
Review

Roles of Leptin in Cancer Progression

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Abstract – Growing evidence suggests a prominent role for leptin in human cancer progression. The intricate pattern of leptin cross-talk with other associated signaling pathways is a critical area of research that will ultimately contribute to comprehending the role of leptin in cancer progression. This review summarizes a portion of the current understanding of leptin signaling, with a critical focus on its contribution to tumor cell invasion and metastasis. Five topics are addressed in this review: (1) Leptin receptor, (2) Leptin signaling, (3) Leptin and cancer, and (4) Leptin and tumor invasion. Due to the complex cellular effects of leptin, a more precise understanding of leptin signaling pathways must still be elucidated. Leptin is clearly a major factor for stimulating tumor progression through a complex spectrum of interplay and cross-talk among various signaling molecules. An understanding of the role of leptin in invasion and metastasis will provide valuable information for establishing strategies to modulate leptin signaling, which should be a high priority for the development of anti-cancer therapeutics.

Keywords: Lepin, Breast cancer, Cancer progression, Invasion, Metastasis, Leptin signaling

INTRODUCTION

Leptin, a product of the obese (ob) gene, is a 16 kD circulating peptide hormone which functions as a regulator of food intake and energy expenditure via hypothalamic-mediated effects (Zhang *et al.*, 1994; Schwartz *et al.*, 1999). Leptin is mainly synthesized and secreted by adipocytes; alternative sources are the stomach, placenta and skeletal muscle (Tartaglia *et al.*, 1995). Leptin exerts its actions by binding to the extracellular domain of transmembrane leptin receptors, ObRs (Tartaglia, 1997; White *et al.*, 1997; Tsuchiya *et al.*, 1999; Hino *et al.*, 2000; Mix *et al.*, 2000). Leptin receptors are recognized in brain, placenta, pancreas, adrenal gland, hematopoietic cells, liver, lung and heart (Ishikawa *et al.*, 2004). The isoforms of leptin receptors contain diverse structures and show different effects when they are bound by leptin (White and Tartaglia, 1996).

Sex hormones, estrogen and testosterone differentially modulate expression of leptin: estrogen enhances leptin

levels (Casabiell *et al.*, 1998; Castracane *et al.*, 1998) while testosterone down-regulates leptin levels (Blum *et al.*, 1997; Elbers *et al.*, 1997; Jockenhovel *et al.*, 1997). Leptin synthesis in adipocytes is affected by various humoral factors related to neoplastic processes. Such factors influencing leptin synthesis include insulin (Cusin *et al.*, 1995; Leroy *et al.*, 1996), glucocorticoids (De Vos *et al.*, 1995; Dagogo-Jack *et al.*, 1997), tumor necrosis factor alpha (TNF- α) (Zhang *et al.*, 2000), prostaglandins (Fain *et al.*, 2000) and reproductive hormones (Machinal-Quelin *et al.*, 2002).

Leptin plays an intricate role in the process of carcinogenesis. It is known that leptin can elevate cell proliferation, cell transformation, aromatase expression, invasion and cell growth. Additionally, studies show that leptin may reduce apoptosis in cancer cells. Binding of leptin to its receptor stimulates intracellular signaling pathways that induce cell growth, and invasion and lead to cancer formation (Garofalo and Surmacz, 2006). Studies have demonstrated that leptin may control cell proliferation in normal and malignant tissues (Tsuchiya *et al.*, 1999; Hino *et al.*, 2000; Mix *et al.*, 2000). Leptin may stimulate the proliferation of normal and hematopoietic cells (Gainsford *et al.*, 1996), non-transformed epithelial cells (Glasow *et al.*,

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1999), and human colon cancer cells (Liu *et al.*, 2001). Furthermore, leptin enhances the invasiveness of pre-malignant colon and kidney epithelial cells *in vitro* (Attoub *et al.*, 2000) and it can control angiogenesis by regulating the activity of vascular endothelial growth factor (VEGF) (Gonzalez *et al.*, 2006).

The present review seeks to emphasize the current understanding of leptin's role in cancer progression, with a focus on breast cancer invasion and metastasis. The following topics are addressed in this review: (1) Leptin receptor - Leptin exerts its effects by binding to leptin receptors, ObRs and receptor isoforms have different intracellular domain structures. (2) Leptin signaling - Diverse signaling modules are responsible for a unique function of leptin. The leptin signaling pathway is essential for progress of carcinogenesis. This review condenses studies on the activation of leptin-mediated signaling molecules, and their roles in leptin-induced cellular responses, especially cell invasion. (3) Leptin and cancer - Many recent studies have demonstrated the role of leptin in carcinogenesis, and leptin has been shown to enhance cell proliferation, cell transformation, aromatase expression, invasion and cell growth *in vitro*. This review will also summarize studies on the contribution of leptin to carcinogenesis, with an emphasis on the function of leptin in human breast cancer cells *in vitro*, and the association between serum leptin levels and breast cancer *in vivo*. (4) Leptin and tumor invasion - Leptin's role in the increased production and secretion of estrogenic compounds, growth factors and angiogenic stimulators by excess fat tissue may be related to affects on tumor growth and cancer metastasis, especially in the case of breast cancer. Several studies on the relationship between leptin and tumor metastasis, and data suggesting a possible association between leptin and tumor invasion through activation of matrix metalloproteinases (MMPs), are also summarized in this review.

LEPTIN RECEPTOR

Leptin receptor, a member of the class I cytokine receptor family, has been found in the brain, placenta, pancreas, adrenal gland, hematopoietic cells, liver, lung and heart in humans (Ishikawa *et al.*, 2004). Currently, six isoforms of the leptin receptors (ObRa-ObRf) have been identified, and leptin receptor isoforms show both differences and similarities in their structures. Five leptin receptor isoforms (ObRa-ObRd and ObRf) have identical extracellular domains which are composed of 816 amino acids, two cytokine-like binding motifs (Trp-Ser-X-Ser-Trp), and a fibronectin type III domain (White and Tartaglia, 1996;

Tsuchiya *et al.*, 1999; Hino *et al.*, 2000; Mix *et al.*, 2000). However, intracellular domains of the leptin receptor isoforms are different. Two categories of the leptin receptor, long and short forms, show different lengths of amino acid chains. The ObRa, ObRc, ObRd and ObRf isoforms are short form receptors in which a short intracellular domain consists of 32-40 residues, and a transmembrane domain is composed of 23 amino acids. The ObRb is a long form receptor with a long intracellular domain composed of -306 amino acids (Hynes and Jones, 2001). It is reported that the ObRb is the only receptor that has full signaling potential (Fong *et al.*, 1998). Short forms of the leptin receptor, which are absent of intracellular domains for recruiting downstream effectors, display decreased or destroyed signaling capabilities (Hileman *et al.*, 2000). Although the exact role of the short forms of ObR is presently unclear, Hileman *et al.* (2000) evidenced that these ObRs function in intra- and transcellular leptin transport. Additionally, the ObRe isoform modulates circulating leptin levels but does not display a direct role in leptin signaling (Huang *et al.*, 2001). Before being bound by leptin, both long and short isoforms of the leptin receptor form homodimers, and when leptin binds to the extracellular membrane domain of the receptor in a 1:1 ratio, it induces activation of various signaling pathways which may contribute to carcinogenesis (Bjorbaek *et al.*, 1997, 2001; Devos *et al.*, 1997; Nakashima *et al.*, 1997; White and Tartaglia, 1999; Sweeney, 2002; Zabeau *et al.*, 2003).

Studies have demonstrated that leptin is required for normal mammary gland development in rodents and humans (Smith-Kirwin *et al.*, 1998; Hu *et al.*, 2002; Neville *et al.*, 2002). Immunohistochemistry has demonstrated expression of leptin and ObR in normal and cancer mammary epithelium (Ishikawa *et al.*, 2004), however, several studies have shown an involvement of leptin in mammary carcinogenesis (O'Brien *et al.*, 1999; Dieudonne *et al.*, 2002; Hu *et al.*, 2002; Laud *et al.*, 2002; Okumura *et al.*, 2002; Cleary *et al.*, 2003). A distinct over-expression of both leptin and ObR was shown in cancer tissue relative to non-cancer epithelium (Ishikawa *et al.*, 2004). In breast cancer, over-expression of both leptin and ObR is observed in human primary and metastatic breast cancer when compared with non-transformed mammary gland (Garofalo *et al.*, 2006). Additionally, evidence shows that in human breast cancer cell lines and breast malignant tumors, leptin and its receptor isoforms ObRa and ObRb, were expressed at the mRNA and/or protein level (O'Brien *et al.*, 1999). Further studies for elucidating the possible correlation between leptin receptors and cancer will contribute to understanding the role of leptin in cancer.

LEPTIN SIGNALING

The leptin signaling pathway has been summarized in several excellent reviews (Zabeau *et al.*, 2003; Garofalo and Surmacz, 2006; Cirillo *et al.*, 2008). Both long and short forms of the leptin receptor share the intracellular domain called "Box 1" which is a Janus-family tyrosine kinase family (JAK) binding domain composed of common 29 amino acid residues. Compared to the short form of the leptin receptor, the long form of the receptor contains a JAK binding site called "Box 2" motif, and possesses a binding site for the signal transducer and activators of transcription (STAT). Although the short form of leptin receptors lacks the STAT3 binding site, the ObRs are still able to recruit and activate JAK2 through Box 1.

Signaling of leptin is mediated by a tetrameric receptor/ligand complex (Devos *et al.*, 1997). Once leptin binds to its receptor, it induces activation of various signaling pathways including JAK/STAT, MAPK (ERK, p38, JNK), IRS1 and SOCS3 which lead to cell proliferation and survival (Cirillo *et al.*, 2008).

JAK/STAT pathway

The receptor/ligand complex resulting from leptin binding causes cross-phosphorylation and activates JAKs. Tyrosine residues of the cytosolic domain of the receptor (ObR1) are rapidly phosphorylated by the activated JAKs. Phosphorylated receptor sites provide recruiting sites for signaling molecules such as members of the STAT family. JAK-mediated phosphorylation of STATs causes homo- or hetero-dimerization, and phosphorylated STATs are released from the receptor. Subsequently, STATs translocate to the nucleus where they can control the transcriptional levels of target genes (Bjorbaek *et al.*, 1997; Devos *et al.*, 1997; White *et al.*, 1997).

One of three tyrosine residues on the leptin receptor, (Tyr¹¹³⁸), is included in the sequences of the STAT3 binding motif (Stahl *et al.*, 1995). Once the leptin receptor is bound by leptin, the Tyr¹¹³⁸ receptor residue is phosphorylated and STAT3 binds to the site via its Src homology (SH)2 domain (Baumann *et al.*, 1996; Ghilardi *et al.*, 1996; Zabeau *et al.*, 2003). Studies on the STAT3 proteins involved in cell growth, survival, proliferation and transformation were summarized by Takeda *et al.* (1997). The target genes of STAT3, including c-myc, cyclin D1, p21waf1, Bcl II and Bcl-xL, were shown to be involved in cell growth and proliferation (Yin *et al.*, 2004). The activation of STAT3 produces a major transducing pathway for the leptin-induced signal in breast carcinoma, and leptin showed maximal activation of STAT3 and mitogen-activated protein

kinase (MAPK) in MCF7 breast carcinoma cells (Dieudonne *et al.*, 2002).

SOCS3 Signaling

In addition to the JAK/STAT pathway, negatively regulated leptin signaling involves suppressor cytokine signaling (SOCS3) proteins. The SH2 domain of SOCS proteins blocks the leptin-mediated signaling pathway by interacting with two tyrosine-phosphorylated residues (Tyr⁹⁸⁵ and Tyr¹⁰⁷⁷) of the receptors or binding to the phosphorylated JAK proteins (Sweeney, 2002). In fact, binding to ObRb induces expression of SOCS3 mRNA in the hypothalamus and SOCS3 proteins then function as a negative feedback loop for the leptin signaling pathway by inhibiting leptin receptor-mediated signal transduction (Bjorbaek *et al.*, 1998). In addition, leptin induced expression of SOCS3 was shown to diminish leptin-induced tyrosine phosphorylation of JAK2 by binding to phosphorylated JAK proteins (Bjorbaek *et al.*, 1999). Two tyrosine residues of the ObRb modulate distinct pathways. Tyr⁹⁸⁵-mediated binding of SHP-2 results in recruitment of GRB-2 to tyrosine-phosphorylated SHP-2, which is required for activation of MAPK in the leptin-induced signaling pathway. Furthermore, this process controls the accumulation of c-fos mRNA. In contrast, Tyr¹¹³⁸-mediated STAT3 activation results in accumulation of the inhibitory SOCS3 protein (Banks *et al.*, 2000).

PI3K and IRS pathways

In the majority of obese humans, high levels of leptin and the activation of insulin signaling coincide. Binding of leptin to its receptor induces activation of JAK proteins, which then phosphorylate insulin receptor substrates 1 and 2 (IRS1 and 2) (Cohen *et al.*, 1996; Bjorbaek *et al.*, 1997; Attoub *et al.*, 2000). Subsequently, phosphorylated IRS1 and 2 bind to phosphatidylinositol 3-kinase (PI3K). Activated IRS1 and 2 then induce phosphorylation of Akt, which functions in cell growth and survival. Szanto and Kahn (2000) demonstrated that leptin and insulin control phosphorylation of the IRS1 and 2 and their interplay with Grb-2 and PI3K in hepatocyte cell lines. Additionally, leptin-induced PI3K stimulation is associated with control of phosphodiesterase (PDE)3B in pancreatic β cells (Zhao *et al.*, 1998).

Leptin-induced stimulation of phospholipase C (PLC)-gamma, protein kinase C (PKC), p38 kinase and nitric oxide (NO) has been demonstrated (Bjorbaek *et al.*, 1997; Sweeney, 2002; Zabeau *et al.*, 2003). The ObRI- induced activation triggers up-regulation of cell proliferation-associated genes such as c-fos, c-jun, junB, egr-1 and socs3, and the

stimulation of an angiogenic factor, (VEGF) (Sweeney, 2002; Zabeau *et al.*, 2003; Frankenberry *et al.*, 2004). Elucidation of the interaction among these signaling pathways, as well as the complex patterns of cross-talk with other signaling pathways, will provide valuable information on the role of leptin in cancer both *in vitro* and *in vivo*.

HER2

The relationship of leptin and the ErbB tyrosine kinase receptors has been elucidated (Normanno *et al.*, 2005, 2006a, b). The ErbB family contains four constituents; the epidermal growth factor receptors: (EGFR-ErbB1), ErbB2 (HER2/Neu), ErbB3 (HER3) and ErbB4 (HER4). Binding of ligand to these receptors induces stimulation of the ErbB tyrosine kinase receptors. Among the ErbB family members, ErbB2 is most directly related to the tumorigenesis of breast cancer (Ma *et al.*, 2003). Approximately 30% of breast tumors show HER2 over-expression, which is often associated with a more aggressive, metastatic phenotype and worse prognosis of breast cancer (Slamon *et al.*, 1987; Yarden, 2001). Stimulation of ErbB receptors activates various intracellular signaling pathways, including PI3K/AKT and the Ras/Raf/MEK/MAPK pathways, and consequently triggers proliferation and survival of breast cancer cells (Normanno *et al.*, 2005, 2006a,b).

In human embryonic kidney HEK 293T cells, both ObRa and ObRb leptin receptor isoforms stimulate ErbB2 activation through transphosphorylation, and this activation stimulates MAPK activation (Eisemberg *et al.*, 2004). Although ObRa shows lesser potential to activate MAPK than ObRb, its ability to increase ErbB2 transphosphorylation and MAPK activation is as potent as that of ObRb in HEK 293T cells (Eisemberg *et al.*, 2004). In ErbB2-ever-expressing SK-BR-3 human breast cancer cells, exogenous leptin causes phosphorylation of ErbB2 on its tyrosine residue, and triggers activation of ERK/MAPK, which is involved in a growth stimulatory effect (Soma *et al.*, 2008). Leptin induces ErbB2 phosphorylation in T47D ER-positive breast cancer cells which express moderate levels of ErbB2, (Soma *et al.*, 2008). Leptin stimulates phosphorylation of EGFR in esophageal (Ogunwobi *et al.*, 2006) and gastric cancer cells (Shida *et al.*, 2005). Saxena *et al.* (2008) reported a bi-directional crosstalk between leptin and IGF-1 signaling, which leads to EGFR transactivation, and this stimulation of EGFR accelerates invasion and migration of triple-negative breast cancer cells which are estrogen receptor negative (ER-), progesterone receptor negative (PR-), and Human Epidermal growth factor Receptor 2 negative (HER2-). Synergistic activation of ObRb and IGF-1 with Akt and MAPK stimulation is induced by com-

bined treatment of both leptin and IGF-1 (Saxena *et al.*, 2008). Treatment of leptin and IGF-1 on triple negative breast cancer cells synergistically transactivates EGFR through ObRb and IGF1-R, and ultimately leads to proliferation in triple negative breast cancer cells. Moreover, invasion was increased in triple negative breast cancer cells by combined treatment with leptin and IGF-1 (Saxena *et al.*, 2008).

VEGF/PI3K and MEK-1/MAPK signaling

Angiogenesis is one of the events in metastasis. Vascular endothelial growth factor (VEGF), is an important arbitrator of tumor-related angiogenesis, contributes to tumor growth, and the transport of nutrients to primary tumors, leading to metastasis (Folkman, 1995). Leptin was shown to modulate angiogenesis by controlling VEGF activity (Gonzalez *et al.*, 2006). An increase of leptin-induced expression of VEGF and its receptor (VEGFR-2), was demonstrated in 4T1 mouse mammary cancer cells, and blocking of leptin signaling slows down expression of VEGF, VEGFR-2, cyclin D1 and breast cancer growth *in vivo* (Gonzalez *et al.*, 2006). The study also demonstrated that leptin-induced up-regulation of VEGF and VEGFR-2 in 4T1 cells requires activation of JAK (Gonzalez *et al.*, 2006). Leptin-mediated stimulation of VEGF and VEGFR-2 also requires induction of PI3K and MEK-1/MAPK signaling. Down-regulation of leptin-induced secretion and VEGFR-2 expression is shown when PI3K is inhibited in 4T1 mouse mammary cancer cells. Similarly, leptin-induced stimulation of VEGFR-2 is totally blocked by inhibition of the MAPK pathway, and a partial decrease of leptin-induced VEGF activation is shown by blockade of the MAPK pathway (Gonzalez *et al.*, 2006).

LEPTIN AND CANCER

Mounting studies provide evidence for the contribution of leptin to carcinogenesis in various types of cancer. The stimulating effects of leptin on cancer cells are shown to be exerted through various signaling pathways.

Breast cancer

Recent studies demonstrate a correlation between leptin and estrogen receptor α (ER α). Co-expression of ER α and ObR has been found in malignant mammary tissue and breast cancer cell lines (Dieudonne *et al.*, 2002; Hu *et al.*, 2002; Laud *et al.*, 2002). Leptin regulates both estrogen synthesis and ER α activity in MCF-7 human breast cells by up-regulating aromatase gene expression (Catalano *et al.*, 2003). Leptin markedly down-regulated ER α ubiq-

uitination in MCF-7 cells treated with antiestrogen compounds, indicating the interference of leptin by antiestrogen *via* post-transcriptional control of ER α (Garofalo *et al.*, 2004). A higher level of ObRI was observed in ER α -positive MCF-7 and T47D breast cancer cells than in ER α -negative MDA-MB-231 and MDA-MB-435 breast cell lines (Garofalo *et al.*, 2004). Studies also indicate up-regulation of leptin-induced DNA synthesis and cell growth, mediated through various signaling pathways including the JAK/STAT, ERK1/2 and PKC- α pathways, in ER α -positive breast cancer cell lines (Dieudonne *et al.*, 2002; Hu *et al.*, 2002; Laud *et al.*, 2002; Okumura *et al.*, 2002; Catalano *et al.*, 2003; Somasundar *et al.*, 2003; Garofalo *et al.*, 2004; Yin *et al.*, 2004). Interestingly, not only leptin-induced cell growth but also leptin-stimulated cell transformation (anchorage-independent growth) is observed in ER α -positive T47D breast cancer cells, but not in normal breast epithelial cells (Hu *et al.*, 2002).

Leptin-mediated signaling pathways induce cell proliferation, cell transformation, aromatase expression and stabilization of ER α expression in breast cancer cells. Garofalo *et al.* (2004) showed that leptin stimulates the Akt/GSK3 survival pathway in breast cancer cells, and affects of leptin on cell cycle have been reported in breast cancer cells. Leptin induces cell cycle progression through enhancement of cdk2 and cyclin D1 levels (Okumura *et al.*, 2002), and inactivates a cell cycle inhibitor, pRb, by hyperphosphorylating that protein (Garofalo *et al.*, 2004). The mitogenic effects of leptin and leptin-dependent activation of STAT3 require steroid receptor coactivator (SRC-1), a member of the p160 family of steroid receptor modulators (Yin *et al.*, 2004). This finding suggests a crosstalk between the steroid receptor and leptin-induced transcriptional activation. Table I summarizes the effects of leptin on breast cancer cells.

It has been suggested that increased stimulation of es-

Table I. Effects of leptin on breast cancer cells

Effects of leptin	Cell model	References
Increased cell proliferation	Human breast cancer cell line: SK-BR3, MCF-7, T47D, MDA-MB-231, ZR75-1	Catalano <i>et al.</i> (2003); Dieudonne <i>et al.</i> (2002); Garofalo <i>et al.</i> (2004); Hu <i>et al.</i> (2002); Laud <i>et al.</i> (2002); Okumura <i>et al.</i> (2002); Soma <i>et al.</i> (2008); Somasundar <i>et al.</i> (2003); Yin <i>et al.</i> (2004);
Stimulated cell growth: interaction of SRC-1 and STAT3 signaling pathway	Human breast cancer cell line: MCF-7, T47D	Yin <i>et al.</i> (2004)
Increased cell transformation (anchorage-independent growth)	Human breast cancer cell line: T47D	Hu <i>et al.</i> (2002)
Activation of the ERK1/2, STAT3, Akt/GSK3, and PKC- α pathways	Human breast cancer cell line: MCF-7, T47D	Catalano <i>et al.</i> (2003); Dieudonne <i>et al.</i> (2002); Garofalo <i>et al.</i> (2004); Hu <i>et al.</i> (2002); Laud <i>et al.</i> (2002); Okumura <i>et al.</i> (2002); Yin <i>et al.</i> (2004)
In creased resistance of apoptosis: leptin/STAT3 upregulates survivin, a member of the inhibitor of apoptosis proteins (IAP) gene family, expression	Human breast cancer cell line: MCF-7	Jiang <i>et al.</i> (2007)
Increased AP-1 activation, upregulation of cdk2, cyclinD1, hyperphosphorylation of pRb Increased aromatase expression via, AP-1 dependent mechanism	Human breast cancer cell line: MCF-7, T47D	Hu <i>et al.</i> (2002); Okumura <i>et al.</i> (2002); Garofalo <i>et al.</i> (2004)
Induced expression of c-myc	Human breast cancer cell line: MCF-7	Yin <i>et al.</i> (2004)
Stablization of ER α expression	MCF-7 human breast cancer cells treated with the antiestrogen ICI 182, 780	Garofalo <i>et al.</i> (2004)
Increased HER2 phosphorylation	Breast cancer cell line: MCF-7, SK-BR3, BT-474, ZR-75-1	Fiorio <i>et al.</i> (2008); Soma <i>et al.</i> (2008)
Stimulated cell invasion: activation of JNK \rightarrow increased MMP-2 activity \rightarrow cell invasion	Human breast cancer cell line: MCF-1	McMurtry <i>et al.</i> (2009)
Increased cell invasion and migration crosstalk between leptin and IGF-1 signaling \rightarrow EGFR transactivation	Human breast cancer cell line: MCF-7, MDA-MB-231, MDA-MB-468, HCC-1806	Saxena <i>et al.</i> (2008)

AP-1: activator protein 1, EGFR: epidermal growth factor receptor, ER: estrogen receptor, ERK: extracellular signal-regulated kinases, MAPK: mitogen-activated protein kinase, GSK: glycogen synthase kinase, HER: human epidermal growth factor receptor, IGF: insulin like growth factor, JNK: Janus-activated kinase, MMP: matrix metalloproteinase, SRC: steroid receptor coactivator, STAT: signal transducer and activators of transcription, PKC: protein kinase C, pRb: phospho-retinoblastoma protein.

trogen in postmenopausal obese women might be the reason for their higher breast cancer risk (Vona-Davis and Rose, 2007). Adipose tissue is the only source of estrogen synthesis in postmenopausal obese women. Since aromatase activity and androstenedione production increase in obesity, the total estrogen amount is higher in obese women. The powerful connection between breast cancer and obesity has been studied for many years and distinct impacts of obesity on carcinogenesis in premenopausal and postmenopausal women are reported (Cleary and Maihle, 1997; Chlebowski *et al.*, 2002; Rose *et al.*, 2002; Stephenson and Rose, 2003; Rose *et al.*, 2004). While enhanced body weight in premenopausal women is inversely correlated with breast cancer risk, obesity in postmenopausal women is a substantial risk factor for breast cancer development (Petridou *et al.*, 2000; Rose *et al.*, 2004). A majority of the studies demonstrate a positive interaction between postmenopausal obesity and increased risk of developing ER/ PR-positive tumors, but not the occurrence of ER/PR-negative tumors (Potter *et al.*, 1995; Enger *et al.*, 2000; Huang and Li, 2000). A positive association has been reported between enhanced upper body obesity, defined by waist to- hip ratio (WHR) >0.8, and poor prognosis in postmenopausal women with ER-positive tumors (Borugian *et al.*, 2003). Harvie and Li, (2003) suggested that excess upper body obesity enhances breast cancer risk regardless of menopausal status. Interestingly, however, the Korean Breast Cancer Society has suggested that being underweight may be a critical factor for cancer recurrence and poor prognosis following breast cancer surgery (Moon *et al.*, 2009).

Despite evidence showing a role for leptin in breast cancer development, associations between circulating leptin and breast cancer or breast cancer risk with different menopausal status remain vague (Petridou *et al.*, 2000; Tessitore *et al.*, 2000; Han *et al.*, 2005) and need to be further investigated (Potter *et al.*, 1995; Cleary and Maihle, 1997; Enger *et al.*, 2000; Huang and Li, 2000; Chlebowski *et al.*, 2002; Rose *et al.*, 2002; Borugian *et al.*, 2003; Harvie *et al.*, 2003; Stephenson and Rose, 2003). Inconsistency in studies of circulating leptin as a risk factor for breast cancer is conceivable due to different experiment designs or different sample preparation methods. Therefore, more carefully designed studies are required to establish a connection between serum leptin and breast cancer.

Colorectal cancer

Several studies show a possible association between the leptin-induced signaling pathway and colon cancer. The presence of mRNAs for ObRs was demonstrated by

RT-PCR in colon cancer cell lines, human colon tumors, polyps and adjacent mucosa, (Attoub *et al.*, 2000; Hardwick *et al.*, 2001; Rouet-Benzineb *et al.*, 2004). In addition, the expression of ObR protein was detected in colon cancer tissues and cell lines (Hardwick *et al.*, 2001).

The presence of leptin-induced signaling pathways in colon cancer cells strengthens the association between leptin and colon cancer. Leptin-induced activation of the NF-kappaB and ERK1/2-dependent pathways induces proliferation of colonic epithelial cells. Stimulation of NF-kappaB by leptin causes a decrease of apoptosis in colon cancer HT-29 cells (Rouet-Benzineb *et al.*, 2004). Activation of ERK1/2 by leptin leads to increased cell growth (Hardwick *et al.*, 2001; Liu *et al.*, 2001; Rouet-Benzineb *et al.*, 2004). Moreover, leptin induces PI-3K, Rho-, and Rac-dependent pathways which contribute to increased cell invasion (Attoub *et al.*, 2000).

Prostate cancer

The association of leptin with prostate cancer is reported in several studies. The presence of ObR was demonstrated in normal prostate epithelia (Cioffi *et al.*, 1996), and benign and malignant prostate epithelial cells (Stattin *et al.*, 2001). Both ObRs and ObRI mRNAs have been detected in malignant prostate cells by RT-PCR (Stattin *et al.*, 2001; Onuma *et al.*, 2003; Somasundar *et al.*, 2004). It has been demonstrated that leptin increases cell growth and survival of prostate cancer cells by acting through either the PI3K/Akt or ERK1/2 pathway (Somasundar *et al.*, 2004). Leptin caused increased cell proliferation *via* JNK activation in PC-3 and DU145 human prostate cancer cells (Onuma *et al.*, 2003; Somasundar *et al.*, 2003; Somasundar *et al.*, 2004). In addition to the mitogenic effects of leptin, Frankenberry *et al.* (2004) reported that leptin functions as both a motility factor and an inducer of pro-metastatic factors such as VEGF, thereby transforming growth factor (TGF-b1), and FGF-b in prostate cancer cells *in vitro*.

Pancreatic cancer

The role of leptin in pancreatic cancer cells in different contexts, such as rat and human, is controversial. In rat insulin-secreting tumor cell lines, leptin suppresses apoptosis and stimulates proliferation (Okuya *et al.*, 2001). Leptin-induced c-fos expression and proliferation were shown in the RINm5F rat insulinoma-derived cell line (Islam *et al.*, 1997). Similarly, leptin induced phosphorylation of STAT3 and STAT5b in the BRIN-BD11 cell line (Briscoe *et al.*, 2001).

In contrast, leptin-induced reduction of cell growth was reported in Mia-PaCa and PANC-1 human pancreatic can-

cer cells (Somasundar *et al.*, 2003). Expression of ObR has not been attempted in human pancreatic cell lines and tissues even though expressions of ObRs and ObRI mRNAs were evaluated in rat insulinoma RIN m5F cells, mouse insulinoma cells BetaCT6 (Islam *et al.*, 1997; Kulkarni *et al.*, 1997) and BRIN-BD11 cells which were derived from fusion between rat beta-cells and rat insulinoma cells, INm5F cells (Briscoe *et al.*, 2001).

Ovarian cancer

The role of leptin in epithelial ovarian cancer (EOC) is not comprehensible at present. Even though the presence of both ObRs and ObRI mRNAs were shown in immortalized ovarian surface epithelium cell lines and in BG-1, OVCAR-3, and SKOV-3 ovarian cancer cell lines, only BG-1 cells demonstrated leptin-induced proliferation via the ERK1/2 MAPK pathway (Choi *et al.*, 2004).

Lung cancer

The association between leptin and lung cancer is not clear. Currently, the presence of ObRI has been reported in human lung tissue and SQ-5 cells derived from human lung squamous cell carcinoma. In SQ-5 cells, leptin in-

duces cell growth via the ERK1/2 pathway (Tsuchiya *et al.*, 1999).

LEPTIN AND TUMOR INVASION

Cancer metastasis, the establishment of primary tumor cells in distant body parts, is a distinct feature of severely malignant cancers, leading to poor clinical outcome, and is a main cause of mortality in women with breast cancer (Jemal, *et al.*, 2007).

Approximately 34% of ObR-positive cancers showed distant metastases with strong immunoreactivity for leptin (Cirillo *et al.*, 2008). Ishikawa *et al.* (2004) demonstrated that patients with over-expression of ObRI in primary breast tumors showed enhanced occurrence of hematogenous metastasis or recurrence of cancer in distinct organs, while patients with ObR-negative tumors showed low expression of leptin, with a good prognosis. In breast cancer, excessive fat tissue up-regulates synthesis and secretion of estrogenic compounds, growth-related factors, and angiogenic stimulators, leading to tumor growth and metastasis (Sierra-Honigsmann *et al.*, 1998; Ahima and Flier, 2000; Miyazawa-Hoshimoto *et al.*, 2004).

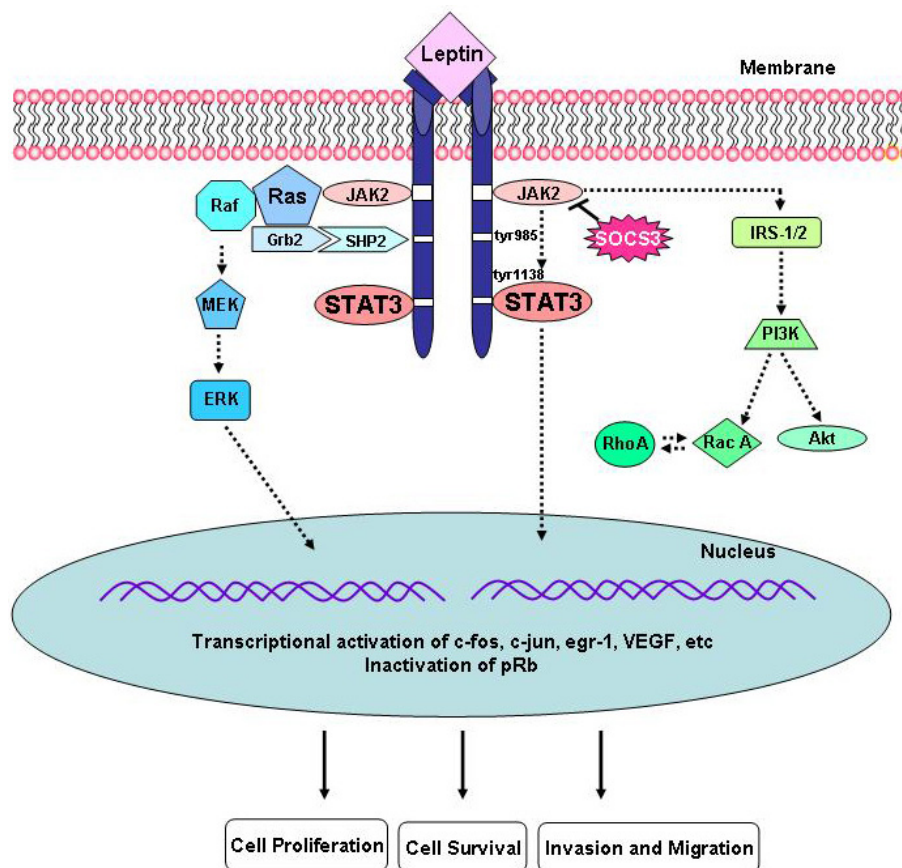


Fig. 1. Signaling pathway activated by leptin. Leptin binds to leptin receptor and activates JAKs. The activated JAKs phosphorylates tyrosine residue of the receptor (ObRI), and phosphorylated sites of the receptor provide recruiting sites for STAT. JAK-mediated phosphorylation of STATs causes their homo- or hetero-dimerization, and they are released from the receptor to translocate to the nucleus where they can control transcriptional levels of target genes. In addition to STATs, leptin-mediated signaling involves activation of a number of downstream targets including Ras/Raf, Rac, MAPKs, IRS and SOCS3 (Sweeney, 2002; Zabeau *et al.*, 2003; Frankenberry *et al.*, 2004; Yin *et al.*, 2004; Garofalo and Surmacz, 2006).

A number of events are involved in the process of metastasis (Ellis and Fidler, 1996). Studies demonstrated that leptin, synthesized and released by adipocytes, modulates neoangiogenesis in cooperation with vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) 2, and also by itself (Bouloumie *et al.*, 1998; Sierra-Honigmann *et al.*, 1998; Cao *et al.*, 2001).

The matrix metalloproteinase (MMP) family has been reported to be significantly connected to tumor invasion and metastasis (Ura *et al.*, 1989; Stetler-Stevenson, 1990; Liotta and Stetler-Stevenson, 1991; Tryggvason *et al.*, 1993; Sato *et al.*, 1994; Park *et al.*, 2001; Kume *et al.*, 2002). The possible association of leptin and metalloproteinases with tumor invasion has been reported (Sierra-Honigmann *et al.*, 1998; Ahima and Flier, 2000; Castellucci *et al.*, 2000; Miyazawa-Hoshimoo *et al.*, 2004). Castellucci *et al.* (2000) reported that leptin dose-dependently enhances secretion of MMP-2 and the activity of MMP-9 in cultured cytotrophoblastic cells. Consistent with this finding, McMurtry *et al.* (2009) demonstrated that activated Janus-activated kinase 2 (JNK) led to enhanced MMP-2 activity, and ultimately caused an increase of MCF-7 human breast cancer cell invasion.

CONCLUSION

Numerous studies show that leptin is involved in a large number of human cancers and plays key roles in tumor cell growth, migration, invasion and angiogenesis which might contribute to breast cancer progression (Garofalo *et al.*, 2006; Surmacz, 2007). Leptin and its receptors are widely over-expressed in many human cancers and this transition in tumors is related to tumor metastasis. Since leptin is evidently a critical factor for stimulating tumor invasion and metastasis, establishing strategies to modulate leptin signaling should be a high priority for the development of anti-metastatic therapeutics. Signaling molecules in leptin activation can serve as potential targets for anti-metastatic therapy. However, strategies for drug development will require cautious and detailed studies designed to unravel the complicated leptin-induced signaling pathways which result in several intricate and multi-functional roles for leptin in tumorigenesis.

This review summarizes a portion of the present understanding of leptin signaling with a major focus on its contribution to breast cancer progression and tumor cell invasion (depicted in Fig. 1). Due to the complex cellular effects of leptin, more precisely defined leptin signaling pathways need to be elucidated. At present, JAK/STAT, SOCS3, PI3K and IRS protein pathways are well estab-

lished as the predominant leptin signaling pathways and understanding the significance of other signaling pathways such as Ras/Raf/MEK/MAPK, requires additional studies.

Although much is known concerning the functions of leptin in tumor progression, more remains to be elucidated to appreciate the implications of a detailed understanding of leptin mediated molecular events leading to cellular conversion to a malignant phenotype. Understanding the role of leptin in invasion and metastasis will provide valuable information for establishing strategies to modulate leptin signaling, which should be a high priority for the development of anti-cancer therapeutics.

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