



TUMOR SUPPRESSOR GENES-THE EPIGENETIC BIOMARKERS IN HEPATOCELLULAR CARCINOMA

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Abstract-Majority of individuals are infected from cancer worldwide. Many types of cancer have been reported in previous years and liver cancer is one of them. 840,000 new cases of liver cancer have been reported worldwide in 2018. Liver cancer is the 5th common disease in men and 9th in women. Smoking, chronic infection, hepatitis (hepatitis B & hepatitis C virus), liver cirrhosis, and genetic/epigenetic changes are the most common risk factor for liver cancer. Epigenetics may be defined as the change in gene expression without any change in DNA sequence. Epigenetic changes include DNA methylation, histone modification, and RNA associate silencing. Promoter hypermethylation of tumor suppressor genes may be considered as a prominent cause of liver cancer. A lot of hypermethylated genes have been reported as a biomarker for the prognosis of liver cancer. Some of these tumor suppressor genes such as BCL6B, RIZ1, SYK, GSTP1, DKK3, FPB1, TIP30, and ZHX2 are described in this paper. **Keywords:** Biomarker, Chronic infection, Epigenetic, Metastasis, Transformation, Tumor suppressor gene (TSGs)

1. INTRODUCTION

Liver is biggest organ in human body. Mainly liver filter the blood that come from digestive path, liver also removes the toxic chemicals from our body and like this also prepare the proteins for our body. Lot of people in worldwide are infected due to malignancy of cancer. In 2018, 9.6 million people have been confined to death due to cancer worldwide while 782000 people in India died from liver cancer. (www.who.int.com, 2018). Liver cancer occur when these cells randomly divide, it results in the development of a tumor. Two types of tumor are there one is benign tumor its cell doesn't divide so does not spread and another one malignant tumor its cells divides number of times so it gets infected to variant part of body, it's a cancerous tumor. Malignant tumor cell divides and infect the other part of our body, the process known as a metastasis. A few months can be expected in the first phase of cancer but after a quick and effective treatment, the lifespan can be extended to 5 years (Forner et al., 2012).

Several types of cancer are formed in liver such as hepatocellular carcinoma, hepatoblastoma, and intrahepatic cholangiocarcinoma etc. Hepatocellular carcinoma is 5th common disease in all cancer and 3rd most malignant cause of death in worldwide; it has good diagnostic and therapy feature while it's difficult to predict it even today (Mitsuro et al., 2014). Hepatocellular carcinoma occurs in the 'hepatocytes' (liver cells), it is a most infected type of primary liver cancer and constitutes 80% of all liver cancer cases. Hepatocellular carcinoma increases as a result of interaction between environmental and genetic factor. The lifespan of hepatocellular carcinoma patients depends on the diagnosis of it. African and Asian people are commonly infected due to the hepatitis B and hepatitis C virus so these people have high risk of increase HCC (Li et al., 2019).

2. EPIGENETICS

Phenomenon of turning out of gene expression without any difference into gene sequence is called epigenetics (Jha et al., 2011). The word 'Epigenetic' was proposed by a renowned developmental biologist C.H. Waddington in 1942. This word is made from two Greek words Epi + Genetics, this Epi refers to "on top of" or "in addition to" in genetics. In epigenetic mechanism, the phenotype is expressed without any changes in genotype; however, they are most often preserved during cell division ceaselessly and reversible probably. Epigenetics is a procedure of secular and local control of the gene expression during the development of complex organisms (Holliday et al., 1996). For the progress and separation of various types of cells, epigenetic changes are essential to be occurred in our body. Epigenetic changes encompass a wide phalanx of chromatin remodeling complex coregulators, effectors, and transcription factors, synthesis of noncoding microRNAs (miRNAs), DNA methylation, as well as covalent modification of histones such as phosphorylation, acetylation, ubiquitination, deacetylation, and simulation that are considered important determinants for regulation of gene expression. These epigenetic and genetic changes work together to increase cancer progress and coordinates at all phases of cancer development (Jones et al., 1999).

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Epigenetic changes contribute like a major tool in all form of the cancer, it is informed by recent records; however, the cancer genetic origin is universally accepted (Feinberg et al., 2006).

This epigenetic mechanism can be of 3 different categories -

2.1 DNA Methylation

DNA methylation refers to 5th position, association with a methyl group to a cytosine occurs DNA modification. Epigenetic changes such as global hypomethylation and specific gene promoter hypermethylation (Fig.1) have been confirmed to be involved in genome variability and tumor suppressor gene silencing separately. Epigenetic change cannot create any change in primary DNA sequence but these changes play an important role in genetic stability and to safe integrity of chromosome. The CpG dinucleotide builds up by cytosine and guanine in DNA sequence. DNA modification has been described as the covalent modification of nucleotides, the cytosine most common methylated nucleotide in human genome after which guanine in the sequence of DNA. In human genome methylated cytosine affects 75% of all CpG dinucleotides, that it in total nucleotides cytosine accounts 1%. The presence of the CpG islands reveal in eukaryotes by analysis of DNA methylation patterns, casually guanine-cytosine-rich regions occur 0.5 to 04 kb length that containing high relative densities.

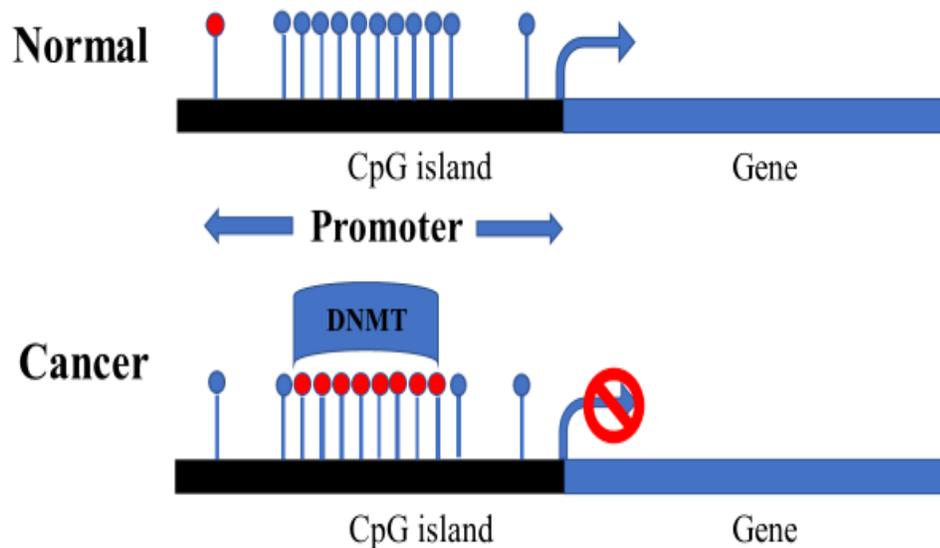


Fig. 2.1 DNA Methylation Profile in Cancer. Liver Cancer Cells Usually Exhibit DNA Hypermethylation at Promoter Sites of Tumor Suppressor Genes, Resulting in Silencing of these Tumor Suppressive Genes

2.2 Histone Modification

Histone modification also known as covalent post-translational modification (PTM) for histone protein which includes phosphorylation, methylation, acetylation. Histone protein present in octamer form inside chromosome wrapper which pack the DNA. DNA damage or repair, chromosome packaging, and transcriptional activation or inactivation these are type of biological mechanism that has been controlled through the histone modification. Chromatin dynamics and transcription, gene silencing, cell cycle progression, differentiation, DNA replication, apoptosis, neuronal repression, and nuclear import are types of cellular mechanism they have been regulated by the histone acetylation process. Histone acetylation has been regulated through the coordination between acetyl co enzyme A and an acetyl group (COCH₃). Histone lysine residues removes hydrolytic group from acetyl group which is catalyzed by histone deacetylases.

2.3 RNA Associated Silencing

miRNA is found 60% in genes of human and other mammals. miRNA plays a contributing role in RNA silencing and post transcriptional regulation of gene expression, and is a micro molecule. Gene expression has been regulated by RNA silencing which is promoting several mechanistic related pathways.

3. PROMOTER HYPERMETHYLATION

DNA segment change their activity through DNA methylation without any change in DNA sequences when methyl group add to 5th position of cytosine ring than known as DNA methylation. The methyl group is attached cytosine through DNA methyltransferase enzyme. The small group of DNA methyltransferase enzyme (DNMT1, DNMT3A and DNMT3B) help in maintaining and establishing the DNA methylation patterns. The cancer cell gene is



inactivated by hypermethylation of CpG Island, this type of change in gene expression was controlled by epigenetic mechanism. CpG island hypermethylation majorly contributes for inactivating the gene and it has been discovered in every type of cancer, and hepatocellular carcinoma is one of them.

4. SOME PROMOTER HYPERMETHYLATED GENES IN HEPATOCELLULAR CARCINOMA (HCC)

4.1 BCL6B

BCL6B is hypermethylated in Chinese population. BCL6B, also known as BAZF, plays a role in the nucleus as a sequence-specific transcriptional repressor (Olabe et al., 1998). BCL6B is a protein that called B-cell lymphoma 6 member B proteins in human. BCL6B gene is located at chromosome 17 in human. P53 signaling inactivated due to BCL6B silencing and inhibits 5-FU for increased human hepatocellular carcinoma. BCL6B may be an HCC biomarker and BCL6B methylation contributes in HCC. It has been proved that BCL6B is normally methylated in hepatocellular carcinoma and the expression of BCL6B was regulated by promoter hypermethylation (Xin Li et al., 2015).

4.2 RIZ1

RIZ1 (PRDM2) was first isolated by screening for proteins. RIZ1 (PRDM2) was named retinoblastoma protein-interacting Zinc finger gene in a screen for proteins binding the retinoblastoma (Rb) protein (Buyes et al., 1995). It is a member of nuclear histone protein methyltransferase super-family. RIZ1 gene methylation was observed in 62% liver cancer. Inactivation of the RIZ1 (PRDM2) gene by promoter hypermethylation has been reported in liver and gastric carcinomas (Fang et al., 2000; Nishida et al., 2008).

4.3 SYK

The SYK gene is known as spleen tyrosine kinase, it has been studied that SYK gene works like a biomarker in hepatocellular carcinoma (Chen et al., 2018). SYK gene was low regulated and examined to be 38% in hepatocellular carcinoma. Gene expressions of SYK observed by immunoblotting and qRT – PCR mechanism in hepatocellular carcinoma (Chen et al., 2018). SYK gene is located at chromosome 9 and its molecular mass is 72066 Da. SYK gene is found to be hypermethylated in Chinese population.

4.4 GSTP1

GSTP1 gene known as π -class glutathione S-transferases. The GSTP1 gene increase hepatitis B virus with hepatocellular Carcinoma. DNA hypermethylation of GSTP1 gene was observed in 65% HCC tissues and 10% associated non-tumor tissues. GSTP1 gene is located at chromosome 11 and its molecular mass is 23356 Da. Phase II enzymes such as glutathione s-transferases (GSTP1, GSTA1) have been suggested to play an important role in protecting cells against damage induced by carcinogens, through regulation of the conjugation of a wide range of xenobiotics for excretion of hydrophilic metabolites (Jakoby et al., 1978; Rebbeck et al., 1997). Significantly increased expression of GSTP1 was demonstrated in early hepatocarcinogenesis (Sakai et al., 2007) and HCC specimens (Niu et al., 2005), compared to their adjacent normal tissues or liver cirrhosis tissues.

4.5 DKK3

It has been investigated that DKK3 gene show mRNA expression in hepatocellular carcinoma (Zhen et al., 2009). DKK3 (Dickkopf WNT signaling pathway inhibitor 3) gene is located at the chromosome 11 and its molecular mass is 38390 Da. As a soluble Wnt inhibitor, Dickkopf-3 (Dkk3) is involved in molecular cancer therapy. Dkk3 binds with LDL-receptor-related protein5/6 (LRP5/6) and destabilizes cytoplasm β -catenin (Li et al., 2005). It has been studied that promoter DKK3 gene contributes as a carcinogen in HCC by hypermethylation because the DKK3 gene expressions increased more than adjacent non-cancerous tissues in hepatocellular carcinoma (Edamoto et al., 2003; Loeppen et al., 2005). Dickkopf f-related protein-3 (DKK3) is one of the proteins expressed during the very early stages of hepatogenesis. DKK3 suppresses Wnt-dependent carcinogenic activity by reducing nuclear and cytoplasmic β -catenin accumulation (Liang et al., 2015)

4.6 FPB1

FPB1 is located at chromosome 9 and its molecular mass is 36842 Da. Fructose-bisphosphates 1 (FBP1), which catalyzes the splitting of fructose-1,6-bisphosphate (F-1,6-BP) into fructose 6-phosphate and inorganic phosphate, is a rate-limiting enzyme involved in gluconeogenesis (Dong et al., 2013). The methylation and expression of FBP1 gene in primary liver cancer has been researched previously. 80% expression of FBP1 gene was observed in HCC (Chen et al., 2011). HepG2, HuH7, SMMC-7721, Sk-Hep1, HCC-LM3, BEL-7402, MHCC-97H and MHCC-97L are the cell lines of liver cancer. In the promoter region, FBP1 binds to the far upstream element to mediate c-Myc gene transcription (Zhang et al., 2013).

4.7 TIP30

It has been researched that the downregulation of TIP30 gene expression effects hepatocellular carcinoma by low dose of sorafenib (Zhigui et al., 2016). TIP30 gene saw their 33% expression in hepatocellular carcinoma. Mutations were frequently found in TIP30 exon 2 in various cancer cells but not in normal cells when the TIP30 sequences in the National Center for Biotechnology Information databases were analyzed (Ito et al., 2003). Somatic missense mutations in the TIP30 gene were identified in human HCC tissue specimens, which resulted in either instability or the abnormal cellular distribution of TIP30 protein in cells (Ito et al., 2003), suggesting that these TIP30 mutations may contribute to the pathogenesis of HCC development through inactivation of TIP30 function.

4.8 ZHX2

It has been researched that ZHX2 (zinc fingers and homeoboxes protein 2) gene show methylation of their 199 base pair sequences in hepatocellular carcinoma (Xuetian et al., 2012), it has been observed by the use of MSRF (methylation-sensitive restriction fingerprinting). Hypermethylation of ZHX2 gene does not express in normal tissues of liver only HepG2 cell line and some HCC tissues occurred CpG island of ZHX2 it has been identify through using bisulfide sequencing. ZHX2 gene is located at chromosome 8 and its molecular mass is 92307 Da. More recent studies indicate that ZHX2 also regulates hepatic enzymes involved in plasma lipid homeostasis, including lipoprotein lipase (Gargalovic et al., 2010). Previously research showed that ZHX2 reduces AFP secretion (Shen et al., 2008) and GPC3 expression (unpublished data) in human HCC cell lines.

CONCLUSION

This study can be utilized as an important tool for prognosis /diagnosis of hepatocellular carcinoma and its risks factor in future. For the better treatment of the cancer patient tumor suppressor genes which have been identified can be utilized as epigenetic biomarkers. Many types of tumor suppressor gene have been reported as biomarkers worldwide and few of them have been described in this paper. We can use TSGs as epigenetic Biomarker which can prove to be an important tool against Hepatocellular Carcinoma all over the world.

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