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To Identify The Relevance Of Setdb1 Dysregulation In Liver Cancer Pathology And The Underlying Processes

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Abstract

Hepatocellular carcinoma (HCC), the most frequent form of liver cancer, is the second biggest cause of cancer-related death worldwide, after lung cancer. Epigenetic changes have recently been implicated in liver cancer, according to growing data. After analysing 591 epigenetic regulators in hepatitis B-associated human HCC with transcriptome sequencing, we discovered that epigenetic regulator dysregulation was frequent in the disease. As a result, we discovered that two histone H3 lysine 9 (H3K9)-specific histone methyltransferases, SETDB1 (SET domain bifurcated 1) and G9a, were highly up-regulated in human HCCs (Euchromatic histone-lysine N-methyltransferase 2, EHMT2). For H3K9 tri- and di-methylation, SETDB1 and G9a are the only two markers that work. Previous epigenetic studies have mostly focused on hypermethylation of promoter DNA. Pathological consequences of histone alterations, in particular the roles of histone methyltransferases such SETDB1 and G9a in human HCC, are still unknown. SETDB1 and G9a, which are overexpressed in human HCC, will be examined to determine their functional significance as well as the underlying mechanisms by which they work.

Multiple HCC sample cohorts corroborated the frequent up-regulation of SETDB1 and found it to be linked with HCC development, cancer aggressiveness, and poor overall survival for patients with HCC. To test this hypothesis, researchers knocked off SETDB1, which prevented HCC cells from proliferating and migrating in culture and from metastasizing to other organs. Many different molecular mechanisms have been proposed to explain why SETDB1 is frequently overexpressed in human HCC, including recurrent gene copy number gain at chromosome 1q21, hyperactivation of SP1 transcription factor, and loss of miR-29, which facilitates SETDB1 overexpression by relieving post-transcriptional repression. Another possibility is that loss of PTEN increases SETDB1 protein levels post-transnationally.

Keywords: HCC, SETDB1, PTEN, methyltransferases, hyperactivation

Introduction

The liver takes up a significant amount of space in the human body and is therefore an important organ. In addition to filtering toxins from the blood and generating bile, the liver also stores glycogen, which can be used as energy in the body. A healthy liver is essential for survival, and there is currently no efficient way to compensate for liver dysfunction.

Hepatocellular carcinoma is a type of cancer that starts in the liver and spreads to other organs. Hepatocellular carcinoma (HCC), cholangiocarcinoma (cancer of the bile duct), and angiosarcoma are the three main cancers included (portal vein cancer). HCC is the most common kind of primary liver cancer, with malignant cells arising from oncogenic altered hepatocytes, accounting for 85% to 90% of cases. Because of this, we focused on HCC in our research.

The fifth most common cancer in men is HCC, while the ninth most common cancer in women is HCC. On the basis of data provided by the International Agency for Cancer Research (IARC), in 2012 there were 782,000 new cases diagnosed and 746,000 deaths (9.1% of all cancer deaths), making this the second leading cause of cancer death globally. When it comes to HCC, the outlook is grim, with a death ratio of 0.95.

HCC is found in many different parts of the world, making it difficult to pinpoint its exact location. Eighty-three percent of HCC cases are from non-Western nations. About half of all new cases in 2012 came from

China. Greater than 20 per 100,000 people were infected in 2012 in East Asia (Chinese Taipei, Hong Kong, Taiwan, and Japan), as well as Sub-Saharan Africa. Southeast Asia, West Africa, and Melanesia are among the regions with a medium incidence. North America, Western Europe, and Australia are among the regions with the lowest incidence rate (5/100,000). However, in the previous three decades, the trend in traditionally high-incidence regions of HCC has declined, while it has increased dramatically in traditionally low-incidence areas. Other main cancer types such as lung, colorectal, and prostate had decreasing incidence during 1974 to 205, whereas the overall age-adjusted incidence of HCC in the US grew from 1.6/100,000 to 4.9/100,000. HCC became a global disease as a result of the US's tripling of the incidence rate, rather than just an East Asian sickness.

The frequency of HCC differs dramatically between ethnic groups, ages, and genders. According to the US SEER database, Asians and Pacific islanders had the highest incidence (11,7/100,000 people) from 2006 to 2010, followed by Hispanics (9.5), African Americans (7.5), and non-Hispanic whites (4.2). The risk of developing HCC rises steadily with time. At diagnosis, patients with HCC are often younger in high-incidence regions than they are in low-incidence regions. This is most likely because of the high occurrence of HBV infection at birth and in the home. A clear sexual difference exists in HCC in almost all populations, with male incidence up to two to four times higher than female. This could be because men are more likely to drink and smoke than women. In addition, different levels of hormones in males and women were suggested as fundamental processes.

HCC incidence and death are very high in Hong Kong. The incidence of HCC in males and women in 2010 was 41/100,000 and 11.1/100,000, respectively, according to the Hong Kong Cancer Registry. In 2012, the mortality rate of HCC was 31.4/100,000 in males and 12/100,000 in women, making it the third leading cause of cancer death in Hong Kong after lung and colorectum cancer

More than $895 billion in direct and indirect health-related economic costs were borne by the HCC in 2010. In the United States, Asia Pacific, and Europe, the median cost of treating HCC patients is estimated at $15,310 ($3370-$84,710) per person, and this number is anticipated to rise. More cost-effective treatment options are therefore urgently required.

Literature Review

The formation of HCC, like many other malignancies, is a multi-step process involving the accumulation of genetic and epigenetic changes that lead to the inactivation of tumour suppressor genes and the activation of oncogenes. HCC. A great deal of research has been done on genetic changes in the last few decades, and we have shown in the sections above how numerous significant changed genes and pathways play a role in HCC (1.1.3). Epigenetic changes, on the other hand, are still unclear in terms of their significance. While chromosomal increase and loss are theoretically reversible processes since genetic alterations influence the DNA sequence of the genome, it is difficult and risky to "correct" these processes, which could lead to safety problems. According to recent research, cancer initiation and development may be influenced by epigenetic changes. Gene transcription and chromatin structure are affected by epigenetic controls, but the DNA sequence remains the same. A complex regulatory network is formed to fine-tune gene expressions because of the various layers of regulation mechanisms involved, both at the transcriptional and post-transcriptional levels. Oncogenes and tumour suppressor genes were expressed in an unbalanced manner in cancer, contributing to the development of cancerous lesions. Epigenetic modifications may be undone, which makes them a good candidate for medication development. Because of this, it will be easier to identify new therapeutic targets and create new drugs for the diagnosis and treatment of HCC if the underlying mechanisms of epigenetic alternations are well understood.

Chromatin remodeling and non-coding RNAs are among the most important epigenetic modifications in

cancer development, including aberrant DNA methylation, histone modification, and chromatin remodeling. As an introduction to human HCC, we'll talk about these three components.

Research Gap

For HCC patients, surgical resection and liver transplantation are the most curative therapy modalities. Due to late presentation of symptoms, most patients are diagnosed with cirrhosis and liver dysfunctions at a late stage, making surgical resection or liver transplantation an impractical option for the majority of them (15%). Other than local ablation therapies, the only treatment options for HCC patients who cannot benefit from curative surgery are radiation therapy and chemotherapy. Most often conducted ablation is radiofrequency ablation (RFA), followed by percutaneous ethanol injection (PET) and microwave coagulation treatment (MCT). These are all local ablation procedures. Local ablation using any of these techniques is best suited for HCC lesions that are small and can be visualised with imaging guidance. PET is based on the toxic impact of ethanol, which causes tumour necrosis while causing the least amount of damage to normal livers in the surrounding area. PET is useful for patients with nodular tumours that are less than 5 centimetres in diameter and have no more than three tumours. PET is not recommended for patients with more than three tumours or other liver disorders such as cirrhosis. To destroy tumour tissue, RFA uses high-frequency alternating current (460-480Hz). Due to its increased safety and success rate, RFA has gradually supplanted PET as a method of local ablation. Patients with HCC who are unable to undergo surgery have only one therapeutic option: transcatheter arterial chemoembolizatoin (TACE). TACE [150] is available to patients who do not have venous invasion or extrahepatic metastases. The only FDA-approved treatment for patients with late-stage primary HCC that has been shown to improve overall survival for three months is sorafenib right now. Small drug Sorafenib inhibits tumour proliferation and angiogenesis by targeting tyrosine protein kinases (VEGFR, PDGFR) and Raf kinases.

Because of the late identification of HCC and the development of substantial co-morbidities, all of the aforementioned therapeutic options have drawbacks. HCC patients must meet specific eligibility requirements before undergoing surgery or local ablation. As a result of HCC patients' rapidly developing chemo-resistance, chemotherapy with either TACE or molecular targeted treatment has a low response rate. As a result, new and improved methods of diagnosis and treatment are eagerly anticipated. Understanding the molecular pathways behind HCC is therefore critical for finding new pharmacological targets and formulating new therapeutic approaches.

Research Objective & Methodology

At the Queen Mary Hospital in Hong Kong, after an HCC tumour resection, primary HCC and surrounding NT liver specimens were obtained. This research utilised MHCC97L, Hep3B, HepG2, and 293FT as well as their associated data and upkeep procedures.

Stable overexpression of shRNA targeting the SETDB1 coding sequence was used to create SETDB1 knockdown cell lines. TTCTGTACTCGAGTACAGAAGTTATCATCTGAGCTTTTTG and shSETDB1#5 CCGGAGTTAGAGACATGGAGAGGTAATACCTCGAGGTATTACCCATGTCTCTAACTTTTG are the shRNA sequences employed in this investigation. pLKO1.1 was the expression vector used to overexpress shRNA. Chapter 2 covered the methods used to create the shSETDB1#2 and shSETDB1#5 overexpression lentiviral vectors. Sigma-Aldrich supplied non-target control shRNA (shNTC) for use as a negative control. Infectious disease virus generation and vector transduction into Hep3B and MHCC97L cell lines with the pLKO1.1.

Transcriptome sequencing (RNA-Seq) was carried out in 16 pairs of primary HCC samples, SETDB1 was knocked down and non-target control Hep3B and MHCC97L cells were used as non-target controls. The sample preparation and data analysis techniques. Gene set enrichment analysis (GSEA) techniques were

used to do the gene signature and pathway analyses.

The process for preparing total RNA and cDNA. A TaqMan gene expression assay and an endogenous HPRT control were used to measure the mRNA expression of SETDB1. TaqMan MicroRNA Reverse Transcription kit with miR-29a and RNU44 specific probes was used to make cDNA for microRNA expression investigation. qRT-PCR was used to measure the level of microRNA expression with a TaqMan microRNA probe. It's a technology that helps people live longer. An anti-SETDB1 rabbit monoclonal antibody was used in immunohistochemistry to detect SETDB1 protein expression in formalin-fixed paraffin-embedded sections and tissue microarrays (1:50, Cell Signaling Technology). Western Blotting with SETDB1-specific rabbit monoclonal antibody revealed the protein level in HCC cell lysate in NET-N buffer (1:1000, Cell Signaling Technology). The loading control was the -tubulin gene, a common housekeeping gene. Using a rabbit monoclonal antibody specific for H3K9me3, H3K4me3, and H3K27me3 to detect the methylation histone proteins in HCC cell histone extracts, we also discovered (1:1000, Cell Signaling Technology). As a loading control, the histone H3 was utilized.

Data Analysis & Findings

DNA methylation, covalent histone modifications, chromatin remodeling, and the production of noncoding RNAs are all regulated by highly intricate networks of epigenetic modifiers. Our hypothesis was that epigenetic changes may be the underlying mechanisms that contribute to liver carcinogenesis due to epigenetic changes being critical in the onset and advancement of many different forms of cancer. In this connection, we used whole-transcriptome sequencing to evaluate the expression profiles of 591 known epigenetic regulators in 16 pairs of HBV-associated primary HCC and their matched NT livers (RNA-Seq). Using unsupervised hierarchical clustering, the expression of 473 epigenetic regulatory genes was analyzed after 118 were found to have poor or undetectable signal (median expression 1 FPKM in both primary HCC and NT-liver samples). There was a clear difference in the expression profiles of epigenetic regulatory genes between human HCC and their NT equivalents, indicating that human HCC is more likely to exhibit changed expression than NT HCC.

A striking difference between the expression of epigenetic regulatory genes in primary HCC and NT liver tissues was found. This finding contrasts with the overexpression of other protein-coding genes. While 341 genes were discovered to be up-regulated in primary HCC, only 10 of the deregulated genes were down-regulated, showing severe dysregulation of epigenetic regulators (FDR 0.05). This finding revealed that epigenetic regulator dysregulation was widespread in human HCC and may have contributed to the genesis, development, and metastasis of human HCC. This is a significant finding.

Conclusion

We found G9a to be one of the most frequently up-regulated epigenetic regulators in human HCCs in this investigation. Increased expression of G9a was found to be closely linked to the progression of HCC, as well as tumour aggressiveness, invasiveness, and migratory potential. On a functional level, we found that inhibiting HCC cell proliferation in vitro and subcutaneous xenograft tumorigenicity with shRNA knockdown and CRISPR/Cas9 deletion G9a. The capacity of HCC cells to migrate was greatly reduced, and cell senescence was promoted, when G9a was depleted. UNC0638 and BIX01294 inhibited G9a pharmacologically, which reduced HCC cell proliferation and altered cell shape. We discovered that chromosomal copy number gain at 6p21 was responsible for the frequent up-regulation of G9a in human HCC. We also discovered that miR-1 suppresses G9a expression. Because miR-1 was missing, the post-transcriptional repression on G9a was relaxed, and this resulted in G9a being overexpressed in human cancer cells. A feed forward regulation loop forms between miR-1 and G9a as a result of G9 up regulation epigenetically repressing miR-1 production. It was discovered that the transcriptome changes in HCC cells

after G9a knockdown were considerably enriched for the IFN- and IFN- signalling pathway as well as HBV sensitive genes by applying RNA-Seq and GSEA analysis More crucially, we discovered that the tumour suppressor RARRES was epigenetically suppressed in HCC and facilitated the growth of tumour cells.

In the world, hepatocellular carcinoma (HCC) is the second-leading cause of cancer death and the fifth most common malignancy among men. Despite advances in treatment procedures including surgical resection and liver transplantation, HCC is still a deadly illness that kills more than 700,000 people each year and kills millions more in the developing world. Cancer metastasis occurs frequently and with great frequency when it is detected late in the disease process. With advanced disease and other comorbidities, including intrahepatic or extrahepatic metastases, most patients are ineligible for curative procedures such as surgical resection and liver transplant. However, even for those who are eligible, the overall survival rate is low, and tumour recurrence is common after surgery. Patients with inoperable HCC need early diagnostic techniques, effective adjuvant treatment, and treatment against tumour recurrence prevention methods. We need to know more about hepatocarcinogenesis' molecular pathophysiology in order to find new therapeutic targets and come up with better ways of diagnosing and treating the disease.

Lysine acetylation, lysine and arginine methylation, threonine and serine phosphorylation, lysine ubiquitination, and lysine sumoylation are the most prevalent modifications of histones. Certain combinations of histone modification patterns are thought to produce various chromatin structures and diverse transcriptional effects on gene expression, according to general belief Histone code" is the name given to this association between combination and effect [158]. An abnormal histone coding disrupts chromatin structure, resulting in erroneous gene expression and genomic instability, which in turn contributes to cancerous cell growth. Because of this, enzymes that catalyze histone changes are crucial in the genesis of cancer.

Since there are new findings suggesting the HMTs play a significant role in cancer initiating and progressing, the research and development of small molecules that target the HMTs has increased dramatically. There are two types of HMT enzymes. Suppressor of variegation, Enhancer of Zeste, Trithorax) is a conserved catalytic domain in one protein family, and human genomes encode 48 HMTs with this domain. The SET domain-free HMT is DOT1L, which is not found in any HMTs. At least 22 of the approximately 50 human genome-encoded lysine HMTs have been linked to cancer or other disorders. In prostate cancer, breast cancer, and bladder cancer, overexpression of the polycomb group transcriptional repressor, EZH2, was reported to be related with cancer progression and metastasis. HMT NSD2 is also linked to poor prognosis and tumour aggressiveness in various forms of cancer. EZH2 was previously found to be overexpressed in human HCC, and this overexpression was linked to a more advanced stage of the tumour and a bad prognosis. In vitro and in vivo, EZH2 knockdown reduced HCC cell proliferation and migration. In addition, we found that the SUV39H1 histone lysine methyltransferase promotes cancer growth in HCC. Other HMTs such SETDB1 and G9a were found to play an oncogenic function in human HCC, which our research confirmed. SETDB1 and G9a both contain HMTs in the SET domain.

References

1. Abdel-Misih, S.R. and M. Bloomston, Liver anatomy. Surg Clin North Am, 2010. 90(4): p. 643-53.
2. Altekruse, S.F., et al., Changing hepatocellular carcinoma incidence and liver cancer mortality rates in the United States. Am J Gastroenterol, 2014. 109(4): p. 542-53.
3. Altekruse, S.F., K.A. McGlynn, and M.E. Reichman, Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. J Clin Oncol, 2009. 27(9): p. 1485-91.

4. Bosch, F.X., et al., Primary liver cancer: worldwide incidence and trends. Gastroenterology, 2004. 127(5 Suppl 1): p. S5-S16.
5. Bruix, J., G.J. Gores, and V. Mazzaferro, Hepatocellular carcinoma: clinical frontiers and perspectives. Gut, 2014. 63(5): p. 844-55.
6. Dunbar, J.K., et al., Increasing survival of hepatocellular carcinoma patients in Scotland: a review of national cancer registry data. HPB (Oxford), 2013. 15(4): p. 279-85.
7. El Khoury, A.C., et al., Economic burden of hepatitis C-associated diseases: Europe, Asia Pacific, and the Americas. J Med Econ, 2012. 15(5): p. 887-96.
8. El-Serag, H.B. and K.L. Rudolph, Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology, 2007. 132(7): p. 2557-76.
9. El-Serag, H.B., Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology, 2012. 142(6): p. 1264-1273 e1.
10. Ferlay, J., et al., Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer, 2015. 136(5): p. E359-86.
11. Gao, S., et al., Declining rates of hepatocellular carcinoma in urban Shanghai: incidence trends in 1976-2005. Eur J Epidemiol, 2012. 27(1): p. 39-46.
12. Gomaa, A.I., et al., Hepatocellular carcinoma: epidemiology, risk factors and pathogenesis. World J Gastroenterol, 2008. 14(27): p. 4300-8.
13. Jemal, A., et al., Global cancer statistics. CA: a cancer journal …, 2011.
14. Li, Z., et al., Foxa1 and Foxa2 are essential for sexual dimorphism in liver cancer. Cell, 2012. 148(1-2): p. 72-83.
15. Liu, C.J. and J.H. Kao, Hepatitis B virus-related hepatocellular carcinoma: epidemiology and pathogenic role of viral factors. J Chin Med Assoc, 2007. 70(4): p. 141-5.
16. Poon, D., et al., Management of hepatocellular carcinoma in Asia: consensus statement from the Asian Oncology Summit 2009. Lancet Oncol, 2009. 10(11): p. 1111-8.
17. Wallace, M.C., et al., The evolving epidemiology of hepatocellular carcinoma: a global perspective. Expert Rev Gastroenterol Hepatol, 2015. 9(6): p. 765- 79.