The Potential Role of Leptin in Tumor Invasion and Metastasis

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Abstract

The adipocyte-released hormone-like cytokine/adipokine leptin behaves differently in obesity compared to its functions in the normal healthy state. In obese individuals, elevated leptin levels act as a pro-inflammatory adipokine and are associated with certain types of cancers. Further, a growing body of evidence suggests that higher circulating leptin concentrations and/or elevated expression of leptin receptors (Ob-R) in tumors may be poor prognostic factors. Although the underlying pathological mechanisms of leptin's association with poor prognosis are not clear, leptin can impact the tumor microenvironment in several ways. For example, leptin is associated with a number of biological components that could lead to tumor cell invasion and distant metastasis. This includes interactions with carcinoma-associated fibroblasts, tumor promoting effects of infiltrating macrophages, activation of matrix metalloproteinases, transforming growth factor-β signaling, etc. Recent studies also have shown that leptin plays a role in the epithelial-mesenchymal transition, an important phenomenon for cancer cell migration and/or metastasis. Furthermore, leptin's potentiating effects on insulin-like growth factor-I, epidermal growth factor receptor and HER2/neu have been reported. Regarding unfavorable prognosis, leptin has been shown to influence both adenocarcinomas and squamous cell carcinomas. Features of poor prognosis such as tumor invasion, lymph node involvement and distant metastasis have been recorded in several cancer types with higher levels of leptin and/or Ob-R. This review will describe the current scenario in a precise manner. In general, obesity indicates poor prognosis in cancer patients.

Graphical Abstract

Leptin-linked Pathological Mechanisms in Tumor Proliferation and Migration

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Conflict of Interest

The authors have no conflict of interest to declare.
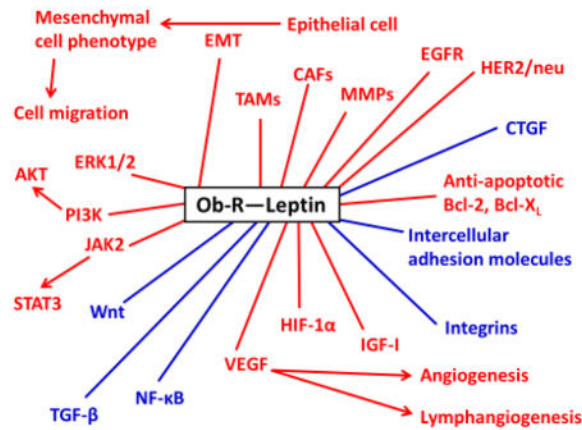
Red: Tumor-promoting effect

Blue: Dual role (tumor–suppressor or –promoting effect depending on the situation)

CAFs: Cancer-associated fibroblasts, CTGF: Connective tissue growth factor, EGFR: Epidermal growth factor receptor, EMT: Epithelial–mesenchymal transition, ERK1/2: Extracellular signal-regulated kinases 1 and 2, HER2: Human epidermal growth factor receptor 2, HIF-1α: Hypoxia-inducible factor-1 alpha, IGF-I: Insulin-like growth factor-I, JAK2: Janus kinase 2, MMPs: Matrix metalloproteinases, NF-κB: Nuclear factor-kappa B, Ob-R: Leptin receptor, PI3K: Phosphatidylinositol 3-kinase, STAT3: Signal transducer and activator of transcription 3, TAMs: Tumor-associated macrophages, TGF-β: Transforming growth factor-beta, VEGF: Vascular endothelial growth factor

Keywords

Invasive cancer; Leptin; Metastasis; Obesity; Prognosis

1. Introduction

Obesity is associated with poor prognosis among cancer patients, apart from its link to other disease processes such as type-2 diabetes, cardiovascular disorders and increased risk of certain malignancies. Hormone-like cytokines or adipokines released from the expanded adipose tissue mass in obesity are thought to be responsible for these pathophysiological changes. One of the important adipokines is leptin, which may influence several biological processes in both normal and disease conditions. Leptin is a 16 kDa protein mainly synthesized by adipocytes that maintains energy homeostasis by influencing the central anorexigenic pathway [1]. However, in obesity, leptin displays diverse abnormal functions. Leptin acts via transmembrane receptors (Ob-R), which include at least 6 alternatively spliced isoforms. The long form Ob-Rb appears to be most important for leptin's weight regulating effects.

The tumor microenvironment, which consists of extracellular matrix (ECM) and various populations of stromal cells such as fibroblasts, adipocytes and macrophages, may influence the behavior of cancer cells through expression of cytokines, growth factors, and proteases

[2]. For instance, both cancer-associated fibroblasts (CAFs) and matrix metalloproteinases (MMPs) are involved in different phases of cancer progression including invasiveness, and metastasis [3,4]. In fact, cancer cells and CAFs crosstalk enhances the production of growth factors, cytokines, chemokines, MMPs, and inflammatory mediators, which eventually facilitates tumor growth [3]. Interestingly, leptin has been demonstrated to be closely associated with activities of CAFs and expression levels of MMPs [5–7]. On the other hand, many reports have revealed leptin's link with a number of ECM components such as fibronectin, transforming growth factor-beta (TGF-β), and connective tissue growth factor (CTGF) as well as several intracellular signaling molecules including AKT, phosphatidylinositol 3-kinase (PI3K), and signal transducer and activator of transcription (STAT) [8–10]. Therefore, leptin is capable of inducing the local autocrine/paracrine cascades within the tumor microenvironment and likely of stimulating neoplastic growth and progression.

The epithelial-mesenchymal transition (EMT) is an intricate biological process by which epithelial cells acquire mesenchymal morphology. This phenomenon is observed during tumor invasion and metastasis, apart from normal embryological development and wound healing. Interestingly, blocking leptin's availability to estrogen receptor positive (ER+) MCF-7 breast cancer cells significantly decreased the number of metastatic lesions in SCID/ beige mice [11]. A growing body of evidence shows that leptin may promote EMT, and a number of mechanisms have been proposed. For example, Choi et al. found that leptin activated hedgehog signaling in hepatic stellate cells, which in turn promoted EMT [12]. Further, a potential cross-talk between leptin and Wnt signaling in EMT has been reported in breast cancer cell-lines [13]. This was demonstrated by an increased accumulation and nuclear translocation of β-catenin by leptin due to a decrease in the formation of glycogen synthase kinase 3β (GSK3β)-liver kinase B1 (LKB1)-axin complex in MCF-7, MDA-MB-468 and MDA-MB-231 breast cancer cells. In addition, using A549 human lung cancer cells, Feng and colleagues reported that leptin significantly upregulated TGF-β that may play an important role in inducing EMT [14]. Leptin-mediated activation of intracellular signaling molecules such as STAT3, AKT, and PI3K could contribute to EMT-linked mechanisms [15,16].

Invasion and distant metastases by cancer cells are the final event that leads to the majority of cancer deaths. Leptin potentially has a potent impact on the spread of cancer to distinct organs. Apart from the pathological effects that have been mentioned above, leptin may influence disease processes through a number of mechanisms such as enhanced cellular proliferation, angiogenesis, and interaction with various cytokines (Fig. 1). In this review, we analyze leptin's role in tumor invasion and metastasis.

2. Obesity-related microenvironment and its influence on tumor progression

A growing body of evidence suggests that adipocytes in the tumor microenvironment play a crucial role in disease progression by providing fatty acids, pro-inflammatory cytokines and proteases [17,18]. Interestingly, cancer cells utilize adipocyte-released fatty acids for energy

production through β-oxidation. Therefore, adequate supply of fatty acids/lipids from cancer-associated adipocytes favors uncontrolled growth and progression of malignancy [17]. A recent study showed that after co-culture with isolated omental adipocytes, MKN-45 and AGS gastric cancer cells exhibited a significant increase in lipid uptake and enhanced invasiveness [19]. Similarly, co-culture of adipocytes with SW480 and DLD1 human colon cancer cells led to a transfer of free fatty acids from the adipocytes to the cancer cells [20]. Furthermore, this study found an accumulation of adipocytes in close association with invasive tumor cells in colon cancer patients. In an *in vitro* study, murine 3T3L1 adipocytes were co-cultured with pancreatic intraepithelial neoplasia and ductal adenocarcinoma cells derived from PKCY mice. Adipocytes promoted proliferation of both cell types [21].

Prostate cancer progression may also be impacted by adipose tissue microenvironment. For example, androgen independent RM1 mouse prostate carcinoma cells were co-cultured with pre-adipocytes or adipocytes as well as with their respective conditioned medium [22]. Both adipocytes and its conditioned media significantly increased RM1 cell proliferation and greater cell migration was observed with pre-adipocyte and adipocyte conditioned media. Moreover, RM1 cell invasion was significantly increased after co-culture with pre-adipocytes and adipocytes. Another study using human prostate cancer cell-lines (DU145, LNCaP, and PC-3) found that conditioned medium from pre-adipocytes increased cell proliferation and invasive activity [23]. Interestingly, Ribeiro et al. reported higher MMP-2 activity in periprostatic adipose tissue compared to pre-peritoneal visceral adipose tissue [24]. In addition, increased proliferation and migration of hormone-refractory PC-3 cells were detected after stimulation with conditioned medium from periprostatic adipose tissue explants.

A number of studies have been conducted on the interactions between breast tumors and adipocytes in the tumor microenvironment. For example, Zhu et al. analyzed 294 cases and concluded that invasive portions of breast cancer were generally situated adjacent to breast adipose tissue [25]. Intriguingly, Fletcher et al. noted that adipocytes associated with the invasive front are reduced in size compared to adipocytes that are farther away [26]. Many investigators have recorded considerable morphological and functional changes of adipocytes which are adjacent to tumors and referred to as cancer-associated adipocytes [27,28].

Additional evidence for an interaction of adipocytes with breast cancer development comes from studies with co-cultures of human breast cancer lines with adipocytes. When MDA-MB-231, MCF-7 and ZR-75-1 human breast cancer cell lines were co-cultured in the presence of adipocytes significant increases in proliferation rate were determined [29]. Furthermore, an enhanced MDA-MB-231 cancer cell invasiveness has been documented when co-cultured with adipocytes [30]. Similarly, Lee et al. observed that adipocytes induced migration and invasion in MCF-7, MDA-MB-435S, and MDA-MB-231 breast cancer cells after co-culture with adipocytes [31]. In this study, increased cell migration and invasion were accompanied by the upregulation of MMP-9 and TWIST1. In an interesting study, MCF-7 cells were co-cultured with SGBS adipocytes (which originated from a patient with Simpson-Golabi-Behmel syndrome, an overgrowth disorder). Gene expression levels of EMT-inducing transcription factors FOXC2 and TWIST1 were increased in the MCF-7 cells

while in the SGBS cells hypoxia-inducible factor-1α (HIF-1α), TGF-β, and lectin-type oxidized LDL receptor 1 (LOX1) mRNA levels were increased [32].

In a somewhat different approach to evaluate the potential impact of adipose tissue in the microenvironment on breast cancer development experiments have been conducted using conditioned medium from isolated normal breast adipocytes as well as cancer associated adipocytes. MCF-7 and MDA-MB-231 cells were used for the purpose of detecting phenotypic changes resulting from culture with the different media. Both breast cancer cell-lines had higher migration in conditioned medium obtained from the cancer-associated adipocytes in comparison to the media from normal breast adipocytes. The conditioned medium from the cancer associated adipocytes had higher levels of monocyte chemoattractant protein-1 (MCP-1) and interleukin-6 (IL-6) than that from the normal breast adipocytes [33].

In the breast tumor microenvironment, adipocytes have been reported to produce a number of biologically active compounds including different cytokines, e.g., MMP-11, plasminogen activator inhibitor-1 (PAI-1), collagen VI, IL-1β, IL-6, tumor necrosis factor – α (TNF-α), insulin-like growth factor-I (IGF-I), and leptin; many of which are metastasis-associated proteins [34–36]. In particular, Wolfson et al. suggested that leptin has a key effect on breast cancer; and in adipocyte-adjacent breast cancer cells, due to stimulation of various regulatory pathways such as Notch, Wnt and Nanog signaling axis [37]. Clearly, the significance of leptin at the adipocyte-breast cancer cell interface, and its effects in the neighboring breast cancer cells are complex (Fig. 2). A potential role of leptin acting to increase aromatase activity in adipose tissue associated with breast cancer is supported by interesting *in vivo* studies. Liu et al. co-injected pre-adipocytes (F442A) with MCF-7 breast cancer cells subcutaneously into SCID mice [38]. The pre-adipocytes developed into fat pads and interestingly tumors also developed in these mice while those with only the MCF-7 cells injected did not. Further investigation indicated that leptin signaling in these adipocytes increased aromatase expression and further that direct injection of leptin into these fat pads increased mRNA for aromatase 6-fold [38]. Their findings suggest that leptin in the tumor microenvironment could play a crucial role in the progress of hormone-dependent breast cancer.

3. Leptin biology and molecules of relevant signal transduction pathways

The leptin gene (*ob*) is located on the long arm of chromosome 7. Initially a 167 amino acid peptide is translated, which forms the mature 146 amino acid peptide after processing through the endoplasmic reticulum [39]. Leptin protein sequences are highly conserved among mammals; however, it exhibits considerable sequence variation among other vertebrates [40]. Due to poor solubility of natural leptin, a mutant leptin has been constructed with a single amino acid substitution of glutamic acid for tryptophan at position 100 [41]. For improved solubility, it has been possible to crystallize this leptin analog W100E. The crystallographic structure has displayed the secondary and tertiary structure of the leptin molecule, which consists of four antiparallel α-helices (A, B, C, and D) and two relatively long interconnected loops [39,41,42]. Two conserved cysteine residues (C96 in the

CD loop and C146 as the C-terminal residue) form a disulfide bridge that is essential for structural stability and thus biological activity [43].

Leptin belongs to the family of class-I cytokines, which also includes biomolecules such as IL-6, leukemia inhibitory factor (LIF), and ciliary neurotrophic factor (CNTF) [44,45]. For this reason, like other members, Ob-R mainly functions via the activation of Janus kinases (JAK) and STAT, particularly through JAK2-STAT3 signal transduction pathway (Fig. 3 and Fig. 4a–c). Finally, activated/phosphorylated STAT3 (p-STAT3) translocate to the nucleus and stimulates transcription. Other pathways that support neoplastic growth include PI3K and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling pathways [46,47]. Evidence to support the role of these proteins in cancer progression will be presented next.

3.1. STAT3 activation in neoplastic process

STAT3 activation has been documented in both solid and hematological malignancies [48,49]. It has been suggested that aberrant signaling of STAT3 is associated with different biological processes of tumor progression such as angiogenesis, cellular proliferation and survival of cells or inhibition of apoptosis leading to invasion and metastasis. Furthermore, STAT3 is involved in EMT by regulating various EMT-related proteins, e.g., Twist, Snail, and vimentin [50–52]. A recent study on intrahepatic cholangiocarcinoma found that patients with high STAT3 expression levels had a poor overall and disease-free survival [53]. After analyzing a large number of gastric cancer cases, both STAT3 and p-STAT3 were shown to be associated with an increased mortality risk [54,55]. In another study, Deng et al. reported that 62.6% (67/107) and 38% (41/107) of gastric cancer tissues exhibited positive immunostaining for STAT3 and p-STAT3, respectively [56]. In a study on gastric cancer conducted by Song et al., immunostaining for STAT3 and p-STAT3 was 81.7% (49/60) and 58% (35/60), respectively [57]. Moreover, their study showed that p-STAT3 played a significant role in prognosis. Similarly, in colon cancer, positive immunostaining rates for STAT3 and p-STAT3 were 72% (36/50) and 76% (38/50), respectively [58]. After reviewing 17 studies and a total of 2,346 colon cancer patients, the authors revealed a positive association between p-STAT3 expression and lymph node metastasis [59].

Likewise, results of another meta-analysis on non-small-cell lung cancer (NSCLC), which included 17 retrospective trials and 1,793 patients, suggested that high STAT3 or p-STAT3 expression was a strong predictor of poor prognosis [60]. Of note, the majority of lung cancers are NSCLC, which includes predominant squamous cell carcinomas and adenocarcinomas. Nevertheless, in a study conducted by Zhao et al., STAT3, and p-STAT3 expressions were observed in 72.1% (49/68) and 58.8% (40/68) of NSCLC cases, respectively [61]. Furthermore, immunohistochemical analyses on 82 surgically resected NSCLC tissues revealed p-STAT3 expression in 59.7% of tumors [62]. The investigators also recorded that p-STAT3 expression was correlated with tumor's degree of differentiation, lymph node metastasis, clinical staging and prognosis after surgical resection.

Liu et al. analyzed 208 breast cancer tissues, and they noticed high level of STAT3 immunoreactivity in 72% (n=150) of cancer samples; whereas high p-STAT3 expression was present in 43.8% (n=91) cases [63]. The expression levels of both STAT3 and p-STAT3 were

increased with higher TNM stage and lymph node metastasis. In another study on 76 breast cancer cases, 66% (n=50) of the cancer specimens showed positive immuno-expression of STAT3, which was significantly associated with the clinical stage, tumor differentiation and lymph node metastasis [64]. Additionally, a key role of STAT3 in the regulation of invasion and metastasis has been revealed by a recent study [65].

3.2. PI3K involvement in cancers

An important intermediate, which functions in diverse hormone signaling pathways including insulin and leptin, is PI3K (Fig. 4d). Possibly, the PI3K signaling pathway is a significant link between obesity, leptin and an increased risk of cancer [66]. In a nutshell, activation of PI3K causes phosphorylation of AKT and stimulation of the mammalian target of rapamycin (mTOR); while the phosphatase and tensin homolog (PTEN) is a negative regulator. Remarkably, PI3K pathway regulates various biological processes such as cellular metabolism, proliferation (Fig. 4e), and apoptosis (Fig. 4f); aberrant responses of these phenomena trigger the malignant pathology (Fig. 3). It is noteworthy that PI3K is composed of a regulatory subunit and a 110 kDa catalytic subunit. The gene encoding PI3K catalytic subunit α (PIK3CA/p110α) is frequently activated by mutation in cancers [67].

PIK3CA mutations represent one of the most common genetic aberrations in breast cancer [68]. In a study on 241 patients with breast cancer, PIK3CA mutations were found in 15.8% cases [69]. On the other hand, Firoozinia et al. analyzed 50 breast cancer samples; 72% samples showed significant amplification of PIK3CA gene, while 24% exhibited protein overexpression [70]. In another study that examined 95 breast cancer tissue samples, PIK3CA gene mutations were present in 25.3% cases [71]; and in a recent study, mutations of PIK3CA were found in 28% (21/75) cases [72].

A number of studies have shown the involvement of PI3K pathway in carcinomas of the gastrointestinal tract. In a recent study on Japanese patients with gastric cancer, PIK3CA mutations were detected in 12% (25/208) [73]. In another study on gastric cancer patients, both PIK3CA gene mutations and amplification were assessed in connection with clinicopathological characteristics and disease outcome [74]. PIK3CA mutations and amplification were found in 7.1% (8/113) and 67% (88/131), respectively. Furthermore, patients with PIK3CA amplification had significantly shorter survival times. A study, which analyzed PIK3CA mRNA levels in both gastric cancer tissues and metastases, observed 2 fold higher mRNA in lymph node metastasis compared to primary tumors [75]. On the other hand, an immunohistochemical analysis of tissue samples from 70 patients with gastric cancer revealed that expression level of PI3K protein was significantly correlated with TNM stage, differentiation grade, lymph node metastasis and distant metastases [76]. In the same way, after analyzing 60 colon cancer specimens immunohistochemically, the investigators concluded that high PI3K expression was associated with cancer metastasis [77]. They also noticed a significant difference in the proportion of PI3K in primary lesions (30%, 18/60) vs metastatic lesions (46.7%, 28/60). Overall, PI3K pathway alteration is common in human cancers, and overexpression of this pathway is associated with the uncontrollable cellular proliferation and growth.

3.3. Overexpression of AKT and ERK in cancers

Different investigators have reported an interaction between PI3K-AKT and ERK signaling pathways [78–80]. A number of studies, which evaluated both AKT and ERK in tumors, recorded similar frequencies of expression [81–83]. In various cancers, high expression rates of both AKT and ERK have been documented [84–88]. Regarding overexpression of AKT and its association with patients' prognosis, conflicting reports have been reported. For example, David et al. noticed that lung cancer patients with strong phosphorylated AKT (p-AKT) expression were more likely to die early [89]. In a study that analyzed endometrial adenocarcinomas, p-AKT expression was significantly higher in women with positive lymph nodes, and higher expression was significantly associated with poor survival [81]. Jia et al. recorded that p-AKT expression correlated with higher histological grade and resistance to chemotherapy in ovarian cancer [90]. On the other hand, a study in colorectal cancer patients showed that p-AKT expression was associated with lower stage and good prognosis [91]. Interestingly, in renal cell carcinoma, Pantuck et al. found that high nuclear p-AKT expression was associated with a favorable prognosis, while high cytoplasmic p-AKT expression was associated with a poor prognosis [92]. Conversely, a study in patients with non-small cell lung cancer observed that higher levels of cytoplasmic p-AKT had a trend toward longer overall survival; whereas higher levels of nuclear p-AKT had a poorer prognosis [93]. In general, investigators have identified an association between ERK activation and poor prognosis such as aggressive neoplastic features, metastasis, and reduced survival [94,95]. In a study among breast carcinoma patients, p-ERK was shown to be associated with higher TNM stage and lymph node metastasis [96]. Similar pathologic characteristics with regard to ERK expression/activation have also been detected in digestive tract cancers. In esophageal carcinoma, increasing p-ERK nuclear and cytoplasmic expression was found to be significantly correlated with tumor grade [97]. Likewise, high nuclear p-ERK expression in colorectal cancer was associated with highly invasive disease [98]. In addition, the investigators showed that nuclear but not cytoplasmic p-ERK expression correlated with the patients' overall survival rate. Perhaps, activation of ERK may increase the metastatic potentiality of cancer cells.

4. Leptin and tumor behavior of the gastrointestinal tract

4.1. Upper gastrointestinal tract

An association between high levels of leptin and risk of esophageal adenocarcinoma in obese individuals has been reported [99]. Adenocarcinoma in the lower part of esophagus most often arises from the epithelium of intestinal metaplasia (Barrett's); while squamous cell carcinoma occurs mainly in the middle and lower regions and originates from non-keratinized stratified epithelium [100]. In a study of esophageal adenocarcinoma increased peri-tumoral adipocyte size was associated with higher leptin expression, angiogenesis, lymphangiogenesis and nodal metastasis [101]. These findings suggest that leptin secreted from peri-tumoral adipocytes may play a key role in the progression of cancer. In esophageal squamous cell carcinoma, leptin immunoexpression also has been demonstrated to be upregulated. Moreover, the expression of leptin was significantly correlated with lymph node involvement and advanced tumor stage in esophageal squamous cell carcinoma [102].

With respect to gastric carcinoma Ob-R expression was correlated with poor survival in 47% of patients with lymph node metastasis, 60% of the patients with advanced gastric cancer, and 41% of the cases defined as Lauren diffuse pathology [103] (Table 1). Of note, gastric cancer has been classified in many different ways such as Lauren, World Health Organization (WHO), and Borrmann classifications [104]. Poorly cohesive carcinomas belong to the diffuse type according to the Lauren classification. By analyzing 61 gastric cancer cases, Zhao et al. observed a significant association between leptin expression and vascular endothelial growth factor (VEGF) expression [105]. Further, leptin expression in these gastric cancer tissues was significantly associated with histology, Borrmann classification, lymph node metastasis and stage. Moreover, in patients with poorly differentiated gastric cancer, a poor prognosis was found for those with a strong expression of leptin. Similarly, Geng et al. recorded that the survival of gastric cancer patients with leptin positive expression was significantly poorer than those with negative expression [106]. They also noted a correlation between the expression of leptin and HER2, both of which were significantly associated with invasion depth, lymph node metastasis, tumor stage and expression of VEGF. Interestingly, Ishikawa et al. found that the immunohistochemical expression levels of both leptin and Ob-R tended to increase as the depth of either gastric tumor invasion or TNM stage increased [107]. Among stages I and II, 31% and 27% of the cases exhibited significant immunostaining for leptin and Ob-R, respectively. Whereas, in stages III and IV, 48% of the cases were strongly positive for leptin and 47% expressed Ob-R. In this study, lymph node metastasis was detected in approximately half of the leptin strong positive and Ob-R positive cases. Both venous and lymphatic invasion was more frequently observed in tumors with high leptin and positive Ob-R expression.

To explain the mechanisms of leptin-mediated invasion and metastasis in gastric cancer, Dong and colleagues reported that leptin promoted gastric cancer cell invasion by upregulating the expression of membrane type-1 matrix metalloproteinase (MT1-MMP or MMP14), which is an ECM degrading protease which plays crucial roles in tumor invasion [108]. Furthermore, they observed that leptin and intercellular adhesion molecule-1 (ICAM-1) were both overexpressed in gastric cancer tissue, and there was a strong positive correlation between them [109]. Both were related with clinical stage and lymph node metastasis. In addition, they demonstrated that leptin induced migration of human gastric carcinoma cells (AGS and MKN-45 cell-lines) by upregulating ICAM-1. It is known that ICAM-1 or CD54 is a transmembrane glycoprotein of the immunoglobulin superfamily and it can facilitate the metastasis of cancer cells by escaping the recognition and attack of immunocytes [110]. Interestingly, leptin expression in gastric adenocarcinoma cell-lines was reported to be correlated with resistance to the chemotherapeutic agent cisplatin [111]. Furthermore, including a leptin antagonist increased the sensitivity of both the cisplatin-resistant gastric cancer AGS Cis5 and esophageal adenocarcinoma OE33 cell-lines to cisplatin, which greatly illustrated the impact of leptin in the disease course (Table 2 [101,111,112]).

Leptin may also be involved with the severity of gall bladder cancer. For example, in a recent *in vitro* study using gallbladder cancer cells the addition of leptin increased the levels of MMPs (−3 and −9) and VEGF (-C and -D, responsible for lymphangiogenesis) [113].

Since these proteins increase proliferation, migration and invasion of cancer cells, leptin could act through them to eventually promote metastasis.

4.2. Lower gastrointestinal tract

At another gastrointestinal cancer site, i.e., colorectal cancer, a relationship between higher serum leptin levels and lymph node involvement has been documented [114,115]. Moreover, after analyzing the data of 130 colorectal cancer patients, Healy et al. found that serum leptin level was associated with microvascular invasion and advanced tumor stage [115]. Similarly, leptin and Ob-R protein expressions in tumor tissue have been found to be correlated with aggressive characteristics of cancer, e.g., tumor grade and stage, depth of bowel wall invasion, lymph node metastasis and distant metastasis [116,117]. Pro-angiogenic and pro-migratory effects of leptin are possibly important in progression of colon cancer. In a study conducted by Ratke and colleagues using SW480, SW620 and HCT116 human colon carcinoma cell-lines, leptin significantly enhanced the migratory activity of these cell-lines through the activation of STAT3 and JAK [118]. The investigators performed three-dimensional collagen-based migration assays, and carcinoma cells exhibited a significant increase of their migratory activity in response to 100 ng/ml of human leptin. It is noteworthy that a positive correlation was observed among leptin, Ob-R, VEGF, and microvessel density (MVD) in colorectal carcinoma [117]. In accordance with the protein expression (i.e., Ob-R) in tumor tissue that has been discussed above, Erkasap et al. documented higher mRNA expression of Ob-R in metastatic growth compared to primary colorectal cancer [119].

Cancers of the gastrointestinal tract are common causes of death worldwide. High levels of leptin released from excessive adipose tissue in obesity may play a key role in different stages of cancer. It is known that leptin can affect several aspects of the gastrointestinal tract such as interactions with local hormones, food absorption and inflammatory condition. Therefore, it is important to understand the precise role of leptin in gastrointestinal tumors for better management and patient outcome.

5. Tumors of endocrine and associated organs

5.1. Pancreas

Adipocyte-derived leptin and insulin which is secreted from the pancreas have fascinating similarities and complex interactions in normal health and conditions such as obesity, leptin resistance and insulin resistance. Overall, an intricate relationship has been observed in energy homeostasis including glucose and lipid metabolism. Leptin resistance in obesity markedly influences the status of insulin sensitivity, and vice versa. Therefore, it is imperative to understand the role of leptin in various disorders of the pancreas, which is a storehouse of several exocrine and endocrine factors.

Fan et al. found an increased expression of leptin's functional receptor Ob-Rb in pancreatic tumor tissue which was significantly associated with the level of MMP-13, lymph node metastasis and TNM stage [120]. Further, in *in vitro* experiments these investigators showed that addition of leptin stimulated the expression of MMP-13 as well as increased the

migration and invasion of PANC-1 human pancreatic cancer cells. Likewise, in a xenograft model with the PANC-1 cell-line, they observed that the overexpression of leptin in tumor tissue significantly increased tumor growth and lymph node metastasis. In this experiment, the investigators used the PANC-1 cells that were transfected with the recombinant lentivirus carrying the human leptin gene; the cell-line stably overexpressed leptin. In another study, Ren et al. found that HIF-1α expression was correlated with Ob-R in pancreatic cancer tissue in xenograft mouse model that was inoculated with PANC-1 cells [121]. This study included human clinical data indicating that overexpression of HIF-1 was associated with lymph node metastasis and overall survival. These findings are consistent with the fact that HIF-1α appears to be involved in tumorigenesis and angiogenesis [122].

5.2. Thyroid gland

Recently there has been an increasing incidence of thyroid cancer. One of the suspected risk factors is the increasing prevalence of obesity [123,124]. Since the level of leptin is elevated in obesity, this adipokine could be involved in the development and progression of this malignancy [124]. The thyroid gland contains two types of endocrine cells – follicular cells that synthesize thyroid hormone, and parafollicular or C cells that produce calcitonin. Papillary carcinoma, the most common type of thyroid cancer, originates from follicular cells; whereas medullary carcinoma develops from C cells. A study on different subtypes of thyroid carcinoma revealed that immunohistochemical expression of Ob-R was significantly correlated with nodal metastasis and advanced stage in medullary thyroid carcinoma. On the other hand, both leptin and Ob-R levels were strongly associated with larger tumor size, nodal metastasis and advanced stage in papillary thyroid carcinoma [125]. Several studies on patients with papillary thyroid cancer documented that expression of leptin and/or Ob-R was directly linked with disease aggressiveness including increased tumor size and lymph node metastasis [126–128]. In addition, it has been found that Ob-R overexpression was significantly associated with the overexpression of anti-apoptotic XIAP and Bcl-X_L proteins as well as with poor disease-free survival in cases with papillary thyroid cancer [128]. In a study on K1 and BCPAP human papillary thyroid cancer cell-lines, tumor cell migration was significantly enhanced by leptin in a dose-dependent manner [129].

6. Female reproductive system cancers

6.1. Gynecologic cancers

Malignancies of the female reproductive system are responsible for significant morbidity and mortality throughout the world. Among all gynecological malignancies, ovarian cancer is the most deadly disease. Due to unique anatomical position of the ovary, dissemination of cancer cells within the peritoneal cavity is common in ovarian cancer. The disease is generally diagnosed at a late stage and prognosis is poor.

Similar to colon cancer [119], metastases from ovarian cancers were found to have higher Ob-Rb expression than what was associated with primary tumors [16]. *In vitro* studies with various ovarian cancer cell-lines further demonstrated that leptin stimulated cell migration and invasion. In a study on endometrial cancer, elevated levels of patients' serum leptin were associated with invasiveness [114]. Similar findings were observed with regard to

immunohistochemical expression of both leptin and Ob-R in endometrial cancer tissue. The expressions of leptin and Ob-R were positively correlated with the invasiveness of cancer, lymph node metastasis, and poorer prognosis [130].

6.2. Breast cancer

Higher breast cancer incidence has been shown to be attributable to increased body mass index (BMI) [131,132]. Leptin is considered as an important adipokine that may modulate this disease process. A number of studies on breast cancer patients have noted an association of higher serum leptin levels with aggressive malignant features. For instance, in Italian women, serum leptin concentrations significantly correlated with tumor size, lymph node involvement and metastasis among ER+ breast cancer cases [133]. Similarly in another study, serum leptin levels were positively correlated with TNM staging, tumor size, lymph node metastasis, and histological grading among postmenopausal women with breast cancer [134]. A number of investigators also included measurement of adiponectin and expressed results in relation to leptin [134–136]. Adiponectin is an adipokine with anti-inflammatory effects unlike the pathophysiological functions of leptin [137]. Nonetheless, Hou et al. observed that reduced serum levels of adiponectin and elevated leptin were associated with lymph node metastasis in breast cancer patients [135]. Chen et al. also reported that an increased serum ratio of leptin/adiponectin (L/A ratio) was positively correlated with tumor size [136].

Similar to serum levels, tumor tissue expressions of leptin and/or Ob-R also indicate the presence of aggressive behavior. For example, Ishikawa and colleagues reported that distant metastasis was detected in Ob-R-positive tumors with leptin overexpression compared to tumors that lacked Ob-R expression or leptin overexpression [138]. Similarly in another study, overexpression of leptin and Ob-R in breast cancer was positively correlated to lymph node metastasis [139]. In this study, analyses of 60 cases of breast cancer and adjacent normal tissue along with benign breast disease tissues revealed that immunostaining intensity for both parameters (co-expression) in cancer tissue was directly proportional to the degree of cancer cell dissemination. Interestingly, Alshaker et al. detected that Ob-R genes were elevated in metastases of ER-negative breast cancer patients [140]. Results from a mouse model have also provided evidence for a role of leptin in the metastatic process in breast cancer. Park et al. observed an absence of Ob-R attenuated tumor progression and lung metastasis through a reduction of ERK1/2 and JAK2/STAT3 pathways in the MMTV-PyMT mammary tumor model of breast cancer [141]. For this purpose, the brain-specific long form of leptin receptor (NseLEPR-B) transgenic mouse was crossed with the MMTV-PyMT mouse strain. Normally, MMTV-PyMT female mice develop mammary adenocarcinomas that metastasize to the lung. Additional support comes from *in vitro* studies. For example, in mouse (4T1, EMT6 and MMT) mammary cancer cell-lines, the addition of leptin induced cell proliferation and migration, as well as upregulated VEGF and its receptor VEGFR-2 [142]. McMurtry et al. reported that leptin increased the invasiveness and MMP-2 activity of the ER+ MCF-7 human breast cancer cell-line [143]. Additionally, in an experiment using breast cancer cells and tumor-associated macrophages (TAMs), leptin was shown to stimulate the expression of IL-18, which is closely linked with inflammatory reactions [144]. In this study, THP-1 human monocytic cell-line and MCF-7, SK-BR-3 and

MDA-MB-231 human breast cancer cell-lines were used. Furthermore, the study documented that leptin could induce IL-8 expression in both breast cancer cells and TAMs.

It is interesting to note leptin's influence on TAMs, which are a crucial component in the tumor microenvironment and the principal source of inflammatory cytokines [144]. Moreover, clinical evidence has revealed a strong correlation between a high density of TAMs and poor prognosis in breast cancer [144]. As such, TAMs could play key roles in cancer progression including EMT. It is noteworthy that macrophages can be classified into two major categories – classically activated M1 macrophages are linked with tumoricidal functions, and alternatively activated M2 macrophages are involved in tumor promotion [145]. Fascinatingly, TAMs have been considered as M2 macrophages, which could promote the EMT [145–147]. A recent study has shown that leptin may promote breast cancer progression by stimulating M2 macrophages [148]. Using a different approach Strong et al. [11] co-cultured adipose stromal/stem cells isolated from obese women with either ER+ MCF-7, ZR75, or T47D human breast cancer cells, which resulted in enhanced expression of EMT and metastasis related genes, SERPINE1, MMP-2, and IL-6. Of note, adipose stromal/ stem cells have the capability to differentiate along the adipocyte and other lineage pathways. Nevertheless, these investigators concluded that leptin from adipose stromal/stem cells could contribute to the aggressiveness of breast cancer in obese women [11]. In a similar way, migration and colonization patterns of MDA-MB-231 and MCF-7 breast cancer cells were studied in co-culture with cancellous or spongy bone tissue fragments. The results showed that breast cancer cells migrated to human bone tissue-conditioned medium in association with increasing levels of leptin and IL-1β, and colonized the bone marrow adipose tissue compartment of cultured fragments [149].

Leptin has been demonstrated to influence numerous biological elements such as signal transduction proteins like JAK and PI3K, cellular receptors such as epidermal growth factor receptor (EGFR) and androgen receptor, and the enzymes aromatase and cytochrome P450 1B1 (CYP1B1): all of these biomolecules could be associated with neoplastic process [10,137]. Perera et al. identified a number of leptin-regulated genes in MCF-7 cells by using a microarray system; and observed that leptin up-regulated the expression of CTGF and the anti-apoptotic genes Bcl-2 and survivin, and also reduced the expression of apoptotic genes [150]. Furthermore, experiments demonstrated a bidirectional crosstalk between leptin and IGF-I signalling that transactivated EGFR and promoted the migration of breast cancer cells [151]. Overall, the results of different clinical, *in vivo* and *in vitro* studies have suggested that leptin could contribute to the aggressiveness of breast cancer.

7. Tumors of other organs

Leptin has been shown to stimulate the migration of human prostate cancer and chondrosarcoma cells [152,153]. In both human prostate cancer and chondrosarcoma cells, one of the underlying mechanisms of leptin-influenced migration was transcriptional up-regulation of αvβ3 integrin expression through Ob-R and activation of associated signaling pathways such as PI3K, AKT, and nuclear factor-κB (NF-κB). Of note, integrins are cell adhesion molecules that connect the cytoskeleton with ECM/other cells. The integrin αvβ3 or vitronectin receptor is linked to many biological processes such as angiogenesis and

tumor metastasis [154]. It may be worth mentioning that obesity has not been shown to be involved in prostate cancer development per se. However, obesity is associated with more aggressive prostate cancer, and this could be mediated by leptin [155].

In a study on patients with renal cell carcinoma, it was observed that serum leptin levels were significantly higher in those with venous invasion. There were significant associations between high Ob-R expression in tumor tissue and the presence of venous invasion, tumor histology, and lymph node metastasis. Patients with higher serum leptin had significantly shorter progression-free survival than patients with lower levels [156].

Investigators have observed that leptin levels were significantly higher in melanoma patients with positive sentinel nodes [157]. Their follow-up analysis revealed more aggressive disease in diabetic patients. It may be worth mentioning that melanoma is a highly aggressive form of skin cancer and the condition is frequently fatal if the tumor is not diagnosed early. An excess of adipose tissue may influence the development of melanoma and disease progression by different obesity-linked phenomena such as type 2 diabetes and enhanced release of leptin [158–161]. In an interesting study on melanoma, RNA was extracted from nodal tissue of 13 tumor-negative sentinel nodes, 10 tumor-positive sentinel nodes (micro-metastases<2 mm), and 11 tumor-negative non-sentinel nodes [162]. Expression levels of leptin were significantly higher in tumor-positive as compared with tumor-negative sentinel nodes.

In a study on lung cancer patients that included both primary and metastatic bone lesions, leptin was present at higher levels in samples associated with diagnosis of lung cancer bone metastatic tissue than lung cancer tissue [14]. In another study, the authors determined the association between the expression level of leptin and the survival of patients suffering from NSCLC [163]. They observed that leptin expression in NSCLC tissue affected the survival time of patients. Therefore it has been suggested that the expression of leptin may be an independent prognostic factor for NSCLC. On the other hand, in a syngeneic murine model study of Lewis lung carcinoma, consumption of a high-fat diet significantly increased plasma leptin in male C57BL/6 mice and resulted in a two-fold increase in the number of lung metastases along with a 50% increase in tumor volume compared to mice fed a low fat diet [164]. These studies have clearly shown that higher concentrations of leptin were associated with tumor progression and this adipokine could serve as an additional parameter for prognosis.

8. Leptin in tumor progression

Deregulated cell proliferation is the key feature in all phases of cancer development. Uncontrolled cell proliferation associated with suppression of apoptosis, proper supply of oxygen and nutrients, and removal of metabolic wastes provide an appropriate condition to support disease progression from in situ neoplastic growth to tissue invasion and metastasis. Remarkably in normal and cancer cells, leptin has been shown to stimulate proliferation. In a study of normal uterine smooth muscle cells collected from myometrial biopsies, leptin induced cell proliferation at both low and high doses (6.25 and 50 ng/ml) [165]. In the same way, leptin was documented to stimulate the proliferation of normal intestinal epithelial cells

(IEC-6 cells) [166]. With regard to the proliferation of cancer cells, the pertinent information has been provided in Table 3 along with a brief etiologic account [116,167–180].

As mentioned above, both inhibition of apoptosis and formation of new blood vessels for adequate supply of oxygen with nutrients and removal of cellular wastes are the essential requirements for the progression of neoplastic disease. Anti-apoptotic effect of leptin in cancer is a frequent observation of many investigators [181–185]. Similarly, several reports have documented that leptin can facilitate the formation of new blood vessels or angiogenesis, along with biosynthesis of pro-angiogenic factors like VEGF [185–187]. It is worth mentioning that without angiogenesis usually a tumor cannot grow beyond 2 mm in diameter [188]. Nonetheless, the presence of vascular channels in the tumor microenvironment definitely promotes the process of metastasis.

Metastasis is a highly complex process, which leads to a number of hypotheses such as progression, transient compartment, and fusion models [189]. The progression model proposes the development of metastatic subpopulations or clone of mutated cells with metastatic potential within the primary tumor. It is known that tumor aneuploidy (chromosome number variation) and genetic heterogeneity/diversity can influence the disease behavior including metastatic capability [190–192]. Apart from genetic changes, epigenetic alterations such as DNA methylation and histone modifications could play a significant role in metastasis [193,194]. Accumulating evidence suggests that both genetic and epigenetic changes can occur in obesity [195–199]. Interestingly, epigenetic regulation of the leptin signaling pathway may cause leptin resistance in obesity [200]. It is noteworthy that an epigenetic phenomenon is the notion of the transient metastatic compartment model [189]. On the other hand, an important aspect of the genetic events is the role of metastasis suppressor genes in secondary tumors. Metastasis suppressors are molecules that inhibit metastasis formation without affecting primary tumor growth [201]. Functionally, leptin is associated with a number of metastasis suppressor molecules, e.g., caspase 8, CD44, kisspeptin, and thioredoxin interacting protein (TXNIP) [172,202–204].

For the development of secondary tumors, cancer cells undergo various steps from local invasion to distant metastasis. This process is involved with numerous intracellular signaling pathways and associated transduction molecules. Some of the well-studied signaling pathways/molecules include: MAPK/ERK, PI3K/AKT, STAT, TGF-β, and Wnt/β-Catenin [205]. Fascinatingly, many of these molecules are linked with the signal transduction pathways of leptin (Table 4 [14,108,109,113,118,120,129,142,144,148,152,153,206–222]).

A highly complex role is played by the tumor infiltrating immune cells, particularly macrophages. In general, studies have indicated that the presence of macrophages within the tumor microenvironment (i.e., TAMs) was associated with poor prognosis in cancer patients [223,224]. Often TAMs display M2 phenotype [225–227]. On the other hand, evidence suggests that obesity can favor M2 polarization [228,229]. Furthermore, leptin has been shown to influence M2 macrophages [148,230]. Nonetheless, macrophages are highly specialized cells having character of plasticity/adaptability in diverse microenvironments. Interestingly, many features of macrophages are also seen in metastatic cancer cells [231]. For instance, carcinomas of the lining epithelial cells can form a solid mass like

mesenchymal tissue. Of note, macrophages arise from the myeloid lineage that originates from mesenchymal cells (derivative of mesoderm). Moreover, many metastatic cancers express aerobic glycolysis (Warburg effect), which may occur in activated macrophages in chronic inflammatory lesions. Additionally, acquiring mesenchymal characteristics in the process of EMT happens during metastasis. Other interesting macrophage-like features of metastatic cells are: they can migrate and extravasate/infiltrate into the surrounding tissues, show phagocytic behavior, and release various pro-angiogenic molecules. All of these features have led to the proposition for the fusion model, which suggests the formation of fusion hybrids between cancer cells and macrophages [189,231]. So, there could be generation of fusion subclones with varying dissemination potentials.

In a recent report, Clawson and colleagues described macrophage-tumor cell fusions that were isolated from the blood of pancreatic ductal adenocarcinoma patients [232]. They observed that fusion cells expressed markers characteristic of pancreatic ductal adenocarcinoma and stem cells, as well as M2-polarized macrophages. In another study, M2 macrophages (derived from U937 cells) were fused with MCF-7 and MDA-MB-231 breast cancer cells [233]. The investigators found that the fusion hybrids had a more aggressive phenotype. It is worth noting that macrophages have the capacity to form fusion cells in physiologic and different pathologic circumstances. Multinucleated cells such as osteoclasts and foreign-body giant cells are thought to be generated by fusion of macrophages. Although the dichotomy of M1 and M2 macrophages is not based on very clear ideas, it has been suggested that M2 macrophages appear at the later phase of a lesion, they stimulate angiogenesis, synthesize ECM components and work towards resolution of inflammation [234]. Obviously, these properties are beneficial for the development of secondary tumors. Overall, excess adipose tissue is an attractive place for macrophages – because, obesity is considered as a state of low grade chronic inflammation. Like other inflammatory conditions, macrophages may influence the disease process or contribute to disease progression in obesity.

9. Conclusions

Leptin could influence cancer cells through numerous phenomena, e.g., inflammation and oxidative stress, cell proliferation, inhibition of apoptosis, angiogenesis, immune modulation, etc. In obesity, cancer cells constantly receive growth promoting stimuli in an environment where pro-inflammatory cytokines or adipokines in particular leptin dominate. Obviously, manipulation of this environment surrounding the neoplastic growth or tumor microenvironment is a challenging task. However, already some potential directions have been proposed such as increased biosynthesis of anti-inflammatory substances like adiponectin, leptin antagonists, and other pharmaceutical agents like metformin.

Leptin antagonists can be divided into 2 major groups: leptin analogs and antibodies against leptin or Ob-R. Leptin interacts with Ob-R through three different binding sites: I-III. Different analogs or muteins are formed by changing the amino acid sequence such as LDFI (in binding site I), LPrA2 (II), and Allo-aca (III). In general, administration of antagonists has been reported to cause weight gain. It is anticipated that orexigenic neuropeptide Y (NPY) will dominate in the hypothalamus in the absence of appropriate leptin signaling.

However, some adverse effects are serious and could be dangerous, e.g., insulin resistance, increased bone mass, disrupted locomotor activity, depressive behavior, and peripheral inflammation [235,236]. All these studies were conducted in experimental animals and for a relatively short duration. In real clinical situations, the treatment is expected to continue for a considerable time period and thus the adverse effects could be substantial. Furthermore, malignancies are often associated with comorbidities, which are common in patients with colon, breast, and lung cancers [237]. Consequently complications in such patients may aggravate in additional disorders such as obesity, insulin resistance or any inflammatory conditions. Development of methods for the local delivery of leptin antagonists at the tumor site is definitely a superior alternative.

Acknowledgments

Abbreviations

AKT	protein kinase B (a serine/threonine kinase)
CAF	cancer-associated fibroblast
CTGF	connective tissue growth factor
ECM	extracellular matrix
EGFR	epidermal growth factor receptor
EMT	epithelial-mesenchymal transition
ER	estrogen receptor
ERK	extracellular signal-regulated kinase
HIF	hypoxia-inducible factor
ICAM	intercellular adhesion molecule
IGF	insulin-like growth factor
IL	interleukin
JAK	Janus kinase
MCP-1	monocyte chemoattractant protein-1
MMP	matrix metalloproteinase
NSCLC	non-small-cell lung cancer
Ob-R	leptin receptor
PI3K	phosphatidylinositol 3-kinase

STAT	signal transducer and activator of transcription
TAM	tumor-associated macrophage
TGF	transforming growth factor
TNF	tumor necrosis factor
TNM	tumor-lymph node-distant metastasis
VEGF	vascular endothelial growth factor

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Biographies

Amitabha Ray

Amitabha Ray completed his graduation in medicine (M.B.B.S.) from University of Calcutta, postgraduation (M.D.) from B.H.U., Varanasi, and Ph.D. from J.M.I., New Delhi, India. He pursued his postdoctoral research in Dr. Cleary's laboratory at the Hormel Institute, University of Minnesota. Currently, he is working with LECOM at Seton Hill University, Greensburg, PA, as an Associate Professor.

Margot P. Cleary

Dr. Margot P. Cleary is a professor at the Hormel Institute, University of Minnesota. She received an undergraduate degree in Chemistry from Regis College in Weston, MA and then did graduate work at Columbia University at the Institute of Human Nutrition earning MS, MPhil and Ph.D. degrees. Dr. Cleary's laboratory was one of the first to study the interactions of body weight with the development of breast and prostate cancers using preclinical models. In addition, she carried out extensive studies on the potential role of leptin as a growth factor linking obesity with breast cancer and proposed that the leptin to adiponectin ratio may be an important determinate in the development of some cancers.

In an interesting study, a leptin antagonist was demonstrated to stimulate appetite and weight gain in chronic kidney disease-associated cachexia in experimental animals [238]. The investigators also observed that the leptin antagonist normalized the expression of pro-inflammatory cytokines such as IL-6 and TNF-α. Cachexia in cancer is associated progressive wasting of body tissues, systemic inflammation, and poor prognosis. Leptin antagonists could be a promising inclusion in the therapy.

Highlights

1. The multifaceted action of leptin involves multiple cell types in the tumor microenvironment and intracellular signaling molecules; cooperation/ interactions between these biomolecules in obesity could accelerate the process of cancer cell migration and metastasis.

2. Approximately half of the cancer burden and two-fifths of cancer deaths are attributable to cancers of the digestive and reproductive systems: usually leptin's role is important in these cancers.

3. Leptin has been demonstrated to affect the prognosis of both adenocarcinomas and squamous cell carcinomas, although obesity is not a risk factor for squamous cell carcinomas.

4. Several cancer dissemination mechanisms such as the involvement of matrix metalloproteinases (MMPs), M2 macrophages, and epithelial-mesenchymal transition (EMT) have been documented to be linked with leptin.
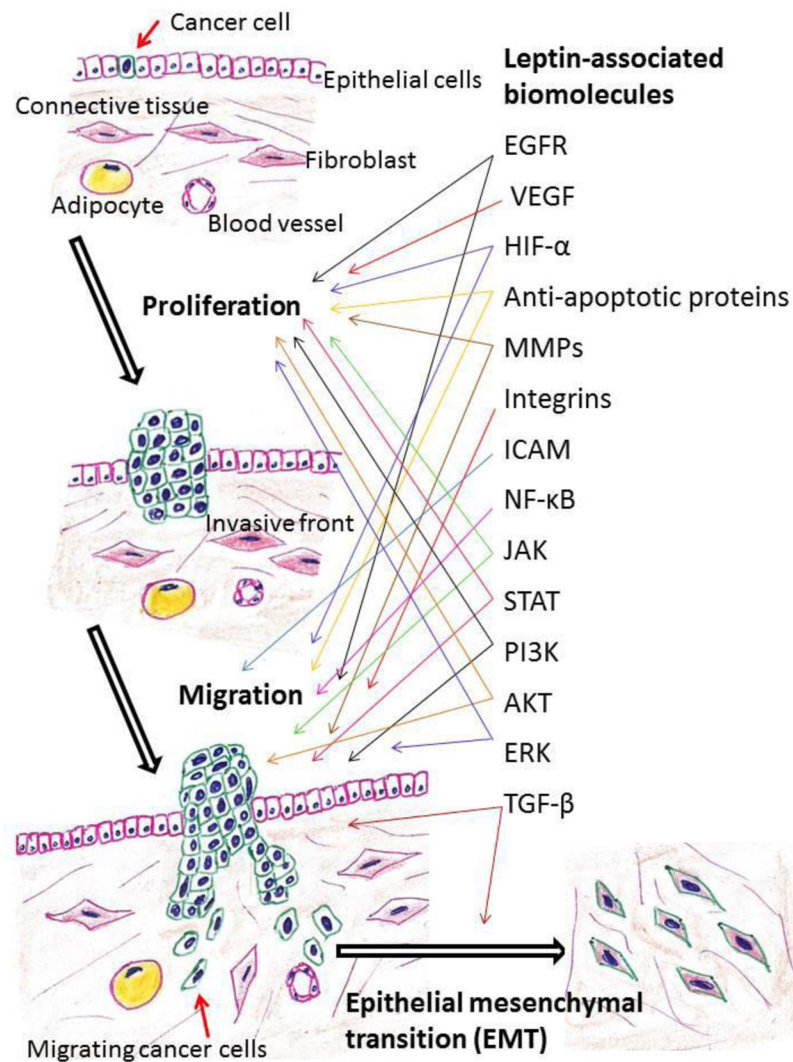
Fig. 1. Leptin-associated biomolecules that may promote cancer cell proliferation and migration
EGFR: Epidermal growth factor receptor, ERK: Extracellular signal-regulated kinases,
HIF-1α: Hypoxia-inducible factor-1 alpha, ICAM: Intercellular adhesion molecule, JAK:
Janus kinase, MMPs: Matrix metalloproteinases, NF-κB: Nuclear factor-kappa B, PI3K:
Phosphatidylinositol 3-kinase, STAT: Signal transducer and activator of transcription, TGF-
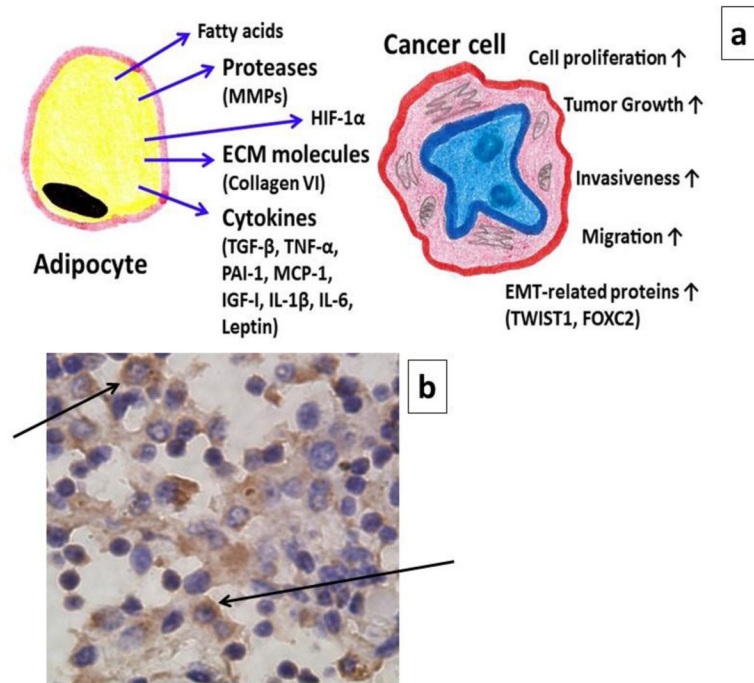β: Transforming growth factor-beta, VEGF: Vascular endothelial growth factor

Fig. 2. Adipocytes in tumor microenvironment

(a) A schematic diagram to show the influence of adipocytes on the progression of cancer.

(b) Immunohistochemical expression of leptin in tumor-adjacent adipose tissue, which was collected from the mammary fat pad of a CD-1 female mouse. In this xenograft model, T47D breast cancer cells were inoculated. The arrows indicate the presence of leptin in the cytoplasm of cells. (↑ - Increase)
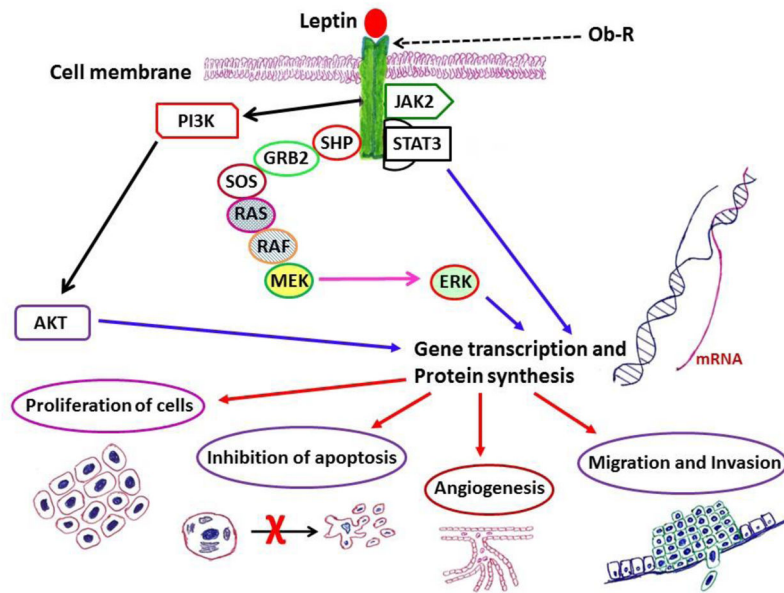
Fig. 3.
Leptin-related intracellular signaling molecules and their association with survival/anti-apoptotic and proliferative potential of cells.
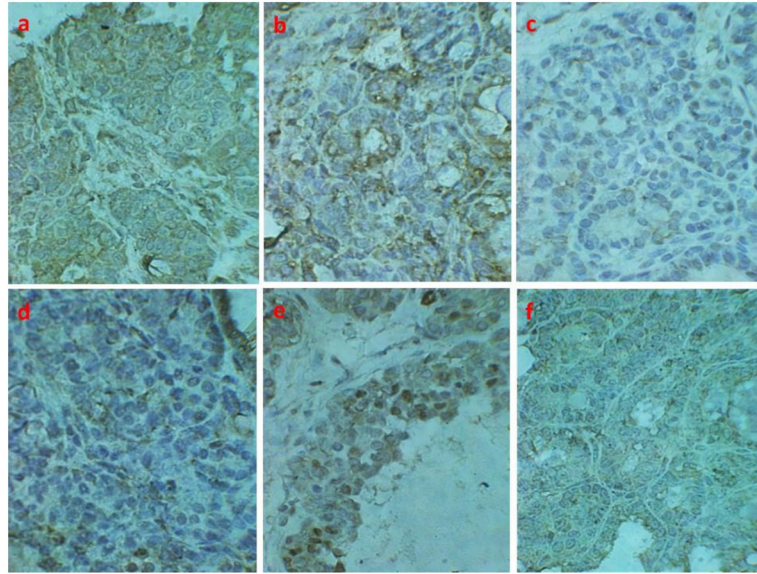
Fig. 4. Immunohistochemical expression of leptin receptor and relevant effector molecules in tissue sections from mammary tumors of TGFα mice

Expression of (a) Ob-Rb, (b) STAT3, (c) p-STAT3, (d) PI3K, (e) cell proliferation marker proliferating cell nuclear antigen (PCNA), and (f) anti-apoptotic protein Bcl-2. Presence of yellowish- to dark-brown color indicates positive staining.

Table 1

Summary of selected clinical studies that recorded a positive association between overexpression of leptin and/or its receptor proteins in tumor tissue and cancer cell invasion or metastasis

Site	Subjects	Findings	Investigators
Breast	76 invasive ductal carcinomas and 32 normal control samples.	Distant metastasis was detected in 21 (34%) of 61 Ob-R-positive tumors with leptin overexpression, but in none of the 15 tumors that lacked Ob-R expression or leptin overexpression.	Ishikawa et al. 2004 [104]
Colon	68 cases of colorectal carcinoma tissue, tumor adjacent tissue and normal colorectal tissue.	Expression of leptin and Ob-R in tumor was correlated with depth of bowel wall invasion, lymph node metastasis, and distant metastasis.	Liu et al. 2011 [117]
Colon	108 patients with colorectal carcinoma.	Leptin/Ob-R expression was significantly associated with TNM stage, lymph node metastasis, and distant metastasis.	Wang et al. 2012 [116]
Esophagus	122 cases of esophageal squamous cell carcinoma and 40 normal esophageal mucosa tissue.	Expression of leptin was significantly correlated with lymph node involvement and advanced tumor stage.	Duan et al. 2014 [102]
Lung	50 patients with lung cancer along with another 50 lung cancer patients with metastatic bone lesions.	Leptin was present at higher levels in bone metastasis cases.	Feng et al. 2013 [14]
Ovary	70 ovarian cancer cases.	Ob-Rb was highly overexpressed in metastases compared to primary tumors.	Kato et al. 2015 [16]
Pancreas	60 pancreatic cancer tissue specimens.	Expression of Ob-Rb was significantly stronger in patients with lymph node metastasis than in those without lymph node metastasis.	Fan et al. 2015 [90]
Stomach	343 cases of gastric carcinoma.	Ob-R expression was correlated with poor survival in 207 patients with advanced gastric cancer (muscle or deeper invasion), 139 of the Lauren diffuse group (poorly differentiated tumor cells scatter throughout the organ), and in 160 patients with lymph node metastasis.	Choi et al. 2015 [103]
Stomach	84 cases from primary gastric carcinoma.	Moderate to strong immnoreactivity for leptin was identified in 57% (n=48) cases. Leptin was positively correlated with lymph node metastasis and clinical stage.	Dong et al. 2014 [109]
Stomach	110 gastric cancer specimens and 96 normal gastric mucosa were analyzed.	Expression of leptin was associated with tumor invasion depth, lymph node metastasis, and expression of HER2 and VEGF.	Geng et al. 2012 [106]
Stomach	207 gastric carcinomas (100 early and 107 advanced carcinomas)	Expression levels of both leptin and Ob-R tended to increase as the depth of tumor invasion or TNM stage increased.	Ishikawa et al. 2006 [107]
Stomach	61 gastric cancer specimens.	Leptin expression was significantly associated with lymph node metastasis and higher stage.	Zhao et al. 2007 [105]
Thyroid	49 papillary thyroid carcinomas.	Coexpression of leptin and Ob-R in primary neoplasms had greater incidence of lymph node metastasis.	Cheng et al. 2010 [95]
Thyroid	93 cases with papillary thyroid carcinoma and 25 cases with medullary thyroid carcinoma.	For papillary thyroid carcinoma, expression of leptin and Ob-R was significantly correlated with lymph node metastasis and advanced stage. For medullary thyroid carcinoma, Ob-R was significantly correlated with lymph node metastasis and advanced stage.	Fan and Li 2015 [93]

Table 2

Relevant leptin antagonists that have been exhibited to prevent aggressive tumor behavior

Authors/Tissue	Antagonist used	Experimental design	Major observations
Bain et al. 2014 [111] Gastroesophageal adenocarcinomas (clinical samples) as well as in vitro experiments on gastric cancer cells and esophageal cancer cells.	Recombinant super human leptin antagonist (SHLA), which is a polypeptide chain containing 146 amino acids.	Cisplatin-resistant gastric cancer cell-line AGS Cis5 and esophageal adenocarcinoma cell-line OE33 were used. Originally, AGS cell-line was derived from a gastric adenocarcinoma of a Caucasian female. In a 96-well plate, cells were grown. Cisplatin and/or SHLA were added. Following incubation, MTT cell proliferation assay was performed.	Leptin antagonist SHLA increased the sensitivity of AGS Cis5 and OE33 cell-lines to cisplatin. SHLA inhibited the growth of OE33 cells when given alone. In both AGS Cis5 and OE33, SHLA inhibited leptin-induced cell proliferation.
Trevellin et al. 2015 [101] Esophageal adenocarcinoma patients, and in vitro study on esophageal adenocarcinoma cells.	Super human leptin antagonist (SHLA).	Treatment of OE33 cells with conditioned media (CM) collected from cultured biopsies of adipose tissue and peritumoral adipose tissue of patients with lymph node metastasis.	After treatment with CM, mRNA levels of two key EMT regulator genes, alpha-smooth muscle actin (α-SMA) and E-cadherin, were increased. However, SHLA diminished the mRNA expression of α-SMA and E-cadherin.
Catalano et al. 2015 [112] In vitro and in vivo experimental models using ERα-positive (MCF-7) and -negative (SK-BR-3) breast cancer cells.	Pegylated LDFI (LDFI-PEG): polyethylene glycol (PEG)-attached to tetrapeptide that mimics the sequence of leptin binding site I. Leptin interacts with Ob-R through 3 different binding sites: I–III. Site I is crucial for the formation of an active leptin–Ob-R complex and in its subsequent activation. Amino acids 39–42 (Leu-Asp-Phe-Ile-LDFI) were shown to contribute to leptin binding site I.	Injected SK-BR-3 breast cancer cells into the intrascapular region of female nude mice (nu/nu Swiss) and followed tumor growth after administration of LDFI-PEG.	After LDFI-PEG treatment, tumor volumes continued to reduce over control for the duration of experiment. At the end of treatment (28 days) it was observed that PEG-LDFI induced a significant tumor growth inhibition compared to vehicle-treated mice. Sections of tumors from PEG-LDFI-treated mice exhibited a reduction in the expression of proliferation marker Ki-67, and phosphorylated levels of STAT3, MAPK and AKT than controls.

AKT: Protein kinase B/a serine/threonine kinase, EMT: Epithelial-mesenchymal transition, MAPK: Mitogen-activated protein kinase, STAT3: Signal transducer and activator of transcription 3

Table 3

Possible mechanisms of leptin-induced cancer cell proliferation

Investigators and Cancer types	Involved intracellular signaling pathways/Mechanisms	Principal mediating factors/phenomena
Blanquer-Rosselló Mdel et al. 2016 [167] MCF-7 breast cancer cells	Lipid metabolism pathways, AMPK	Leptin favored the use of glucose for biosynthesis and lipids for energy production
Chen et al. 2013 [168] OVCAR-3 ovarian cancer cells	PI3K/AKT and MEK/ERK1/2 pathways	Leptin enhanced the expression of regulators of cell proliferation and apoptosis inhibition, cyclin D1 and Mcl-1
Chin et al. 2017 [169] SKOV-3 and OVCAR-3 ovarian cancer cells	PI3K, STAT3, ERK1/2	Leptin increased FSH, stimulated ERα and the expression of ERα-responsive genes
Dubois et al. 2014 [170] MCF-7 and T47D breast cancer cells	Estrogenic pathway, leptin autocrine/paracrine signaling loop	Leptin stimulated proliferation, overexpression of leptin, Ob-R, ER, and aromatase
Habib et al. 2015 [171] PC-3 prostate cancer cells	Estrogenic pathway	Leptin increased the expression of ERα, aromatase and CYP1B1, and decreased the expression of ERβ and COMT
Harbuzariu et al. 2017 [172] BxPC-3, MiaPaCa-2, Panc-1, AsPC-1 pancreatic cancer cells	Notch signaling pathway, leptin autocrine/paracrine signaling loop	Leptin increased cell cycle progression and proliferation
Kim et al. 2017 [173] MCF-7 breast cancer cells	STAT3, AKT, ERK, JNK, PKA, cAMP	Leptin increased COX-2 expression and PGE2 production, increased aromatase expression
Liu et al. 2013 [174] Ishikawa endometrial cancer cells	Estrogenic pathway	Leptin stimulated cell proliferation via enhancing aromatase expression and estradiol synthesis
Nepal et al. 2015 [175] HepG2 hepatoma and MCF-7 breast cancer cells	p53/FoxO3A axis	Leptin augmented the expression of autophagy-related genes, including beclin-1, Atg5 and LC3 II, which caused increase in cell number and suppression of apoptosis
Ptak et al. 2013 [176] OVCAR-3 ovarian cancer cells	Increase in cyclin D1, cyclin A2, and a decrease in p21WAF1/CIP1, Bad, TNFR1, and caspase 6	Leptin promoted cell proliferation and downregulated apoptotic pathway
Qian et al. 2015 [177] MCF-7 and tamoxifen-resistant cells	ERK1/2, STAT3	Leptin enhanced proliferation, and CCND1 (cyclin D1) gene transcription by inducing the binding of ERα to the promoter of CCND1 gene
Shouman et al. 2016 [178] MCF-7 breast cancer cells	Estrogenic pathway	Leptin increased CYP1B1 expression and DNA adducts, and diminished COMT protein expression
Wang et al. 2012 [116] HCT-116 colon cancer cells	PI3K/AKT/mTOR pathway	Leptin stimulated proliferation and inhibited apoptosis
Xu et al. 2013 [179] SKOV3 and A2780 ovarian cancer cells	STAT5	Leptin promoted cell proliferation in cooperation with microRNA-182 and microRNA-96 (targeting FoxO3)
Yoon et al. 2014 [180] LS174T, HCT-116 and CaCo-2 colon cancer cells	JAK and ERK signaling pathways	Leptin increased the number of cells

AKT: v-Akt murine thymoma viral oncogene or protein kinase B (a serine/threonine kinase), AMPK: AMP-activated protein kinase, Atg5: autophagy protein 5, cAMP: cyclic AMP, COMT: catechol-o-methyltransferase, COX-2: cyclooxygenase-2, CYP1B1: cytochrome P450 1B1, ER: estrogen receptor, ERK: extracellular signal-regulated kinase, FoxO3A: forkhead box O3, FSH: follicle-stimulating hormone, JAK: Janus kinase, JNK: c-Jun N-terminal kinase, LC3: light chain 3 (microtubule-associated), Mcl-1: myeloid cell leukemia 1, MEK: mitogen-activated protein kinase kinase, mTOR: mechanistic/mammalian target of rapamycin, Ob-R: leptin receptor, p21WAF1/CIP1: cyclin-dependent kinase inhibitor, PGE2: prostaglandin E2, PI3K: phosphatidylinositol-3-kinase, PKA: protein kinase A, STAT3: signal transducer and activator of transcription 3, TNFR1: tumor necrosis factor receptor 1

Table 4

Proposed mechanisms of cancer cell migration and metastasis under the influence of leptin

Investigators and Cancer types	Nature of neoplastic progression	Mechanisms or involved intracellular signaling pathways	Observed phenomena
Cao et al. 2016 [148] MCF-7 and MDA-MB-231 breast cancer cells, and xenograft model	Migration and metastasis	p38 MAPK and ERK1/2	Leptin-induced tumor-associated M2 macrophage-derived IL-8 promoted the migration and invasion/ metastasis of cancer cells in both in vitro and xenograft model
Cheng et al. 2011 [129] K1 and B-CPAP papillary thyroid cancer cells	Migration	AKT and ERK pathways	Leptin enhanced the migratory activity
Ding et al. 2016 [206] HepG2 hepatocellular carcinoma and MCF-7 breast cancer cells	Migration	STAT3, ERK1/2, and AKT	APPL1 positively mediated leptin signaling and promoted leptin-induced proliferation and migration of cancer cells
Dong et al. 2013 [108] AGS, MKN-28 and MKN-45 gastric cancer cells	Migration	AKT and ERK1/2	Leptin upregulated MT1-MMP expression and enhanced the interaction of MT1-MMP with KIF1B, which contributed to cancer cell invasion
Dong et al. 2014 [109] AGS and MKN-45 gastric cancer cells	Migration	Rho/ROCK pathway	Leptin enhanced cancer cell migration by upregulating ICAM-1
Fan et al. 2015 [120] PANC-1 and AsPC-1 pancreatic cancer cells, and clinical samples	Migration and metastasis	JAK2/STAT3 pathway	Leptin upregulated the expression of MMP-13
Fava et al. 2008 [207] Intrahepatic cholangiocarcinoma HuH-28 cells	Migration	STAT3 and ERK1/2	Leptin increased the growth and migration of cancer cells
Feng et al. 2013 [14] A549 lung cancer cells	Metastasis	TGF-β	Leptin promoted the metastasis by inducing EMT in a TGF-β-dependent manner
Frankenberry et al. 2004 [208] androgen-independent DU145 and PC-3 prostate cancer cells	Migration	MAPK and PI3K (mainly)	Leptin enhanced cancer cell migration, and induced expression of VEGF, TGF-β1, and bFGF
Ghasemi et al. 2017 [209] OVCAR3, SKOV3 and CaoV-3 ovarian cancer cells	Migration	RhoA/ROCK, PI3K/AKT, JAK/STAT pathways and NF-κB activation	Leptin induced cancer cell invasion via upregulating uPA
Ghasemi et al. 2017 [210] SKOV3 and OVCAR3 ovarian cancer cells	Migration	ERK and JNK pathways	Leptin regulated cancer cell invasion by promoting MMP-7 expression
Guo and Gonzalez-Perez 2011 [142] Mouse 4T1, EMT6 and MMT breast cancer cells	Migration	JAK2/STAT3, MAPK, PI3K/mTOR, p38 MAPK and JNK signaling pathways	Leptin upregulated cell proliferation/migration and pro-angiogenic factors Notch, IL-1 and VEGF/VEGFR-2
Huang et al. 2011 [152] PC-3, DU145, and LnCaP prostate cancer cells	Migration	IRS-1, PI3K, AKT, and NF-κB	Leptin increased the migration of cancer cells. Moreover, leptin upregulated αvβ3 integrin expression
Huang et al. 2017 [211] MCF-7 and T47D breast cancer cells	Migration	PI3K/AKT/SREBP2 pathway	Leptin enhanced the proliferation, migration and invasion of cancer cells via ACAT2 upregulation
Knight et al. 2011 [212] MCF-7 and MDA-MB-231 breast cancer cells	Migration	EGFR, Notch1, and survivin	Leptin increased the migration potential of cancer cells

Investigators and Cancer types	Nature of neoplastic progression	Mechanisms or involved intracellular signaling pathways	Observed phenomena
Li et al. 2016 [144] THP1 human monocytes, and MCF-7, SK-BR-3 and MDA-MB-231 breast cancer cells, and xenograft model	Migration and metastasis	NF-κB signaling in TAMs, and PI3K-AKT/ATF-2 signaling in cancer cells	Leptin stimulated IL-18 expression in both TAMs and breast cancer cells
Martín et al. 2017 [213] Human astrocytoma 1321N1 cells	Migration	EGFR, and ERK, AKT/mTOR pathways	Leptin and sPLA2-IIA increased growth and migration in these cells
Mendonsa et al. 2015 [214] Murine Panc02 and human Panc1 pancreatic cancer cells	Migration	PI3K/AKT pathway	Leptin increased migration of cancer cells
Mishra et al. 2017 [215] Breast epithelial and cancer cells: MCF10A, MCF10AT1, MCF-7 and MDA-MB-231	Metastasis	TGF-β1 pathway	Leptin-mediated changes represented EMT and a cancer stem cell-like phenotype
Noda et al. 2015 [216] LNCaP, DU145 and PC-3 prostate cancer cells	Migration	PI3K/AKT	Leptin increased the cell proliferation, migration, and invasion. Leptin also increased the phosphorylation of FOXO1, the expression of cyclin D1, and decreased the expression of p21 protein
Ratke et al. 2010 [118] SW480, SW620, and HCT-116 colon cancer cells	Migration	JAK, STAT3, Src kinase, FAK, PI3K, PKCδ	Leptin enhanced the migratory activity of cancer cells
Saxena et al. 2007 [217] HepG2 and Huh7 hepatocellular carcinoma cells	Migration	JAK/STAT3, ERK, and PI3K	Leptin promoted cancer cell growth, invasiveness, and migration
Sobrinho Santos et al. 2017 [218] SCC-9 and SCC-4 oral squamous cell cancer cells, and clinical samples	Migration	E-cadherin, Col1A1, MMP-2 and -9, mir-210 and HIF-1α	Leptin favored higher cell proliferation, migration, and reduced apoptosis. Furthermore, leptin decreased caspase-3 mRNA expression
Wang et al. 2013 [219] MCF-7 breast cancer cells	Migration	JAK/STAT and PI3K/AKT signaling pathways	Leptin promoted migration and invasion. Leptin also increased the expression of MMP-9 and TGF-β
Wei et al. 2016 [220] MCF-7, SK-BR-3, and MDA-MB-468 breast cancer cells	Migration and metastasis	PI3K/AKT signaling pathway	Leptin promoted EMT in breast cancer cells via upregulation of PKM2
Yang et al. 2009 [153] JJ012 and SW1353 chondrosarcoma cells	Migration	IRS-1, PI3K, AKT and NF-κB	Leptin increased migration and expression of αvβ3 integrin in human chondrosarcoma cells
Yeh et al. 2009 [221] C6 glioma cells	Migration	p38 MAPK and NF-κB pathways	Leptin enhanced the migration and invasion of glioma cells. Leptin also upregulated the expression of MMP-13
Yuan et al. 2014 [222] MCF-7 breast cancer cells	Migration	ERK pathway	Leptin increased the proliferation and migration of cancer cells
Zou et al. 2016 [113] Gallbladder cancer cell subline GBC-SD, xenograft model, and clinical samples	Migration	JAK2/STAT3/SOCS3	Leptin promoted the proliferation, migration and invasion of cancer cells. Leptin upregulated VEGF-C and VEGF-D levels and activated MMP-3 and MMP-9

ACAT2: acetyl-CoA acetyltransferase 2, AKT: v-Akt murine thymoma viral oncogene or protein kinase B (a serine/threonine kinase), APPL1: adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 (involved in the regulation of cell proliferation), ATF-2: activating transcription factor 2, bFGF: basic fibroblast growth factor, Col1A1: collagen type I alpha 1, EGFR: epidermal growth factor receptor, EMT: epithelial-mesenchymal transition, ERK: extracellular signal-regulated kinase, FAK: focal adhesion kinase, FOXO1: forkhead box O1, HIF-1α: hypoxia-inducible factor 1α, ICAM-1: Intercellular adhesion molecule-1, IL: interleukin, IRS: insulin receptor substrate, JAK: Janus kinase, JNK: c-Jun N-terminal kinase, KIF1B: kinesin family member 1B (involved in the transport of materials), MAPK: mitogen-activated protein kinase, mir-210: mir-210 microRNA (predominant hypoxia-responsive miRNA), MMP: matrix metalloproteinase, MT1-MMP: membrane

type 1-matrix metalloproteinase, mTOR: mechanistic/mammalian target of rapamycin, NF-κB: nuclear factor-kappa B, PI3K: phosphatidylinositol-3-kinase, PKC: protein kinase C, PKM2: pyruvate kinase M2 isoform, Rho: G protein under the Ras homologue gene family, RhoA: Rho family small GTPase-member A, ROCK: Rho-associated coiled-coil-forming protein kinase, SOCS3: suppressor of cytokine signaling-3, sPLA2-IIA: secreted phospholipase A2-IIA, Src: avian sarcoma viral oncogene homolog, SREBP2: sterol regulatory element-binding protein 2, STAT3: signal transducer and activator of transcription 3, TAM: tumor-associated macrophage, TGF-β: transforming growth factor beta, uPA: urokinase plasminogen activator, VEGF: vascular endothelial growth factor, VEGFR-2: vascular endothelial growth factor receptor 2.