Obesity-related hepatocellular carcinoma: roles of risk factors altered in obesity

Chuan Shen¹, Cai-Yan Zhao¹, Rongxin Zhang², Liang Qiao³

¹Department of Infectious Disease, the Third Hospital of Hebei Medical University, Shijiazhuang, 050051, China, ²Research Center of Basic Medical Sciences and Department of Immunology, Tianjin Medical University, Tianjin 300070, China, ³Storr Liver Unit, Westmead Millennium Institute, The Western Clinical School of the University of Sydney, Westmead Hospital, Westmead, NSW 2145, Australia

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1. ABSTRACT

Epidemiological data have demonstrated that the prevalence of either obesity or hepatocellular carcinoma (HCC) is increasing worldwide during past decades, and obesity has been unequivocally shown to be a risk factor for HCC. It has been reported that a significant proportion of HCC in obesity develops in cryptogenic cirrhosis, which is largely associated with the progression of nonalcoholic fatty liver disease, especially nonalcoholic steatohepatitis. Since the HCC is a highly malignant tumor with a poor prognosis, a better understanding of the molecular mechanisms may help researchers to explore new approaches for preventing and treating the obesity-related HCC, and thereby facilitating a substantial reduction of morbidity and mortality. In this article, we reviewed the mechanisms underlying the relationship between obesity and HCC, with an emphasis on the roles of insulin/insulin-like growth factor axis, adipose tissue derived hormones, oxidative stress, and liver stem cells. In addition, we will discuss the impact of life-style modification on obesity-related HCC.

2. INTRODUCTION

The World Health Organization (WHO) estimated that more than 1.5 billion adults (20 years or older) worldwide are overweight and of whom over 500 million people are obese, as defined by the body mass index (BMI) equal or greater than 25 or 30 kg/m², respectively.(1) Obesity is a part of the metabolic syndrome which includes several other conditions such as hypertension, stroke, type 2 diabetes (T2DM), dyslipidemia and nonalcoholic fatty liver disease (NAFLD). Epidemiological evidence over the recent years has demonstrated that obesity is a risk factor for the development of many cancers such as those derived from endometria, prostate, postmenopausal breast, kidney, colon, pancreas, esophagus, and liver.(2-12) Many factors altered in obesity are considered important for causing obesity-associated cancers. These factors include increased blood levels of insulin, insulin-like growth factors (IGFs), leptin, interleukin (IL)-6, IL-17, tumor necrosis factor-alpha (TNF-alpha) and decreased adiponectin.(13-17) This review focuses on the relationship between obesity and
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hepatocellular carcinoma (HCC), with an emphasis on the molecular mechanisms by which obesity predisposes to HCC, as well as the effect of lifestyle modification on preventing HCC in obese people.

3. EPIDEMIOLOGICAL EVIDENCE LINKING OBESITY AND HCC

HCC is the fifth most frequent malignancy, with an incidence rate of 10.8/100,000 and a mortality rate of 10/100,000 worldwide. (18) China is the high prevalence area for HCC, accounting for approximately 55% of all cases worldwide. (19) The incidence of HCC is constantly rising in many western countries, including USA, United Kingdom, Australia, and France. (20-23)

Although the risk factors for most HCC cases are well established (HBV and HCV infection, and alcohol intake), in 5-30% of HCC cases the etiological factors are not readily identifiable. (24) In parallel with the rising HCC incidence, the prevalence of obesity has been increasing markedly over the past few decades. In a cohort study involving 362,552 Swedish men, (10) the relative risk for HCC in obese individuals (BMI>30 kg/m²) was 3.1 fold higher than that in people with normal weight. A positive dose-response pattern of higher liver cancer risk with increasing BMI was observed. In a study conducted in 23,820 resident Taiwanese, (25) those with obesity (BMI≥30 kg/m²) was independently associated with a 4.13 times higher in HCC risk if they are HCV infected, and 2.36 times higher in HCC risk if they are without HBV or HCV infection, suggesting a synergistic oncogenic effect between obesity and HCV infection. It was also estimated that the synergistic effects of obesity, diabetes and HBV or HCV infection result in a 100-fold increase in the risk of HCC development. (25) HCC in obese subjects is associated with increased mortality. In a 16-year follow-up study of more than 900,000 U.S. adults, the risk of cancer-related death from liver cancer was 4.52 times higher for severe obese men (BMI≥35kg/m²) than subjects with normal weight (BMI 18.5-24.9 kg/m²). (5) All these data suggest that obesity affects the incidence and mortality of HCC.

It is unclear why obese subjects are more susceptible to develop HCC. It was reported that a higher incidence of HCC frequently occurred in obese patients with cryptogenic or alcoholic cirrhosis. (26-28) In a study conducted in USA involving 105 consecutive patients with HCC, (29) HCV infection and cryptogenic cirrhosis are the two commonest causes of HCC (51% and 29%, respectively). Clinically, cryptogenic cirrhosis is largely attributed to the presence of NAFLD, in particular, nonalcoholic steatohepatitis (NASH), (27, 29-30) the more aggressive form of NAFLD. NAFLD is now believed to be the most common cause of abnormal liver function tests in clinical setting. The causal relation between NASH and HCC has been reported both epidemiologically and experimentally. (31-36) Most obese people have abnormal livers one way or another. For example, it was reported that approximately 60% of patients with NAFLD have simple steatosis, 25% have NASH, and 3%-5% have cirrhosis, whereas only 10%-15% of obese patients are expected to have normal livers. (37) On the other hand, 30%-100% NAFLD subjects are believed to have obesity. It has been reported that HCC occurs in 0.5% patients with NAFLD and up to 2.8% of patients with NASH. (38-39) The histological evidence of NASH was found to be present in 20% of patients with cryptogenic cirrhosis 4.3±1.6 years prior to the diagnosis of HCC. (29) In a cross-sectional multicenter study in Japan, obesity (defined as BMI≥25 kg/m²) was present in 62% of 87 patients with histologically proven NASH who developed HCC. (32) Clearly, obesity and NASH are closely linked with HCC. Indeed, progression from NASH to HCC has also been recapitulated in mice fed on a choline-deficient L-amino acid-defined (CDAA) diet for 84 weeks. (40)

4. UNDERLYING MOLECULAR MECHANISMS LINKING OBESITY AND HCC

The possible molecular mechanisms underlying obesity-associated HCC as we discussed in this review article that were briefly summarized in Figure 1.

4.1. Role of Insulin/IGF axis in obesity-related HCC

IGFs, including IGF-I and IGF-II, are the polypeptides structurally homologous to insulin. They are mainly synthesized and secreted by the liver in response to growth hormone (GH). (41) Binding of IGF-I and IGF-II to cell surface IGF-I receptor (IGF-IR) results in activation of intrinsic tyrosine kinase domain, thus triggering various signaling pathways, such as mitogen activated protein kinase (MAPK) pathway and phosphoinositide 3-kinase (PI3K/Akt) pathway. (42) Two major signaling pathways mediating cell proliferation and differentiation. IGFs can also bind to the insulin receptor. (43) IGFs in the circulation can specifically bind to six high-affinity IGF-binding proteins (IGFBP 1-6), thereby regulating the availability and biological actions of IGFs by preventing IGFs/IGF-IR interaction and increasing IGFs half-time. (44)

4.1.1. Insulin/IGF axis and obesity

The causal relationship between insulin/IGFs axis and obesity remains largely unclear. Accumulating evidence has demonstrated that production of IGFBPs is reduced by prolonged high insulin level, therefore leading to increased bioavailability of IGFs. (45-47) Furthermore, IGFBP-1 and -2 appear to be inversely associated with indices of obesity. (46, 48) Conflicting data on the circulating level of IGFs in obese subjects have been reported. For example, elevated free IGF-I or percentage of bioactive IGF-I were observed in obese individuals. (46, 48) whereas other reports showed that in obese individuals the circulating level of IGF-I was normal or lower, as compared to normal-weight individuals. (47) A non-linear relationship between IGF-I and IGF-1/IGFBP-3 ratio with BMI was recently reported, suggesting that IGF-I varies substantially over a wide range of body weight. (49) Thus, the role of insulin and IGFs in obesity or overweight is not fully established.
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Figure 1. Possible molecular mechanisms in obesity-related HCC. Obesity leads to enhanced oxidative stress, increased insulin/insulin-like growth factor (IGF-II) production and aberrant adipokines secretion. The products of oxidative stress such as 8-hydroxydeoxy guanosine (OH8dG), 8-nitroguanin and malondialdehyde (MDA)-DNA adducts can initiate carcinogenesis due to their ability to damage DNA and biomembrane. Reactive oxygen species (ROS) induced calcium release can activate protein kinase C (PKC), and disrupt association of Kelch ECH associating protein 1 (Keap 1) and nuclear factor erythroid 2-related factor 2 (Nrf2). ROS can also activate transcription factor activator protein 1 (AP-1) through c-Jun NH2-terminal kinase (JNK)/p38 mitogen activated protein kinase (MAPK) pathway. In addition, ROS can lead to activation of nuclear factor kappa B (NF-kappa B) and hypoxia-inducible factors (HIFs). All these factors (i.e. PKC, Nrf2, NF-kappa B and HIFs) can regulate specific gene transcription associated with cell proliferation or apoptosis. Binding of free insulin-like growth factor II (IGF-II) to insulin growth factor-I receptor (IGF-IR) can stimulate carcinogenesis through phosphoinositide 3-kinase (PI3K/Akt) pathway or MAPK pathway. IGF-II can also stimulate insulin receptor (IR)-mediated signaling. Tissue inhibitor of metalloproteinase 1 (TIMP-1) is able to reduce matrix metalloproteinases (MMP)-mediated proteolysis of insulin growth factor binding protein 3 (IGFBP-3), leading to an increased production of free IGF-II. Leptin and adiponectin exert seemingly “opposite” functions in HCC development. Leptin can enhance cell proliferation through activation of protein kinase B (PKB)/Akt signaling pathway. In contrast, adiponectin can suppress tumor growth and increase cell apoptosis through tuberous sclerosis complex 2 (TSC2)/mammalian target of rapamycin (mTOR) pathway or JNK/caspase 3 pathway, as a result of phosphorylation of 5'-adenosine monophosphate activated protein kinase (AMPK). Adiponectin can also affect the role of leptin by inhibiting activation of STAT3 or Akt, as well as by increasing the expression of suppressor of cytokine signaling 3 (SOCS3). These pathways also modulate cyclin D1-dependent cell cycle transition.

4.1.2. Possible role of insulin/IGF axis in HCC

Insulin resistance (IR) is a key mechanism in the development of obesity, and hyperinsulinemia is frequently present in individuals with excessive body weight. In addition to its effects on metabolic homeostasis, insulin can act as a crucial mitogen that may result in carcinogenesis and metastasis of several cancers. One cohort study has indicated that fasting hyperinsulinemia not only signifies a poorer prognosis in the early stage of HCC, but also indicates a higher recurrence rate in the curative HCC. In addition, mice with obesity and T2DM are often associated with IR, and a higher incidence of HCC in these mice has been reported. Consistent with the in vivo data, insulin has been found to suppress apoptosis and enhance metastasis of HCC cells in vitro through activation of protein kinase B (PKB)/Akt signaling pathway.

Dysregulation of IGFs-signaling in HCC has been reported to be predominantly mediated by IGF-II. Enhanced expression level of IGF-II at both mRNA and protein levels have been reported in HCC. The altered expression level of IGF-II in HCC was attributed to the silencing of IGF-II gene promoter P1 or the shift from adult P1 promoter to fetal P2, P3 and P4 promoters, or the hypomethylation of IGF-II gene promoter P3 or P4. Indeed, an allelic imbalance at the IGF-II locus has been found to contribute to over-expression of the IGF-II gene in human precancerous lesions and HCC. On the other hand, in vivo and in vitro studies have demonstrated that IGF-II mediated growth of HCC can be selectively blocked by antibodies or antisense oligodeoxynucleotides specific to IGF-II, further suggesting that IGF-II gene regulation is aberrant in HCC.

Angiogenesis is an important mechanism in HCC development. IGF-II has been showed to facilitate angiogenesis as it could induce the synthesis of vascular endothelial growth factor (VEGF), thereby favoring tumor formation and development. In addition, in vitro experiments in HCC cell lines have showed that the hypoxia environment may stimulate the production of IGF-II, which can enhance the DNA binding activity of
transcription factor Egr-1 on the IGF-II P3 promoter.(67) Clinically, a higher level of plasma IGF-II could predict the occurrence of metastatic HCC after transcatheter arterial chemoembolization (TACE).(68)

The mitogenic role of IGFs in the development of HCC may be partially attributed to the reduced production of IGFBPs. Significant down-regulation of IGFBP-1, -2, -3, and -4 expressions was detected in human HCC.(63, 69-72) The reduced level of IGFBPs may substantially potentiate and -4 expressions was detected in human HCC.(63, 69-72) The reduced expression of IGFBP-3 in HCC, possibly as a result of promoter hypermethylation,(70) is significantly associated with higher portal venous invasion and poorer prognosis.(73) To further support these observations, it was found that treatment of HCC cells with IGFBP-3 in vitro significantly prevents cell proliferation, which is associated with reduced IGF-1 mitogenic activity and decreased expression of IGF-IR downstream effectors such as MAPK, Elk-1, Akt-1 and PI3K.(63)

IGFs may be interacting with some other molecules to facilitate HCC development. Tissue inhibitor of metalloproteinase 1 (TIMP-1) is an inhibitor of matrix metalloproteinases (MMP) and its expression was found to be down-regulated in HCC.(56) A recent study has also identified MMP-2 as a mediator of the invasive phenotype downstream of IGF-IR signaling.(74) TIMP-1 has been shown to reduce MMP-mediated degradation of IGFBP-3, thereby lowering dissociable IGF-II level and its activity.(75) Thus, proteinolysis of IGFBPs regulates IGFs bioavailability, which favors cell survival and HCC development.

In addition to aberrant expression level of IGFs, abnormal expression of IGF-IR may also be involved in the development of HCC. It has been found that IGF-IR is constitutively expressed at low level in normal hepatocytes,(76) but its expression is up-regulated in human HCC tissues(77-78) and HCC cell lines.(79) Specific targeting of IGF-IR using antisense oligonucleotides can markedly inhibit the proliferation of HCC cells in vitro and prevent tumor growth and metastasis in vivo.(80) Of interest, simultaneously targeting both IGF-IR and epidermal growth factor receptor (EGFR) may overcome acquired resistance of HCC cells to IGF-IR inhibition.(78)

4.2. Role of adipose tissue-derived hormones (adipocytokines) in the development of HCC

4.2.1. Leptin

Leptin is an adipose-derived hormone encoded by ob gene. Subjects with overweight or obesity tend to have an increased production of leptin. It regulates body weight and metabolic homeostasis by controlling appetite and energy expenditure.(81-82) Leptin is also involved in other biological processes such as hematopoiesis,(83) inflammation/immunity,(84) osteogenesis,(85) cell proliferation(86) and tissue angiogenesis.(87) The possible role of leptin in the development of liver cancer may also be reflected by some published in vivo studies. In human HCC, the expression level of leptin/leptin receptor was found to be correlated to the extent of tumor angiogenesis.(92-93) In animal studies, intact leptin signaling was found to be necessary in the natural course of chronic liver condition such as NASH. In Zucker rats, which are known to naturally carry leptin receptor mutation and thus lack leptin signaling, dietary induced NASH was not associated with liver fibrosis nor do these rats develop HCC.(94) These data imply that leptin/leptin receptor signaling may be necessary for the malignant transformation of hepatocytes.

4.2.2. Adiponectin

Adiponectin is an important protective adipocytokine that is abundantly expressed and secreted by visceral adipocytes. Apart from adipose tissue, liver cells are also responsible for adiponectin production.(95-96) In circulation, adiponectin exists as three major isofoms: trimers, hexamers, and larger multimers (also known as high molecular weight complex).(97) Multimeric isofom appears to be the most active one that is responsible for many of the physiological functions of adiponectin.(98-99) Three adiponectin receptor isofoms have been identified: AdipoR1, AdipoR2 and T-cadherin. These three receptors have distinct tissue distributions and binding of adiponectin to these receptors may activate different cellular functions through activating multiple signaling pathways.(100-101)

Clinical epidemiological data have showed a consistent inverse association between adiponectin and obesity-related disorders, such as T2DM.(102) coronary
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artery disease.(103) and NAFLD.(104) In obese and overweight individuals who have lower plasma adiponectin levels, there is an increased risk for several types of malignancies.(105-109) Reduced plasma or hepatic adiponectin levels have been found in HCC patients,(110-111) and adiponectin expression inversely correlates with tumor size.(111) Clearly, there is a close correlation between reduced adiponectin level and the development of HCC. However, what is not certain is whether reduced adiponectin is a cause or effect of HCC.

Many in vivo studies support a tumor suppressor role of adiponectin in the development of liver cancer. Compared with wild-type mice, administration of CDAA diet to adiponectin knockout mice enhanced the progression from NASH to cirrhosis and hepatic adenomas.(112)

Evidence of adiponectin act as a tumor suppressor gene also comes from in vitro studies. Adiponectin affects all biological processes that are important for cancer formation, such as cell proliferation, apoptosis, and angiogenesis. Adiponectin was able to directly suppress the growth of HCC cells through stimulating c-Jun NH2-terminal kinase (JNK) activation and c-Jun phosphorylation, as well as inhibiting STAT3 DNA binding activity.(113) Inhibition of cell proliferation by adiponectin has also been found to be through increased phosphorylation of 5'-adenosine monophosphate activated protein kinase (AMPK) and activation of tumor suppressor tuberous sclerosis complex 2 which down-regulates phosphorylation of mammalian target of rapamycin.(111) The same study also demonstrated that adiponectin was able to induce apoptosis in HCC cells through activation of AMPK/JNK/caspase-3 signaling pathway.(111) Thus, AMPK appears to be a main effector that mediates the anti-proliferative and pro-apoptotic effects of adiponectin on HCC cells. However, more direct evidence of the tumor suppressor role of adiponectin comes from direct administration of adiponectin in vitro and in vivo. Purified adiponectin significantly inhibited liver tumor growth, invasiveness, and angiogenesis.(110) The reported mechanisms for these effects include down-regulation of Rho kinase/interferon-inducible protein 10/angiopoietin 1/MMP 9/VEGF signaling pathway, and inhibition of lamellipodia on the surface of liver cancer cells.(110)

During the development of HCC, leptin and adiponectin may function in a seemingly “opposite” manner. Adiponectin was shown to inhibit transition of HCC cells from G1 to S phase via decreasing cyclin D1 expression,(111) whereas leptin was found to stimulate the G2-M transition during cell cycle progression. Recent evidence indicate that adiponectin can inhibit leptin-induced tumor growth by reducing STAT3 and Akt phosphorylation and increasing suppressor of cytokine signaling (SOCS3), a negative regulator of leptin signal transduction.(114)

Collectively, increased production of leptin and decreased secretion of adiponectin during obesity may substantially predispose the obese individuals to develop HCC.

5. ROLE OF OXIDATIVE STRESS IN OBESITY ASSOCIATED HCC

Obesity is inevitably associated with systemic chronic oxidative stress.(115-116) Three mechanisms have been proposed for increased oxidative stress in obesity. Firstly, obesity increases the mechanical and metabolic loads on the myocardium, thus increasing myocardial oxygen consumption and production of reactive oxygen species (ROS) such as superoxide, hydroxyl radical and hydrogen peroxides from the increased mitochondria respiration.(117) Secondly, adipokines released by the adipose issues, such as TNF-alpha, IL-6, leptin, adiponectin, and resistin, have been documented to play roles in oxidative stress.(118-123) Lastly, increased lipid peroxidation is frequently present in nutritious obesity.

Hepatocarcinogenesis is a multistep process. Oxidative stress may play a vital role in the first two steps of carcinogenesis: initiation and promotion stages. In the initiation stage, the initiated mutated cells usually contain a fixation DNA mutation. ROS have been documented to cause DNA damage. The most abundant cellular product of oxidative damage is 8-hydroxydeoxy guanosine (OH8dG), which can pair with cytosine or adenine to form G:A or G:C mismatch. If the mutagenic replication of OH8dG as templates, G→T substitutions would occurred, while if the OH8dG misincorporation as substrates, A→C substitutions would take place.(125) Similarly, reactive nitrogen species (RNS) can cause nitrative DNA damage to form 8-nitroguanin which is a mutagenic DNA lesion, leading to G→T transversions.(126)

Apart from causing DNA damage, free radicals also damage the cellular biomembranes, leading to lipid peroxidation. One of the major products of lipid peroxidation is malondialdehyde (MDA), which reacts with several nucleic acid bases to form dG, dA, and dC adducts.(127) MDA-DNA adducts can induce mutations in oncogenes and tumor suppressor genes. In vitro studies have showed that MDA-DNA adduct levels correlate with altered cell cycle control in cultured cells.(128)

The promotion stage is a reversible process characterized by the clonal expansion of initiated cells to form identifiable focal lesions. The expansion of initiated cells is mainly the result of enhanced cell proliferation and/or reduced apoptosis. ROS is involved in this stage through altering the structure or function of major transcription factors that are related to proliferation, migration, cell cycle progression, cell survival and cell death.

ROS promotes the proliferation of the initiated cells and thus favors tumor formation through several mechanisms. (1) ROS induces calcium release from intracellular compartments, resulting in the activation of kinases, such as protein kinase C (PKC). Active PKC has been well-documented to regulate a variety of cellular...
functions such as proliferation, cell cycle progression, cell differentiation, cytoskeletal organization, cell migration, and apoptosis.\textsuperscript{(129)} (2) ROS can react with and change the conformation of Kelch ECH associating protein 1 (Keap 1), resulting in the disruption of the interaction between Keap1 and nuclear factor erythroid 2-related factor 2 (Nrf2), and liberation of Nrf2.\textsuperscript{(130)} Active PKC also causes phosphorylation of Nrf2.\textsuperscript{(131)} Activated Nrf2 may translocate to the nucleus triggering the transcriptional expression of a broad spectrum of protective enzymes including those involved in xenobiotic detoxification, nitrooxidative response, and proteome maintenance.\textsuperscript{(132)} All these would favor cancer cell survival.\textsuperscript{(133)} (3) ROS may activate the transcription factor activator protein 1 (AP-1) through reacting with cysteine64 or up-regulating PKC.\textsuperscript{(134)} Activation of AP-1 has been reported to increase cell proliferation via up-regulating cyclin D1\textsuperscript{(135)} or suppressing p21.\textsuperscript{(136)} (4) ROS is a potent activator for nuclear factor kappa B (NF-kappa B),\textsuperscript{(137)} a critical transcription factor involved in the regulation of cell survival, differentiation, inflammation, and growth.\textsuperscript{(138)} Activation of NF-kappa B has been well-linked to carcinogenesis through mechanisms such as promotion of cell proliferation, invasion, and angiogenesis, as well as inhibition of apoptosis.\textsuperscript{(138)} (5) ROS may stimulate the production of and activate hypoxia-inducible factors (HIFs),\textsuperscript{(139)} thus favoring cancer formation via enhanced cell proliferation and angiogenesis.

6. ROLE OF LIVER STEM CELLS IN OBESITY RELATED HCC

Liver stem cell (LSC) represents a cell compartment, which has a bipotential plasticity that can differentiate into hepatocytes or bile duct epithelia.\textsuperscript{(140)} Rodent oval cells or human progenitor cells are regarded as facultative LSC in most of the available data. This cell compartment predominantly locates in the peripheral branches of the biliary tree known as the Canals of Hering.\textsuperscript{(141-143)} In addition, small hepatocytes,\textsuperscript{(144)} marrow stem cells,\textsuperscript{(145)} and embryonic stem cells\textsuperscript{(146)} are also thought to be candidates for LSC. When liver undergoes severe injuries or hepatocyte replication is inhibited, the quiescent LSC is activated and participates in liver regeneration.

There are two major hypotheses concerning the cellular origin of HCC: from dedifferentiation of mature hepatocyte or from maturation arrest of the LSC.\textsuperscript{(147)} It has been shown that the oval cells distributed inside and outside the HCC nodes in rodent livers.\textsuperscript{(148-149)} Both human hepatic adenomas and HCCs highly express progenitor cell biomarkers, such as cytokeratin (CK) 7, 8, 18, and 19, chromogranin-A, OV-6, neural cell adhesion molecule, CD44, CD133, and Nestin.\textsuperscript{(150-153)} Furthermore, depletion of tumor suppressor gene p53 led to generation of immortalized and tumorigenic oval cells, and injection of these cells into athymic nude mice cause xenograft HCC.\textsuperscript{(154)} On the other hand, inactivation of oncogene myc resulted in HCC cells differentiating into hepatocytes and biliary cells.\textsuperscript{(155)} Recently, a side population (SP) cell exhibiting characteristics of both hepatocyte and cholangiocyte has been identified in Huh7 and PLC/PRF/5 HCC cell lines, and these SP cells showed extreme tumorigenic potential in non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice.\textsuperscript{(156)} All these data supported the possibilities that LSC may be the sources of malignant phenotype of HCC.

Majority of evidence linking LSC and obesity comes from the data of fatty liver and NASH. Significant accumulation of liver oval/progenitor cells has been shown in several rodent models and humans of fatty liver and NASH.\textsuperscript{(157-161)} In such condition, excessive ROS production and absence of antioxidants induced by oxidative stress can promote replicative senescence of mature hepatocyte, thereby leading to the adaptive activation and expansion of oval/progenitor cells.\textsuperscript{(157-158)} Furthermore, it is also speculated that some mutagenic reactive aldehyde products of lipid peroxidation such as 4-hydroxynonenaldehyde and MDA potentiate oval/progenitor cells towards neoplastic differentiation possibly by p53 gene mutation.\textsuperscript{(162)} Obese individuals show increased production of several inflammatory cytokines such as TNF-alpha and IL-6,\textsuperscript{(163-164)} which could stimulate proliferation and transformation of oval cells that may give rise to HCC.\textsuperscript{(165-166)}

Interestingly, although peroxisome proliferator-activated receptors (PPARs) usually provide potential benefits for obesity and NAFLD,\textsuperscript{(167-170)} the phenotype of oval cells promoted by PPAR-alpha activator (WY14643) has been found to be similar to that of neoplastic features in cells induced by peroxisome proliferators known as nongenotoxic carcinogens.\textsuperscript{(171)} In contrast, evidence from  in vivo and in vitro studies have demonstrated that an agonist of PPAR-gamma (ciglitazone) resulted in an inhibition of proliferation induction of apoptosis of liver oval cells during chronic liver injury,\textsuperscript{(172)} suggesting a possibly preventative role of PPAR-gamma in liver carcinogenesis.

There are evidence that insulin/IGFs axis may participate in LSC activation. IGF-II has been shown to induce proliferation of malignantly transformed liver oval cells (M22 cells) via an autocrine mechanism when binding to IGF-IR  in vitro; however, transfection of an IGF-II complementary DNA into normal oval cells (OC/CDE 22 cells) does not cause a malignant transformation.\textsuperscript{(173)} This finding suggests that IGF-II may only exert mitogenic effect on malignant liver oval cells themselves. Additionally, proliferation and differentiation of liver oval cells can be negatively regulated by IGFBP-3 via TGF-beta pathway both  in vivo and in vitro, suggesting that decreased IGFBP-3 may potentiate aberrant proliferation of oval cells.\textsuperscript{(174)} Recently, the liver oval cells have also been shown to differentiate into pancreatic endocrine hormone-producing cells,\textsuperscript{(175-176)} and these cells can synthesize and secrete insulin in response to glucose stimulation.\textsuperscript{(176)} Future studies are required to clarify whether oval cell-derived insulin-producing cells in obesity-related disorders may
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increase the probability of hepatic oncogenesis due to increased production of insulin.

7. EFFECTS OF LIFESTYLE MODIFICATION ON PREVENTING OBESITY RELATED HCC

Obesity-related HCC mostly derives from NASH progression. It is of great importance that proper treatment for NASH may reduce the incidence of HCC. Weight loss by dietary intervention and physical exercise has been regarded as the first-line therapy for NASH.(177) A study performed by Suzuki et al examined the influence of lifestyle modification on improvement of serum alanine aminotransferase (ALT) levels in 348 patients with NAFLD after one or two years follow-up.(178) The results showed that weight loss (≥5%) with subsequent weight maintenance resulted in significant improvement and persistent normalization of serum ALT levels.(178) This relationship has also been concluded from a recent Korean cohort.(179) Hickman et al followed up 43 patients with chronic liver disease (including 10 subjects with clinically or histologically diagnosed NAFLD), of which 31 completed a 15 months diet and exercise intervention, to investigate the long-term effect of weight loss on liver biochemistry and serum insulin levels in obese patients with liver disease. They found that modest weight loss by an appropriate diet and exercise resulted in persistent improvements of serum ALT, insulin levels and quality of life.(180) Additionally, in another study by Hashizume et al, who observed 9 patients with HCC and one with intrahepatic cholangiocarcinoma in NASH. They found that none had HCC relapse after curative therapy, and that might be associated with weight loss by the active therapeutic intervention of nutritional care and physical exercise, indicating that weight loss may improve the prognosis of HCC in NASH.(181) Therefore, lifestyle modifications, mainly gradual weight loss, a low calorie diet, and aerobic exercise, are necessary to prevent the progression of NASH-related HCC.

8. CONCLUSIONS

Numerous studies have showed a strong association of obesity with increased risk of HCC. The increased prevalence of obesity in our society and increased incidence of HCC certainly warrant rigorous investigations on several critical questions: how obesity is causally linked to HCC? What intervention can be employed to reduce the fat toxicity on liver cells during obesity? Can HCC be prevented through dietary or other life style modification in obese individuals? Despite numerous studies being conducted over the last few decades, the molecular mechanisms underlying obesity-related HCC still remain to be fully elucidated. Only after a better understanding of the molecular mechanisms involved in the obesity-related carcinogenesis is fully clarified that more effective preventive or therapeutic approaches for HCC may be developed.

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**Abbreviations:** BMI: body mass index; HCC: hepatocellular carcinoma; IGF: insulin-like growth factor; IGFBP: insulin-like growth factor binding protein; LSC: liver stem cell; MAPK: mitogen-activated protein kinase; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; ROS: reactive oxygen species

**Key Words:** Hepatocellular carcinoma, Obesity, Nonalcoholic steatohepatitis, Insulin-like growth factors, Adipokine, Review

**Send correspondence to:** Cai-Yan Zhao, Department of Infectious Disease, The Third Hospital of Hebei Medical University, 139 Ziqiang Road, Shijiazhuang 050051, China, Tel: 86-311-88602150, Fax: 86-311-87023626, E-mail: zhaocy2005@163.com