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Review Article

The Role of Epigenetic System in Hepatocellular Carcinogenesis

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Abstract

Liver cancer (hepatocellular carcinoma or HCC) is a major lethal cancer worldwide. Epigenetic and genetic alterations contribute to HCC initiation and progression. The epigenetic system in the human body consists of two main categories, the processes and the workers or factors. Epigenetic processes, like DNA methylation, histone modifications, regulate the genes expression without altering DNA sequences, by the action of internal factors like non-coding RNAs, DNA methylation enzymes and transcription factors. Disruptions in epigenetic processes or factors can lead to alter gene function and disrupts many cell processes leading to Cancer transformation. A better understanding of the underlying epigenetic alterations during carcinogenesis is provided us to discover epigenetic biomarkers for detection, prognosis, risk assessment, and HCC monitoring.

Keywords: Epigenetics; HCC; DNA Methylation; Non-Coding RNA; Histone Modifications

Abbreviation

AFB1: AFlatoxin B1; AFP: Alpha-Fetoprotein; APC: Adenomatosis Polyposis Coli; AR: Androgen Receptor; ARF1: ADP-ribosylation Factor 1; BHMT: Betaine Homocysteine Methyl Transferase; Bmf: Bcl2 Modifying Factor; CASD1: CAS1 Domain Containing 1; CBS: Cystathione β Synthase; cccDNA: Covalently Closed Circular DNA; CCRK: Cell Cycle-Related kinase; CDH1: E-cadherin; CDK4: Cyclin-Dependent protein kinase 4; CHFR: Checkpoint With Forkhead And Ring Finger Domains; CYP2E1: Cytochrome P450 family 2 subfamily E member 1; DKK2: Dickkopf WNT signalling pathway inhibitor 2; DNMTs: DNA methyltransferases; DNMT1: DNA methyltransferases1; EGCG: Epigallocatechin gallate; E-cadherin: Epithelial Cadherin; EZH2: Enhancer of Zeste 2 Polycomb Repressive Complex 2 Subunit; FZD7: Frizzled Class Receptor 7; GNMT: Glycine-N methyltransferase; H3K27ac: Acetylation of histone 3 at lysine 27; H3K36me3:Trimethylation of histone 3 at lysine 36; H3K4me3: Trimethylation of histone 3 at lysine 4; H3K9ac: Acetylation of histone 3 at lysine 9; HBV: Hepatitis B virus; HBx: HBV encoded protein x; HCC: Hepatocellular carcinoma; HCC-AFB: Hepatocellular carcinoma induced by AFB; HCC-HBV: Hepatocellular carcinoma induced by HBV; HCC-HCV: Hepatocellular carcinoma induced by HCV; HDACs: Histone deacetylases; MLH1: DNA Mismatch Repair Protein Mlh1; MSH2: DNA mismatch repair protein Msh2; MSH6: DNA mismatch repair protein Msh6; HOTAIR: HOX Transcript Antisense RNA; PMS2: Postmeiotic Segregation Increased 2; IARC: International Agency for Research on Cancer; IL-6: Interleukin-6; LINE1:Long Interspersed Elements -1; lncRNA: Long non-coding RNA; LOI: Loss of Imprinting; LSD1: Lysine-Specific Histone Demethylase 1; MAP3K4: Mitogen-Activated Protein Kinase Kinase Kinase 4; MBD: Methyl CpG-Binding Domain Proteins; MDM2: Mouse Double Minute 2 homolog; MEOS: The microsomal ethanol oxidizing system; MGMT: O6-Methylguanine DNA Methyltransferase; MMP14: Matrix Metallopeptidase 14; MMRs: The Mismatch Repair system proteins; MS: Methionine Synsthase; NADH: Nicotinamide Adenine Dinucleotide dehydrate; NDMA: N-Nitrosodimethylamine; NF-κB: Nuclear Factor Kappa B Subunit 1; NNK: 4-N-methyl-N-nitrosamino-1-3-pyridyl-1-butanone; P14 –ARF: CDK4 Inhibitor P14-ARF; p16- INK4: CDK4 Inhibitor P16-INK4; PARP1: Poly(ADP-Ribose) Polymerase 1; PCMP: Polycomb complexes proteins; PdG: Propanodeoxyguanosine; pgRNA: Long

pregenomic RNA; Pol II: RNA Polymerase II; PSA: Prostate-Specific Antigen; PTMs: Post-Translational Modifications; RASSF1A: RAS Association Domain Family Protein 1 Isoform A; RC DNA: Relaxed Circular partially double-stranded DNA; ROS: Reactive Oxygen Species; SAM: S-Adenosyl Methionine; SAH: S-Adenosyl Homocysteine; SFRP1: Secreted Frizzled Related Protein 1; SOCS-1: Suppressor of cytokine signaling 1; SOCS-3: Suppressor of cytokine signaling 3; STAT3: Signal Transducer And Activator Of Transcription 3; SYK: Spleen Associated Tyrosine Kinase; TGF-β1: Transforming growth factor-beta 1; TMPRSS2: Transmembrane Serine Protease 2; UBE2C: Ubiquitin Conjugating Enzyme E2 C; WIF-1: Wnt inhibitory factor 1.

Introduction

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. It is the third lethal cancers causing death in the world. In the developing countries like in Eastern Asia, sub-Saharan Africa, most of the HCC cases occur due to the chronic infection of hepatitis B virus (HBV), but in the developed countries like USA, infection with hepatitis C virus (HCV) is the most causes of HCC [1].

Beside virus infections, there are a lot of risk factors causing HCC progress and development such as aflatoxins, excessive alcohol consumption, smoking, and diabetes. These risk factors have the ability to cause both genetically and epigenetically alterations once the body affected by one or more of them. These alterations can cause activation of oncogenes and can suppress the tumor suppressor genes, leading to development of hepatocellular carcinoma [2].

In hepatocellular carcinogenesis, processes such as cell signalling, apoptosis, transcription, and DNA repair are affected leading to disturbance in cell survival, growth, transformation and maintenance [1].

In fact, epigenetics are how genes introduce themselves into the cells to produce a specific phenotype. HCC is developed due to epigenetic and genetic alterations. Researches on epigenetics are necessary to understand the etiology of HCC and established biomarkers used for early detection and monitoring the progress of the disease. Good understanding of the causes of the disease gives the opportunity to evaluate novel effectiveness and beneficial treatment.

What is epigenetic?

Epigenetic processes are normal processes used by the body to control its genes by expression or silencing. Study any alteration occurred to the gene expression without altering DNA sequence is called epigenetics [3]. Holliday [4] defined epigenetics as "the study of the mechanisms of temporal and spatial control of gene activity during the development of complex organisms".

Accordingly, epigenetics are the way that causes genes to behave or "express themselves". In normal situations, when epigenetics are working they makes only the necessary genes needed in the cell activity to be expressed [3].

Epigenetic alterations are tissue specific only. Through this fact the genome and risk factors of lifestyle and the environment play together in the body causing the specificity of tissue toxicity, pharmacokinetics, and pharmacotoxicity. Genetic alterations can be measured at any point, but epigenetics alterations must be measured at certain time points [5].

The epigenetic alterations are dynamic and very respond to the environmental alterations. Cancer is a genetics and epigenetics disease. HCC is like other cancers affected by epigenetic modifications; where epigenetic components participate in all the liver carcinogenesis stages. The epigenetic alterations affected on the oncogenes and tumor suppressor genes, leading to alterations in cell proliferation, cell growth, cell survival, apoptosis, cell adhesion, and body metabolism [2].

The epigenetic system has two wings fly by them: 1) the epigenetic processes which are DNA methylation, histone modification, chromatin remodelling and chromosome looping, 2) epigenetic factors which are the agents responsible for doing modifications and they are two types one is internal factors such as noncoding RNA and, transcription factors, the other is external factors mainly is environmental factors such as virus infection, food preventative and aflatoxins.

Epigenetic processes dysregulation in HCC

DNA Methylation

DNA methylation is a primary mechanism of epigenetic regulations of genome function. Adding methyl group, using "S-adenosyl methionine" (SAM) as a methyl donor, to the cytosine (5^{th}) carbon ring at the 5′ position of a CpG islands is called methylation pro-

cess. Approximately 70% of all CpG are methylated in the repetitive DNA elements, but only a few of the CpG sites are unmethylated, located within the CpG islands found over the promoter and first exon regions of housekeeping genes and tumor suppressor genes. In normal cells, DNA methylation is the protection process of the genomic integrity. Nevertheless, in cancer cells, the methylation pattern is altered causing tumor prognosis. The methylation modifications occur by three ways hyper-methylation, hypomethylation, and loss of imprinting (LOI) [6,7].

Hyper-methylation

DNA hyper-methylation is the addition of a methyl group to specific sites, occurs specially in the CpG islands found in the promoters. It is now established that tumor suppressor genes are hyper-methylated in cancer cells. Moreover, each cancer subtype is specified by methylated genes sets causing silencing of these genes. In addition, DNA methylation can inhibit gene expression directly by suppressing the binding of specific transcription factors and indirectly by attaching to the "methyl CpG-binding domain" (MBD) Proteins [7]. The promoter of the tumour suppressor genes, that controls many biological processes such as cell growth, cell cycle and DNA repair, are found to be hyper-methylated at CpG islands loci causing development and progress HCC. As shown in

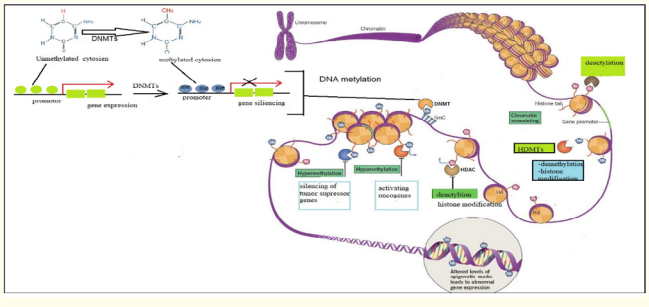


Figure 1: Epigenetic dysregulation process.

Cell growth affected by hyper-methylation in HCC

The cytokine which regulates the JAK/STAT signalling pathway it was silenced by hyper-methylating its promoter. This observation was found in more than 60% of HCC cases. Multiple tumor-related genes are silenced in HCC, moreover, increasing in methylation of several genes was observed in the cancer progression [8].

Silencing of "Suppressor of cytokine arcinoma 1" (SOCS-1) and "Suppressor of cytokine arcinoma 3" (SOCS-3) genes by hyper-methylating their promoters were revealed in liver cirrhosis, fibrosis stage and HCC. SOCS-1 and SOCS-3 are cytokines mediated the JAK/STAT signalling cell growth and differentiation pathway [9].

Cell cycles is affected by hyper-methylation in HCC

HCC can be developed when the promoters of the genes that govern the cell cycles pathways are hyper-methylated. For example, in INK4a-ARF pathway, "CDK4 Inhibitor P16-INK4" (P16-INK4a) and "CDK4 Inhibitor P14-ARF" (p14-ARF) shown to be hyper-methylated causing loss of their function. These two proteins are regulating cell-cycle proteins like, "cyclin-dependent protein kinase 4" (CDK4) and p53. P16-INK4a binds to CDK4 causing loss of its ability to react with cyclin D1. The cell cycle phases are controlled by cyclin D1 gene which is binding to "Mouse double minute 2 homolog" (MDM2). P14-ARF prevents the degradation of p53 and induces cell cycle arrest [2]. The CpG islands found in the promoter of these proteins was reported to be hyper-methylation in 73% of HCC, but 29% in chronic HBV and up to 23% in HCV [2].

DNA repair is affected by hyper-methylation in HCC

The mismatch repair system (MMR) proteins were silenced due to the high methylation of their promoters like "DNA Mismatch Repair Protein Mlh1" (MLH1), "DNA mismatch repair protein Msh2" (MSH2), "DNA mismatch repair protein Msh6" (MSH6) and "PMS2 Postmeiotic Segregation Increased 2" (PMS2) genes leading to disrupt DNA repair process and stimulating HCC development [6]. Moreover, O6-methylguanine DNA methyltransferase (MGMT) was found to be down-regulated when its promoter was hyper-methylated producing carcinogenesis cascade. MGMT is highly activated in the liver; it protects cells from DNA damage caused by mutagenic and cytotoxic agents [7].

Hypo-methylation

Loss of DNA methyl group, referred to "DNA hypo-methylation", it is accompanied by genomic instability and cancer progression. Moreover, DNA hypo-methylation contributes to oncogenesis by causing loss of parental allele (LOI). Hypo-methylation of highly repeated DNA sequences is associated with cancers. These repeats are like "Tandem centromeric satellite α", "juxta centromeric (centromere-adjacent) satellite 2", and "long interspersed elements (LINE)-1" which are the most hypo-methylated repeats contribute with cancer [10].

In tumor cells, global hypo-methylation and hyper-methylation working together in the CpG islands promoter. Stefanska [11] say

that, 3700 hypo-methylated gene promoters were discovered in HCC tumour samples which mediate cell proliferation, adhesion, cell signalling, mobility and invasion for example "ADP-ribosylation factor 1" (ARF1), "CAS1 Domain Containing 1" (CASD1), "Mitogen-Activated Protein Kinase Kinase Kinase 4" (MAP3K4), "Matrix Metallopeptidase 14" (MMP14) and "RAS Like Proto-Oncogene A" (RALA) genes [11].

Methionine metabolism modifications and DNA methylation in HCC

The methionine synthesis pathway is central to liver function. Methionine cycle is considered as the machine of methyl group in the liver factory. In the liver, SAM "S-Adenosyl methionine" is an enzyme considered as the major methyl donor required for the methylation of nucleic acids, phospholipids, histones, biogenic amines, and proteins. More than half of the dietary methionine is converted to SAM [12]. Therefore, an optimal supply of SAM or removal of "S-Adenosyl homocyteine" SAH is essential for normal establishment of genome-wide DNA methylation patterns [12].

SAM synthesis is depressed in chronic liver disease. Moreover, decrease expressions of "betaine homocysteine methyltransferase" (BHMT), "cystathione β synthase" (CBS), "Glycine-N methyltransferase" (GNMT) and "methionine synsthase" (MS) genes are observed in tissues of HCC more than normal tissues [13].

Histone modifications

Histone modifications are running in the chromatin structure and regulate gene expression. Unlike DNA methylation, histone modifications can lead to either activation or repression depending upon which target genes are modified and the type of modifications. Multiple post-translational modifications occur mainly to the histones N- terminal tail. These modifications are acetylation, methylation and phosphorylation among other modifications [5].

Histone acetylation modifications in HCC

Histones acetylation is running in enhancer genes and promoter regions leading to increase in their transcription activation by acetylating the histone H3 at lysine 9 "H3K9ac" and lysine 27 "H3K27ac". The balance between histone methyltransferase and histone demethylase regulate transcription process of the target gene. Where, in normal cells the tri-methylation of lysine 4 and 36 in H3 "H3K4me3" and "H3K36me3" generally leads to activating transcription. On the other hand, the tri-methylation of histone H3 in lysine 9 "H3K9me3", 27 "H3K27me3" and 20 "H3K20me3" are associated with repression of transcription at target gene loci [14]. Tri-methylation of H3K27 is occurred by "Polycomb complexes proteins" (PCMP). It was reported that alterations in the activities of "histone deacetylases" HDACs resulting in altered acetylation of histone, leading to several diseases including cancer. Where, it is found that HDACs inhibits the transcription of p21 by removing acetylation from histones, resulting in cell-cycle activation and cell proliferation leading to HCC [15].

Histones methylation modifications in HCC

The level of H3K27 tri-methylation is significantly increased in HCC tissues compared with non-tumors liver tissues considering their prognostic values of HCC development and progression. Beside that, the increased level of H3K27 tri-methylation in HCC significantly correlated with large tumor size, poor differentiation, advanced clinical stage, vascular invasion and shortened survival time of patients with HCC [16].

"Enhancer of Zeste 2 Polycomb Repressive Complex 2 Subunit" (EZH2) is one of the (PCMP) proteins, it methylates H3K27. It was found to be up-regulated in HCC tissue not only but also presented with cancer progression, invasion and proliferation. EZH2 regulates a big network of genes encoding many biological activities. For example, EZH2 can inhibit the expression of Wnt inhibitors in Wnt/ β-catenin pathway promoting β-catenin dependent carcinogenesis [16].

Nucleosome Positioning

Nucleosome is the basic unit of chromatin which consist of the DNA wrapped around the octamer histones. Chromatin activity, is influenced by the histone-modifying enzymes and chromatin remodelling complexes, and initiates transcrip¬tion, replication, and DNA repair processes. Any change in histone-modifying molecules and chromatin complexes leads to development of many cancers [5]. In normal cells, the nucleosome complex is removed from tumor suppressor genes promoters such as MLH1 causing de-methylation and activation of these suppressors. But in cancer cells, the nucleosome complex is depletion causing hyper-methylation and silencing of tumor suppressor genes leading to cancer development [17].

Chromosomal Looping

The DNA strand on the same chromosome sticky with each other forming chromatin loop this occurs when the stretched genomic sequence are closer to each other. Chromosomal looping af-

fects gene expression, where silencing or activation of the genes is affected by the formation of chromosomal looping. In cancer cell, exhibits the formation of chromosomal loops between distal "Androgen Receptor" AR-binding sites and the proximal promoters of a few target genes including "Prostate-Specific Antigen" (PSA), "Transmembrane Serine Protease 2" (TMPRSS2), and "Ubiquitin Conjugating Enzyme E2 C" (UBE2C) is leading to a cancer progress and development [18].

Epigenetics factors dysregulation in HCC

The internal factors

Non-coding RNA

The non-coding regions of DNA are responsible for regulation of many biological activities. It is now believed that about 80% of the human genome transcribed as non-coding RNA (ncRNA) molecules. The ncRNAs are two types one is small under 200 nucleotides and the other is large up 200 nucleotides. These RNAs are essential for normal development and their alterations may cause cancer [19].

MiRNAs

MiRNA is the most famous important regulators of gene expression. Because of its role in carcinogenesis, the circulating miRNAs in serum is considered as cancer biomarkers. The understanding to the Links between miRNA and the development of human malignancies are increased making miRNA profiles used to classify human cancers and cancer development [20].

Cell proliferation, suppresses apoptotic cell death, angiogenesis, and metastasis are regulated by a big net of MiRNA. As shown in table 1. MiRNAs have different expression modes in HCC either by up-regulation as oncogenes or by down-regulation as tumor suppressors. For example, miR-122 is found to modulate and facilitate replication of hepatitis C virus, suggesting that miR-122 is a potential target for antiviral intervention. MiR-122 and miR-221 regulate the cell cycle by regulating cyclin1 or CDK [20].

Moreover, miR- 221 targets pro-apoptotic proteins "Bcl2 Modifying Factor" (Bmf) which helps HCC cells to avoid apoptosis. MiR-221 and miR-222 regulate p27. MiR-221, as oncogenic player, target the cyclin-dependent kinase inhibitor, p57 and causing down-regulation of both p27 and p57 into HCC-derived cells and up-regulated them [21].

On the other hand, miR29 can promote HCC apoptosis by targeting the Bcl-2 and Mcl-1, the anti-apoptotic proteins. The invasion and metastasis of HCC are regulated by miRNAs like miR-106b and cell migration via activating epithelial-mesenchymal transition process. While, Let-7g, miR-139, and miR-195 suppress metastasis and HCC progression [21].

Long Non-Coding RNAs

Long non-coding RNA (lncRNA) is greater than ~200 nucleotides in length. lncRNA transcription is regulated by histone modification and chromatin modulating. lncRNAs interact with different transcription factors to prevent them from binding to their target genes. Not only but also, they may rearrange chromatin acting as enhancers or may act as sponges to bind proteins or microRNAs [22].

The multiple functions of lncRNA in transcriptional, post-transcriptional, and epigenetic regulation of gene expression make them become a new player in tumorigenesis. Cui [23] found that 31 and 41 lncRNA loci were located in genomic regions with recurrent DNA gains or losses, respectively, suggesting that the dysregulation of lncRNAs may be involved in HCC, cancer cell metastasis and HCC recurrence. As shown in table 2.

"HOX transcript antisense RNA" (HOTAIR) is one of lncRNA acts on HOXD locus in the target genes causing inhibition of their expressions. On the level of chromatin, HOTAIR binds to "polycomb repressive complex 2" (PRC2) and "Lysine-Specific Histone Demethylase 1" (LSD1) proteins, causing trimethylation of H3 K27 and de-methylation of H3 K4 [24]. Down-regulation of HOTAIR is accompanied by depletion in cell growth and epithelial–mesenchymal transition. In the solid tumor cells including HCC, HOTAIR was found to be over-expressed leading to high progress of the tumor. Furthermore, HOTAIR is found to be expressed in cancer stem cells, where, it would play a dramatic role in resistance to chemotherapy [25].

Human circRNA as a new player in HCC

Human circRNA is a by-product derived from RNA processing pathway. It consists of 2 to 3 exons ranging from several hundreds to several thousand nucleotides in length, and it was found in very low concentrations in the blood. It has been discovered since 1991 and from this time with the help of the advanced molecular techniques in RNA sequencing, a lot of circRNAs have been discovered [10].

The role of the circRNA has been discovered, some of them act as "miRNA sponge" and regulate the gene expression through re-

miRNAs	Target genes	Mode of miRNA actions	miRNA expression
miR-21	PTEN, RECK, PDCD4	Anti-apoptotic activity, promotes metastasis and Invasion	↑↑
miR-106b	E2F1, RhoGTPases, RhoA, RhoC	Promotes cell migration and actin stress fibre Formation	↑↑
miR-17-5p	p38, MAPK pathway, E2F-1, c-MYC	Promotes malignancy and metastasis	↑↑
miR- 151	RhoGDIA, FAK,	Promotes tumour metastasis and invasion	↑↑
miR-122	CyclinG1, ADAM10, SRF, IGF1R, PTTG1, PBF, CUTL1,NDRG3,MDR-1	Responsible for inhibition of virus replication and cell proliferation	↓↓
miR-143	FNDC3B	Promotes tumour metastasis	↑↑
miR-210	VMP1	Promotes hypoxia induced epithelial to mesenchymal transition	↑↑
miR-29	MEG3, Bcl-2, Mcl-1	Promotion of apoptosis and inhibition of tumour growth	↓↓
let-7	cMyc, p16, Bcl-xl, COLIA2	Inhibit cell growth and proliferation	↓↓
miR-26a	Cyclin D2, Cyclin E2, Cyclin E1,CDK6, IL-6	Inhibit metastasis, invasion and tumour growth	↓↓
miR-221	CDKN1B/p27,CDKN1C/p57, DDIT4,PTEN, Bmf, TIMP3, PPP2R2A	Anti-apoptotic, help in metastasis and tumour growth.	↑↑
miR-1	FoxP1, MET, HDAC4	Inhibition of cell growth and reduced replication potential	↓↓
miR-195	cyclin D1, CDK6, E2F3, LATS2,VEGF, VAV2, CDC42, IKKα and TAB3, TNF-α/NF-κB pathway	Inhibit metastasis, G1/S transition, angiogenesis and helps in apoptosis.	↓↓
miR-45	OCT4, IRS1, IRS2, IGF signaling,HDAC2	Inhibit cell proliferation, migration and invasion	↓↓
miR-224	API-5, CDC42, CDH1, PAK2, BCL-2,MAPK1, PPP2R1B.	Promote cell proliferation, migration, invasion, and inhibit cell apoptosis	↑↑

Table 1: Some of the expressed miRNA and their target genes (2).

flecting its effect on miRNA by removing the inhibitory action and activating the expression of the target gene. CircRNA is a cytoplasmic RNA and a small subset of circRNA has been reported to exist on the nucleus coming from introns affecting the transcription by binding to "RNA Polymerase II" (Pol II) [26].

In cancer including HCC, the level of circRNA found to be changed, which means that it has a role in carcinogenesis. CircRNA is used as a prognostic biomarker because of its specific to the cell type and tissue and its great stability. As shown in table 3.

Long Interspersed Element 1 (LINE-1)

Long interspersed element 1 (LINE-1 or L1) is one of the non-coding RNA; it represents approximately 17% of the human genome. Due to its "retrotranspose" activity which it gains by "copy-and-paste" process LINE-1 effects the target genes [27]. Consequently, it is considered that the active LINE-1 retrotransposition can has an oncogenesis activity in humans, contributing in the instability of the target genes and tumorigenesis. Rahrmann and Largaespada, [27]. delineated that in normal cells the retrotransposi-

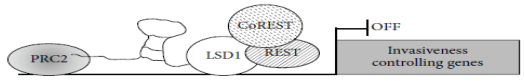
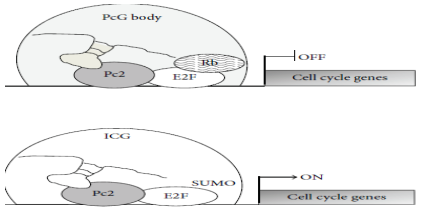
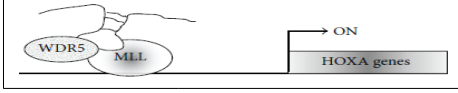
LncRNA	Epigenetic mechanisms in HCC
HOTAIR	
H19	
HEIH	
MALAT1	
MEG3	
HOTTIP	

Table 2: Some lncRNAs and their roles in HCC [24].

tionally activity of LINEs-1 were silenced due to hyper-methylation of their promoters, also histone modifications, and host defence factors like APOBEC3G expression are causing block to LINE-1 promoter. Consistently, it has been reported that LINE-1 up-regulated in cancer [28]. Like other epigenetics agents LNE-1 retrotransposition is affected by environmental factors, such as carcinogenesis compounds, oxidative stress and viral infectio. Many studies have shown that LINE-1 stimulate carcinogenic genes expressions in HCC. In addition, studies of the effects HBV on HCC induction, the transcription of HBV genes were found to be associated with the transcription of LINE-1 and both associated with hepatocellular carcinoma [28].

Epigenetic External factors

The response to the environmental factors is the main player in the epigenetics modifications causing cancers and these environmental factors including chemicals, physicals and biological agents [29].

Tobacco smoke, arsenic, cadmium, nickel and ionizing and UV radiation are some of the epigenetics deregulators. The lifestyle of the individual can also cause epigenetics changes by interacting with the genome [29].

Many carcinogens compound are strongly believed to promote tumour development through the induction of epigenetics changes, genetics alterations or the both together. It is very important to note that, person's genetics polymorphisms and epigenetics make-up will determined which person will be at risk to develop cancer due to his environmental response. According to, the "International

circRNA	Regulation effects	Associated clinical features	Target miRNA
hsa_circ_0001649	Downregulation	Tumor size	Undetermined
hsa_circ_0004018	Downregulation	Tumor size, AFP, clinical stage	miR-30e, miR-92a-1, miR-647, miR-660
hsa_circ_0005986	Downregulation	Tumor size, MVI, clinical stage	miR-129
circZKSCAN1	Downregulation	Tumor number, MVI, differentiation grade	Undetermined
cSMARCA5	Downregulation	Survival, recurrence	miR-17, miR-181b
circMTO1	Downregulation	Survival	miR-9
hsa_circ_0005075	Upregulation	Tumor size	miR-23a/b, miR-93, miR-581
ciRS-7(CDR1as)	Upregulation	Undetermined	miR-7
circ_0067934	Upregulation	Clinical stage, survival	miR-1324
circHIPK3	Upregulation	Undetermined	miR-1324
circRNA_100338	Upregulation	Survival	miR-141-3p

Table 3: Some circRNA and their effects on HCC (43).

Agency for Research on Cancer" (IARC) Monographs Programme identifies environmental and lifestyle factors that are causing human carcinogens, more than 100 chemicals have been proved and classified as carcinogenic to human, for example, physical agents, biological agents [30].

The preservative food industry

The by-product of several industrial processes like "N-Nitrosodimethylamine" (NDMA) is toxic to the liver specially and to other organs in general and is considered as carcinogen. It is present at very low levels as preservative in the food industry. It is also used in the laboratories to induced HCC in rats for the cancer researches [31].

NDMA induces HCC by influencing the inflammation signalling in the liver through up-regulate the interleukin-6 (IL-6) gene in male mice. IL-6 in turn increases the Androgen Receptor (AR) gene expression level and activity. The expressed AR suppresses p53 gene and promote hepatocarcinogenesis [32]. AR regulates the progression of HCC, by two ways 1) through binding to its response element on the "transforming growth factor-beta 1" (TGF-β1) gene promoter and regulate its expression. 2) AR stimulates the cell cycle-related kinase (CCRK) expression in a cascade of processes ending by promoting the cell proliferation via activating β-catenin signal and stimulating the expression of the epidermal growth factor receptor and cyclin-D1 [33].

Aflatoxins contamination

Aflatoxins are the most famous carcinogenesis metabolites coming from fungi "Aspergillus flavus" and "Aspergillus parasiticus" which contaminate our staple foods specially ground nuts. Aflatoxins have a very lethal effect on liver leading to hepato-carcinogenesis. The main known effect of aflatoxins is causing mutagenesis for DNA sequence. Where in the liver, aflatoxin B1"AFB1" metabolites, are reacting with DNA forming AFB1-guanine adducts, and reacting with proteins forming AFB1-albumin and other protein adducts [34]. The DNA adducts convert guanine (G) to thymine (T) causing a mutagenesis. AFB1 motivates the malignant transformation. P53 is the most target gene for the mutagenesis effect of AFB1 where R249S mutation was found in 64% of hepatocellular carcinoma induced by AFB (HCC-AFB) cases [2,34].

But in the past few years, several studies found association between the hyper-methylation of several genes promoters, like "RAS Association Domain Family Protein 1 Isoform A" (RASSF1A), p16, MGMT, and GSTP1, and AFB-DNA adduct [1]. These studies suggested that beside mutagenesis effect, AFB1 has an epigenetic effect associated with HCC development. They suggest that the mechanism of action as epigenetic agent of AFB1 may be due to its binding to the methylated CpG islandsand de-methylate it initiates the activity of this gene leading to tumorigenesis such in case of, LINE 1 and SAT1 which found to be hypo-methylated in HCC-AFB tumor cells instead of normal cells [1].

Other studies suggested that AFB1 can bind to chromatin structure inducing DNA damage and histone modifications. In a study done by, Zhu [35], they found that H3 was hyperphosphorylated at Ser10 (p-H3S10) and Ser28 (p-H3S28) in AFB1-HCC L02 cell line. This hyper-phosphorlation suppresses cell growth forming tumor. Moreover, they found that this hyperphosphorlation motivates the cells to keep the intensive chromatin structure and prevent DNA damage and induced cell transformation. Nicety, this histone modification abolished the combination of p-H3S10 to the promoter of DNA repair genes like, "Poly (ADP-Ribose) Polymerase 1" (PARP1) and MLH1 [36].

Alcohol consumption

Alcohol is one of the most lethal compounds, causing a dramatically effects on the body in general and liver organ in particular. Alcohol consumption is associated with liver cirrhosis and cancer. A lot of studies in the world reported that, chronic alcoholic consumption is associated with the primary liver cancer (HCC) development and progress. But how alcohol affects hepatic metabolism and the liver disease progression is still unclear. As a fact, the majority of the up taken alcohol is metabolized in the liver and only a small part is absorbed and metabolized in the stomach [37].

Alcohol is catabolism in hepatocytes cytosol, by the "microsomal ethanol oxidizing system (MEOS)" producing two metabolites "nicotinamide adenine dinucleotide (NAD)" and "nicotinamide adenine dinucleotide dehydrate (NADH)". Increase NADH/NAD+ ratio resulting in accumulating triglycerides in the liver causing alcoholic steatosis. These reactions and accumulation of their by-products in the liver disrupt the lipid metabolism through inhibiting fatty acid oxidation (FAO), lipogenesis, inhibit gluconeogenesis, increased ketone bodies production and increased hyperuricemia [38]. In fact, acetaldehyde as hepatotoxic catabolic bi-products stimulates liver injury, inflammation, DNA damage and cell death. The cytochrome P-450 family is a member MEOS, which regulate ROX pathway in

the body. In response to high alcohol concentrations, cytochrome P450 family 2 subfamily E member 1 (CYP2E1) gene expression level is strongly up-regulated in the liver causing high increase in intracellular oxidative stress and lipid per-oxidation [37]. The induction of CYP2E1 during alcohol metabolism damages mitochondrial function and increase "Reactive oxygen species" (ROS) causing DNA damage in the liver cells. Moreover, CYP2E1 inhabits autophagic process due to alcohol consumption causing lipid accumulation in the liver [37,38].

Acetaldehyde has a mutagenesis effect and carcinogenesis potential is gained from its production of DNA damage or through interfering with DNA synthesis and disrupts DNA repair [39]. Considerably, acetaldehyde changes DNA chemically by reacting with it producing "N2-ethylidenedeoxyguanosine" (N2-ethylidene-dGuo). Unfortunately, there isn't a repair system for such error in the body indicating a mutagenic potential. Moreover, it was detected that in the liver of alcoholic person, two molecules of acetaldehyde react with DNA producing propanodeoxyguanosine (PdG), which is suggested to have a mutagenic effects, but its mechanisms are still unclear. Not only but also, acetaldehyde can suppress DNA repair through hindrance of the activity and expression of the O6-methylguanine methyltransferase (MGMT) [39].

Smoking

Tobacco smoking is considered as an independent risk factor participating in the initiation and development of liver cancer. The tobacco carcinogen "4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone" (NNK) beside more than 73 other carcinogens products induce a lethal damage to liver cells. It was established that high exposure to NNK increases HCC aggressiveness with severe inflammatory, cell infiltration, accumulating fat in the liver, hepatomegaly, and high "Nuclear Factor Kappa B Subunit 1" (NF-κB) expression and rising bilirubin. NNK is activated by the cytochrome p450 (CYP) enzyme system to give it the tumorigenic activity. The activated NNK molecules have the ability to react with DNA forming adducts causing DNA single strand damage in the liver cells (152). NNK is an activated ligand for the "α7 nicotinic acetylcholine receptor" (α7 nAChR) gene, where its activation initiates the development and progression of HCC [40].

Viral infection

Worldwide, hepatitis B virus (HBV) infection is the most common chronic virus infection. HBV infection is the tenth causing death due to cirrhosis or hepatocellular carcinoma (HCC). The HBV relaxed circular partially double-stranded DNA (RCDNA) is inserted into hepatocellular nucleus, then converted into a circular episome, called covalently closed circular DNA (cccDNA) which transcribed to double-stranded genome called long pregenomic RNA (pgRNA) [41].

The presence of cccDNA in the nucleus, and its functional chromatin dynamics, is involved in HBV transcriptional activation. For the nuclear cccDNA "transcription HBV encoded protein x" (HBx) induces several epigenetic modifications in the host cell causing arcinoma positioning, DNA methylation and "histone post-translational modifications" (PTMs). HBx up-regulates "DNA methyltransferases" DNMT genes during their transcription active state [42].

DNA methylation was altered through the early stages of HBV infection. Dramatically, some studies have reported that the host cell may use hyper-methylation and histone acetylation as a defence mechanisms to modify the HBV`s DNA to hinder the viral RNA replication and protein expression but this also leads to the hypermethylation of its own CpG islands, specially those are located in the promoters of tumor suppressor genes, for example p16-INK4A, GSTPI and some miRNAs [43].

Unlike HBV, HCV is an RNA virus. HCV protein initiates the human hepatocytes in mice lead to HCC. The Down-regulation of p16 and the up-regulation of "Signal Transducer and Activator of Transcription3" (STAT3) are two of transcriptional changes that occur in liver cells in HCV infection. Where, HCV protein up-regulates "DNA methyltransferases1" DNMT1 causing hyper-methylation and silencing of "Secreted Frizzled Related Protein 1" (SFRP1), "Epithelial Cadherin" (E-cadherin) and "CDK4 Inhibitor p16-INK4A (P16-INK4)" genes which found to be associated with high HCC aggressiveness [44].

Clinical Applications

Epigenetic biomarkers

Like other types of cancer, the early detection of HCC improves the response of the patients to treatment and can enclose the mortality of the tumor. "Alfa fetoprotein" (AFP) level is the only current blood taste used to detected HCC, but unfortunately, it is limited because of its low sensitivity and accuracy. In fact, AFP levels couldn't be detected in many patients with cirrhotic liver developing HCC [45]. Other point of view, compared to tissue biopsy, a biomarker in the body fluid is needed for many reasons for example, the collection of peripheral blood is minimally invasive than taken a tumor

biopsy. It is easier and less aggressive to collect blood samples at any time to monitoring the tumors progress and patients therapy response rather than taken biopsy or even imaging where the very small tumour is not apparent or couldn't determinate on imaging [45].

The price of using epigenetic markers, that it can be tested in body fluids and are specific, accurate and saving time with no need for excessive precautions while collecting the samples.

DNA methylation pattern as HCC biomarkers

The difference in DNA methylation patterns between normal tissues and tumor tissues makes it is possible to use it to differentiate between the HCC tumor tissues and non HCC liver tissues. Moreover, the hyper-methylation of certain gene promoter have found to be associated with HCC progression and development such as, "Frizzled Class Receptor 7" FZD7, P16, CDKN2A and RASSFIA and they can be used as differentiating tools between tumor cells and normal cells [2,5].

It is worth mentioning that, the difference in methylation pattern between the HCC stages can be also used to differentiate between the stages and monitoring the HCC progress, where DNMT1 hyper-methylation is accompanied by poor tumor differentiation. "Spleen Associated Tyrosine Kinase" (SYK) and "Checkpoint with Forkhead and Ring Finger Domains" CHFR hyper-methylation occur mainly in the advanced stages of HCC. On the other hand, P15, P16 and RSSFIA genes hyper-methylation occur since the beginning of HCC development [42].

Also, the "specific methylation signature" for each HBV and HCV make it possible to use DNA methylation to differentiate between each one of them beside that each one of them has its specific methylation pattern in a specific HCC stage [18]. For example, high hyper-methylation of P15, "adenomatous Polyposis Coli Protein (APC)", "STAT1" and "SOCs-1" were found to be associated with HCC-HCV not with HCV alone. Moreover, "DCC Netrin 1 Receptor" (DCC), "CSPG2" and "N-Acetyltransferase family (NATs)" hyper-methylation is specific in "hepatocellular carcinoma induced by HBV" HCC-HBV. While PAX6 hyper-methylation is specific for "hepatocellular carcinoma induced by HCV" HCC-HCV [43].

Non-coding RNAs as HCC biomarkers

The expression level of several noncoding RNAs can be used for diagnosis and prognosis of disease. For example, low expression of miR-122 is associated with poor prognosis of HCC [44]. Upregulation of HOTTIP and HOXAIR are associated with poor HCC, tumor progression, and metastasis [43]. Short interspersed nuclear elements (SINE) and (LINE) hypomethylation and activation are mainly occur in HCC development.

Epigenetic therapy and prevention

Cancer epigenetics alterations are site-specific. As a positive aspect, epigenetic changes can be reversed and are good goal for therapeutic approaches [45].

The "US Food and Drug Administration (USFDA) " have been approved four epigenetic therapies targeting two main epigenetic modifications, class one target the methylation process called " DNA methyltransferase (DNMT) inhibitors" and the other class target the histone modifications called " histone deacetylase (HDAC) inhibitors". DNMT inhibitors are "5- azacytidine (Vidaza)" and "5-aza-2′-deoxycitidine (Decitabine)" the two drugs are cytosine analogism. They have an inhibition activity against blood cancers, but on the other hand, these activities are low in case of solid tumors [46]. Dramatically, these agents have very toxic side effects, where they alter the methylation pattern in both non-tumor cells and, tumor cells causing very lethal effects. But there are other agents under research with more specificity and less toxicity are "DNMT anti-sense (MG98)" and "small molecule RG108 inhibitors" [46]. The HDAC inhibitors are ""suberoylanilide hydroxamic acid" (SAHA)" (vorinostat) and "romidepsin" (F-228) have been approved by USFDA. Clinical trials show that HDAC inhibitors can suppress the HDAC activity in cancer cells and more importantly, they have the ability to stop the tumor development. As shown in table 4.

Other HDAC inhibitors such as "butyrate", "trichostatin A (TSA)", "oxamflatin" and "MS-275" are regulated many cell cycle genes, by acting on silencing cyclin-dependent kinase they inhibit cell-cycle causing enclosing the development of the tumors [43].

Using of miRNAs as potential therapeutic targets has been examined in several studies` due to its important presentation in the carcinogenesis progress and development. Oka [47]. uses GTXs or NGTXs as treatment agent for HCC in mouse, where they targeted three miRNAs, miR-22-3p, miR-409-3p, and miR-543-3p. They were significantly down-regulated in GTX-treated mouse liver.

Nutritional Epigenetics

Due to the dramatically lethal effects of chemotherapy, it is necessary to search for other therapeutic strategies without or even

with fewer side effects. Therefore, the trend to develop treatments from nature has become an urgent reality. So, many in vivo and in vitro studies were performed to understand anti-hepatocarcinoma effects of polyphenolic compound "curcumin", they found that curcumin activates "DNA methyltransferases" (DNMTs) genes by removing the methyl group from their promoters in turn they reactivated their related cancer genes through demethylated them [48].

In a study done by Ahmed [32], curcumin was used as treatment for HCC initiated by the using NDMA as carcinogenesis compound, curcumin downregulated AR gene expression in the HCC liver tissue by inhibit the IL-6.

Epigallocatechin gallate (EGCG) compound which is rich in green tea, have a DNA demethylation effect on hyper-methylation tumor-suppressor genes by which they can arrest the progression and proliferation of HCC. Bioactive polyphenol resveratrol and HDAC inhibitors are an natural products present in different plants such as "broccoli (sulforaphane)", "grapes (resveratrol)", "blueberries (piceatannol)", and "garlic (allyl mercaptan)" they initiate apoptosis in liver cancer cells protecting them from chemopreventive effects [43].

Epigenetics detection tools

DNA methylation and histone modifications are the most processes affect gene expression without causing mutations in DNA sequence. Bisulfite technique is used to detect DNA methylation; using the theory that methylated cytosine can't convert to uracil. Therefore, this technique uses sodium bisulfite to convert unmethylated cytosine to uracil and then the methylation pattern can be detected by using different molecular tools such as, methylation-dependent restriction enzymes (MDRE), Methylation Specific PCR (MSP) and Illumina Infinium HumanMethylation450 (HM450) bead chip assay [49].

Immunoprecipitation (ChIP) is the most common technique used to investigate the histone modifications chromatin. The theory of this technique is to design a specific antigen to the DNA target protein and specific antibody for the modified protein then the interaction between them produces a precipitation. This precipitation is an indicator for histone modifications and could be detected by using microarray analysis (ChIPchip), sequencing (ChIP-seq), or quantitative PCR. But this method has disadvantaged, where to design a specific antibody sometimes is very difficult [50].

Deregulated expression level of several noncoding RNAs can be used for diagnosis and prognosis of disease. The high-throughput technologies such as cDNA microarray, RNA sequencing (RNA-seq), NGS technology, Sanger sequencing and quantitative real-time PCR (qPCR) are techniques used to detect miRNA and other noncoding RNA alterations and expressions [49].

Conclusion and Recommendations

The importance of epigenetic in HCC has been recognized and the field has forced rapidly over the years. Study epigenetic process at the global level is more effective in prognostic value than a single gene and it become possible by using the advancement in technology and the use of new high-throughput methods like next-generation sequencing which give a chance to study DNA methylation status of human cells at nucleotide resolution.

This revolution of cancer epigenetic gives us clear understanding about epigenetic biomarkers for early detection, prognosis, and designing better HCC treatment strategies.

Therefore, it was recommend using epigenetic as biomarkers to diagnosis and monitoring cancer in general and HCC in particular. Also, it was recommended to wide the research in the natural epigenetics as a viable solution to chemotherapy.

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