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Review Article

Disturbances of the Hepatic Epigenome in Hepatocellular Carcinoma: the Role of Monozygotic Twins

Weledji EP*

Department of Surgery, Faculty of Health Sciences, University of Buea. S.W. Region, Cameroon, West Africa

***Corresponding author:** Elroy Patrick Weledji, Faculty of Health Sciences, University of Buea, Holy Family Foundation, Livanda Kongo Hill, PO Box 126, S.W. Region, Cameroon, West Africa

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Abstract

The specific genetic abnormality that leads to Hepatocellullar Carcinoma (HCC) is not yet known. Carcinogenesis and cancer progression does not only evolve from the mutation of DNA codings but from any effect on its expression i.e. epigenecity. There are several levels of epigenetic dysregulation involved in carcinogenesis and the stepwise progression towards metastasis or incurable disease. The advancement of next generation DNA sequencing has increased the understanding of the genetic and molecular pathology of liver cancer. By avoiding the confounding influence of consecutive genetic backgrounds, age, and cohort effects, epigenetic studies on monozygotic twins with HCC would improve the understanding of HCC. Unlike genetic events, epigenetic alterations are reversible and thus potentially considered to be an alternative option in cancer treatment protocols. Mechanisms of epigenetic control may also offer an alternative path to acquiring stable oncogenic traits. Electronic searches of the medline (PubMed) database, Cochrane library, and science citation index were performed to identify original published studies on liver epigenome, hepatic carcinogenesis and familial hepatocellular carcinoma. The aim was to briefly report on how monozygotic twins would be an excellent model for elucidating the disturbances of the hepatic epigenome in the manifestation of familial hepatocellular carcinoma.

Keywords: Hepatocellular; Carcinoma; Epigenome; Hepatitis; Familial; Monozygotic twins

Introduction

Hepatocellular Carcinoma (HCC) is the fifth most common cancer worldwide with greater than 80% found in endemic areas of hepatitis B in Africa and E. Asia. The World Health Organisation (WHO) 2012 reports an age-standardised death rate of 38-100/100,000 inhabitants [1,2]. 70-90% develop on a background of cirrhosis. Hepatitis B Virus (HBV) infection provides a 100- fold increase in risk. Alcoholic cirrhosis and hepatitis C are the common causative factors in the Western world. The specific genetic abnormality that leads to HCC is not yet known [3]. A classical Mendelian inheritance is limited to the rare monogenic diseases such as haemochromatosis, tyrosinaemia type 1 and alpha1 antitrypsin deficiency [4]. The rare fibrolamellar variant of HCC which mostly occurs in non-hepatitis B endemic areas is due to a heterozygous deletion on chromosome 19 that encodes a functional chimeric protein (DNAJB1-PRKACA) [5]. It is characterized by being less aggressive with a normal Alpha-Fetoprotein (AFP) tumor marker and a female predominance [6]. Serum neurotensin, a new tumour marker may discriminate it from HCC especially as a negative or normal value of AFP tumor marker does not exclude an HCC [7,8]. Being rare, familial fibrolammelar clustering has never been reported. A multifactorial inheritance including novel DICER1 germline mutation and altered liver zonation contribute to the risk of HCC, regardless of viral hepatitis infection (Figure 1) [9]. In cancerous cells, more genes are affected by epigenetic changes than genetic changes. Different Single Nucleotide Polymorphisms (SNPs) may increase the risk of HCC and the effect on various biological pathways may predispose to the manifestations of other risk factors such as aflatoxin (from fermented crops) [10,11]. Global gene expression profiling reveals SPINK 1 as a potential hepatocellular carcinoma marker [12]. The over expression of the apolopoprotein family (ApoA1, ApoA2, ApoC3, ApoE) and serum amyloid A protein in comparative proteomic profiles from liquid biopsies in hepatocellular carcinoma familial aggregation indicate genetic factors are involved [13]. Assuming that these molecular processes may occur earlier in carcinogenesis and excreted in the circulatory blood, the identification of such sensitive markers may help to facilitate earlier diagnosis of HCC at a curable stage.

Pathophysiology

In cirrhosis, HCC occurs due to chronic injury, regeneration and dysplasia (Figure 2). Necroinflammation and telomere (TERC/TERCT mutation) shortening are hallmarks of the early stages of chronic liver disease that finally lead to fibrosis and cirrhosis. The initial acute inflammation is protective, whereas chronic inflammation initiates necroinflammation, tissue remodeling and oxidative microenvironment [14]. The enzyme c-Jun N-terminal kinase (JNK) is a central mediator in integrating multiple metabolic signals critical in cellular homeostasis when conditions challenge the endoplasmic reticulum [15]. Oxidative bursts trigger DNA damage and genomic aberrations that finally culminates in neoplasia. This is corroborated by the fact that some regenerating nodules in the cirrhotic liver show atypical cells that progress towards dysplasia and culminate as a neoplastic lesion. Thus, HCC is accompanied with telomerase activation and accumulation of genetic and epigenetic alterations. TGF-B signaling and T cell activation is seen during the

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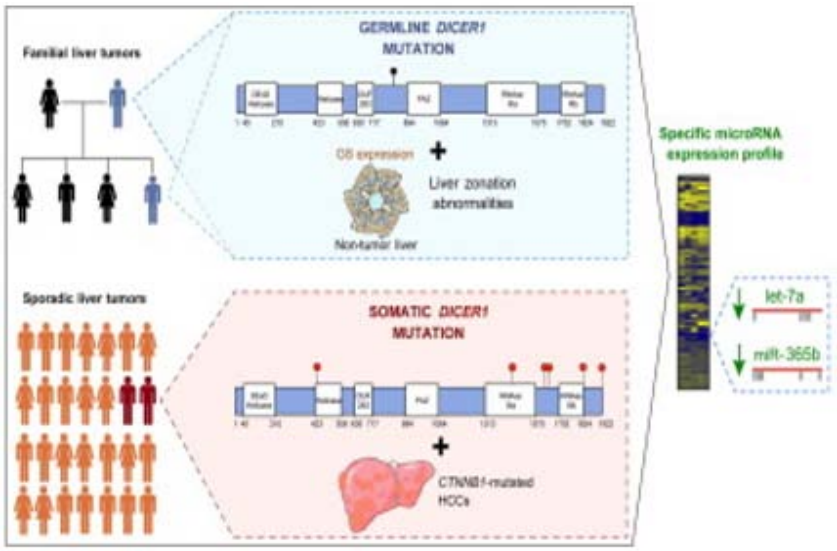

Figure 1: Germline and somatic DICER1 mutations in familial and sporadic liver cancer.

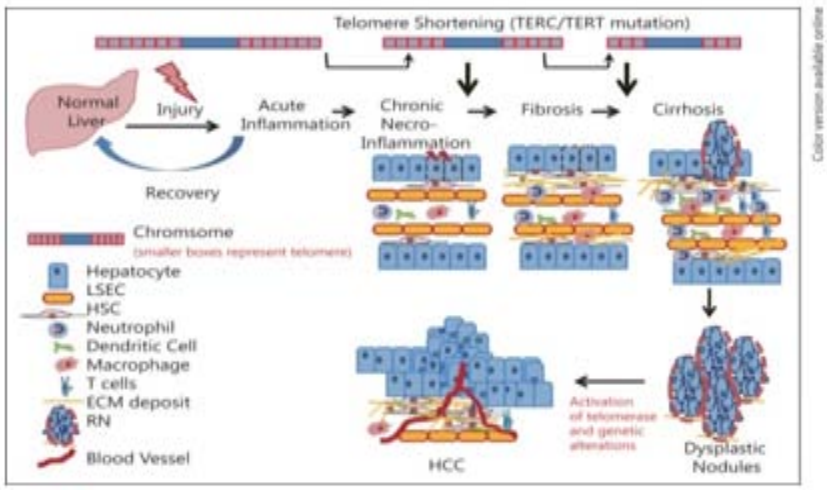

Figure 2: Diagrammatic representation of various pathological changes associated with liver disease progression [14].

acute and end stage of liver disease, whereas attenuation is noted during the chronic stage of infection (Figure 3) [14-16].

These *inflammasomes* activate caspase which cleaves the pro-inflammatory cytokines Interleukin (IL)-4/ IL-16 to their active forms. Through activation of transcription factors by an unknown mechanism, they provide a hepatic milieu fertile for cellular transformation [14]. It takes 10 years to develop chronic hepatitis, 20 years to develop cirrhosis and 30 years to progress to HCC. Therefore, HCC usually affects patients aged 50-70 years [17]. However, there is an earlier onset (25-40 years) in HBV endemic areas such as Africa and in patients with a family history [18-20]. This is associated with earlier exposure and aggressiveness. In some of these cases, HBV can be directly oncogenic by incorporating into host DNA and rapidly cause HCC in the absence of cirrhosis (Figure 4) [14,18,21].

In endemic areas where HCC is common, HBV infection is considered to be important risk factor for Familial Clusters of HCC (FHCC), especially as the maternal transmission of HBsAg and susceptibility to HCC is suggested by the pattern of involvement

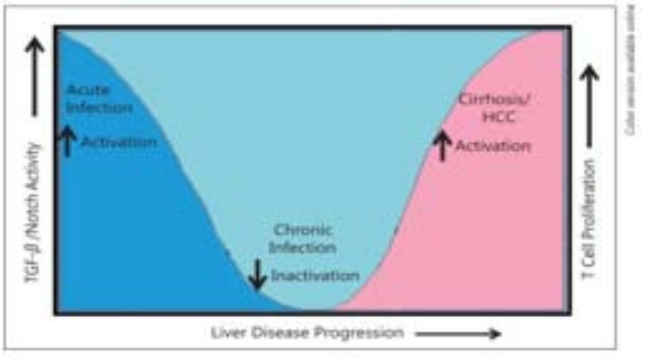

Figure 3: Graphical representation showing Notch/TGF-B signaling and T cell activation during liver disease progression [14,16].

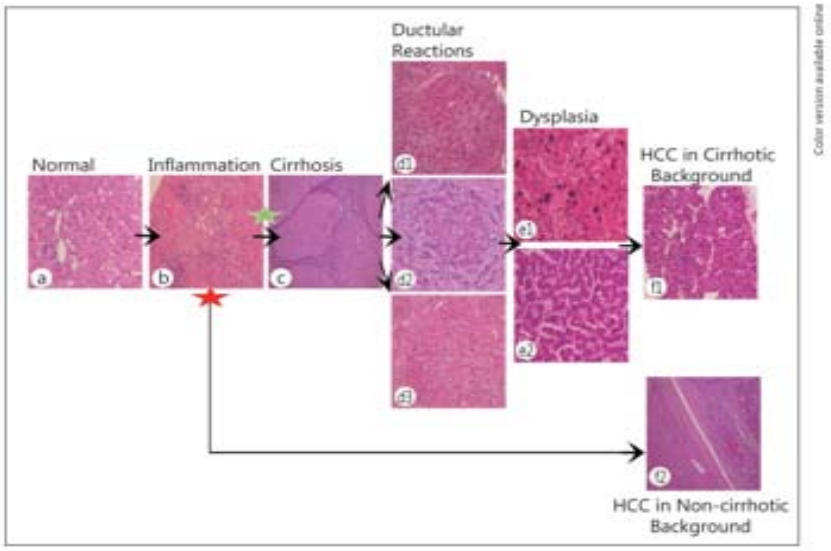

Figure 4: Chronological representation of histopathological events during progression toward HCC [14].

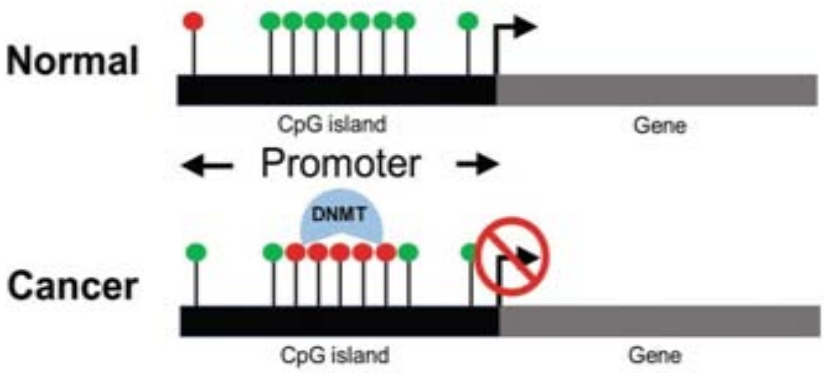

Figure 5: DNA methylation profile in cancer [34].

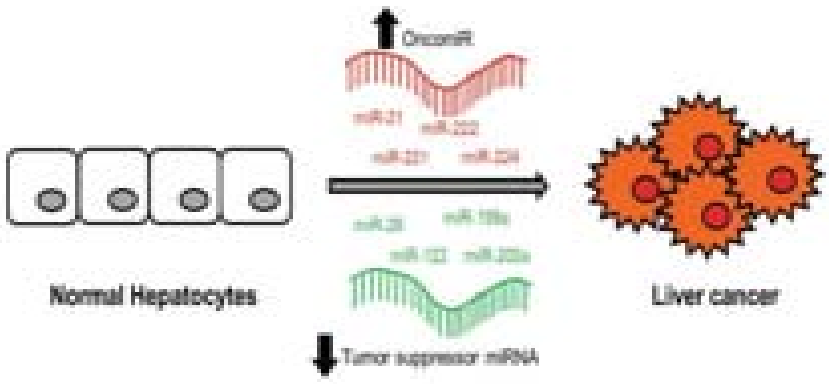

Figure 6: Micro RNAs in cancer [34].

[22,23]. However, studies from the western world concluded that a family history of HCC increases the risk of familial HCC independently of hepatitis [24,25], but hepatitis B/C serum markers is associated with an over 70 - fold elevated HCC risk [24]. The highest risk for HCC may occur in families in which a hereditary component is acting in concert with environmental factors such as HBV, aflatoxin or other epigenetic factors [26,27]. As familial clusters of HCC are usually described in areas with endemic HBV infection, limited attention has been given to the role of primary genetic and epigenetic factors. Even in the presence of HBV infection, familial HCC seemed

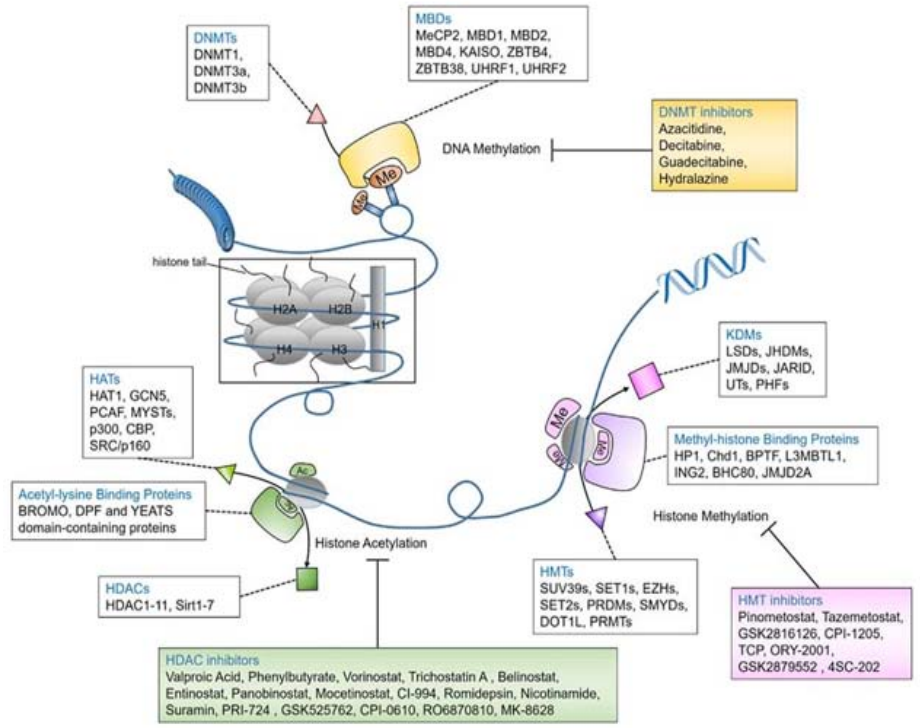

Figure 7: Schematic diagram of epigenetic regulation of DNA methylation, histone acetylation, and histone methylation [52]. (DNMTs: DNA methyltransferases; TETs: Ten-Eleven Translocation enzymes; HATs: Histone Acetylases; HAACs: Histone Deacytylases; HMTs: Histone Methyl Transferases; HDMs: Histone Demethylating Enzymes; niLL: Biphenotypic (mixed lineage) leukaemia).

Table 1: Summary of the genetic and Epigenetic factors of Hepatocellular Carcinoma.

Genetics	Epigenetics
Genetic mutations	Life time exposures (childhood-ageing)
• **Chromosomal rearrangements**	• DNA interaction • Mutated epigenetic regulators (CTNNB1, TERT promoters, TP53 gene) • Epigenetic alterations • DNA repair mechanisms (DNA methylation) • Histone modifications(methylation, acetylation, phosphorylation) • Higher order chromatin regulation.
• **Non-coding RNAs (miRNAs/ lncRNAs)**	• Cancer phases • Tumour-promoting inflammation • Deregulated metabolism • Avoiding immune destruction • Replicative immortality
• **Multifactorial inheritance** • **DICER1 mutation** • **Single nucleotide polymorphisms**	
• **Fibrolamellar variant (deletion of Chromosome 19)**	
• **Classical Mendelian inheritance (rare monogenic diseases)- haemochromatosis, tyrosinaemia type 1, alpha1 antitrypsin deficiency**	

statistically unlikely to occur by chance [28]. Environmental and/or genetic factors are involved. Genetic and epigenetic contributions may also explain the presence of healthy or non-healthy carriers of HBsAg, and the carrier state being commoner in males [29-31].

Epigenetic modifications

As few genetic mutations are required in neoplastic transformations, there is the importance of the added influence of epigenomic alterations. Epigenetics are the reversible molecular changes of the DNA that do not arise from alteration of the DNA proper [32]. Alterations in epigenetic modifications in cancer regulate various cellular responses including cell proliferation, apoptosis, invasion and senescence. Through DNA methylation, histone modification, chromatin remodeling and non-coding RNA regulation, epigenetics play an important role in tumourogenesis. These main aspects of epigenetics are the reversible effects on gene

silencing and activation via epigenetic enzymes and related proteins. There are several levels of epigenetic dysregulation involved in carcinogenesis and the stepwise progression towards metastasis or incurable disease [33]. Epigenetic events may regulate expression of many hundreds of oncogenes e.g. K-ras gene involved in control of cell growth, Adenomatous Polyposis Coli (APC) gene central to ordered cell motility, the p53 gene for the repair of DNA and induction of programmed cell death, CDH 1, c-MYC, BRCA1/2 gene, etc. These events are independent of DNA alterations. For example, the inactivation of Mismatch Repair (MMR) given by promoter methylation is not related to any inherited factor. The liver epigenome is extremely sensitive to its highly variable environment- from chronic inflammation –fibrosis-accumulation of mutation in regeneration- dysplasia to liver cancer. The epigenetic alterations and influence occur through life-time exposures, DNA interaction and the cancer evolutionary phases. The life-time exposure factors include HBV infection, metabolic risk factors (obesity, excessive alcohol), nutrition, physical activity, microbiome, toxins, aflatoxins, drugs/medications, pregnancy/in-utero, childhood, ageing and Asian ethnicity [34]. The DNA interaction includes (i) mutated epigenetic regulators (CTNNB1, TERT promoters, TP53 gene), (ii) epigenetic alteration of (a) DNA repair mechanisms- DNA methylation (hyper/hypo), (ii) histone modification (methylation/acetylation/phosphorylation), and (iii) higher order chromatin regulation (Table 1). Liver cancer cells typically exhibit post-transcriptional DNA hypermethylation at promoter sites (CpG islands) of tumour suppressor genes resulting in silencing of these genes (Figure 5) [32-34]. For example CDKN2A tumour suppressor gene is frequently silenced by DNA hypermethylation [35]. Hypomethylation of multiple CpG sites although not common in HCC lead to chromosomal and genomic instability with high risk of local recurrence and metastasis after surgical resection [36,37]. Molecular consequences from DNA methylation are increased tumour growth, invasion, metastasis and field susceptibility to malignant change. Aberrant DNA methylation and copy number are associated significantly with poorer survival [38]. Micro- RNA-122 (miR-122) over expression contributes to liver tumorigenesis [39]. Elevation of oncogenic miRNAs (OncomiRs) which is indirectly linked to DNA methylation results in silencing of tumour suppressor genes while down regulation of tumour suppressor RNAs leads to reduced inhibition of oncogenes [40]. These consequently lead to the development of liver cancer (Figure 6). Micro-RNA-122 expression is found to be aberrant in IDH1/2 mutant HCC, with reduced expression throughout tumour tissues which is associated with poor survival. It is thought that that miR-122 has tumour suppressor functions by modulating the effects of TP53 through Mdm2 [41,42]. The influence in cancer evolution from the breakthrough, expansion and invasion phases include (a) the tumour-promoting inflammation that promotes growth and invasion, (b) deregulated metabolism that promotes angiogenesis and metastasis, (c) avoiding immune destruction that leads to evasion of immune cells and metastasis, and (d) replicative immortality from evasion of apoptosis [32].

Using monozygotic twins

Although Monozygotic (MZ) twins have very similar epigenetic profiles, detailed examination of MZ individuals has revealed that they have surprisingly high rates of disease discordance for many common disorders including metabolic disease, autoimmune disease and cancer. The older the monozygotic twins the greater the discordance [43]. This difference in disease occurrence between MZ twins is typically interpreted as the result of environmental factors although there will be some stochactic effects [44]. By avoiding the confounding influence of consecutive genetic backgrounds, age and cohort effect, epigenetic studies in monozygotic twins would improve the understanding of HCC. Dahms et al in 1971 reported hepatoma in familial cholestatic cirrhosis of childhood in twin brothers [45]. In 2002, Debir et al [46] reported simultaneous HCC in identical twin brothers and Caglari et al in 2016 reported HCC in identical twins in Chile [47]. With the advent of new generation DNA sequencing [48] the identification of host factors such as Single Nucleotide Polymorphisms (SNPs) that may relate to various biological pathways involved in liver carcinogenesis such as aflatoxins should be much easier and robust in monozygotic twins with HCC [10,43]. Epigenetic variation analysis in these twins would identify the susceptibility loci that may be sensitive to modification by the environment.

Therapeutic implications

Unlike genetic events, epigenetic alterations are reversible and thus potentially considered to be an alternative option in cancer treatment protocols [49]. Liquid biopsies provide access to the tumour genome by investigating circulating tumor cells, circulating cell free DNA, exosomes, microRNA and other genomic and proteomic biomarkers. Clinical applications would include diagnostic, prognostic or predictive markers. Circulating tumour alteration for DNA methylation provides an accurate test for detecting HCC and aids treatment stratification [32]. Therapy effect can be monitored and cancer clones followed for response or resistance to therapy, either as a before/ after effect of surgery, or as drug-related responses [50].There are few clinical trials of agents targeting epigenetic alteration for HCC. DNA methylation inhibitor-*DNA Methyl Trasferase (*DNMT) which is an analogue of the nucleoside cytosine (azycytidine) incorporates into DNA and prevents methylation in myeloplastic disorders [32]. It sensitizes HCC cells to sorafenib, the tyrosine kinase inhibitor which significantly improves median survival in patients with advanced disease [18,34]. The concomitant use of Histone Deacetylase (HDAC) inhibitor alongside sorafenib gives promising results in small trials [49,51]. Future goals should include therapeutic strategies to prevent chronic inflammation and to direct tissue homeostasis towards the normal. For example, to exploit NF-xβ, JNK STAT3 and monocyte polarization as therapeutic targets to prevent chronic inflammation and disease progression [14]. Cheng Y et al [52] summarized the inhibitors of or drugs targeted at the aberrant functioning epigenic enzymes in DNA methylation, histone acetylation and histone methylation during tumour progression (Figure 7). Although a positive family history is associated with earlier appearance and aggressiveness of HCC, a first degree family history is a prognostic factor for better oncological outcome and prolonged survival in early resectable tumours [53]. This may be due to no underlying liver disease or at most, the presence of a Child A cirrhosis [18].

Prevention is better than cure

HCC prone families of the familial type may provide a powerful model for studying preventive and therapeutic strategies [27]. The

hepatitis vaccination should be given the highest priority to those individuals where the HCC yield is increased [54,55]. As the impaired immunity of the baby increases the risk of developing persistent hepatitis B carriage, the optimum timing for immunization in conjunction with the administration of hepatitis B immunoglobulin at a contralateral site should be immediately after birth or within 12 h [23,54,56]. It is also important to screen all first degree relatives of patients with HCC in order to detect and treat early, asymptomatic disease [27,53].

Conclusion

A greater attention should be given to the role of primary genetic and epigenetic factors and their interactions in the manifestation of HCC. Monozygotic twins with HCC are suitable models for elucidating the disturbances of the hepatic epigenome and the susceptible loci to the environmental factors. Studies would lead to robust epigenetic markers for the detection and risk assessment of cancer. Novel epigenetic therapies may improve drug targeting and delivery, optimize dosing schedules and improve the efficacy of pre-existing treatment modalities. Mechanisms of epigenetic control may offer an alternative path to acquiring stable oncogenic traits. A primary neonatal hepatitis B immunization scheme would greatly reduce the incidence of HCC in endemic areas.

References

1. Ferlay J, Soerjomatram L, Ervik M, Dikshit R, Eser S, Marchers C, et al. GLOBOCAN 2012 V1.0 Cancer incidence and mortality worldwide: IARC Cancer Base No 11(Internet) Lyon, France, International Agency for Research on Cancer 2013.
2. Bosch FX, Ribes J, Diaz M, Cleries R. Primary liver cancer: worldwide incidence and trends. Gastroenterology 2004;127: 5-16.
3. Bosetti C, Turati F, La Vecchia C. Hepatocellular carcinoma epidemiology. Best Pract Res Clin Gastroenterol 2014; 28: 753-770.
4. Weledji EP. Paediatric metabolic conditions of the liver. EMJ Hepatol 2015; 3: 55-62.
5. Honeyman J, Simon EP, Robins N, Chiaronie-Clarke R, Darcy GE, Isabel IP, et al. Detection of a recurrent DNAJB1-PRKACA chimeric transcript in fibrolamellar hepatocellular carcinoma. Science 2014; 343: 1010-1014.
6. Torbenson M. Fibrolamellar carcinoma: 2012update, Scientifica (Cairo) 2012; 2012: 743790.
7. Ward SC, Waxman S. Fibrolamellar carcinoma: a review with focus on genetics and comparison to other malignant primary liver tumours. Semin. Liver Dis. 2011; 3191: 61-70.
8. Forner A, Reig M, Bruix J. Alpha-fetoprotein for hepatocellular carcinoma diagnosis: the demise of a brilliant star. Gastroenterology 2009; 137: 26-29.
9. Caruso S, Calderaro J, Lettuce E, Nault JC, Couch G, Boulai A, et al. Germline and somatic DICER 1 mutations in familial and sporadic liver tumours. J. Hepatol 2017; 66: 734-742.
10. Nahon P, Zeuman-Rossi J. Single nucleotide polymorphisms and risk of HCC in cirrhosis. J of Hepatol 2012; 57: 663-674.
11. Peers FJ, Boach FX, Kaldor JM. Aflatoxin exposure, hepatitis B virus and liver cancer in Swaziland. Int J Canc. 1987; 39: 545-553.
12. Marshall A, Lukk M, Kutter C, Davies S, Alexander G, Odom DT. Global gene expression profiling reveals SPINK 1 as a potential hepatocellular carcinoma marker. Plos One 2013; 8.
13. Zhong DN, Ning QY, Wu JZ, Zang N, Wu JL, Hu DF, et al. Comparative proteomic profiles indicating genetic factors may be involved in hepatocellular carcinoma familial aggregation. Cancer Sci 2012; 103: 1833-1838.
14. Ramakrishna G, Rastogi A, Trahanpati N, Sen B, Khosia R, Savin SK. From cirrhosis to hepatocellular carcinoma: new molecular insight on inflammation and cellular senescence. Liver cancer 2013; 2: 357-363.
15. Hotamisligil GS. Role of endoplasmic reticulum stress and c-Jun NH2 – terminal kinase pathways in inflammation and origin of obesity and diabetes. Diabetes 2005; 54: 573-578.
16. Trehanpati N, Shrivastav S, Shivakumar B, Knosta R, Bhardwaj S, Chaturvedi J, et al. Analysis of notched TGF-β signaling expression in different stages of disease progression during hepatitis B virus infection. Clin Transl Gastroenterol 2012; 3.
17. Fattovitch G, Brollo I, Giustina G. Natural history and prognostic factors for chronic hepatitis type B. Gut 1991; 32: 294-298.
18. Weledji EP, Enoworock G, Ngowe NM, Nsagha D. How grim is hepatocellular carcinoma. Ann Med Surg 2014; 3: 71-76.
19. Park CH, Jeong SH, Yim HW,Kim JD, Bae SH, Chol YJ, et al. Family history influences the early onset of hepatocellular carcinoma. World J Gastroenterol 2012; 18: 2661-2667.
20. Liu L, Li L, Zhou S, Jiang Q, Chen S Gao Y, et al. Familial correlations of onset age of hepatocellular carcinoma: a population-based case-control family study. Plos One 2014.
21. Weledji EP, Nsagha D, Enoworock G, Mouladje M. Familial hepatocellular carcinoma in an endemic area: two new case report. BMC Res, Notes 2015; 8: 415.
22. Harvey VJ, Woodfield G, Robert JC. Maternal transmission of hepatocellular carcinoma. Cancer 1984; 54: 1360-1363.
23. Blumberg B London NT. Hepatitis B virus and the prevention of primary hepatocellular cancer. N Eng J Med 1981; 304: 782-784.
24. Turati F, Edefonti V, Talamini R, Ferraroni M, Malvezzi M, Bravi F, et al. Family history of liver cancer and hepatocellular carcinoma. Hepatology 2012; 55: 1416-1425.
25. Hassan MM, Spitz MR, Thomas MB, Curley SA, Patt YZ, Vauthey JN, et al. The association of family history of liver cancer with hepatocellular carcinoma: a case - control study in the United States. J. Hepatol 2009; 50: 334-341.
26. Lok ASF, Lai CI. Factors determining the development of hepatocellular carcinoma in hepatitis B surface antigen carriers. Cancer 1988; 7: 1287-1291.
27. Weledji EP. Familial hepatocellular carcinoma: "A model for studying preventive and therapeutic measures". Ann Med Surg 2018; 35: 129-132.
28. Gilmore LT, Harrison JM, Parkins RA. Clustering of hepatitis B virus infection and hepatocellular carcinoma in a family. J Soc Med 1981; 74: 843-845.
29. Blumberg B, Friedlander A, Woodside A, Sutnick AI, London WT, et al. Hepatitis and Australian antigen, Autosomal recessive inheritance of susceptibility to infection in humans. Proc. Natl. Acas. Sci. USA 1969; 62: 1108-1115.
30. Hsu HC, Lin WS, Tsai MJ. Hepatitis B surface antigen and hepatocellular carcinoma in Taiwan. With special reference to types and localization of HBsAg in the tumour cells. Cancer 1983; 52: 1825-1832.
31. Hsu HC, Chang MH, Liaw SH, Ni,YHChen HL. Changes in hepatitis B surface antigen variants in carrier children before and after universal vaccination in Taiwan. Hepatology 1999; 30: 1312-1317.
32. Drake TM, Soreide K. Cancer epigenetics in solid organ tumours: A primer for surgical oncologists. Eur J Surg Oncol 2019; 45: 736-746.
33. Wang Q, Wang G, Lui C, He X. Prognostic value of CpG island methylation phenotype among hepatocellular carcinoma patients: a systematic review and meta-analysis. Int J Surg 2018; 54: 92-99.
34. Toh TB, Lim JJ, Chow EK. Epigenetics of hepatocellular carcinoma. Clin Trans Med 2019; 8: 13.
35. Cancer genome Atlas Research Network. Electronic address wbe and cancer genome Atlas research N. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. Cell 2017; 169: 1327-1341.

36. Nakamura M, Chica T, Kanayama K, Kanzaki H, Saito T, Kusakabe Y, Kati N, et al. Epigenetic dysregulation in hepatocellular carcinoma: an up - to - date review. Hepatol Res: Official J Japan Soc Hepatol 2019; 49: 3-13.

37. Eden A, Gaulde F, Waghmare A, Jaeniscg R. Chromosomal instability and tumours promoted by DNA hypomethylation. Science 2003; 300: 455.

38. Issa JP. CpG island methylator phenotype in cancer. Nat Rev Cancer 2004; 4: 988-993.

39. Saito Y, Liang G, Pineal P, Volina S, McJunkin K, Marchio A, et al. miR-221 overexpression contributes to liver tumorigenesis. Proc Natl Acad Sci USA 2010; 107: 264-269.

40. Saito Y, Liang G, Egger G, Friedman JM, Chuang JC, Coetzee GA, et al. Specific activation of microRNA-127 with down regulation of the proto-oncogene BCL6 by chromatin modifying drugs in human cancer cells. Cancer cell 2006; 9: 435-443.

41. Simerzin A, Zorde-Khralevsky E, Rivkin M, Adam R, Zueman-Rossi J, Couch G, et al. The liver-specific microRNA-122, the complementary strand of microRNA-122, acts as a tumour suppressor by modulating the p53/mouse double minute 2 homolog circuitry. Hepatology 2016; 64: 1623-1636.

42. Tsai WC, Hsu PW, Lai TC, Chau GY, Lin CW, Chen CM, et al. MicroRNA-122, a tumour suppressor microRNA that regulates intrahepatic metastasis of hepatocellular carcinoma. Hepatology 2009; 49: 1571-1582.

43. Roos L, Spector T, Bell C. Using epigenetic studies in monozygotic twins to improve our understanding of cancer. Epigenomics 2014; 6: 299-309.

44. Mandio TA. Bringing genome-wide association findings into clinical use. Nat Rev Genet 2013; 1498: 549-558.

45. Dahms BB. Hepatoma in familial cholestatic cirrhosis of childhood: its occurrence in twin brothers. Arch Pathol Lab Med 1979; 103: 30-33.

46. Demir G, Belentepe S, Ozgurogia M, Celik AF, Sayhan N, Tekin S, et al. Simultaneous presentation of hepatocellular carcinoma in identical twin brothers. Med Oncol 2002; 19: 113-116.

47. Caglevic C, Silva S, Mahave M, et al. Hepatocellular carcinoma on identical twins in Chile: case report. Ecancermedical science 2016; 10: 708.

48. Rakyan VK, Down TA, Balding DJ, Beck S. Epigenome- wide association studies for common human diseases. Nat Rev Genet 2011; 12: 529-541.

49. Anestopoulous I, Voulgaridou GP, Georgakilas AG, Franks R, Pappa A, Panaviotidos MI. Epigenetic therapy as a novel approach in hepatocellular carcinoma. Pharmacol Ther 2015; 145: 103-119.

50. Nordgard O, Tjensvoll K, Gilje B, Soreide K. Circulating tumour cells and DNA as liquid biopsies in gastrointestinal cancer. Br J Surg 2018; 105.

51. Bitzer M, Horner M, Gianni EG, Ganten TM, Worns MA, Siveke JT, et al. Resminostat plus sorafenib as second-line therapy of advanced hepatocellular carcinoma-the SHELTER study. J Hepatol 2016; 65: 280-288.

52. Cheng Y, He C, Wang M, Ma X, Mo F, Yang S, et al. Targeting epigenetic regulators for cancer therapy: mechanisms and advances in clinical trials. Signal Transduct Target Ther 2019; 4: 62.

53. An J, Chang S, Kim H, Song GW, Shim JH. The clinical behavior and survival of patients with hepatocellular carcinoma and a family history of the disease. Cancer Medicine 2019; 0: 1-10.

54. European Association for the study of the liver, European organization for research and treatment of cancer, EASL-EORCTC clinical practice guidelines management of hepatocellular carcinoma. Eur J Canc. 2012; 5: 599-641.

55. Dienstag JT, Cladd ES, Atull KB, Szimuvers W. Hepatitis B vaccine given to chronic carriers of hepatitis B surface antigen. Ann Intern Med 1982; 96: 573-579.

56. Zuckerman AJ. Hepatitis B vaccine. Viral Ther 1986; 13: 1-4.