

Leptin signaling molecular actions and drug target in hepatocellular carcinoma

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Abstract: Previous reports indicate that over 13 different tumors, including hepatocellular carcinoma (HCC), are related to obesity. Obesity-associated inflammatory, metabolic, and endocrine mediators, as well as the functioning of the gut microbiota, are suspected to contribute to tumorigenesis. In obese people, proinflammatory cytokines/chemokines including tumor necrosis factor-alpha, interleukin (IL)-1 and IL-6, insulin and insulin-like growth factors, adipokines, plasminogen activator inhibitor-1, adiponectin, and leptin are found to play crucial roles in the initiation and development of cancer. The cytokines induced by leptin in adipose tissue or tumor cells have been intensely studied. Leptin-induced signaling pathways are critical for biological functions such as adiposity, energy balance, endocrine function, immune reaction, and angiogenesis as well as oncogenesis. Leptin is an activator of cell proliferation and anti-apoptosis in several cell types, and an inducer of cancer stem cells; its critical roles in tumorigenesis are based on its oncogenic, mitogenic, proinflammatory, and pro-angiogenic actions. This review provides an update of the pathological effects of leptin signaling with special emphasis on potential molecular mechanisms and therapeutic targeting, which could potentially be used in future clinical settings. In addition, leptin-induced angiogenic ability and molecular mechanisms in HCC are discussed. The stringent binding affinity of leptin and its receptor Ob-R, as well as the highly upregulated expression of both leptin and Ob-R in cancer cells compared to normal cells, makes leptin an ideal drug target for the prevention and treatment of HCC, especially in obese patients.

Keywords: hepatocellular carcinoma, leptin, leptin antagonist, leptin signaling, tumor angiogenesis, drug target

Introduction

It has been reported that over 400 million people are obese worldwide, and the number is projected to reach 700 million by 2015.¹ According to the reports from the International Association for the Study of Obesity, approximately one-fourth of European men and women are obese and approximately one-half of European men and one-third of European women are overweight.² In the United States, where adult obesity is 30%–35%, the obesity epidemic also poses threats to public health.^{3–6} The escalation of obesity and overweight has become a global problem in the past decade. Accumulating evidence indicates that the obese state shares some characteristics with chronic low-grade inflammation, which deliberates various diseases, particularly cardiovascular disease,^{7,8} chronic kidney disease,^{9–11} dyslipidemia,¹² hypertension,^{13,14} liver disease,^{15–17} type 2 diabetes,¹⁸ as well as a number of tumors.^{19–21} Many tumors, including gynecologic tumors (breast, ovarian, cervical, uterine cancer), digestive system tumors (esophageal, stomach, colon or rectal, liver, gall bladder, pancreatic), and hematologic tumors (multiple myeloma and non-Hodgkin lymphoma), as well as others, such as kidney and glioma, are found to be correlated with obesity.^{19,20,22–24}

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It is estimated that being overweight or obese contributes to 20% of cancer deaths in the United States.²⁵

Although obesity has been considered as an increased risk for many cancers, the molecular mechanisms by which obesity affects cancer incidence is still unclear. Obesity-associated inflammatory, metabolic, and endocrine mediators, as well as the functioning of the gut microbiota, are suspected to contribute to tumorigenesis. Among obese people, proinflammatory cytokines/chemokines including tumor necrosis factor- α (TNF- α), interleukin (IL)-1, IL-6, insulin and insulin-like growth factors (IGFs), adipokines, plasminogen activator inhibitor-1, adiponectin, and leptin are found to play a crucial role in the initiation and development of cancer.^{26–30} The gut microbiota, including altered microbial metabolism, is able to contribute to the generation of procarcinogenic toxic metabolites; increased extraction of energy and nutrient availability leading to metabolic dysregulation contributes to tumor initiation and progression.^{31–33} Among the above molecules, leptin is the most abundant adipokine. Since it was first cloned in 1994,³⁴ this cytokine-like hormone, controlling adipocyte mass and energy balance by binding to the leptin receptor (Ob-R), has been the subject of intensive studies in cancer development.

Hepatocellular carcinoma (HCC) is the most typical liver cancer. Approximately three-quarters of total liver cancer worldwide are associated with HCC, which is the major histological subtype of liver cancer burden worldwide, and complicating cirrhosis due to chronic viral infection or toxic injury remains the third leading cause of cancer death in the world. HCC is increasingly diagnosed among individuals with obesity and related disorders. A systematic review and meta-analysis, along with other evidences, linked obesity to increased risk of common and less common malignancies, such as HCC.³⁵ A number of epidemiological studies have reported that overweight and/or obesity are associated with a greater risk of HCC compared to the general population.^{36–39} A significant increase in serum leptin levels and a positive correlation between the serum levels of leptin and α -fetoprotein in cirrhotic HCC group were also observed in HCC patients.⁴⁰ The serum leptin levels were also found to be considerably higher in patients with HCC than in normal healthy controls in another study.⁴¹

In light of the increasingly reported role of leptin in several types of cancer,^{42–47} this review is focused on the updated knowledge on the oncogenic role of leptin signaling in the occurrence and development of HCC, clinical significance, and development of specific drug targets in HCC. Additionally, leptin-induced angiogenic ability and molecular

mechanisms in HCC cells are also discussed. The stringent binding affinity of leptin/Ob-R, the overexpression of leptin/Ob-R, and its targets in cancer cells make leptin a unique drug target for the prevention and treatment of HCC, particularly in obese patients.

Cellular and molecular structure and function of leptin and the leptin receptor

Leptin, coded by the *LEP* gene, is a small, 167-amino acid, nonglycosylated protein. The name of “leptin” is derived from the Greek word “leptos,” which means “thin”. The biological function of leptin in energy homeostasis was determined by normalization of hyperphagy and obese phenotypes using recombinant leptin administration in rodents and humans.^{48,49} Leptin also plays critical roles in the regulation of immune response, growth, reproduction, glucose homeostasis, and angiogenesis.^{50–53} The N-terminal region (94 amino acids) in leptin protein is essential for both its biological and receptor binding activities.⁵⁴ The binding of leptin to Ob-R is capable of inducing the extracellular domains of Ob-R to form a homodimer, which constitutes the functional unit responsible for leptin-mediated signals.

Ob-R belongs to a member of the class I cytokine receptor superfamily.⁵⁵ This superfamily of receptors needs auxiliary kinases for activation because they lack autophosphorylation capabilities. So far, six leptin receptor isoforms generated by mRNA alternative splicing have been discovered⁵⁶: shorter isoforms with less biological activity (OB-RS) and the long isoform (OB-RL or OB-Rb) with full intracellular signaling capabilities.^{47,55} All Ob-R forms have the common large extracellular domain of Ob-R (816 amino acids).⁴⁷ In contrast, all Ob-R forms have variable lengths of cytoplasmic tail (300 amino acid residues).^{57,58} Ob-R binding to leptin induces its conformational changes that recruit Janus kinases (JAKs), which in turn phosphorylate Ob-R and activate signal transducers and activators of transcription (STATs).⁴⁷ In addition to the JAK2/STATs signaling pathway, leptin binding to Ob-R also induces canonical (phosphoinositide 3-kinase [PI-3K]/protein kinase B [Akt], mitogen-activated protein kinase [MAPK]/extracellular regulated kinase 1 and 2 [ERK 1/2]), and noncanonical signaling pathways (AMPK, JNK, PKC, and p38 MAPK) in diverse cell types. The long form (Ob-Rb) has a long intracellular domain which is essential for intracellular signal transduction. Only Ob-Rb in the leptin receptor isoforms contains an intact intracellular domain and has the ability to activate the intracellular JAK/STAT pathway on ligand binding.^{47,59} Importantly, leptin-mediated

STAT3 (signal transducer and activator of transcription 3) signaling needs Tyr-1138 of Ob-Rb for its action.^{60–62} In addition, leptin-induced signals occur in normal peripheral tissues, but the high level of leptin in obesity could amplify leptin signaling, thereby finally inducing the development of obesity-associated cancers.

Expression of leptin and Ob-R in human HCC

Wang et al⁶³ examined, using immunohistochemical staining, leptin expression in 36 cases of adjacent nontumorous liver tissues (36/36, 100%) with moderate (++) to strong (+++) intensity and in 72.22% (26/36) of HCC with weaker (+) intensity ($P < 0.05$). However, they suggested that further studies were needed to determine the inhibitory and/or activating role of leptin in the etiology, carcinogenesis, and progress of human HCC.⁶³ In another report,⁶⁴ high leptin expression was demonstrated in 60.3% of patients with HCC and was not correlated to Ki-67 expression, but it is significantly correlated to intratumor microvessel density (high vs low; 59.2 [standard deviation 3.2] vs 44.2 [19.5], $P = 0.004$). However, leptin expression was determined as a predictor for improved overall survival of patients with HCC (odds ratio [OR] 0.16; 95% confidence interval [CI] 0.03–0.87; $P = 0.033$) using a multivariate Cox's proportional hazards model. Interestingly, high Ob-R expression was detected in 53% of HCC patients and was also significantly correlated to intratumor microvessel density (high vs low; 59.4 [3.2] vs 44.7 [3.7]; $P = 0.004$).⁶⁴ In addition, high Ob-R expression was associated with a better overall survival ($P = 0.027$) using the Kaplan–Meier survival curve.⁶⁴ Multivariate analysis also showed that Ob-R expression was a significant determinant for HCC (OR 0.02, 95% CI 0.01–0.85; $P = 0.041$).⁶⁴ In a recent study,⁶⁵ the overexpression rate of leptin and Ob-R in 81 HCC patients was 56.8% and 35.8%, respectively. Ob-R overexpression was significantly correlated to the tumor size and TNM stage ($P < 0.05$), but not to age, body mass index, α -fetoprotein, hepatitis B surface antigen status, tumor grade, vascular invasion, or liver cirrhosis ($P \geq 0.05$). Leptin overexpression showed no significant correlations to the above clinicopathological factors ($P \geq 0.05$). In vitro, leptin and Ob-R are simultaneously expressed in the HCC cell line HepG2.⁶⁶ Leptin increased HepG2 cell proliferation in a concentration- and time-dependent manner. The effect of promotion of cell proliferation by leptin is due to the increment of DNA synthesis and enhancement of mitotic activity.

The results of the previous studies were inconsistent and contradictory. The existing conflicts among different studies might be due to the use of different tools or different stages

of tumor tissues. Therefore, leptin may be involved in the occurrence and development of HCC, and the specific role and mechanism needs further research.

Interaction between leptin signaling and oncogenic pathways in HCC

In the central nervous system, particularly in the hypothalamus, which is a site of high Ob-Rb mRNA expression, many of the effects are attributable to leptin. Alternative splicing and proteolytic cleavage events also produce a circulating extracellular domain of Ob-R, which may affect the stability of circulating leptin.^{47,51} The cell-membrane-bound short-form receptors may also have potentially important roles, including the endocytosis and transport of leptin across the blood–brain barrier. High levels of leptin in obese patients are not able to suppress feeding and decrease body weight (BW)/adiposity. The proposed mechanisms of leptin resistance include perturbations in developmental programming, alterations in cellular Ob-Rb signaling, alterations in the transport of leptin across the blood–brain barrier, and others.⁶⁷ In peripheral tissues, high levels of circulating leptin could also overregulate the signaling and expression of active Ob-R. These phenomena lead to the deregulation of leptin signaling, thereby significantly contributing to HCC progression through its crosstalk with multiple signaling pathways, as discussed in breast cancer⁴⁷ or colorectal cancer.⁶⁸

The PI-3K/Akt pathway, an assembly of membrane-localized complexes, plays a central role in a variety of multiple biological processes such as cell motility, proliferation, survival, and angiogenesis in tumor cells including HCC.^{69–72} The PI-3K/Akt pathway also plays a major role in tumor growth factor (TGF)- β -induced epithelial–mesenchymal transformation (EMT), notably through the regulation of translation and cell invasion during carcinogenesis.^{73,74} In addition, many of the transforming events in HCC are a result of the enhancement or deregulation of PI3-K/Akt pathway.^{72,75} A great number of studies have already established the central role of leptin-induced regulation of the PI-3K/Akt signaling pathway in several types of cancer including HCC (Figure 1).^{47,76–85}

Mounting evidence has shown that the STAT3 is a frequent biochemical aberrant in the development, progression, and maintenance of cancer cells.^{86,87} STAT3 regulates a variety of genes involved in the regulation of critical functions, including immune responses, cell proliferation, differentiation, angiogenesis, apoptosis, and metastasis. STAT3 can function either as an oncoprotein or a tumor suppressor depending on the specific genetic background or in different

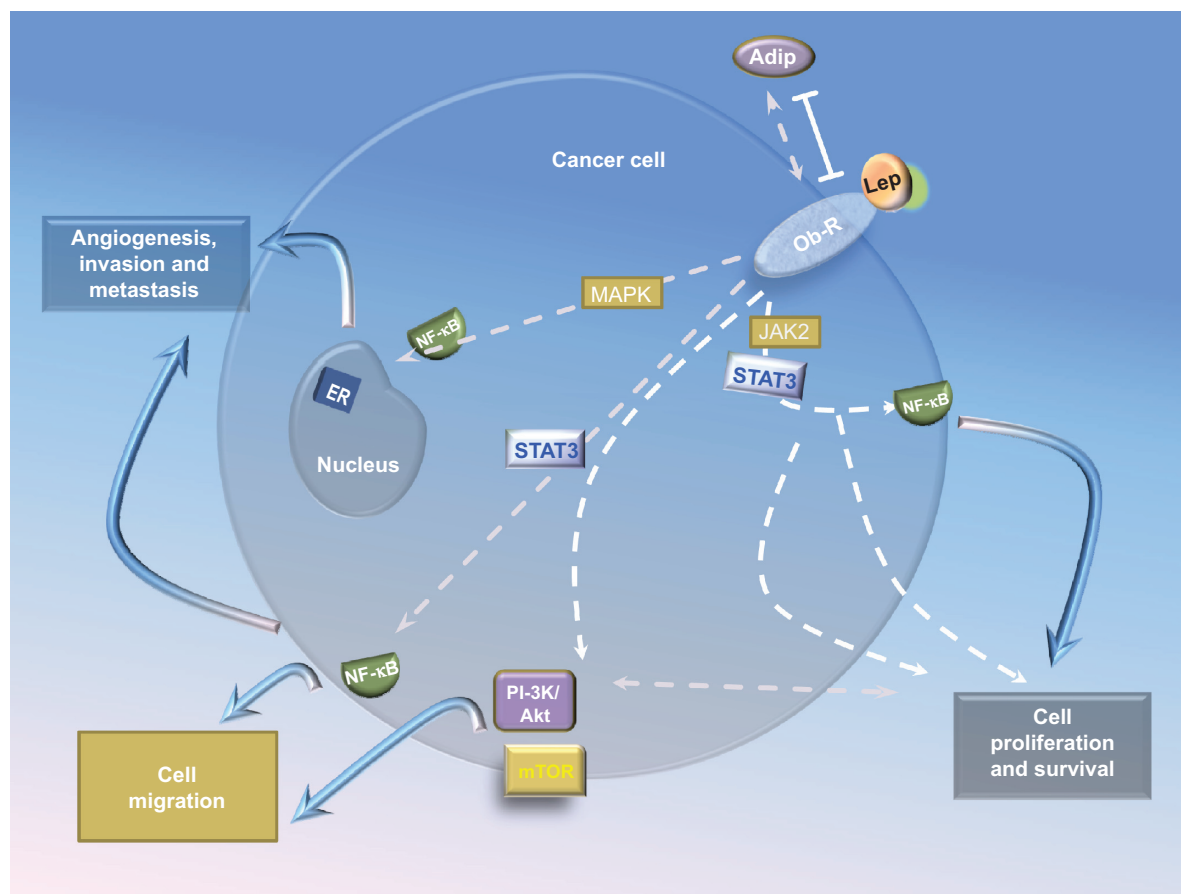


Figure 1 Crosstalk between leptin signaling and signaling pathways in HCC.

Notes: Leptin binding to the receptor Ob-R in HCC cells activates canonical (JAK2/STAT3, MAPK, and PI-3K) signaling pathways. Leptin-induced JAK2/STAT3, MAPK, PI-3K/mTOR, p38, and JNK signaling. PI-3K/Akt induces phosphorylation of mTOR. MAPK activation plays an important role in activating ERK 1/2, p38, and JNK, which in turn induce NF-κB activation. Levels of proinflammatory/pro-angiogenic molecules can also be induced by leptin signaling pathways. Solid and dashed arrows indicate the main and alternative mechanisms of leptin actions.

Abbreviations: HCC, hepatocellular carcinoma; JAK2, Janus kinase 2; MAPK, mitogen-activated protein kinase; PI-3K, phosphoinositide 3-kinase; mTOR, mammalian target of rapamycin; Akt, protein kinase B; ERK 1/2, extracellular regulated kinase 1 and 2; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; STAT, signal transducer and activator of transcription; Adip, adipose; Lep, leptin; ER, estrogen receptor.

contexts of cancer biology.⁸⁸ However, for many tumors, increased levels of activated STAT3 have been associated with worsened prognosis, indicating that STAT3 could be an attractive molecular target for the development of novel malignancy therapeutics.^{89,90}

The STAT3 pathway mediates leptin actions on food intake, weight gain, glucose metabolism, and neuroendocrine function, but does not influence fertility and glycemic control.⁹¹ In malignant cells, the STAT3 pathway is involved in leptin actions in cell migration,^{85,92} proliferation,^{93–98} and anti-apoptosis.^{82,99,100} Leptin-STAT3 regulates the genes cyclooxygenase (*COX*-2),⁸³ *cyclin D1* (kinase and regulator of cell cycle D1),^{93,95} human telomerase reverse transcriptase (*hTERT*),¹⁰¹ *VEGF*,^{102,103} *leptin*,¹⁰² and *survivin*.¹⁰⁴ STAT3 could also regulate nuclear factor kappa-light-chain-enhancer of activated B cells,^{105,106} IL-1, Notch,^{107,108} canonical WNT,^{109,110} and VEGFR-2,^{107,108} and thereby regulate tumor angiogenesis.

Leptin could crosstalk with signaling pathways which are involved in the pathogenesis of nonalcoholic fatty liver disease, which is a risk disease of HCC.^{111–113} Leptin is able to contribute to the development of insulin resistance, steatosis, proinflammation, and liver fibrosis.^{46,114} Leptin injections have been shown to result in the increased expression of procollagen-I, TGF-β1, and smooth muscle actin which is a marker of activated hepatic stellate cells, and eventually to increased liver fibrosis.¹¹⁵

Leptin could also crosstalk with signaling pathways which involve in the development of fibrosis. Aleffi et al identified the effect of leptin on fibrogenic cells is the induction of vascular endothelial growth factor (VEGF) via oxygen-independent activation of hypoxia-inducible factor 1a, which is a master switch of the angiogenic response.¹¹⁶ Their results strongly suggest the fibrogenic role of leptin in the liver.

Therapeutic potential for leptin/Ob-R signaling molecules

Evidence ever more strongly implicates that leptin/Ob-R signaling is correlated to many cancer types and point toward new drug targets. Leptin binds only to Ob-R. Moreover, the extracellular activation of Ob-R is obtained only upon leptin binding to its extracellular region.⁴⁷ Interestingly, this family of receptors is capable of binding only to leptin or leptin-modified peptides, indicating the potential use of leptin antagonists and/or other inhibitors in blocking Ob-R signals.^{47,117}

Previous studies have shown that blocking of leptin signaling could cause decreased growth and development of mammary tumors derived from mice and humans.^{118,119} Tumor growth and the expression of VEGF-A/VEGFR-2 were markedly reduced in orthotopic mouse models using a pegylated leptin peptide receptor antagonist (PEG-LPrA2).^{118,119} The BW or appetite of a large number of normal lean (male and female) CD-1 and BALB/c mice did not change during a several months of using PEG-LPrA2. Surmacz's group reported similar results in the same orthotopic xenograft model using a different leptin antagonist (Allo-aca).¹²⁰ Allo-aca induced 6%–10% BW increase, but it significantly extended the mouse survival time for 1–2 weeks and did not show systemic toxicity when tested for toxicity effects in normal CD-1 mice.¹²⁰ Recently, the same group tested a number of Allo-aca analogs. d-Ser was a peptidomimetic and distributed only in the periphery of experimental animals. This novel peptide d-Ser may serve as a prototype to develop new therapeutics because it significantly inhibited leptin-dependent proliferation of Ob-R-positive cancer cells in vitro at 1 nM concentration without exhibiting any partial agonistic activity.¹²¹ The above results indicate that inhibition of leptin signaling by leptin antagonists may serve as a novel adjuvant for the treatment of HCC.

Several groups have developed antibodies targeting leptin signaling. Zabeau et al produced and evaluated a number of neutralizing nanobodies targeting Ob-R.¹²² Three classes of neutralizing nanobodies targeting different Ob-R subdomains, ie, the Ig-like and fibronectin type III domains and cytokine receptor homology 2, were identified. Among them, only nanobodies directed against the cytokine receptor homology 2 domain inhibited leptin binding. Ross and Strasburger's groups developed monoclonal antibodies (mAbs) against human Ob-R and verified their antagonistic activity using an LEP-signaling bioassay.^{123,124} 9F8, the most promising mAb showed dose-dependent antagonist activity using the LEP bioassay. However, all the above-mentioned antibodies have not been used in cancer therapy.

In summary, although there have already been compounds or antibodies targeting leptin/Ob-R that showed significant in vitro or in vivo anticancer effect, they have not been utilized in clinical settings. The major reason might be the low efficiency and specificity of some compounds or antibodies. In addition, the side effects of these drugs are not completely known and require further studies.

Conclusion

Obesity-associated inflammatory, metabolic, and endocrine mediators are suspected to play a role in tumorigenesis. Body fat and adipocyte size are clearly correlated to high leptin levels in obesity and in overweight individuals or populations. High leptin level is a hallmark of obesity, which has been correlated to the incidence and progression of several malignancies including HCC. In vitro studies have clearly demonstrated the role of leptin in HCC proliferation, migration, and angiogenesis. In addition, leptin signaling and its crosstalks with many signaling pathways, such as PI-3K/Akt and STAT3, play critical roles in HCC cell growth, invasion, angiogenesis, and metastasis. There are still a number of gaps to fill in the field of leptin signaling in HCC, especially further identification of the molecular mechanisms of leptin-signaling-mediated regulation of HCC. There are conflicting data concerning the correlation between tissue leptin level and HCC risk. Although several groups have developed antibodies targeting leptin signaling, all these antibodies have not been used in cancer therapy. Novel opportunities could emerge from the discovery of leptin crosstalk with other oncogenic pathways, inflammatory and angiogenic cytokines, and their links to obesity-related cancers.

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Disclosure

The authors declare no conflicts of interest in this work.

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