modifications combinedly regulate the functioning of the genome by altering its structural dynamics of chromatin.[1] The interplay of these modifications creates an 'epigenetic landscape' on which aberrant changes occurs in mammalian genome that results in carcinogenesis and other pathologic conditions. Epigenetic regulations in routine physiology are as follows:

Epigenetic mechanisms in normal cells:
DNA methylation- by
Gene silencing
Chromatin organization
Histone modification- by-
Labile heritable modification
Gene silencing
Chromatin remodelling by-
Gene silencing
Tissue-specific expression

BOX 1

1.DNA methylation-

As indicated by its name, its a chemical process.[4] In this process, covalent modification of either DNA or of histone proteins occurs which play central roles in multiple epigenetic inheritance.[5] DNA Methylation occurs by two ways- The first way is post translational modification of the amino acids that make up histone proteins.[5] And the second way is among cytosine and guanidine nucleotide (i.e. CpG),a methyl group is additionally included to the DNA in this process, which are regulatory region of many genes (approximately 40% of the promoters of human genes). Due to this alteration, appearance and structure of DNA modified that result in the altered nuclear transcription of the cell.[2] It is also used in imprinting of DNA to differentiate paternally and maternally inherited genes. Hence, DNA methylation controls various genetic activities and their modification alter genetic architecture of the nucleus.[6] These CpG islands are usually not methylated in normal cells. many of them belonging to tumour suppressor genes, become abnormally methylated and thus silenced in cancer promoting carcinogenesis.[6]

2. Histone modification

Histones are positively charged proteins that strongly adhere to negatively charged DNA, by physical attraction, to form chromatin. When histones are modified, impact of this modification alter the arrangement of chromatin as the determining factor of transcribed chromosomal DNA. Alteration in histones can be occurs in following 4 ways:

i) ***Histone acetylation****:* Chemical process, as the name suggest, an acetyl group is added to amino acid lysine located in the histone, which is usually associated with active chromatin (euchromatin).

ii) ***Histone methylation****:* Chemical process, as the name suggest, methyl group is added to amino acid +lysine in the histone but this is associated with both active and inactive chromatin

iii) ***Histone phosphorylation****:* Chromatin process, amino acid serine is phosphorylated which alters the chromatin to allow DNA transcription in active form during mitosis or change it to inactive form of chromatin. Histone phosphorylation plays an important role in DNA damage response.

iv) ***Histone ubiquitination:*** Ubiquitin is a 76-aminoacid protein that is ubiquitously distributed and serves the purpose of signalling and plays a crucial role in DNA damage repair response to maintain the genomic integrity.

3. Chromatin remodelling Chromatin remodelling is also an important epigenetic mechanism. Reversible post-translational modifications in histones modulate chromatin structure by restructuring nucleosomes and this process is ATP-dependent. Chromatin remodelling is essential for epigenetic regulation of transcription, genome stability, DNA damage response, X chromosomes inactivation and for epigenetic memory.

Role of Epigenetics in tumor- formation

Several genetic aberrations along with epigenetic alteration which modulates important cellular pathways, gives rise to the cancer, a multifactorial disease.[7] These genetic and epigenetic alterations interplay at all stages of cancer development, working together to give rise and promote cancer progression, depicted in **Figure 1**.

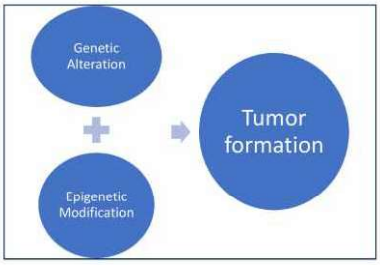

Figure:1 Genetic alterations and Epigenetic modifications combined together which results in tumor-formation

Hallmarks pathognomic of cancer, includes sustained proliferative signalling, activation of invasion and metastasis, evading immune destruction, apoptotic resistance phenotype, genome instability and mutation, cellular energetics dysregulation, evading growth suppressors, induction of angiogenesis, vascular or lymphatic system invasion, and tumor-promoting inflammatory microenvironment.[7] Many of the above mentioned hallmarks have direct related to epigenetic alterations, affecting the expression of proto-oncogenes/tumor suppressors, which results in formation of tumor.Epigenetic agents includes prions,RNA and micro-RNA in which prions are a kind of protein which are able to form an infectious state by inducing genome- modification without phenotypic change .[3]

Aberrant DNA Methylation- Aberrant DNA methylation pattern in CpG islands was the first and most significant step towards the abnormal epigenetic changes occurred in cancerous cells as a beginning of carcinogenesis.[4]Aberrant methylation of CpG islands leads to silencing of tumor suppressor genes and molecules involved in the cell differentiation.[4] Aberrant DNA- methylations basically acts by two ways- either Hypo-methylation or hypermethylation. Genetic effects of both, hypomethylation as well as hypermethylation depicted in **Table 1**. Among cancer cells, DNA *hyper*-methylation is associated with gene *silencing* and DNA *hypo*-methylation with gene *expression*, both of which are crucial incident in the initiation of carcinogenesis process.[8]

Molecular consequences resulting from DNA-Methylation

- *Genome wide patterns of hypomethylation & hypermethylation regulate expression of many hundreds of genes, independent of DNA alteration*
- *Hypermethylation of promoter sequences of tumor suppressor genes and hypomethylation of proto-oncogenes*
- *Reprogramming at the epigenetic level of cellular phenotype and promotes tumor-growth, invasion, and metastasis*
- *Methylation can also regulate the expression of microRNAs, including those which regulates the expression of DNA methyltransferases*
- *Adds a further level of epigenetic modification, in addition to methylation alone*

Table :1

Micro-RNA- Micro RNAs are deemed as a class of small, noncoding, and endogenous RNAs that play a crucial role in gene expression through transcription prevention by induction of a group of regulatory molecules. The miRNA can also be important in the controlling of the DNA methylation and histone modifications.[4]

Histone modifications- Histone modifications significantly play an important role in epigenetic regulation. Histones are dynamic proteins that can become methylated or acetylated on specific amino acid residues, which correlates with active or repressive transcription[8]. An octamer of histones makes up the nucleosome, acts as a template of chromatin-modification. The nucleosomes have lysine-rich histone tails extending outward from the four constituent core histone proteins (H2A, H2B, H3, and H4).[8] Reversible modifications occurs on these histone tails to alter chromatin structure and thus, gene expression. Therefore, histone modification has an imp role in the regulation of gene expression, hence it makes it a promising target for disease treatment. Epigenetic events are important in normal biological processes as well as in tumorigenesis and that the epigenetic status is usually widely altered during cancer initiation.[9]

Hence genomic methylation, histone modification and Micro-RNAs combinedly affects the genomic expression **(Figure: 2)**

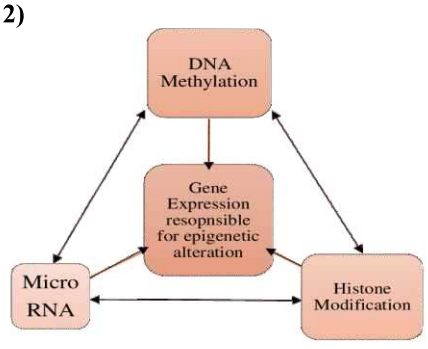

Figure 2: Diagrammatic presentation of DNA methylation, histone modification & the effects of micro-RNA on gene-expression, responsible for epigenetic alterations

Proposed theories, based on epigenetic alteration of tumor-progression-

1. The cancer stem cell (CSC) model
2. The stochastic or clonal evolution model

BOX 2

There are two theories which are proposed for tumor-formation which are based on epigenetic alterations. According to, **The cancer stem cell (CSC) model**, Stem cell acts as base-model on which tumour-formation occurs.[2] This theory suggests that the initial events in cancer initiation, which occur in normal stem or progenitor cells, results in neoplasm -formation.[2] Another theory, **the stochastic or clonal evolution model** states that, that the initial oncogenic change is adopted progressively by non-Cancer stem-cells, which further results in cancer formation or evolution of cancer oocurs.

After alterations occurs in genomic and epigenetic processes it led to Tumorigenesis. The genomic changes occur as a result of alteration in gene-expression. Affected genes are categorized into: tumor promoter genes (TPG) or oncogenes that promote cell growth and reproduction, and tumor suppressor genes (TSG) that inhibit cell division and survival. Enhanced activity of oncogenes as well as supressed activity of tumour suppressor genes (TSGs) results in formation of cancer. Disturbance in DNA methylation and histone modification related to CSC model.

Epigenetic factors responsible for cancer formation-
1. Stromal fibroblastic cells
2. Silencing of RasGAPs gene
3. Tumor micro environmemt
4. Lifestyle factors

BOX 3

Role of stromal fibroblastic cells in activation of cancer

Cancer associated fibroblasts (CAFs)are the dominant cell type in tumor microenvironment, with both pro- and anti-tumorigenic capacity.[4] Studies showed that, CAFs have an inherent 'memory to maintain tumor-induction in the absence of the constant signals from cancer cells for a period of time and promote tumerogenesis.[5] Hence it established from multiple studies that, cancer is the end-product of both genetic and epigenetic modifications.[10] Apart from induction of stromal fibroblasts, microenvironment-mediated epigenetic perturbations also have an significant role in neoplastic development.[5] **Figure 3** illustrates general epigenetic changes involved in fibroblast and microenvironment of cell, which alter cancer epithelial proliferations and subsequently give rise to a tumor.[5]

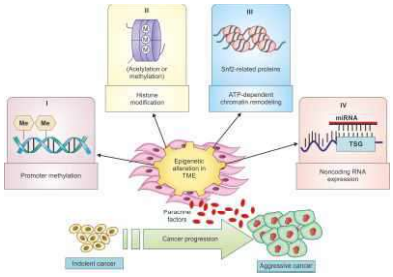

Figure 3: Role of epigenetic changes in fibroblasts in tumor microenvironment lead to cancer progression in paracrine manner

Role of tumor micro environmemt, in activation of cancer

Tumour micro-environment exert great impact on epigenetic-alterations.[1] Epigenetic reprogramming, especially noncoding RNAs works as a template in modifying the tumor-microenvironment. They initiates and regulates tumorigenesis by regulating the on and off states of oncogenes and TSGs. The tumor microenvironment also plays a significant part in metastasis seeding and outgrowth of tumor.

Suppressing of RasGAPs due to epigenetic, in activation of cancer

Tumor-suppressor genes can be inactivated by epigenetic-mechanism as well as by other mechanism, tabulated in **Table 2,** which is critical in origin a of tumor.[11] An example would be Ras, which is tumor-supressor gene, when mutated, as a result ,it increases the activity of the gene product to stimulate growth. Hence, aberrant alteration in Ras signalling is a major factor in contribution to tumorigenesis.[5] More than 30% of all human neoplasms exhibits an oncogenic form of Ras proteins, which is made up of a small family of three closely related proteins (K-Ras, H-Ras, or N-Ras).The silencing of the RasGAP genes by promoter methylation results in the tumor development. Epigenetic modulate expression of a certain genotype in a different phenotype.[12]

Tumor-Suppressor Genes (TSGs), inactivated by-

- *Through mutation, which results in disability of its function*
- *Due to loss of heterozygosity, which results in complete loss of gene.*
- *A gene can be switched off in a somatically heritable fashion by aberrant epigenetic alterations.*

Table :2

Role of lifestyle factors in activation of cancer

Sometimes several lifestyle factors have been contributed in modification of epigenetic patterns, such as diet, obesity, physical activity, tobacco smoking, alcohol consumption, environmental pollutants, psychological stress, and working on night shifts.[13] Impact of life-style factors on epigenetics in this way, have been influenced multiple biological process and these genomic changes ultimately cause alterations in somatic cells regardless of their differentiation status.[14]

Epigenetic biomarker & its role in cancer-detection

Detection of aberrant DNA methylation, acts as a biomarker, which is an important tool for prognostic and diagnostic purposes. These biomarkers can be detected from body

secretions such as blood, saliva, or urine. These secretions can be used to diagnosis of tumors at the early stage of disease. There are reasons to suggest the methylated tumor suppressor genes as a good diagnostic marker as depicted in **Figure 4**. Although still it is quite impossible to identify high- and low-risk patients or early/advanced stage of disease,

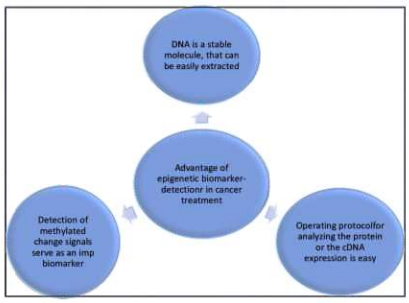

Figure 4: Advantage of epigenetic biomarker in the field of cancer detection

based only on circulating or tissue biomarkers in cancer as several epigenetic changes occurs before histopathological changes are detected.[6] Hence focus should be on early detection, accurate prediction of disease progression, and frequent monitoring for successful management of cancer.[6] Genes such as ATM and BRCA1,BRCA2 confirms link between breast cancer risk and methylation changes.[11] Recently liquid biopsies also plays a promising role in detection of epigenetic changes.[15] However,only detecting epigenetics aberrations cannot help in cancer-diagnosing proper, and it would further must be confirmed with other necessary screening tests. Recent studies on epigenetics proved their two favourable features of heritable and reversible regulations, hence researches on epigenetics is a definitive mode of treatment in this deadly disease . **Table 3** depicts the summary of role played by Epigenetics in tumor-formation. Advanced research in the field of cancer-treatment showed that, epigenetic changes are reversible in nature, which allow great potential in current therapeutic development by targeting epigenetic changes.

Summary of Role of Epigenetics in tumor- formation
• *Disturbance in DNA- Methylation*
• *Activation of Onco-genes*
• *Suppression in suppressor -genes*
• *Altered tumor-micro-environment.*
• *Epigenetic regulation may be the key machinery to achieve metastatic properties.*

Table :3

Conclusion

In normal physiologic condition, there is complexity and interplay of epigenetic regulation occurs, but when aberrant expression or functional irregulation occurs, it could give rise to cancer.

Abbreviations

TPG - Tumor promoter genes
TSG - Tumor suppressor genes
CSC - Cancer stem cell
TME - Tumor-microenvironment
DNA – Deoxynucleic Acid
RNA – Ribonucleic Acid
BRCA1 -gene- Breast Cancer Gene
CAF-Cancer Associate Fibroblast

Disclosures

The authors declare no financial disclosure or conflicts of interest.

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