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Promoter Hypermethylation of Tumor Suppressor Genes in Lung Cancer.

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ABSTRACT

Lung cancer is commonest and deadly disease. Tobacco smoking remains its most significant etiologic factors. In approximately 50% of the cases the patient is non-smoker. Because of this it is indicated that tobacco and smoking do not seem to be the sole cause for cancer. Epigenetic changes conjointly play a very important role in the generation of the lung cancer. Hypermethylation of CpG islands of promoter region in tumor suppressor gene results in their inactivation that ends up in numerous kinds of cancer, as well as lung cancer. This review encompasses the promoter hypermethylation of tumor suppressor genes in lung cancer and its possible reversal using demethylating agents.

Keywords: Lung Cancer, Epigenetic changes, Promoter hypermethylation, Reversal of the Tumor Suppressor Gene.

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INTRODUCTION

With more than 1.61 million cases recognized annually lung carcinoma is that the leading cause behind cancer, loss of life among men and second main cause in the back of loss of life in women worldwide [1]. Morphologically the lung cancer is divided into small cell lung carcinoma (SCLC), non-small cell lung carcinoma (NSCLC) and adenocarcinoma (AC), with non-small cell carcinoma (NSCLC) accounting for 80% of all cases [2]. The medical accomplishment in two decades has provided least effect on the treatment of the most cancers, the overall five year survival rate of NSCLC is roughly 15%.

Lung carcinoma is blend of interaction between genetic, epigenetic and environmental factors. In Epigenetic alterations the promoter DNA methylation occurs commonly that ends up in gene silencing. Initial recognition of lung carcinoma could change the sickness result, the survival rate will show increment drastically. In the practice to enhance early identification, numerous imaging and cytology-based procedures have been tried, none of them has yet been profoundly powerful, either in light of constrained affectability or the restrictive cost they bear to general wellbeing frameworks [3]. Hypermethylation of the promoter region of the tumor suppressor gene and several different cancers associated genes is the vital mechanism which leads to many kind of cancers.

Recently, the simultaneous resolution of many of precise, phenotypically described cancer-related CpG methylation marks result in positive resolution through modern biotechnological tools, making an allowance for fast and reliable high-throughput epigenetic profiling of human tissue CpG methylation [4]. The identification of the biomarkers specific to the lung cancer and development of non-invasive technique for detection of the biomarkers will enhance the diagnostic capability in the earlier stage of the cancer.

Epidemiology

Earlier than the start of manufacturing of tobacco in the late 19th century, lung carcinoma was an unprecedented cancer. The Relative Risk Ratio (RR) for the lung cancer was found to be 2.00 stated through several registries based studies [5-7]. Among the other cancers the lung cancer has been the most diagnosed cancer since afterwards 1985. Incidence of lung cancer was higher among men than any other cancer followed by prostate cancer and stomach cancer, in the case of women it had been fourth most diagnosed cancer beside breast cancer, cervical cancer and colorectal cancer. The approximation of total lung cancer detection for both sexes was found to be same in comparison study between developed and developing countries [8]. Globally lung carcinoma finally ends up with 1.38 million deaths, developing it a number one cause of mortality. In 2012, lung cancers killed an expected 1,098,700 men and 4,91,200 women worldwide, similar to 24% and 14% of all cancers deaths in men and women, respectively. lung cancers is that the leading reason for cancer death in 87 countries in men and 26 countries in women, with the latter ordinarily restricted to excessive economic advantage countries [9] global, 5% of lung carcinoma instances have been diagnosed among individuals elderly 0 to 44 years, 14% inside the 45 to 54 age group, 25 in the 55 to 64 age group, and 55 among the ones aged 65 years and over. 26 the proportions had been pretty uniform for each sexes [10]. In India 63,000 new lung carcinoma instances are pronounced annually [11].

Histology

Lung cancer consists of two major sub types which are non-small cell lung cancer (NSCLC) which is responsible for about 80 % of lung cancers and small cell lung cancer (SCLC) representing approximately remaining 20 % of it [12]. Non-small cell lung carcinoma (NSCLC) are sub divided in three categories of squamous cell, adenocarcinoma and large cells. SCLC is most regularly centrally situated tumour arising up from bronchial epithelium [13]. Histologically, NSCLC can be classified into adenocarcinoma, this is the most diagnosed form (40 % prevalence), followed by squamous cell carcinoma (25 % prevalence), and large cell carcinoma which represents only 10 % of the cases [14]. The existence of Non-small cell lung carcinoma (NSCLC) and adenocarcinoma (AC) are seen to be more commonly observed.

Major causes

The consumption of Tobacco, chewable and smoke are amongst the major cause for the cancer. Along with cigarette smoking multistep process involving the genetic and epigenetic changes [15]. Approximately more than 60 cancer causing agents incorporates by tobacco smoke, and amongst those, about 20 carcinogenic agents are truly associated with the Lung cancer generation. Some of these compounds consist of the tobacco-specific nitrosamine 4-(methylnitrsosamino)-1-(3-pyridyl)-1-butanone and polycyclic aromatic hydrocarbons, leads to DNA adduct formation causes genetic mutation [16]. Intake of wood smoke has been found to be causing methylation of p16 gene which causes lung cancer [17]. In different studies it has been shown that various factors are there which are responsible for the occurrence of the lung cancer for eg. urban air pollution (having concentration of 7.5 $\mu g/m^3$ for $P.M_{2.5}$ causes lung cancer) [18]. Intake of indoor smoke produced by burning of coals and other solid fuels has also been found to be the reason for lung cancer [19]. On the dietary factors low intake of fruit and vegetables also act as a risk factor for the lung cancer [20].

Epigenetic changes

The term 'epigenetics' suggests 'outside conventional genetics'. Epigenetics was originally outlined by C.H. Waddington [21]. Epigenetics describes the study of the stable alteration within the sequence functionality that happens throughout the various stages of the proliferation of the cell. The 5-carbon of the DNA base cytosine in the 5' -CpG-3' dinucleotide sequence present in the CpG islands of promoter regions go through methylation reactions carried out by DNA cytosine methyltransferases, DNA methylation is mediated via three DNA methyltransferases (DNMT): DNMT1 – 3a and 3b. DNMT1 binds to hemimethylated DNA to maintain methylation patterns after DNA replication [22]. These enzymes plays vital role in epigenetic regulatory mechanisms in mammals [23]. Epigenetic changes play a vital role in the cell cycle regulation and differentiation along with these changes prevent genes from genetic mutations. The functioning of genome is regulated and prevented by epigenetic changes, by altering the native structural dynamics of chromatin. This results in amendment within the accessibility and also the compactness of genome. In traditional cell functioning, the integration of viral genome in sequence is prevented because of epigenetic changes, to achieve this several epigenetic changes embrace DNA methylation, covalent histone modification.

DNA promoter hypermethylation

DNA methylation is the most studied epigenetic modification. It plays a crucial role within the regulation of the gene expression and chromatin design by providing a stable gene silencing mechanism. Changes that occur in the pattern of DNA methylation leads to cancer initiation and progression. These were the first epigenetic alterations identified in cancer [24-25]. In mammals DNA methylation is directly related to the covalent modification of the cysteine residue within the CpG dinucleotides. CpG rich DNA stretches do not seem to be found within the whole genome however they are principally present on the promoter region of the gene, referred to as the CpG island that is the region of enormous repetitive sequences (e.g. centromeric repeats, retrotransposome elements etc.) [26]. 60% of the human gene promoter region approximately is occupied by CpG islands that are located at the 5' end of the gene [27]. Inhibition of the transcription factor through methyl-binding domain protein (MBDs) which restrict the biding of transcription factor, due to the DNA methylation [28]. These MBDs are present in the transcriptional corepressor complexes (for e.g. histone deacetylase (HDAC) and histone methyltransferase) which are cause for the chromatin redesigning and gene silencing [29]. However, some CpG island promoters become methylated throughout development leading to future transcriptional silencing.

Covalent histone modification

Histone modifications are proposed to play a vital role in determining cellular identity, through Specific patterns present in different kind of cells provides specific function the cell controlling the expression of these gene [30]. The misregulation of histone modification, which occurs due to deregulation of factors that mediate the modification installation, interpretation and/or removal, actively contributes to cancer [31]. The structure and activity of the various chromatin region is decided by variety of a 'histone code'. Some modifications of histone protein include methylation, acetylation, phosphorylation, sumoylation, and ubiquitination which leads to activation or deactivation of the chromatin [32]. Histone modification changes the accessibility of the gene inflicting changes in gene regulation. Various kinds of modification in methylation/demethylation and

acetylation/deacetylation of core histone leads to lung cancer. For example, hypoacetylation of H4K12/H4K16, decreased H4K20Me3 levels and hyperacetylation of H4K5 and H4K8 were observed in lung cancer cells compared to adjacent normal respiratory epithelial cells [33]. Studies have shown a global loss of acetylated H4-lysine 20 trimethylation (H4K20me3) and H4- lysine 16 (H4K16ac) [34]. Loss of function occurs due to histone acetylation mediated by HDACs which causes gene repression. Overexpression of HDACs are found in various types of cancer [35-36] and thus, have become major target for the epigenetic therapy.

Role of the epigenetic changes in cancer

The epigenomics of traditional cells undergoes intensive distortion in cancer [37]. These epimutations, result in wide genetic alteration, registering a vital role within the cancer initiation and progression [38]. The cancer epigenome is defined by global changes within the DNA methylation and histone modification pattern, gene expression the organic phenomenon that ends up in the event of the cancer stem cells. Epimutation ends up in the silencing of the tumor suppressor gene and additionally within the conjunction deletion of the gene that therefore gives the second hit for the cancer development. This hypothesis is thought because the 'two-hit' model and was projected by Alfred Knudson [39]. The epimutation additionally ends up in the conversion of the protooncogene to oncogenes that ends up in cancer. The cancer cells have an aberrant methylation standing that causes global hypomethylation and promoter hypermetylation of TSG (tumor suppressor gene) [40-41].

Gene which are found to be hypermethylated in lung cancer

Through over a decades of the research, scientists have targeted those genes that are found to be hypermethylated and are the cause behind occurrence of lung cancer. The genes which have been found to be hypermethylated in lung cancer are shown in **Table 1**.

Genes	Reference
• GSTT1	Raimondi *et al., 2006*
• DNMT1	Xing *et al., 2008*
• DNMT3b	
• p16	Belinsky *et al.,* 1998
• FHIT	Topaloglu *et al.,* 2004
• H- CADHERIN	
• RARβ	
• CDH1	
• APC	
• NEUROG2	Geng *et al.,* 2012
• NID2	
• MGMT	Schiemann *et al.,* 2005
• CDKN2A	
• GST.P1	
• CDKN2A	
• RARβ2	
• ARF	
• RASSF1A	Grote *et al.,* 2006
• RARβ2	
• SHOX2	Schmidt *et al.,* 2010
• A5C	Brock *et al.,* 2008
• DAPK	

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• APL	
• CDH13	Sato *et al.,* 1998
• BRMS1	Yang *et al.,* 2011
• PITX2	Dietrich *et al.,* 2012
• HS3512	Anglim *et al.,* 2008
• KLK10	Zhang *et al.,* 2011
• SFRP1	
• SOX 17	Son, 2011
• CDH13,	Yang *et al.,* 2011
• BRMS1	
• PITX2	Dietrich *et al.,* 2012
• PAX5β,	Belinsky *et al.,* 2007
• GATA5	
• BLU	Hsu *et al.,* 2007
• ASCL2	Anglim *et al.,* 2008
• CDX2	Tsou *et al.,* 2007
• HOXA1	
• OPCML	
• SHOX2	Kneip *et al.,* 2011
• PAX5α	Belinsky *et al.,* 2004
• GATA 5	
• MGMT	
• NY-ESO	Gure *et al.,*
• LAGE-1	
• MAGEE-A1	
• MAGE-AB	
• MAGE-A4	
• SSX4	
• p73	Esteller *et al., 2001*
• GSTP1	
• hMLH1	
• $p15^{INK4b}$	
• EGFR	Mitsuo *et al., 2007*
• HER2	
• C-KIT	
• BRAF	
• RAS	
• BCL2	
• EMR3	Heather *et al., 2012*
• CPA4	
• S100A2	
• GML	
• MKRN3	
• TNF	
• HDAC1	

• SPP1	
• CYP2E1	
• RUNX3	
• RIZI	Du *et al., 2001*
• FUSI	Konda *et al., 2001*
• SEMA 3B	Tomizawa *et al., 2001*
• C/EBPα	Tada *et al., 2006*

Table 1: Genes found to be hypermethylated in lung cancer

Reversal of the hypermethylated gene

The epigenetic changes are reversible changes. Thus through the application of several enzyme inhibitors reversal of the activity of the gene may be achieved and normal functioning of the gene can be restored in the cell thus reversing the cancer. Through years of research and study several drugs and inhibitors are found which have potential for reversal of hypermethylation. To achieve more practical and accurate therapeutics the chromatin granule modifiers are held to be potential targets for therapeutics development against the cancer [69-70]. Food and Drug Administration (FDA) and European Medicines Evaluating Agency (EMEA) have approved chromatin granule regulator as an important aspect for treatment. Drugs which have been found to have potential of causing reversal of promoter hypermethylation in lung cancer are shown in **Table 2**.

Drugs	**References**
• Histone deacetylase (HDAC). • Inhibitors Janus Kinase 2 (JAK2) inhibitors.	Hatzimicheal & Crook, 2013 Dawson *et al.,* 2012
• DNA methyltransferase inhibitors (DNMTi)	Liu *et al.,* 2013
• Azacytidine (5-azacytidine, Vidaza) • decitabine (5-aza'-2-deoxycytidine, Dacogen)	Hatzimicheal & Crook 2013, Liu *et al.,* 2013 & Tang *et al.,* 2009
• Valproic acid	Perrino *et al.,* 2008
• Azacytidine	Komashko & Farnham, 2010
• LBH589, scriptaid, valproic acid, apicidin, • OSU-HDAC-44 and • MAS-275	Brazelle *et al.,* 2010
• tyrosine kinase inhibitor (TKi) therapy	Zhang *et al.,* 2009
• Trichostatin A (TSA)	Platta *et al.,* 2007
• Romidepsin	Karthik *et al.,* 2014
• **Vorinostat (suberanilohydroxamic acid (SAHA))**	**Petta V., *et al., 2013***
• Pan-ERBB Tyrosine Kinase inhibitor(CI-1033)	Mitsuo *et al., 2007*
• SRC inhibitor (Dosatinib)	
• Raf kinase inhibitor (Sorafenib)	
• P 13 K inhibitor (CY294002)	
• Tyrosine kinase inhibitors (gefitinib, etrotonib)	

• Panobinostat	Crisanti *et al., 2009*
• Hydralazine	Jakopovic *et al. 2013*
• Decitabine	
• **Azacytidine** • **Gefitinib (an EGFR TKi)** • **Trichostatin A (TSA)**	**Noro, R., *et al., 2007***

Table 2: Drug used for the reversal of the promoter hypermethylation of gene.

CONCLUSION

Epigenetic changes like promoter hypermethylation cause inactivation of tumor suppressor genes in cancer. The hypermethylation of these genes could also be used as a diagnostic biomarker in the diagnosis of the lung cancer. The epigenetic study of these genes may offer a reliable and quick diagnosis of the cancer at a pre- malignant state, therefore reducing the high fatality rate (95%). These changes are reversible and therefore are of paramount importance. As compared to the genetic changes that are non-reversible, epigenetic changes are dynamic and therefore are reversed through use of various agents thereby providing a non-invasive, accurate and quick treatment. Various drugs are used for effective treatment of carcinoma and reversal of hypermethylation of the genes concerned. It has been determined that these chemicals and drugs have potential harmful effects on human health and might cause cytotoxic effects. Efforts are being made to develop efficient and effective drugs from natural compounds that cause less side effects to human health.

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