**Explore the role of the epigenome and/or other gene expression regulatory mechanisms in the development of cancer. Discuss in detail one possible epigenetic/post-transcriptional/post-translational target for cancer treatment, and identify the successes and drawbacks of this treatment.**

**INTRODUCTION**

Epigenetics is defined as stable heritable changes in the genome which plays an important role in highly regulated processes such as development. Epigenetics has been investigated as early as 1961 when Lyon hypothesis was formulated showing that the inactive X chromosome was highly methylated. A lot of research has been done to study the molecular basis of epigenetics which include DNA methylation, histone modification and much recently discovered small RNAs. DNA methylation occurs at specific cytosine residues in the promoter region of the gene thereby attenuating the expression of nearby genes. Some of the implications of cytosine methylations are chromosomal stability, genomic imprinting, X chromosome inactivation, cell differentiation during development, cancer progression and aging. Histone modification is the post translational modification of DNA associated basic proteins mainly by acetylation, methylation and phosphorylation. Among these histone acetylation is widely studied. It involves the participation of two enzymes namely histone acetyl transferase (HAT) and histone deacetylase (HDAC). Acetylation removes a positive charge from the amino acid which changes the conformation of chromatin to a more open form thereby enhancing transcription. Thus histone acetylation increases the rate of gene expression. The silencing of genes by RNA interference is yet another epigenetic modification. miRNAs are short single stranded RNA molecules which are approximately 22-23 nucleotides in length and are known to control gene expression post transcriptionally. The expression of certain microRNAs is under the control of DNA methylation and histone acetylation. CpG island hypermethylation epigenetically silences miRNAs and has been shown to cause cancers.

**DNA METHYLATION**

DNA methylation is a covalent modification of cytosine residues followed by guanine residues which occurs in CG rich regions in the promoter sequence known as CpG islands. The cytosine is methylated at the C-5 position by a group of enzymes called DNA methyltransferases(DNMTs) from the donor S-adenosyl –L-methionine (SAM). Till date three DNMTs have been discovered (DNMT1, DNMT3A, and DNMT3B). Normal DNA repair mechanism by DNA uracil glycosylase enzyme can recognize uracil residues formed from spontaneous deamination of cytosine. But DNA repair mechanism cannot recognize thymine formed from 5-methyl cytosine deamination. A transition mutation C↔T occurs which introduces a mutation in the genome. DNA methylation usually results in a loss of function mutation. In familial cancers germline mutations are common where as in sporadic cancers somatic mutations are frequent. DNA methylation is known to accumulate in the genome in long evolutionary time spans. Hyper methylation and hypo methylation of genes are both causes of malignancies. A vast amount of data has been derived from experimental approaches in DNA methylation and has been deposited in several biological databases. Small but significant proportion of de novo methylation patterns in the promoter regions of developmental genes has been found to play an important role in genomic imprinting and X chromosome inactivation. The selection of particular regions in the genome where DNA methylation occurs is still a mystery in epigenetics.

**CANCER AND DNA METHYLATION**

The development of cancer is a multistep process. Normal state of cells is maintained by the action of several factors and a shift from the normal homeostasis leads to uncontrolled cell proliferation. Promoter hyper methylation is a common mechanism operating in familial cancers. All DNMTs are known to act co-operatively in cancer progression. It has been reported that approximately 13% of sporadic breast cancers and 5-30% of ovarian cancers present with hyper methylation of the BRCA1 gene and 40-90% of sporadic colorectal cancer has hyper methylation of the MGMT (O6-methylguanine methyltransferase) gene. In studies of p53 gene it has been observed that 50% of inactivating mutations occurred at the methylated cytosine residues. The following genes are found to be hypo methylated in many forms of sporadic cancers Rb (retinoblastoma), APC (colorectal cancer), ARF (colorectal cancer), CDKN2B (leukemia), CDKN2A (various cancers), VHL (renal cancer), HMLH1 (colorectal, gastric and endometrial cancers) and ER-α (breast, colorectal and other). CpGhyper methylation is also known to be inherited in many cases. Microarray analysis of pancreatic cancer samples has revealed the overexpression of CLDN4, LCN2, YWHAS (14-3-3σ), TFF2, MSLN, and PSCA. These genes were found to be hypo methylated. Novel biomarkers can be developed during early stages of cancer development by utilizing DNA methylation. Such an approach has been adopted in pancreatic cancer and BNC1 and ADAMTS1 are now considered as biomarkers for the same. Promoter hyper methylation of these genes can be assayed to detect early pancreatic cancer. Different DNA methylene mapping methods such as bisulfite sequencing are available to detect DNA methylation in tissue samples.

**METHYLATION AS A TARGET FOR CANCER TREATMENT**

Epigenetic changes are known to occur during the beginning of cancer development and are known to provide a suitable microenvironment for cancer progression. Epigenetic deregulation eventually leads to alterations in the blueprint of gene expression activating oncogenes and suppressing tumor suppressor genes. Such changes can be regarded as an on/off switch that can be made use of in the treatment of cancers by reversing or reprogramming the cancerous phenotype. Epigenetic changes can be reversed in many genes and this has been considered as a good target for cancer treatment.Azacitidine and decitabine are two drugs which exploit epigenetics and have been clinically approved for treatment in pre-leukemic disorder and myelodysplastic syndrome. Both these compounds are cytosine analogs and are known to inhibit DNMTs. S-110 is a decitabine analog that is known to be more stable and is in clinical trials to evaluate cytotoxicity.They have widespread functions ranging from cancer cell differentiation,DNA damage, formation of covalent adducts between DNMTs and azanucleoside-substituted DNA, immune modulatory effects, inhibition of NFκB anti-apoptotic pathway etc. They are known to alter multiple signaling pathways thereby preventing DNA damage. Clinical trials for Azacitidine and decitabine are being done in several other cancers to test their efficacy which showed minimum side effects. The patients who showed absolute responses were found to be highly demethylated and showed high levels of drugs in circulation. Drugs targeting epigenome are not proved to be effective in solid tumors due to reduced cellular drug uptake. Another challenge is to combine other therapies with epigenetics to find a better cure to cancers.

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Currently, there are two main hypotheses to explain why cancer cells exhibit the Warburg effect (also known as aerobic glycolysis).  Choose one of these viewpoints and provide supportive evidence for your argument (these viewpoints outlined in the introduction of the paper below).

(Refer to Kim et al. 2009)

Cancer cells adopt an anaerobic way of respiration even in the presence of oxygen. They show a tendency to avoid oxidative phosphorylation. This paradoxical behavior of cancer cells is regarded as the Warburg effect. In the paper by Kim et. al. (2009) two hypothesis have been put forth regarding the Warburg effect.  According to the first hypothesis the relationship between Warburg effect has been described to correlate with greater proliferation. Second hypothesis states that the Warburg effect has an effect in reducing cellular oxidative stress, repairing cellular damage resulting from aggressive proliferation and increased metabolism. Cancer cells possess a unique way of escaping from the surrounding cells and from the immune system. This escape has been evolutionarily taken as an advantage by these cells. The end product of glycolysis is pyruvate. The hypoxic environment is induced by genes such as HIFα which diverts pyruvate from entering into oxidative phosphorylation. Hence pyruvate metabolizes to lactate. This creates an acidic environment. This is favorable to the tumor microenvironment. The presence of acidic environment prevents attack by host immune system. Warburg effect can be utilized for the diagnosis of cancer.

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**Table 2.9** (Weinberg) documents examples of epidemiological correlations between cancer incidence and environmental/lifestyle factors that have no apparent causal relationship.  Choose one of these relationships to explore in more detail and provide evidence supporting or refuting a causal relationship between the lifestyle/dietary factor/medical condition and the risk of a particular type of cancer.

Cancer is one of those diseases which has an epidemiological backgrounds. Almost all cancers have been known to have a predisposition of the familial or habitual background. An investigation of the risk factors which lead to cancer development and progression is essential in the case of familial and habitual cancers. In many such cases cancer may be preventable to some extent through education or changing habits. For example smoking causes lung cancer. However all people who smoke do not develop lung cancer and all people with lung cancer do not smoke. Thus we cannot blindly predict that smoking is a causal habit of lung cancer. But people who smoke have a greater risk of developing lung cancer. A population which is continuously exposed to tobacco smoke creates mutation in certain genes which leads to the development of neoplastic transformation in the lung alveoli. Other effects of tobacco smoke include oral cancer. The combined effects of tobacco smoke along with chewing gutkas causes oral cancer.

There is also an increased risk of lung cancers in passive smokers. The carcinogenic hydrocarbons present in tobacco smoke causes alterations in metabolism of xenobiotic, DNA damage, affects cell cycle and apoptosis and restructures the cytoskeleton to favors neoplastic transformation into a tumor microenvironment. Thus the aims of cancer epidemiology are to study the distribution of cancer, promote insights in to mechanisms of cancer progression and assess efficacy of preventive mechanisms.

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.  Don’t like these questions? Write your own! (but please stay on topic)  Your original question should address at least one of the articles from the  additional reading list.

Comment on the action of any one tumor suppressor gene

Tumor suppressor genes suppress cell division and growth. We will consider Rb gene as an example. A loss of function of Rb locus is involved in retinoblastoma and other forms of cancer including osteosarcomas and small cell lung cancers. This gene is located in the band q14 of chromosome 13. Protein RB interacts with a variety of cellular proteins (E2F and cyclins D and E) and tumor antigens. RB is a nuclear protein which is specifically phosphorylated at the end of G1 phase by cyclin- CDK complexes. It is dephosphorylated during mitosis and remains in this state till it is phosphorylated again at G1/S boundary. UnphosphorylatedRb specifically binds several proteins including E2F and cyclins D and E. E2F is a group of transcription factors. According to one model, unphosphorylatedRb binds to E2F and thereby inactivates it. E2F group of transcription factors activates such genes whose products are essential for S phase. Thus unphosphorylatedRb prevents entry of cells into S phase and consequently cell division. In addition RB-E2F complex directly represses transcription of some target genes. Phosphorylation of Rb at G1/S boundary abolishes its ability to sequester E2F which can now activate its target genes. Further the repression of genes that are targets for RB-E2F complex is also relieved. This allows the cell to enter the S phase.

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