[Frontiers in Bioscience 17, 2356-2370, June 1, 2012]

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

Chuan Shen[1], Cai-Yan Zhao[1], Rongxin Zhang[2], Liang Qiao[3]

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

TABLE OF CONTENTS

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/m^2, 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

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/m^2) 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/m^2) 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/m^2) than subjects with normal weight (BMI 18.5-24.9 kg/m^2).(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/m^2) 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)

Apparently, increased epidemic of obesity in general population may increase the proportion of patients with both NASH and cryptogenic cirrhosis and may thus be responsible for higher incidence of HCC.

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 via 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-I/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.

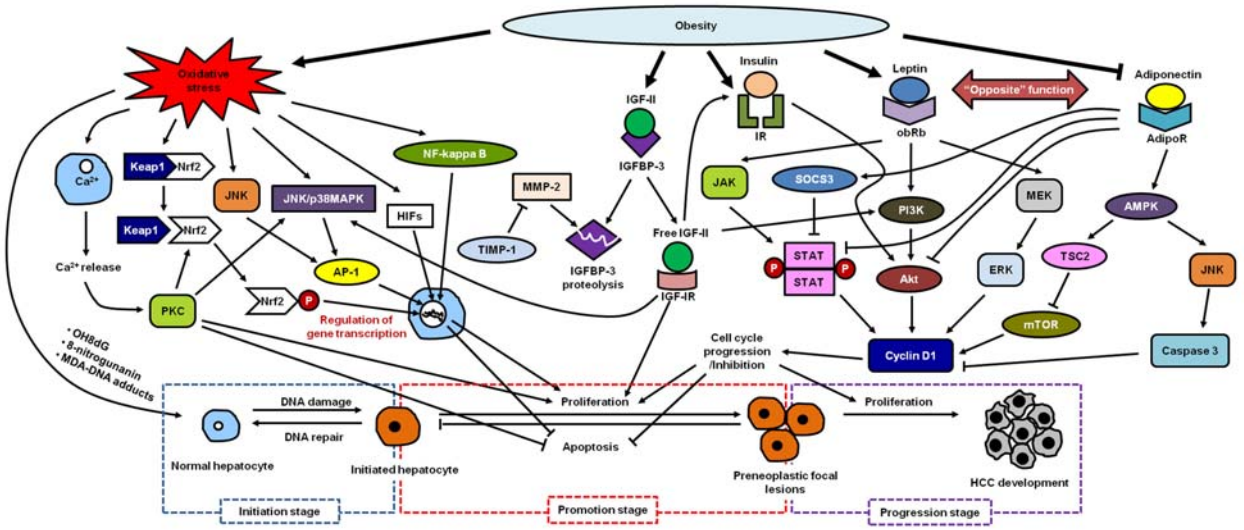

Figure 1. Possible molecular mechanisms in obesity-related HCC. Obesity leads to enhanced oxidative stress, increased insulin/insulin-like growth factor (IGF-II) production and aberrant adipokines secretion. The products of oxidative stress such as 8-hydroxydeoxy guanosine (OH8dG), 8-nitrogunanin and malondiadlehyde (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 Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3), PI3K/Akt, and extracellular signal-regulated kinase (ERK) signaling pathways. 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.(50) Mice with obesity and T2DM are often associated with IR, and a higher incidence of HCC in these mice has been reported.(51) 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.(52-53)

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.(54-56) 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,(57-59) or the hypomethylation of IGF-II gene promoter P3 or P4.(60-61) 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.(62) 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(63-64) or antisense oligodeoxynucleotides(65) 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),(66) 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 an increased bioavailability of IGFs. In particular, 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-I 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, proteolysis 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) Binding of leptin to its receptor triggers a cascade of signal events through activation of different signaling pathways, including cell proliferation, cell survival, and differentiation. However, not all receptors are functional in the leptin signaling. Among the six isoforms of leptin receptor (obRa-f) that have been identified so far, only the obRb isoform with long intracellular domain mediates intracellular signals.(88)

Many *in vitro* studies have reported that leptin can regulate HCC growth by influencing cell proliferation and apoptosis. Leptin increases proliferation of human HCC cells through concomitant activation of Janus kinase (JAK)/signal transducer and activator of transcription (STAT), PI3K/Akt, and extracellular signal-regulated kinase (ERK) signaling pathways.(89) On the other hand, inhibition of these pathways effectively blocks leptin-mediated invasion and migration of HCC cells.(89) The pro-proliferative role of leptin may be through its acceleration on the cyclin D1-mediated cell cycle progression in human HCC cells, as inhibitors specific for JAK2, PI3K/Akt, and mitogen-activated protein kinase kinase (MEK)/ERK1/2 can block this progression.(90) In addition to the above mechanisms, the proliferative effect of leptin was reported to be related to promotion of DNA synthesis and enhancement of mitotic activity, leading to increased HCC cells progressing to G2-M phase of the cell cycle.(91) Apart from its pro-proliferation effect, leptin was shown to prevent HCC cells from transforming growth factor-beta (TGF-beta) 1 induced apoptosis, likely through attenuating the effect of pro-apoptotic gene Bax.(90)

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 isoforms: trimers, hexamers, and larger multimers (also known as high molecular weight complex).(97) Multimeric isoform appears to be the most active one that is responsible for many of the physiological functions of adiponectin.(98-99) Three adiponectin receptor isoforms 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

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 NH_2-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),(124) 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-nitrogunanin 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 malondiadlehyde (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.(129) (2) ROS can react with and change the conformation of Kelch ECH associating protein 1 (Keap 1), resulting the disruption of the interaction between Keap1 and nuclear factor erythroid 2-related factor 2 (Nrf2), and liberation of Nrf2.(130) Active PKC also causes phosphorylation of Nrf2.(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, nitroxidative response, and proteome maintenance.(132) All these would favor cancer cell survival.(133) (3) ROS may activate the transcription factor activator protein 1 (AP-1) through reacting with cysteine64 or up-regulating PKC.(134) Activation of AP-1 has been reported to increase cell proliferation via up-regulating cyclin D1(135) or suppressing p21.(136) (4) ROS is a potent activator for nuclear factor kappa B (NF-kappa B),(137) a critical transcription factor involved in the regulation of cell survival, differentiation, inflammation, and growth.(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.(138) (5) ROS may stimulate the production of and activate hypoxia-inducible factors (HIFs),(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.(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.(141-143) In addition, small hepatocytes,(144) marrow stem cells,(145) and embryonic stem cells(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.(147) It has been shown that the oval cells distributed inside and outside the HCC nodes in rodent livers.(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.(150-153) Furthermore, depletion of tumor suppressor gene p53 leaded to generation of immortalized and tumorigenic oval cells, and injection of these cells into athymic nude mice cause xenograft HCC.(154) On the other hand, inactivation of oncogene myc resulted in HCC cells differentiating into hepatocytes and biliary cells.(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.(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.(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.(157-158) Furthermore, it is also speculated that some mutagenic reactive aldehyde products of lipid peroxidation such as 4-hydroxynonenal aldehyde and MDA potentiate oval/progenitor cells towards neoplastic differentiation possibly by p53 gene mutation.(162) Obese individuals show increased production of several inflammatory cytokines such as TNF-alpha and IL-6,(163-164) which could stimulate proliferation and transformation of oval cells that may give rise to HCC.(165-166)

Interestingly, although peroxisome proliferator-activated receptors (PPARs) usually provide potential benefits for obesity and NAFLD,(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.(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,(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.(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.(174) Recently, the liver oval cells have also been shown to differentiate into pancreatic endocrine hormone-producing cells,(175-176) and these cells can synthesize and secrete insulin in response to glucose stimulation.(176) Future studies are required to clarify whether oval cell-derived insulin-producing cells in obesity-related disorders may

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 been also 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.

9. ACKNOWLEDDGEMENT

Dr. R Zhang is supported by Major Research Program of National Natural Science Foundation of China (Grant ID: 91029705). Dr. L Qiao is supported by the Robert W. Storr Bequest to the Sydney Medical School Foundation and Career Development and Support Fellowship Future Research Leader Grant of the Cancer Institute NSW (Grant ID: 08/FRL/1-04). The authors declare no conflicts of interests.

11. REFERENCES

1.http://www.who.int/mediacentre/factsheets/fs311/en/index.html.

2. C. M. Kitahara, E. A. Platz, L. E. Freeman, A. W. Hsing, M. S. Linet, Y. Park, C. Schairer, A. Schatzkin, J. M. Shikany and A. Berrington de Gonzalez: Obesity and thyroid cancer risk among U.S. men and women: a pooled analysis of five prospective studies. *Cancer Epidemiol Biomarkers Prev* 20, 464-472 (2011)

3. T. Mijovic, J. How and R. J. Payne: Obesity and thyroid cancer. *Front Biosci (Schol Ed)* 3, 555-564 (2011)

4. G. D. Batty, M. J. Shipley, R. J. Jarrett, E. Breeze, M. G. Marmot and G. D. Smith: Obesity and overweight in relation to organ-specific cancer mortality in London (UK): findings from the original Whitehall study. *Int J Obes (Lond)* 29, 1267-1274 (2005)

5. E. E. Calle, C. Rodriguez, K. Walker-Thurmond and M. J. Thun: Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 348, 1625-1638 (2003)

6. Research WCRFAIfC. Food, nutrition, physical activity, and the prevention of cancer: a global perspective, AICR, Washington DC (2007)

7. J. Polesel, A. Zucchetto, M. Montella, L. Dal Maso, A. Crispo, C. La Vecchia, D. Serraino, S. Franceschi and R. Talamini: The impact of obesity and diabetes mellitus on the risk of hepatocellular carcinoma. *Ann Oncol* 20, 353-357 (2009)

8. K. Rapp, J. Schroeder, J. Klenk, S. Stoehr, H. Ulmer, H. Concin, G. Diem, W. Oberaigner and S. K. Weiland: Obesity and incidence of cancer: a large cohort study of over 145,000 adults in Austria. *Br J Cancer* 93, 1062-1067 (2005)

9. S. Y. Pan, K. C. Johnson, A. M. Ugnat, S. W. Wen and Y. Mao: Association of obesity and cancer risk in Canada. *Am J Epidemiol* 159, 259-268 (2004)

10. C. Samanic, W. H. Chow, G. Gridley, B. Jarvholm and J. F. Fraumeni Jr: Relation of body mass index to cancer risk in 362,552 Swedish men. *Cancer Causes Control* 17, 901-909 (2006)

11. A. G. Renehan, M. Tyson, M. Egger, R. F. Heller and M. Zwahlen: Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet* 371, 569-578 (2008)

12. J. A. Thomas 2nd and S. J. Freedland: Obesity and prostate cancer: collateral damage in the battle of the bulge. *Front Biosci (Schol Ed)* 3, 594-605 (2011)

13. G. Paz-Filho, E. L. Lim, M. L. Wong and J. Licinio: Associations between adipokines and obesity-related cancer. *Front Biosci* 16, 1634-1650 (2011)

14. X. F. Huang and J. Z. Chen: Obesity, the PI3K/Akt signal pathway and colon cancer. *Obes Rev* 10, 610-616 (2009)

15. J. M. Howard, G. P. Pidgeon and J. V. Reynolds: Leptin and gastro-intestinal malignancies. *Obes Rev* 11, 863-874 (2010)

16. T. Gislette and J. Chen: The possible role of IL-17 in obesity-associated cancer. *ScientificWorldJournal* 10, 2265-2271 (2010)

17. J. Chen, A. Katsifis, C. Hu and X. F. Huang: Insulin decreases therapeutic efficacy in colon cancer cell line HT29 via the activation of the PI3K/Akt pathway. *Curr Drug Discov Technol* 8, 119-125 (2011)

18.http://globocan.iarc.fr/factsheets/populations/factsheet.asp?uno=900.

19. S. L. Ye: Expert consensus on standardization of the management of primary liver cancer. *Zhonghua Gan Zang Bing Za Zhi* 17, 403-410 (2009)

20. S. F. Altekruse, 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* 27, 1485-1491 (2009)

21. M. G. Law, S. K. Roberts, G. J. Dore and J. M. Kaldor: Primary hepatocellular carcinoma in Australia, 1978-1997: increasing incidence and mortality. *Med J Aust* 173, 403-405 (2000)

22. A. M. Benhamiche, C. Faivre, A. Minello, F. Clinard, E. Mitry, P. Hillon and J. Faivre: Time trends and age-period-cohort effects on the incidence of primary liver cancer in a well-defined French population: 1976-1995. *J Hepatol* 29, 802-806 (1998)

23. S. D. Taylor-Robinson, G. R. Foster, S. Arora, S. Hargreaves and H. C. Thomas: Increase in primary liver cancer in the UK, 1979-94. *Lancet* 350, 1142-1143 (1997)

24. S. H. Caldwell, D. H. Oelsner, J. C. Iezzoni, E. E. Hespenheide, E. H. Battle and C. J. Driscoll. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. *Hepatology* 29, 664-669 (1999)

25. C. L. Chen, H. I. Yang, W. S. Yang, C. J. Liu, P. J. Chen, S. L. You, L. Y. Wang, C. A. Sun, S. N. Lu, D. S. Chen and C. J. Chen: Metabolic factors and risk of hepatocellular carcinoma by chronic hepatitis B/C infection: a follow-up study in Taiwan. *Gastroenterology* 135, 111-121 (2008)

26. V. Ratziu, L. Bonyhay, V. Di Martino, F. Charlotte, L. Cavallaro, M. H. Sayegh-Tainturier, P. Giral, A. Grimaldi, P. Opolon and T. Poynard: Survival, liver failure, and hepatocellular carcinoma in obesity-related cryptogenic cirrhosis. *Hepatology* 35, 1485-1493 (2002)

27. E. Bugianesi, N. Leone, E. Vanni, G. Marchesini, F. Brunello, P. Carucci, A. Musso, P. De Paolis, L. Capussotti, M. Salizzoni and M. Rizzetto: Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 123, 134-140 (2002)

28. S. Nair, A. Mason, J. Eason, G. Loss and R. P. Perrillo: Is obesity an independent risk factor for hepatocellular carcinoma in cirrhosis? *Hepatology* 36, 150-155 (2002)

29. J. A. Marrero, R. J. Fontana, G. L. Su, H. S. Conjeevaram, D. M. Emick and A. S. Lok: NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. *Hepatology* 36, 1349-1354 (2002)

30. J. M. Hui, J. G. Kench, S. Chitturi, A. Sud, G. C. Farrell, K. Byth, P. Hall, M. Khan and J. George: Long-term outcomes of cirrhosis in nonalcoholic steatohepatitis compared with hepatitis C. *Hepatology* 38, 420-427 (2003)

31. E. Bugianesi: Non-alcoholic steatohepatitis and cancer. *Clin Liver Dis* 11, 191-207, x-xi (2007)

32. K. Yasui, E. Hashimoto, Y. Komorizono, K. Koike, S. Arii, Y. Imai, T. Shima, Y. Kanbara, T. Saibara, T. Mori, S. Kawata, H. Uto, S. Takami, Y. Sumida, T. Takamura, M. Kawanaka and T. Okanoue: Characteristics of patients with nonalcoholic steatohepatitis who develop hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 9, 428-433; quiz e50 (2011)

33. M. S. Ascha, I. A. Hanouneh, R. Lopez, T. A. Tamimi, A. F. Feldstein and N. N. Zein: The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology* 51, 1972-1978 (2010)

34. E. Hashimoto, S. Yatsuji, M. Tobari, M. Taniai, N. Torii, K. Tokushige and K. Shiratori: Hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *J Gastroenterol* 44 Suppl 19, 89-95 (2009)

35. A. E. Hill-Baskin, M. M. Markiewski, D. A. Buchner, H. Shao, D. DeSantis, G. Hsiao, S. Subramaniam, N. A. Berger, C. Croniger, J. D. Lambris and J. H. Nadeau: Diet-induced hepatocellular carcinoma in genetically predisposed mice. *Hum Mol Genet* 18, 2975-2988 (2009)

36. S. Watanabe, Y. Horie and A. Suzuki: Hepatocyte-specific Pten-deficient mice as a novel model for

nonalcoholic steatohepatitis and hepatocellular carcinoma. *Hepatol Res* 33, 161-166 (2005)

37. B. A. Neuschwander-Tetri and S. H. Caldwell: Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 37, 1202-1219 (2003)

38. L. A. Adams, J. F. Lymp, J. St Sauver, S. O. Sanderson, K. D. Lindor, A. Feldstein and P. Angulo: The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 129, 113-121 (2005)

39. M. Ekstedt, L. E. Franzen, U. L. Mathiesen, L. Thorelius, M. Holmgvist, G. Bodemar and S. Kechagias: Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 44, 865-873 (2006)

40. A. Denda, W. Kitayama, H. Kishida, N. Murata, M. Tsutsumi, T. Tsujiuchi, D. Nakae and Y. Konishi: Development of hepatocellular adenomas and carcinomas associated with fibrosis in C57BL/6J male mice given a choline-deficient, L-amino acid-defined diet. *Jpn J Cancer Res* 93, 125-132 (2002)

41. I. Ueki, G. T. Ooi, M. L. Tremblay, K. R. Hurst, L. A. Bach and Y. R. Boisclair: Inactivation of the acid labile subunit gene in mice results in mild retardation of postnatal growth despite profound disruptions in the circulating insulin-like growth factor system. *Proc Natl Acad Sci U S A* 97, 6868-6873 (2000)

42. R. T. Kurmasheva and P. J. Houghton: IGF-I mediated survival pathways in normal and malignant cells. *Biochim Biophys Acta* 1766, 1-22 (2006)

43. A. Denley, G. V. Brierley, J. M. Carroll, A. Lindenberg, G. W. Booker, L. J. Cosgrove, J. C. Wallace, B. E. Forbes and C. T. Roberts Jr: Differential activation of insulin receptor isoforms by insulin-like growth factors is determined by the C domain. *Endocrinology* 147, 1029-1036 (2006)

44. C. Duan: Specifying the cellular responses to IGF signals: roles of IGF-binding proteins. *J Endocrinol* 175, 41-54 (2002)

45. J. U. Weaver, J. M. Holly, P. G. Kopelman, K. Noonan, C. G. Giadom, N. White, S. Virdee and J. A. Wass: Decreased sex hormone binding globulin (SHBG) and insulin-like growth factor binding protein (IGFBP-1) in extreme obesity. *Clin Endocrinol (Oxf)* 33, 415-422 (1990)

46. S. Y. Nam, E. J. Lee, K. R. Kim, B. S. Cha, Y. D. Song, S. K. Lim, H. C. Lee and K. B. Huh: Effect of obesity on total and free insulin-like growth factor (IGF)-1, and their relationship to IGF-binding protein (BP)-1, IGFBP-2, IGFBP-3, insulin, and growth hormone. *Int J Obes Relat Metab Disord* 21, 355-359 (1997)

47. N. Attia, W. V. Tamborlane, R. Heptulla, D. Maggs, A. Grozman, R. S. Sherwin and S. Caprio: The metabolic syndrome and insulin-like growth factor I regulation in adolescent obesity. *J Clin Endocrinol Metab* 83, 1467-1471 (1998)

48. J. Frystyk, D. J. Brick, A. V. Gerweck, A. L. Utz and K. K. Miller: Bioactive insulin-like growth factor-I in obesity. *J Clin Endocrinol Metab* 94, 3093-3097 (2009)

49. A. Lukanova, S. Soderberg, P. Stattin, R. Palmgvist, E. Lundin, C. Biessy, S. Rinaldi, E. Riboli, G. Hallmans and R. Kaaks: Nonlinear relationship of insulin-like growth factor (IGF)-I and IGF-I/IGF-binding protein-3 ratio with indices of adiposity and plasma insulin concentrations (Sweden). *Cancer Causes Control* 13, 509-516 (2002)

50. S. Miuma, T. Ichikawa, N. Taura, H. Shibata, S. Takeshita, M. Akiyama, Y. Motoyoshi, E. Ozawa, M. Fujimoto, H. Kawashimo, H. Miyaaki, K. Equchi and K. Nakao: The level of fasting serum insulin, but not adiponectin, is associated with the prognosis of early stage hepatocellular carcinoma. *Oncol Rep* 22, 1415-1424 (2009)

51. L. B. Lemke, A. B. Rogers, P. R. Nambiar and J. G. Fox: Obesity and non-insulin-dependent diabetes mellitus in Swiss-Webster mice associated with late-onset hepatocellular carcinoma. *J Endocrinol* 199, 21-32 (2008)

52. I. Wanke, M. Schwarz and A. Buchmann: Insulin and dexamethasone inhibit TGF-beta-induced apoptosis of hepatoma cells upstream of the caspase activation cascade. *Toxicology* 204, 141-154 (2004)

53. H. L. Qi, Y. Zhang, J. Ma, P. Guo, X. Y. Zhang and H. L. Chen: Insulin/protein kinase B signalling pathway upregulates metastasis-related phenotypes and molecules in H7721 human hepatocarcinoma cell line. *Eur J Biochem* 270, 3795-3805 (2003)

54. E. Cariani, D. Seurin, C. Lasserre, D. Franco, M. Binoux and C. Brechot: Expression of insulin-like growth factor II (IGF-II) in human primary liver cancer: mRNA and protein analysis. *J Hepatol* 11, 226-231 (1990)

55. T. Nussbaum, J. Samarin, V. Ehemann, M. Bissinger, E. Ryschich, A. Khamidjanov, X. Yu, N. Gretz, P. Schirmacher and K. Breuhahn: Autocrine insulin-like growth factor-II stimulation of tumor cell migration is a progression step in human hepatocarcinogenesis. *Hepatology* 48, 146-156 (2008)

56. H. M. El Tayebi, W. Salah, I. H. El Sayed, E. M. Salam, A. R. Zekri, N. Zayed, E. S. Salem, G. Esmat and A. I. Abdelaziz: Expression of insulin-like growth factor-II, matrix metalloproteinases, and their tissue inhibitors as predictive markers in the peripheral blood of HCC patients. *Biomarkers* 16, 346-354 (2011)

57. K. Uchida, M. Kondo, S. Takeda, H. Osada, T. Takahashi, A. Nakao and T. Takahashi: Altered transcriptional regulation of the insulin-like growth factor 2 gene in human hepatocellular carcinoma. *Mol Carcinog* 18, 193-198 (1997)

58. T. Sohda, K. Yun, K. Iwata, H. Soejima and M. Okumura: Increased expression of insulin-like growth factor 2 in hepatocellular carcinoma is primarily regulated at the transcriptional level. *Lab Invest* 75, 307-311 (1996)

59. X. Li, Z. Nong, C. Ekstrom, E. Larsson, H. Nordlinder, W. J. Hofmann, C. Trautwein, M. Odenthal, H. P. Dienes, T. J. Ekstrom and P. Schirmacher: Disrupted IGF2 promoter control by silencing of promoter P1 in human hepatocellular carcinoma. *Cancer Res* 57, 2048-2054 (1997)

60. S. H. Tang, D. H. Yang, W. Huang, H. K. Zhou, X. H. Lu and G. Ye: Hypomethylated P4 promoter induces expression of the insulin-like growth factor-II gene in hepatocellular carcinoma in a Chinese population. *Clin Cancer Res* 12, 4171-4177 (2006)

61. Y. Li, G. Meng, L. Huang and Q. N. Guo: Hypomethylation of the P3 promoter is associated with up-regulation of IGF2 expression in human osteosarcoma. *Hum Pathol* 40, 1441-1447 (2009)

62. T. Aihara, S. Noguchi, Y. Miyoshi, H. Nakano, Y. Sasaki, Y. Nakamura, M. Monden and S. Imaoka: Allelic imbalance of insulin-like growth factor II gene expression in cancerous and precancerous lesions of the liver. *Hepatology* 28, 86-89 (1998)

63. H. Huynh, P. K. Chow, L. L. Ooi and K. C. Soo: A possible role for insulin-like growth factor-binding protein-3 autocrine/paracrine loops in controlling hepatocellular carcinoma cell proliferation. *Cell Growth Differ* 13, 115-122 (2002)

64. D. T. Dransfield, E. H. Cohen, Q. Chang, L. G. Sparrow, J. D. Bentley, O. Dolezal, X. Xiao, T. S. Peat, J. Newman, P. A. Pilling, T. Phan, I. Priebe, G. V. Brierley, N. Kastrapeli, K. Kopacz, D. Martik, D. Wassaf, D. Rank, G. Conley, Y. Huang, T. E. Adams and L. Cosgrove: A human monoclonal antibody against insulin-like growth factor-II blocks the growth of human hepatocellular carcinoma cell lines in vitro and in vivo. *Mol Cancer Ther* 9, 1809-1819 (2010)

65. S. B. Lin, S. H. Hsieh, H. L. Hsu, M. Y. Lai, L. S. Kan and L. C. Au: Antisense oligodeoxynucleotides of IGF-II selectively inhibit growth of human hepatoma cells overproducing IGF-II. *J Biochem* 122, 717-722 (1997)

66. K. W. Kim, S. K. Bae, O. H. Lee, M. H. Bae, M. J. Lee and B. C. Park: Insulin-like growth factor II induced by hypoxia may contribute to angiogenesis of human hepatocellular carcinoma. *Cancer Res* 58, 348-351 (1998)

67. S. K. Bae, M. H. Bae, M. Y. Ahn, M. J. Son, Y. M. Lee, M. K. Bae, O. H. Lee, B. C. Park and K. W. Kim: Egr-1 mediates transcriptional activation of IGF-II gene in response to hypoxia. *Cancer Res* 59, 5989-5994 (1999)

68. B. C. Song, Y. H. Chung, J. A. Kim, H. C. Lee, H. K. Yoon, K. B. Sung, S. H. Yang, K. Yoo, Y. S. Lee and D. J. Suh: Association between insulin-like growth factor-2 and metastases after transcatheter arterial chemoembolization in patients with hepatocellular carcinoma: a prospective study. *Cancer* 91, 2386-2393 (2001)

69. Y. Gong, L. Cui and G. Y. Minuk: The expression of insulin-like growth factor binding proteins in human hepatocellular carcinoma. *Mol Cell Biochem* 207, 101-104 (2000)

70. T. Hanafusa, Y. Yumoto, K. Nouso, H. Nakatsukasa, T. Onishi, T. Fujikawa, M. Taniyama, S. Nakamura, M. Uemura, Y. Takuma, E. Yumoto, T. Higashi and T. Tsuji: Reduced expression of insulin-like growth factor binding protein-3 and its promoter hypermethylation in human hepatocellular carcinoma. *Cancer Lett* 176, 149-158 (2002)

71. S. M. Luo, W. M. Tan, W. X. Deng, S. M. Zhuang and J. W. Luo: Expression of albumin, IGF-1, IGFBP-3 in tumor tissues and adjacent non-tumor tissues of hepatocellular carcinoma patients with cirrhosis. *World J Gastroenterol* 11, 4272-4276 (2005)

72. K. Subramaniam, L. L. Ooi and K. M. Hui: Transcriptional down-regulation of IGFBP-3 in human hepatocellular carcinoma cells is mediated by the binding of TIA-1 to its AT-rich element in the 3'-untranslated region. *Cancer Lett* 297, 259-268 (2010)

73. S. Aishima, Y. Basaki, Y. Oda, Y. Kuroda, Y. Nishihara, K. Taguchi, A. Taketomi, Y. Maehara, F. Mosoi, Y. Maruyama, A. Fotovati, S. Oie, M. Ono, T. Ueno, M. Sata, H. Yano, M. Kojiro, M. Kuwano and M. Tsuneyoshi: High expression of insulin-like growth factor binding protein-3 is correlated with lower portal invasion and better prognosis in human hepatocellular carcinoma. *Cancer Sci* 97, 1182-1190 (2006)

74. Y. W. Chen, V. Boyartchuk and B. C. Lewis: Differential roles of insulin-like growth factor receptor- and insulin receptor-mediated signaling in the phenotypes of hepatocellular carcinoma cells. *Neoplasia* 11, 835-845 (2009)

75. D. C. Martin, J. L. Fowlkes, B. Babic and R. Khokha: Insulin-like growth factor II signaling in neoplastic proliferation is blocked by transgenic expression of the metalloproteinase inhibitor TIMP-1. *J Cell Biol* 146, 881-892 (1999)

76. J. F. Caro, J. Poulos, O. Ittoop, W. J. Pories, E. G. Flickinger and M. K. Sinha: Insulin-like growth factor I binding in hepatocytes from human liver, human hepatoma, and normal, regenerating, and fetal rat liver. *J Clin Invest* 81, 976-981 (1988)

77. X. Liu, W. Jiang, F. Aucejo, R. Kim, C. Miller, M. Byrne, R. Lopez and L. Yerian: Insulin-like growth factor I receptor beta expression in hepatocellular carcinoma. *Hum Pathol* 42, 882-891 (2011)

78. C. Desbois-Mouthon, A. Baron, M. J. Blivet-Van Eggelpoel, L. Fartoux, C. Venot, F. Bladt, C. Housset and O.

Rosmorduc: Insulin-like growth factor-1 receptor inhibition induces a resistance mechanism via the epidermal growth factor receptor/HER3/AKT signaling pathway: rational basis for cotargeting insulin-like growth factor-1 receptor and epidermal growth factor receptor in hepatocellular carcinoma. *Clin Cancer Res* 15, 5445-5456 (2009)

79. M. C. Cantarini, S. M. de la Monte, M. Pang, M. Tong, A. D'Errico, F. Trevisani and J. R. Wands: Aspartyl-asparagyl beta hydroxylase over-expression in human hepatoma is linked to activation of insulin-like growth factor and notch signaling mechanisms. *Hepatology* 44, 446-457 (2006)

80. R. X. Lin, Z. Y. Wang, N. Zhang, C. W. Tuo, Q. D. Liang, Y. N. Sun and S. Q. Wang: Inhibition of hepatocellular carcinoma growth by antisense oligonucleotides to type I insulin-like growth factor receptor in vitro and in an orthotopic model. *Hepatol Res* 37, 366-375 (2007)

81. Y. Zhang, R. Proenca, M. Maffei, M. Barone, L. Leopold and J. M. Friedman: Positional cloning of the mouse obese gene and its human homologue. *Nature* 372, 425-432 (1994)

82. A. M. Brennan and C. S. Mantzoros: Drug Insight: the role of leptin in human physiology and pathophysiology--emerging clinical applications. *Nat Clin Pract Endocrinol Metab* 2, 318-327 (2006)

83. B. D. Bennett, G. P. Solar, J. Q. Yuan, J. Mathias, G. R. Thomas and W. Matthews: A role for leptin and its cognate receptor in hematopoiesis. *Curr Biol* 6, 1170-1180 (1996)

84. G. Matarese, S. Moschos and C. S. Mantzoros: Leptin in immunology. *J Immunol* 174, 3137-3142 (2005)

85. D. Fan, Z. Chen, Y. Chen and Y. Shang: Mechanistic roles of leptin in osteogenic stimulation in thoracic ligament flavum cells. *J Biol Chem* 282, 29958-29966 (2007)

86. M. Artwohl, M. Roden, T. Holzenbein, A. Freudenthaler, W. Waldhausl and S. M. Baumgartner-Parzer: Modulation by leptin of proliferation and apoptosis in vascular endothelial cells. *Int J Obes Relat Metab Disord* 26, 577-580 (2002)

87. S. Anagnostoulis, A. J. Karayiannakis, M. Lambropoulou, A. Efthimiadou, A. Polychronidis and C. Simopoulos: Human leptin induces angiogenesis in vivo. *Cytokine* 42, 353-357 (2008)

88. D. Cirillo, A. M. Rachiglio, R. la Montagna, A. Giordano and N. Normanno: Leptin signaling in breast cancer: an overview. *J Cell Biochem* 105, 956-964 (2008)

89. N. K. Saxena, D. Sharma, X. Ding, S. Lin, F. Marra, D. Merlin and F. A. Anania: Concomitant activation of the JAK/STAT, PI3K/AKT, and ERK signaling is involved in leptin-mediated promotion of invasion and migration of hepatocellular carcinoma cells. *Cancer Res* 67, 2497-2507 (2007)

90. C. Chen, Y. C. Chang, C. L. Liu, T. P. Liu, K. J. Chang and I. C. Guo: Leptin induces proliferation and anti-apoptosis in human hepatocarcinoma cells by up-regulating cyclin D1 and down-regulating Bax via a Janus kinase 2-linked pathway. *Endocr Relat Cancer* 14, 513-529 (2007)

91. J. Zhou, W. Lei, L. Shen, H. S. Luo and Z. X. Shen: Primary study of leptin and human hepatocellular carcinoma in vitro. *World J Gastroenterol* 14, 2900-2904 (2008)

92. S. N. Wang, Y. T. Yeh, S. F. Yang, C. Y. Chai and K. T. Lee: Potential role of leptin expression in hepatocellular carcinoma. *J Clin Pathol* 59, 930-934 (2006)

93. D. Ribatti, A. S. Belloni, B. Nico, M. Di Comite, E. Crivellato and A. Vacca: Leptin-leptin receptor are involved in angiogenesis in human hepatocellular carcinoma. *Peptides* 29, 1596-1602 (2008)

94. M. Kitade, H. Yoshiji, H. Kojima, Y. Ikenaka, R. Noguchi, K. Kaji, J. Yoshii, K. Yanase, T. Namisaki, K. Asada, M. Yamazaki, T. Tsujimoto, T. Akahane, M. Uemura and H. Fukui: Leptin-mediated neovascularization is a prerequisite for progression of nonalcoholic steatohepatitis in rats. *Hepatology* 44, 983-991 (2006)

95. J. R. Jonsson, A. R. Moschen, I. J. Hickman, M. M. Richardson, S. Kaser, A. D. Clouston, E. E. Powell and H. Tilg: Adiponectin and its receptors in patients with chronic hepatitis C. *J Hepatol* 43, 929-936 (2005)

96. A. M. Wolf, D. Wolf, M. A. Avila, A. R. Moschen, C. Berasain, B. Enrich, H. Rumpold and H. Tilg: Up-regulation of the anti-inflammatory adipokine adiponectin in acute liver failure in mice. *J Hepatol* 44, 537-543 (2006)

97. U. B. Pajvani, X. Du, T. P. Combs, A. H. Berg, M. W. Rajala, T. Schulthess, J. Engel, M. Brownlee and P. E. Scherer: Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications fpr metabolic regulation and bioactivity. *J Biol Chem* 278, 9073-9085 (2003)

98. H. Waki, T. Yamauchi, J. Kamon, Y. Ito, S. Uchida, S. Kita, K. Hara, Y. Hada, F. Vasseur, P. Froguel, S. Kimura, R. Nagai and T. Kadowaki: Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. *J Biol Chem* 278, 40352-40363 (2003)

99. Y. Liu, R. Retnakaran, A. Hanley, R. Tungtrongchitr, C. Shaw and G. Sweeney: Total and high molecular weight but not trimeric or hexameric forms of adiponectin correlate with markers of the metabolic syndrome and liver injury in Thai subjects. *J Clin Endocrinol Metab* 92, 4313-4318 (2007)

100. T. Yamauchi, J. Kamon, Y. Ito, A. Tsuchida, T. Yokomizo, S. Kita, T. Sugiyama, M. Miyagishi, K. Hara, M. Tsunoda, K. Murakami, T. Ohteki, S. Uchida, S. Takekawa, H. Waki, N. H. Tsuno, Y. Shibata, Y. Terauchi, P. Froguel, K. Tobe, S. Koyasu, K. Taira, T. Kitamura, T.

Shimizu, R. Nagai and T. Kadowaki: Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 423, 762-769 (2003)

101. T. Kadowaki and T. Yamauchi: Adiponectin and adiponectin receptors. *Endocr Rev* 26, 439-451 (2005)

102. K. Hotta, T. Funahashi, Y. Arita, M. Takahashi, M. Matsuda, Y. Okamoto, H. Iwahashi, H. Kuriyama, N. Ouchi, K. Maeda, M. Nishida, S. Kihara, N. Sakai, T. Nakajima, K. Hasegawa, M. Muraguchi, Y. Ohmoto, T. Nakamura, S. Yamashita, T. Hanafusa and Y. Matsuzawa: Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 20, 1595-1599 (2000)

103. C. A. Skrabal, J. Czaja, K. Honz, R. Emini, A. Hannekum and R. Friedl: Adiponectin--its potential to predict and prevent coronary artery disease. *Thorac Cardiovasc Surg* 59, 201-206 (2011)

104. L. L. Jiang, L. Li, X. F. Hong, Y. M. Li and B. L. Zhang: Patients with nonalcoholic fatty liver disease display increased serum resistin levels and decreased adiponectin levels. *Eur J Gastroenterol Hepatol* 21, 662-666 (2009)

105. T. N. Spyridopoulos, E. T. Petridou, A. Skalkidou, N. Dessypris, G. P. Chrousos and C. S. Mantzoros: Low adiponectin levels are associated with renal cell carcinoma: a case-control study. *Int J Cancer* 120, 1573-1578 (2007)

106. L. Dal Maso, L. S. Augustin, A. Karalis, R. Talamini, S. Franceschi, D. Trichopoulos, C. S. Mantzoros and C. La Vecchia: Circulating adiponectin and endometrial cancer risk. *J Clin Endocrinol Metab* 89, 1160-1163 (2004)

107. E. Petridou, C. Mantzoros, N. Dessypris, P. Koukoulomatis, C. Addy, Z. Voulgaris, G. Chrousos and D. Trichopoulos: Plasma adiponectin concentrations in relation to endometrial cancer: a case-control study in Greece. *J Clin Endocrinol Metab* 88, 993-997 (2003)

108. S. S. Tworoger, A. H. Eliassen, T. Kelesidis, G. A. Colditz, W. C. Willett, C. S. Mantzoros and S. E. Hankinson: Plasma adiponectin concentrations and risk of incident breast cancer. *J Clin Endocrinol Metab* 92, 1510-1516 (2007)

109. E. K. Wei, E. Giovannucci, C. S. Fuchs, W. C. Willett and C. S. Mantzoros: Low plasma adiponectin levels and risk of colorectal cancer in men: a prospective study. *J Natl Cancer Inst* 97, 1688-1694 (2005)

110. K. Man, K. T. Ng, A. Xu, Q. Cheng, C. M. Lo, J. W. Xiao, B. S. Sun, Z. X. Lim, J. S. Cheung, E. X. Wu, C. K. Sun, R. T. Poon and S. T. Fan: Suppression of liver tumor growth and metastasis by adiponectin in nude mice through inhibition of tumor angiogenesis and downregulation of Rho kinase/IFN-inducible protein 10/matrix metalloproteinase 9 signaling. *Clin Cancer Res* 16, 967-977 (2010)

111. N. K. Saxena, P. P. Fu, A. Nagalingam, J. Wang, J. Handy, C. Cohen, M. Tighiouart, D. Sharma and F. A. Anania: Adiponectin modulates C-jun N-terminal kinase and mammalian target of rapamycin and inhibits hepatocellular carcinoma. *Gastroenterology* 139, 1762-1773, 73 e1-5 (2010)

112. Y. Kamada, H. Matsumoto, S. Tamura, J. Fukushima, S. Kiso, K. Fukui, T. Igura, N. Maeda, S. Kihara, T. Funahashi, Y. Matsuzawa, I. Shimomura and N. Hayashi: Hypoadiponectinemia accelerates hepatic tumor formation in a nonalcoholic steatohepatitis mouse model. *J Hepatol* 47, 556-564 (2007)

113. T. Miyazaki, J. D. Bub, M. Uzuki and Y. Iwamoto: Adiponectin activates c-Jun NH2-terminal kinase and inhibits signal transducer and activator of transcription 3. *Biochem Biophys Res Commun* 333, 79-87 (2005)

114. D. Sharma, J. Wang, P. P. Fu, S. Sharma, A. Nagalingam, J. Mells, J. Handy, A. J. Page, C. Cohen, F. A. Anania and N. K. Saxena: Adiponectin antagonizes the oncogenic actions of leptin in hepatocellular carcinogenesis. *Hepatology* 52, 1713-1722 (2010)

115. N. I. Khan, L. Naz and G. Yasmeen: Obesity: an independent risk factor for systemic oxidative stress. *Pak J Pharm Sci* 19, 62-65 (2006)

116. M. D'Archivio, G. Annuzzi, R. Vari, C. Filesi, R. Giacco, B. Scazzocchio, C. Santangelo, C. Giovannini, A. A. Rivellese and R. Masella: Predominant role of obesity/insulin resistance in oxidative stress development. *Eur J Clin Invest* (2011)

117. J. F. Turrens: Mitochondrial formation of reactive oxygen species. *J Physiol* 552, 335-444 (2003)

118. S. Ghosh, R. Tewari, D. Dixit and E. Sen: TNFalpha induced oxidative stress dependent Akt signaling affects actin cytoskeletal organization in glioma cells. *Neurochem Int* 56, 194-201 (2010)

119. O. El-Assal, F. Hong, W. H. Kim, S. Radaeva and B. Gao: IL-6-deficient mice are susceptible to ethanol-induced hepatic steatosis: IL-6 protects against ethanol-induced oxidative stress and mitochondrial permeability transition in the liver. *Cell Mol Immunol* 1, 205-211 (2004)

120. L. Tacchini, G. Cairo, C. De Ponti, M. Massip, J. Rosello-Catafau and C. Peralta: Up regulation of IL-6 by ischemic preconditioning in normal and fatty rat livers: association with reduction of oxidative stress. *Free Radic Res* 40, 1206-1217 (2006)

121. M. Mossalam, J. H. Jeong, E. D. Abel, S. W. Kim and Y. H. Kim: Reversal of oxidative stress in endothelial cells by controlled release of adiponectin. *J Control Release* 130, 234-237 (2008)

122. J. Fukushima, Y. Kamada, H. Matsumoto, Y. Yoshida, H. Ezaki, T. Takemura, Y. Saji, T. Igura, S. Tsutsui, S.

Kihara, T. Funahashi, I. Shimomura, S. Tamura, S. Kiso and N. Hayashi: Adiponectin prevents progression of steatohepatitis in mice by regulating oxidative stress and Kupffer cell phenotype polarization. *Hepatol Res* 39, 724-738 (2009)

123. V. Balasubramaniyan, J. Kalaivani Sailaja and N. Nalini: Role of leptin on alcohol-induced oxidative stress in Swiss mice. *Pharmacol Res* 47, 211-216 (2003)

124. H. Tanaka, N. Fujita, R. Sugimoto, N. Urawa, S. Horiike, Y. Kobayashi, M. Iwasa, N. Ma, S. Kawanishi, S. Watanabe, M. Kaito and Y. Takei: Hepatic oxidative DNA damage is associated with increased risk for hepatocellular carcinoma in chronic hepatitis C. *Br J Cancer* 98, 580-586 (2008)

125. K. C. Cheng, D. S. Cahill, H. Kasai, S. Nishimura S and L. A. Loeb: 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G----T and A----C substitutions. *J Biol Chem* 267, 166-172 (1992)

126. S. Kawanishi and Y. Hiraku: Oxidative and nitrative DNA damage as biomarker for carcinogenesis with special reference to inflammation. *Antioxid Redox Signal* 8, 1047-1058 (2006)

127. K. Stone, M. B. Ksebati and L. J. Marnett: Investigation of the adducts formed by reaction of malondialdehyde with adenosine. *Chem Res Toxicol* 3:33-38 (1990)

128. C. Ji, C. A. Rouzer, L. J. Marnett and J. A. Pietenpol: Induction of cell cycle arrest by the endogenous product of lipid peroxidation, malondialdehyde. *Carcinogenesis* 19, 1275-1283 (1998)

129. W. S. Wu: The signaling mechanism of ROS in tumor progression. *Cancer Metastasis Rev* 25, 695-705 (2006)

130. N. Wakabayashi, A. T. Dinkova-Kostova, W. D. Holtzclaw, M. I. Kang, A. Kobayashi, M. Yamamoto, T. W. Kensler and P. Talalay: Protection against electrophile and oxidant stress by induction of the phase 2 response: fate of cysteines of the Keap1 sensor modified by inducers. *Proc Natl Acad Sci U S A* 101, 2040-2045 (2004)

131. H. C. Huang, T. Nguyen and C. B. Pickett: Phosphorylation of Nrf2 at Ser-40 by protein kinase C regulates antioxidant response element-mediated transcription. *J Biol Chem* 277, 42769-42774 (2002)

132. T. W. Kensler, N. Wakabayashi and S. Biswal: Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu Rev Pharmacol Toxicol* 47, 89-116 (2007)

133. A. Singh, V. Misra, R. K. Thimmulappa, H. Lee, S. Ames, M. O. Hogue, J. G. Herman, S. B. Baylin, D. Sidransky, E. Gabrielson, M. V. Brock and S. Biswal: Dysfunctional KEAP1-NRF2 interaction in non-small-cell lung cancer. *PLoS Med* 3:e420 (2006)

134. L. Chang and M. Karin: Mammalian MAP kinase signalling cascades. *Nature* 410, 37-40 (2001)

135. J. R. Brown, E. Nigh, R. J. Lee, H. Ye, M. A. Thompson, F. Saudou, R. G. Pestell and M. E. Greenberg: Fos family members induce cell cycle entry by activating cyclin D1. *Mol Cell Biol* 18, 5609-5619 (1998)

136. L. Bakiri, D. Lallemand, E. Bossy-Wetzel and M. Yaniv: Cell cycle-dependent variations in c-Jun and JunB phosphorylation: a role in the control of cyclin D1 expression. *EMBO J* 19, 2056-2068 (2000)

137. C. Pantano, N. L. Reynaert, A. van der Vliet and Y. M. Janssen-Heininger: Redox-sensitive kinases of the nuclear factor-kappaB signaling pathway. *Antioxid Redox Signal* 8, 1791-1806 (2006)

138. G. Sethi, B. Sung and B. B. Aggarwal: Nuclear factor-kappaB activation: from bench to bedside. *Exp Biol Med (Maywood)* 233, 21-31 (2008)

139. T. Klimova and N. S. Chandel: Mitochondrial complex III regulates hypoxic activation of HIF. *Cell Death Differ* 15, 660-666 (2008)

140. J. Kano, T. Ishiyama, N. Nakamura, T. Iijima, Y. Morishita and M. Noguchi: Establishment of hepatic stem-like cell lines from normal adult porcine liver in a poly-D-lysine-coated dish with NAIR-1 medium. *In vitro Cell Dev Biol Anim* 39, 440-448 (2003)

141. T. A. Roskams, N. D. Theise, C. Balabaud, G. Bhagat, P. S. Bhathal, P. Bioulac-Sage, E. M. Brunt, J. M. Crawford, H. A. Crosby, V. Desmet, M. J. Finegold, S. A. Geller, A. S. Gouw, P. Hytiroglou, A. S. Knisely, M. Kojiro, J. H. Lefkowitch, Y. Nakanuma, J. K. Olynyk, Y. N. Park, B. Portmann, R. Saxena, P. J. Scheuer, A. J. Strain, S. N. Thung, I. R. Wanless and A. B. West: Nomenclature of the finer branches of the biliary tree: canals, ductules, and ductular reactions in human livers. *Hepatology* 39, 1739-1745 (2004)

142. C. Fougere-Deschatrette, T. Imaizumi-Scherrer, H. Strick-Marchand, S. Morosan, P. Charneau, D. Kremsdorf, D. M. Faust and M. C. Weiss: Plasticity of hepatic cell differentiation: bipotential adult mouse liver clonal cell lines competent to differentiate in vitro and in vivo. *Stem Cells* 24, 2098-2109 (2006)

143. M. B. Herrera, S. Bruno, S. Buttiglieri, C. Tetta, S. Gatti, M. C. Deregibus, B. Bussolati and G. Camussi: Isolation and characterization of a stem cell population from adult human liver. *Stem Cells* 24, 2840-2850 (2006)

144. C. Shibata, T. Mizuguchi, Y. Kikkawa, T. Nobuoka, H. Oshima, H. Kawasaki, M. Kawamoto, T. Katsuramaki, T. Mitaka and K. Hirata: Liver repopulation and long-term function of rat small hepatocyte transplantation as an alternative cell source for hepatocyte transplantation. *Liver Transpl* 12, 78-87 (2006)

145. F. C. Popp, P. Piso, H. J. Schlitt and M. H. Dahlke: Therapeutic potential of bone marrow stem cells for liver diseases. *Curr Stem Cell Res Ther* 1, 411-418 (2006)

146. J. Cai, Y. Zhao, Y. Liu, F. Ye, Z. Song, H. Qin, S. Meng, Y. Chen, R. Zhou, X. Song, Y. Guo, M. Ding and H. Deng: Directed differentiation of human embryonic stem cells into functional hepatic cells. *Hepatology* 45, 1229-1239 (2007)

147. S. Sell: Cellular origin of cancer: dedifferentiation or stem cell maturation arrest? *Environ Health Perspect* 101 Suppl 5, 15-26 (1993)

148. C. H. Fang, G. Q. Zhang, X. Y. Zhu and J. Q. Gong: Distribution of oval cells and c-myc mRNA expression in mouse hepatocarcinogenesis. *Hepatobiliary Pancreat Dis Int* 3, 433-439 (2004)

149. C. H. Fang, J. Q. Gong, W. Zhang: Function of oval cells in hepatocellular carcinoma in rats. *World J Gastroenterol* 10, 2482-2487 (2004)

150. L. Libbrecht, R. De Vos, D. Cassiman, V. Desmet, R. Aerts and T. Roskams: Hepatic progenitor cells in hepatocellular adenomas. *Am J Surg Pathol* 25, 1388-1396 (2001)

151. X. R. Yang, Y. Xu, B. Yu, J. Zhou, S. J. Qiu, G. M. Shi, B. H. Zhang, W. Z. Wu, Y. H. Shi, B. Wu, G. H. Yang, Y. Ji and J. Fan: High expression levels of putative hepatic stem/progenitor cell biomarkers related to tumour angiogenesis and poor prognosis of hepatocellular carcinoma. *Gut* 59, 953-962 (2010)

152. A. Tsuchiya, H. Kamimura, Y. Tamura, M. Takamura, S. Yamagiwa, T. Suda, M. Nomoto and Y. Aoyagi: Hepatocellular carcinoma with progenitor cell features distinguishable by the hepatic stem/progenitor cell marker NCAM. *Cancer Lett* 309, 95-103 (2011)

153. A. Suetsugu, M. Nagaki, H. Aoki, T. Motohashi, T. Kunisada and H. Moriwaki: Characterization of CD133+ hepatocellular carcinoma cells as cancer stem/progenitor cells. *Biochem Biophys Res Commun* 351, 820-824 (2006)

154. M. L. Dumble, E. J. Croager, G. C. Yeoh and E. A. Quail: Generation and characterization of p53 null transformed hepatic progenitor cells: oval cells give rise to hepatocellular carcinoma. *Carcinogenesis* 23, 435-445 (2002)

155. C. M. Shachaf, A. M. Kopelman, C. Arvanitis, A. Karlsson, S. Beer, S. Mandl, M. H. Bachmann, A. D. Borowsky, B. Ruebner, R. D. Cardiff, Q. Yang, J. M. Bishop, C. H. Contag and D. W. Felsher: MYC inactivation uncovers pluripotent differentiation and tumour dormancy in hepatocellular cancer. *Nature* 431, 1112-1117 (2004)

156. T. Chiba, K. Kita, Y. W. Zheng, O. Yokosuka, H. Saisho, A. Iwama, H. Nakauchi and H. Taniguchi: Side population purified from hepatocellular carcinoma cells harbors cancer stem cell-like properties. *Hepatology* 44, 240-251 (2006)

157. T. Roskams, S. Q. Yang, A. Koteish, A. Durnez, R. DeVos, X. Huang, R. Achten, C. Verslype and A. M. Diehl: Oxidative stress and oval cell accumulation in mice and humans with alcoholic and nonalcoholic fatty liver disease. *Am J Pathol* 163, 1301-1311 (2003)

158. S. Yang, A. Koteish, H. Lin, J. Huang, T. Roskams, V. Dawson and A. M. Diehl: Oval cells compensate for damage and replicative senescence of mature hepatocytes in mice with fatty liver disease. *Hepatology* 39, 403-411 (2004)

159. V. M. de Lima, C. P. Oliveira, V. A. Alves, M. C. Chammas, E. P. Oliveira, J. T. Stefano, E. S. de Mello, G. G. Cerri, F. J. Carrilho and S. H. Caldwell: A rodent model of NASH with cirrhosis, oval cell proliferation and hepatocellular carcinoma. *J Hepatol* 49, 1055-1061 (2008)

160. J. Y. Cho, K. S. Suh, W. Y. Shin, H. W. Lee, N. J. Yi, M. A. Kim, J. J. Jang and K. U. Lee: Expansion of hepatic progenitor cell in fatty liver graft after living donor liver transplantation. *Transpl Int* 23, 530-537 (2010)

161. Y. Jung and A. M. Diehl: Non-alcoholic steatohepatitis pathogenesis: role of repair in regulating the disease progression. *Dig Dis* 28, 225-228 (2010)

162. W. Hu, Z. Feng, J. Eveleigh, G. Iyer, J. Pan, S. Amin, F. L. Chung and M. S. Tang: The major lipid peroxidation product, trans-4-hydroxy-2-nonenal, preferentially forms DNA adducts at codon 249 of human p53 gene, a unique mutational hotspot in hepatocellular carcinoma. *Carcinogenesis* 23, 1781-1789 (2002)

163. H. S. Park, J. Y. Park and R. Yu: Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-alpha and IL-6. *Diabetes Res Clin Pract* 69, 29-35 (2005)

164. P. A. Kern, S. Ranganathan, C. Li, L. Wood and G. Ranganathan: Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab* 280, E745-751 (2001)

165. Y. Tang, K. Kitisin, W. Jogunoori, C. Li, C. X. Deng, S. C. Mueller, H. W. Ressom, A. Rashid, A. R. He, J. S. Mendelson, J. M. Jessup, K. Shetty, M. Zasloff, B. Mishra, E. P. Reddy, L. Johnson and L. Mishra: Progenitor/stem cells give rise to liver cancer due to aberrant TGF-beta and IL-6 signaling. *Proc Natl Acad Sci U S A* 105, 2445-2450 (2008)

166. V. B. Matthews, E. Klinken and G. C. Yeoh: Direct effects of interleukin-6 on liver progenitor oval cells in culture. *Wound Repair Regen* 12, 650-656 (2004)

167. M. A. Abdelmegeed, S. H. Yoo, L. E. Henderson, F. J. Gonzalez, K. J. Woodcroft and B. J. Song: PPARalpha expression protects male mice from high fat-induced nonalcoholic fatty liver. *J Nutr* 141, 603-610 (2011)

168. A. Tsuchida, T. Yamauchi, S. Takekawa, Y. Hada, Y. Ito, T. Maki and T. Kadowaki: Peroxisome proliferator-activated receptor (PPAR)alpha activation increases adiponectin receptors and reduces obesity-related inflammation in adipose tissue: comparison of activation of PPARalpha, PPARgamma, and their combination. *Diabetes* 54, 3358-3370 (2005)

169. A. M. Sharma and B. Staels: Review: Peroxisome proliferator-activated receptor gamma and adipose tissue--understanding obesity-related changes in regulation of lipid and glucose metabolism. *J Clin Endocrinol Metab* 92, 386-395 (2007)

170. C. Y. Zhao, L. L. Jiang, L. Li, Z. J. Deng, B. L. Liang and J. M. Li: Peroxisome proliferator activated receptor-gamma in pathogenesis of experimental fatty liver disease. *World J Gastroenterol* 10, 1329-1332 (2004)

171. C. Kaplanski, C. J. Pauley, T. G. Griffiths, T. T. Kawabata and B. J. Ledwith: Differentiation of rat oval cells after activation of peroxisome proliferator-activated receptor alpha43. *Cancer Res* 60, 580-587 (2000)

172. B. Knight, B. B. Yeap, G. C. Yeoh and J. K. Olynyk: Inhibition of adult liver progenitor (oval) cell growth and viability by an agonist of the peroxisome proliferator activated receptor (PPAR) family member gamma, but not alpha or delta. *Carcinogenesis* 26, 1782-1792 (2005)

173. N. Zhang, K. Siegel, M. Odenthal, R. Becker, F. Oesch, H. P. Dienes, P. Schirmacher and P. Steinberg: The role of insulin-like growth factor II in the malignant transformation of rat liver oval cells. *Hepatology* 25, 900-905 (1997)

174. N. C. Steiger-Luther, H. Darwiche, S. H. Oh, J. M. Williams and B. E. Petersen: Insulin-like growth factor binding protein-3 is required for the regulation of rat oval cell proliferation and differentiation in the 2AAF/PHX model. *Hepat Med* 2, 13-32 (2010)

175. M. Vorobeychik, K. Bloch, R. Zemel, L. Bachmetov, R. Tur-Kaspa and P. Vardi: Immunohistochemical evaluation of hepatic oval cell activation and differentiation toward pancreatic beta-cell phenotype in streptozotocin-induced diabetic mice. *J Mol Histol* 39, 463-468 (2008)

176. L. Yang, S. Li, H. Hatch, K. Ahrens, J. G. Cornelius, B. E. Petersen and A. B. Peck: In vitro trans-differentiation of adult hepatic stem cells into pancreatic endocrine hormone-producing cells. *Proc Natl Acad Sci U S A* 99, 8078-8083 (2002)

177. M. R. Kashi, D. M. Torres and S. A. Harrison: Current and emerging therapies in nonalcoholic fatty liver disease. *Semin Liver Dis* 28, 396-406 (2008)

178. A. Suzuki, K. Lindor, J. St Saver, J. Lymp, F. Mendes, A. Muto, T. Okada and P. Angulo: Effect of changes on body weight and lifestyle in nonalcoholic fatty liver disease. *J Hepatol* 43, 1060-1066 (2005)

179. H. K. Kim, J. Y. Park, K. U. Lee, G. E. Lee, S. H. Jeon, J. H. Kim and C. H. Kim: Effect of body weight and lifestyle changes on long-term course of nonalcoholic fatty liver disease in Koreans. *Am J Med Sci* 337, 98-102 (2009)

180. I. J. Hickman, J. R. Jonsson, J. B. Prins, S. Ash, D. M. Purdie, A. D. Clouston and E. E. Powell: Modest weight loss and physical activity in overweight patients with chronic liver disease results in sustained improvements in alanine aminotransferase, fasting insulin, and quality of life. *Gut* 53, 413-419 (2004)

181. H. Hashizume, K. Sato, H. Takagi, T. Hirokawa, A. Kojima, N. Sohara, S. Kakizaki, Y. Mochida, T. Shimura, Y. Sunose, S. Ohwada and M. Mori: Primary liver cancers with nonalcoholic steatohepatitis. *Eur J Gastroenterol Hepatol* 19, 827-834 (2007)

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