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Leptin Signaling in Breast Cancer: An Overview

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ABSTRACT

The adipocyte-derived peptide leptin acts through binding to specific membrane receptors, of which six isoforms (obRa-f) have been identified up to now. Binding of leptin to its receptor induces activation of different signaling pathways, including the JAK/STAT, MAPK, IRS1, and SOCS3 signaling pathways. Since the circulating levels of leptin are elevated in obese individuals, and excess body weight has been shown to increase breast cancer risk in postmenopausal women, several studies addressed the role of leptin in breast cancer. Expression of leptin and its receptors has been demonstrated to occur in breast cancer cell lines and in human primary breast carcinoma. Leptin is able to induce the growth of breast cancer cells through activation of the Jak/STAT3, ERK1/2, and/or PI3K pathways, and can mediate angiogenesis by inducing the expression of vascular endothelial growth factor (VEGF). In addition, leptin induces transactivation of ErbB-2, and interacts in triple negative breast cancer cells with insulin like growth factor-1 (IGF-1) to transactivate the epidermal growth factor receptor (EGFR), thus promoting invasion and migration. Leptin can also affect the growth of estrogen receptor (ER)-positive breast cancer cells, by stimulating aromatase expression and thereby increasing estrogen levels through the aromatization of androgens, and by inducing MAPK-dependent activation of ER. Taken together, these findings suggest that the leptin system might play an important role in breast cancer pathogenesis and progression, and that it might represent a novel target for therapeutic intervention in breast cancer. J. Cell. Biochem. 105: 956–964, 2008.

KEY WORDS: LEPTIN; BREAST CANCER; SIGNAL TRANSDUCTION

Evidence suggests that different hormones and peptide growth factors might cooperate in promoting mammary carcinogenesis. Leptin, the product of the obesity (ob) gene, is a circulating peptide hormone acting as a regulator of food intake via hypothalamic-mediated effects [Zhang et al., 1994; Schwartz et al., 1999]. Leptin is principally synthesized and secreted from adipocytes; secondary sources are placenta, stomach and skeletal muscle. Leptin expression is induced by obesity, insulin, TNF-α and glucocorticoids and it is negatively regulated by β-adrenergic agonists and thiazolidinediones [Tartaglia et al., 1995]. In obese humans, high plasma levels of leptin are correlated with increased fat mass [Considine et al., 1996] and with the development of resistance to insulin [Kahn and Flier, 2000]. In fact, obesity is not related to deficiency of leptin but rather to resistance to this hormone [Bjorbaek and Kahn, 2004]. Higher concentrations of serum leptin in obese individuals are associated with increased release from adipocytes [Hamilton et al., 1995; Kolaczynski et al., 1996]. Notably, serum leptin levels are higher in women than in men [Havel et al., 1996]. This phenomenon could be explained through a different regulation of leptin expression by sex hormones. It has been shown that estrogen up-regulates [Casabiell et al., 1998; Castracane et al., 1998] leptin expression, whereas testosterone down-regulates it [Blum et al., 1997; Elbers et al., 1997; Jockenhovel et al., 1997]. Leptin affects many peripheral organs, behaving as a mitogen, survival factor, metabolic regulator or angiogenic factor depending on the target tissue [Wauters et al., 2000].

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Leptin acts through binding to the extracellular domain of specific membrane receptors. Up to now, six isoforms of the leptin receptor have been identified (obRa-obRf). However, the physiological relevance of each leptin receptor is still under investigation. Importantly, it has been demonstrated that leptin may regulate cell proliferation in several normal and malignant tissues. Expression of leptin receptor has been found in cells of lung and gastric carcinoma and in leukemia cells [Tsuchiya et al., 1999; Hino et al., 2000; Mix et al., 2000]. Furthermore, it has been shown that leptin stimulates the proliferation of normal and hematopoietic cells [Gainsford et al., 1996], non-transformed epithelial cells [Glasow et al., 1999], and of human colon cancer cells [Liu et al., 2001]. Leptin is also able to stimulate the invasiveness of premalignant colon and kidney epithelial cells in vitro [Attoub et al., 2000].

Leptin and its receptor isoforms obRa and obRb have been found to be expressed at the mRNA and/or protein level in human breast cancer cell lines and breast malignant tumors [O'Brien et al., 1999]. The expression of leptin has also been reported in the human normal mammary gland, and several lines of evidence suggest that tumorigenesis is associated with an increase in leptin expression by epithelial mammary cells [O'Brien et al., 1999].

These observations and epidemiologic studies regarding obesity as a risk factor in human breast tumorigenesis suggest that leptin may contribute to the malignant transformation of breast epithelial cells. In this review article, we will summarize the current knowledge on leptin signaling with particular regard to its potential involvement in the pathogenesis and progression of breast cancer.

LEPTIN SIGNALING

The leptin receptor belongs to the class-I cytokine receptor family. It shares the highest sequence similarity with the receptor for granulocite colony-stimulating factor (G-CSF) and with the glycoprotein 130 (gp130) family receptors, including gp 130, leukemia inhibitory factor (LIF) and oncostatin (OSM) receptors. Six different isoforms of the leptin receptor have been identified (obRa-obRf). The extracellular domain of five isoforms (obRa-obRd, obRf) is identical. It consists of 816 aminoacids, two cytokine-like binding motifs (Trp-Ser-X-Ser-Trp) and a fibronectin type III domain [White and Tartaglia, 1996]. The obRa, obRc, obRd, and obRf isoforms are also characterized by a short intracellular domain formed of 32–40 residues and a transmembrane domain consisting of 23 aminoacids. Only the obRb contains a long intracellular domain, comprising approximately 306 aminoacids [Hynes and Jones, 2001]. Several studies, employing mutant receptor, demonstrated that only the long intracellular domain of obRb has full signaling potential [Fong et al., 1998].

The short forms of leptin receptors, lacking major domains recruiting downstream effectors, show a diminished or abolished signaling capability; they are ubiquitously expressed, but their function is not clear even if there are evidences that the short isoforms of leptin receptors can be involved in intra- and transcellular leptin transport [Hileman et al., 2000]. Finally, the obRe is a soluble receptor and it does not play a direct role in leptin signaling but it controls leptin circulating levels [Huang et al., 2001].

Both long and short isoforms are able to form homodimers in absence of leptin [Devos et al., 1997; Nakashima et al., 1997; White and Tartaglia, 1999]. Thus dimerization does not play a regulatory role in the activation of the receptor even if the formation of dimers into leptin receptors seems to be essential for signaling [Devos et al., 1997]. Leptin binds its receptor in 1:1 ratio leading to the formation of a tetrameric receptor/ligand complex [Devos et al., 1997].

Binding of leptin to its receptor induces activation of different signaling pathways, including the JAK/STAT, MAPK, IRS1, and SOCS3 signaling pathways, which mediate its effects on cell proliferation and survival.

JAK/STAT PATHWAY

Like all other class-I cytokine receptor, the obRs lack any intrinsic kinase activity, and employs to signal cytoplasmatic-associated kinases of the Janus-family tyrosine kinase (JAK) family. All the leptin receptor isoforms share in the intracellular domain a common 29 aminoacid sequence representing a "Box 1" JAK binding domain. This site, strongly conserved among most members of the cytokine receptor family, is located in the juxtmembrane domain within the first 20 cytoplasmatic residues of the receptor. The long leptin receptor isoform (obRb) also possesses a less conserved "Box 2" motif, located between the first 50–60 aminoacids of the cytoplasmatic domain, and a signal transducer and activators of transcription (STAT) binding site (Fig. 1). ObRa lacks a STAT3 docking site but is still able to bind and activate JAK2 through Box 1. However, Bjorbaek et al. [1997] demonstrated that obRa is able to induce activation of JAK2, IRS-1 and MAPK in transient transfection models.

The tetrameric complex resulting from leptin binding, leads to the cross-phosphorylation and activation of JAKs. The activated JAKs rapidly phosphorylate tyrosine residues of the cytosolic domain of the receptor (obRb). Such phosphorylated residues provide binding

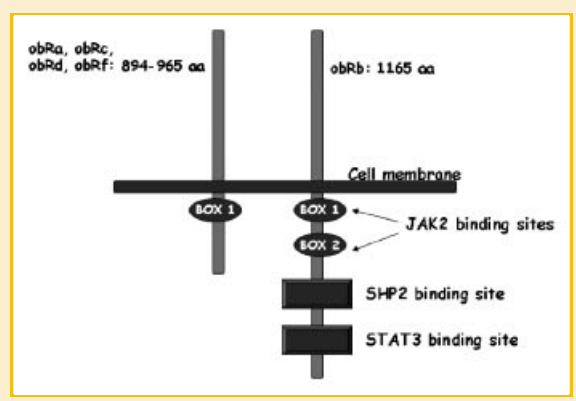

Fig. 1. Schematic representation of obR isoforms. All the isoforms show the same extracellular domain, but they differ for the length of the intracellular domain. The long isoform (obRb) of the receptor contains two JAK2 binding site (Box1 and Box2), a SHP2 binding motif and a STAT3 binding site. The short isoforms (obRa, obRc, obRd, obRf) have only the Box1 domain. The obRe soluble form, not shown, lacks the transmembrane and intracellular domains.

sites for signaling molecules including members of the STAT family. STATs themselves are also subjected to JAK-mediated phosphorylation, which induce their homo- or hetero-dimerization, release from receptor complex and subsequent translocation to the nucleus, where they can modulate transcription of specific target genes (Fig. 2) [Bjorbaek et al., 1997; Devos et al., 1997; White et al., 1997].

In humans, the obRb shares three tyrosine residues, each of one may be associated with specific signaling pathways. It has been demonstrated that the sequence surrounding Tyr^{1138} (YXXQ) in ObRb constitutes a consensus STAT-3 binding motif [Stahl et al., 1995]. Banks et al. [2000] demonstrated that Tyr^{1138} becomes phosphorylated as a consequence of ligand binding. After the phosphorylation of this site, STAT3 is recruited via its SH2 (Src homology) domain. Activation, homo-dimerization and nuclear translocation of STAT3 finally leads to specific gene induction [Baumann et al., 1996; Ghilardi et al., 1996; Zabeau et al., 2003].

STAT3 protein has been shown to be an important mediator of cell growth. It plays an essential role during embryonic development, cell survival, and proliferation. STAT3 activation is often associated with cell transformation [Takeda et al., 1997]. At the molecular level, STAT3 acts as a transcription activator and regulates the expression of a number of genes, such as c-*myc*, *cyclin D1*, *p21waf1*, *Bcl*II, and *Bcl-xL*, which are critically involved in cell growth and proliferation [Yin et al., 2004]. It has been demonstrated that leptin induces the expression of the c-*myc* gene and the association of STAT3 with the c-*myc* promoter, suggesting an important role of STAT3 in the biological and pathobiological activities of leptin. STAT3 interacts with, and recruits members of the p160 family such as SRC-1, but not GRIP1 and AIB1, to its target gene promoter [Yin et al., 2004].

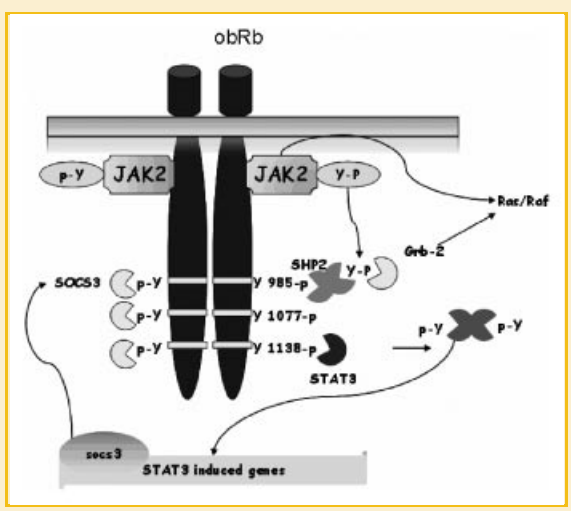

Fig. 2. Leptin receptor possesses three conserved Tyr residues in its cytoplasmatic domain. JAK2, located nearby the membrane, is activated after ligand binding and is able to phosphorylate these Tyr residues. Y1138, membrane-distal, is a binding site for STAT3. The membrane-proximal Y985 and Y1077 are involved in regulation and attenuation of leptin signal though interaction with different signaling proteins.

Phosphorylation-dependent activation of STAT3 is a major transduction pathway for leptin signaling in breast carcinoma. In MCF-7 breast cancer cells, 20 nM was the lowest leptin concentration giving the maximal STAT3 and MAPK activation. Moreover, exposure of MCF-7 cells for 5 min to both leptin (20 nM) and the specific MAPK inhibitor UO126 resulted in a complete abolition of the positive effect of leptin on the p42/44 MAPK isoform phosphorylation [Dieudonne et al., 2002].

SOCS3 SIGNALING

The suppressor cytokine signaling (SOCS) proteins have been shown to negatively regulate cytokine-induced signaling. Many cytokines, such as Interleukin-6 (IL-6), growth hormone and erythropoietin induce the expression of one or more SOCS isoforms, thus realizing a negative feedback loop. SOCS proteins contain a central SH2 domain, which may allow these proteins to inhibit signaling by binding to phosphorylated JAK proteins or through direct interaction with tyrosine phosphorylated receptors.

SOCS-3 is an inducible suppressor of leptin signaling. In fact, leptin specifically induces expression of SOCS3 mRNA in region of the hypothalamus through binding to the long form of the leptin receptor (obRb) [Bjorbaek et al., 1998]. In addition, forced expression of SOCS3 blocks leptin receptor-mediated signal transduction in mammalian cell lines, demonstrating its function as a negative feedback loop for leptin signaling (Fig. 2). SOCS3 blocks leptin receptor activation through binding to the Tyr^{985} and Tyr^{1077} residues of the receptor (Fig. 2) [Sweeney et al., 2000].

How leptin regulates SOCS3 expression and by which mechanism SOCS3 inhibits leptin signal transduction is not yet deeply understood. It has been demonstrated that leptin pre-treatment induces resistance to this growth factor in CHO cells stably expressing the long form of the leptin receptor [Bjorbaek et al., 1998]. In these cells, leptin induces expression of SOCS3 and SOCS3 protein levels remained elevated for more than 20 h following leptin treatment. Leptin-induced resistance involves inhibition of leptin receptor tyrosine phosphorylation without affecting receptor surface expression. In agreement with these findings, forced expression of SOCS3 attenuates leptin-induced tyrosine phosphorylation of JAK2 [Bjorbaek et al., 1999].

The obRb residue Tyr^{985} controls the tyrosine phosphorylation of SHP-2, whereas Tyr^{1138} controls STAT3 activation. The mechanisms by which obRb controls downstream MAPK activation, and c-*fos* and SOCS3 message accumulation has been investigated. Banks et al. [2000] demonstrated that Tyr^{985}-mediated recruitment of SHP-2 does not alter tyrosine phosphorylation of Jak2 or STAT3 but results in GRB-2 binding to tyrosine-phosphorylated SHP-2 and is required for the majority of MAPK activation during obR signaling. Tyr^{985} and MAPK activation similarly mediate c-*fos* mRNA accumulation. In contrast, SOCS3 mRNA accumulation requires Tyr^{1138}-mediated STAT3 activation. Thus, the two obR tyrosine residues that are phosphorylated during receptor activation, mediate distinct signaling pathways as follows: SHP-2 binding to Tyr^{985} positively regulates the MAPK and c-*fos* pathway, instead STAT3 binding to Tyr^{1138} mediates the accumulation of the inhibitory SOCS3 protein [Banks et al., 2000].

PI3K AND IRS PROTEIN PATHWAYS

It has been demonstrated that a strong correlation exists between leptin and insulin signaling, since high levels of activation of leptin and insulin signaling pathways occur coincidentally in the majority of obese humans. Phosphorylation of the insulin receptor substrates 1 and 2 (IRS1 and 2) as well as their interaction with Grb-2 and phosphatidylinositol 3-kinase (PI3K) have been shown to be modulated cooperatively by leptin and insulin in various hepatocytic cell lines (Fig. 3) [Szanto and Kahn, 2000]. Stimulation of PI3K by leptin is also involved in the regulation of phosphodiesterase (PDE)3B in pancreatic β cells [Zhao et al., 1998], Na, K-pump in fibroblasts [Sweeney et al., 2000], and invasiveness of colon epithelial cells [Attoub et al., 2000]. Leptin alone had no effect on the insulin signaling pathway in Fao hepatoma cells, but leptin pretreatment transiently enhanced insulin-induced tyrosine phosphorylation of IRS-1 and its binding to PI3k, while produced an inhibition of tyrosine phosphorylation of IRS-2 that resulted in reduced binding to PI3K [Szanto and Kahn, 2000]. Leptin alone also induced serine phosphorylation of Akt and glycogen synthase kinase 3 but to a lesser extent than insulin, and the combination of these hormones was not additive [Szanto and Kahn, 2000]. These results suggest complex interactions between the leptin and insulin signaling pathways that can potentially lead to differential modification of the metabolic and mitotic effects of insulin exerted through IRS-1 and IRS-2 and the downstream kinases that they activate.

Finally, in several cell lines [Cohen et al., 1996; Bjorbaek et al., 1997; Attoub et al., 2000] leptin induced phosphorylation of IRS proteins through JAK's activation. For example, the phosphorylation of IRS-1 was increased by leptin in human embryonic kidney cells HEK293 cells coexpressing leptin receptor, JAK2 and IRS1 [Bjorbaek et al., 1997].

LEPTIN AND BREAST CANCER

A strong relationship between breast cancer and obesity has long been recognized for many years. Interestingly, there is a substantial difference in the impact of obesity on carcinogenesis in pre-menopausal and postmenopausal women [Rose et al., 2004]. While in premenopausal women increased body weight seems to be inversely related to breast cancer risk, in postmenopausal women obesity represents a significant risk factor for breast cancer development [Rose et al., 2004]. In postmenopausal obese women, adipose tissue is the only source of estrogen production by aromatization of C19 steroid androstenedione. As there is increased aromatase activity and androstenedione production in obesity, the total pool of estrogen is higher in obese women. The adipose tissue-derived hormone estrone is readily prepared for peripheral conversion to the more biologically potent estradiol. Obesity also affects the binding of plasma estradiol to the sex-hormone binding globulin (SHGB). Therefore, increased estrogen stimulation in postmenopausal obese women might be the cause of higher breast cancer risk [Vona-Davis and Rose, 2007].

Evidence suggests that leptin might be involved in the pathogenesis of different type of human carcinoma, including breast cancer [Garofalo and Surmacz, 2006; Surmacz, 2007]. In particular, several actions of leptin, including the stimulation of tumor cell growth, migration and invasion, and enhancement of angiogenesis, may play a relevant role in breast cancer progression [Garofalo et al., 2006; Surmacz, 2007].

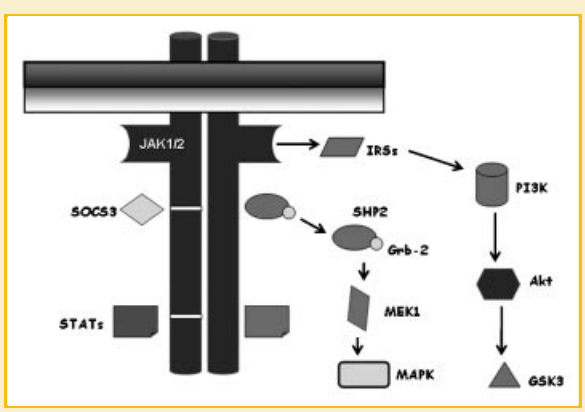

Fig. 3. Signaling pathways activated by leptin.

EXPRESSION OF LEPTIN AND LEPTIN RECEPTORS

Expression of leptin and its receptors in breast cancer has been addressed by using either immunohistochemistry or reverse transcriptase-PCR. In particular, antibodies and primers that recognize common domains of the obR receptors have been used. Therefore, if not otherwise specified, the data regarding receptor expression are related to all the different isoforms.

Both leptin and obR were found to be significantly overexpressed in human primary and metastatic breast cancer as compared with non-transformed mammary gland [Garofalo et al., 2006]. In primary tumors, the levels of expression of leptin positively correlated with obR, and both molecules were most abundant in less differentiated G3 tumors [Garofalo et al., 2006]. In agreement with these findings, Ishikawa et al. [2004] also found a significant correlation between the levels of expression of leptin and obR in primary breast carcinoma. Expression of leptin was found in both normal and transformed mammary epithelial cells, although overexpression of leptin was found to occur in 92% of the primary breast carcinomas as compared with normal epithelium [Ishikawa et al., 2004]. Importantly, distant metastases were detected in 34% of obR-positive cancers with strong immunoreactivity for leptin, but in none of the tumors that lacked either obR expression or leptin overexpression.

Taken together, these data suggest that an autocrine loop involving leptin and its receptor might be operative in breast cancer, and that it might sustain the ability of breast cancer cells to form metastasis. However, the various isoforms of leptin receptor might play a different role in breast cancer.

Laud et al. [2002] investigated the expression of the long and short isoforms of leptin receptor in 20 tumoral breast tissues and two human breast cancer cell lines (T47-D and MCF-7). They showed the expression of both leptin receptor transcripts in all tumoral tissues examined. By in situ hybridization experiments, they localized

leptin receptors in proliferating breast cancer epithelial cells [Laud et al., 2002]. More recently, Revillion et al. [2006] measured the expression of leptin and its two main receptors (obRa and obRb) in 322 breast cancer. They found that all the tumors expressed both receptors and 318 of 322 expressed leptin. A positive correlation was observed between the expression of leptin, obRb and estrogen receptor (ER), and between obRa and progesterone receptor (PR). The leptin receptors were expressed at high levels in more differentiated tumor as compared with undifferentiated carcinomas, and such difference was more evident for obRa. More importantly, patients with elevated obRa expression showed longer-relapse-free survival, whereas an high obRb/obRa ratio was associated with shorter-relapse-free survival. Multivariate analysis confirmed the positive prognostic value of obRa.

REGULATION OF LEPTIN EXPRESSION

Different factors have been shown to affect the expression of leptin and its receptors in breast cancer cells. Garofalo et al. [2006] addressed the mechanisms that regulate leptin/obR overexpression in breast cancer by using ER-positive MCF-7 and ER-negative MDA-MB-231 breast cancer cells. These authors demonstrated that insulin stimulated the expression of leptin and obRb mRNAs in both cell lines, whereas estradiol and insulin like growth factor (IGF)-1 produced significant effects on the expression of leptin and its receptor only in MCF-7 cells. In this regard, a cross-talk between estrogen and IGF-1 has been demonstrated in ER+ breast cancer cells [Cascio et al., 2007]. Interestingly, Garofalo et al. [2006] also found that hypoxia was able to stimulate the synthesis of transcripts for leptin and obRb in breast cancer cells. Indeed, the human leptin gene promoter contains several regulatory motifs among which eight hypoxia-responsive elements (HRE) that can recruit hypoxia-inducible factor (HIF) [Grosfeld et al., 2002; Meissner et al., 2003]. HIF is a transcription factor involved in the nutrient stress signaling, that is able to promote metastatic processes by activating tumor neoangiogenesis, and increasing cell motility and invasion [Pousseygur et al., 2006]. HIF is an heterodimer formed of two subunits: HIF-1α, an oxygen-regulated instable unit, and HIF-1β constitutively expressed. HIF-1α expression is significantly increased under hypoxic conditions, but it can also occurs in normoxia upon activation of the mTOR pathway [Pousseygur et al., 2006]. It has been hypothesized that increased leptin expression in tumor cells might be related to accumulation of nuclear HIF-1α, resulting in increased HIF loading on leptin regulatory sequences. In this regard, Cascio et al. [2008] and Bartella et al. [2008] recently confirmed that leptin expression in breast cancer cells can be induced by hyperinsulinemia, hypoxia and/or hypoxia-mimetic treatments. In particular, these authors showed that in MCF-7 and in MDA-MB-231 cells, nuclear accumulation of HIF-1α in response to insulin occurred in a dose-dependent manner. The greatest nuclear HIF-1α levels under physiological hypoxia were seen at 16 h, and under 100 nM of fosgene ($COCl_2$) treatment, which mimics hypoxia, at 4 h [Cascio et al., 2008]. Similar conditions stimulated maximal leptin mRNA and leptin protein expression [Cascio et al., 2008]. Finally, in breast cancer cells nuclear HIF-1α was able to associate with several leptin promoter domains containing HREs [Bartella et al., 2008; Cascio et al., 2008].

ROLE OF LEPTIN IN BREAST CANCER CELL PROLIFERATION

A growing body of evidence indicates that proliferation of both estrogen-dependent and estrogen-independent breast cancer cells is enhanced by addition of leptin [Hu et al., 2002; Laud et al., 2002; Okumura et al., 2002; Somasundar et al., 2003]. For example, physiologic concentrations of leptin (25–100 ng/ml) activated the STAT3 and MAPK pathways resulting in increased proliferation of T-47D breast cancer cells and HBL100 non-transformed mammary epithelial cells [Hu et al., 2002]. Interestingly, leptin did not enhance the anchorage-independent growth of non-transformed HBL100 mammary epithelial cells [Hu et al., 2002]. It has also been demonstrated that leptin stimulates the growth of estrogen receptor positive MCF-7, T47-D and ZR-75-1 human breast cancer cells in a time- and dose-dependent manner [Dieudonne et al., 2002; Hu et al., 2002; Okumura et al., 2002; Somasundar et al., 2003; Yin et al., 2004]. Activation of STAT3 and MAPK signaling was induced by treatment with leptin in these cells [Dieudonne et al., 2002; Yin et al., 2004]. However, blockade of STAT3 phosphorylation with a specific inhibitor, AG490, abolished leptin-induced proliferation of MCF-7 cells, whereas inhibition of MAPK activation with UO126 did not result in any significant changes in leptin-induced cell proliferation [Yin et al., 2004]. Ray et al. [2007] reported a growth stimulatory effect of leptin in T-47D cells and in the estrogen receptor negative MDA-MB-361, MDA-MB-231 and SK-BR-3 cell lines. Leptin concentrations of 25, 50, and 100 ng/ml were effective in promoting cell growth at 24 h time point, while only 50 and 100 ng/ml concentrations maintained the same effect at 48 h [Ray et al., 2007]. These findings were in agreement with those proposed by Laud et al. [2002].

Taken together, these findings suggest that leptin receptors are expressed in a wide range of tumor cells, and that leptin might have effects on the phenotype of normal and malignant mammary epithelial cells [Otvos et al., 2008]. However, the levels of expression of the receptors and the activation of different signaling pathways or other molecular alterations might affect the response to leptin. In this regard, leptin had no growth-stimulatory activity on HTB-26 and HTB-131 breast cancer cell lines at 1–100 ng/ml [Oelmann et al., 2003]. Actually, the growth of these two cell lines was slightly inhibited by leptin; this phenomenon was independent from the expression of the transcripts for leptin or its receptors in the cells.

LEPTIN AND ESTROGEN

Evidence suggests that the leptin system is involved in the pathogenesis and progression of estrogen dependent breast carcinoma. In fact, leptin can enhance aromatase activity, with the potential for the promotion of estrogen production from androstenedione in adipose tissue, and hence the stimulation of estrogen-dependent breast cancer progression [Catalano et al., 2003]. Furthermore, leptin enhances activation of ERα through the MAPK pathway in MCF-7 and HeLa cells [Catalano et al., 2004]. Interestingly, leptin was found to increase the estradiol-induced activation of ERα, thus suggesting that leptin and estrogen might cooperate in sustaining the growth of estrogen-dependent breast cancer cells.

Finally, leptin might be involved in the resistance of breast cancer cells to anti-estrogen therapies. In fact, Garofalo et al., [2004]

demonstrated that leptin is able to attenuate the effects of the antiestrogen ICI 182,780 (fulvestrant) in MCF-7 breast cancer cells. Treatment of these cells with ICI 182,780 led to fast degradation of membrane ERα, which reduced nuclear expression of the receptor and ERα-dependent transcription, and produced significant growth inhibition. Leptin was able to counteract the cytostatic activity of ICI 182,780, as well as the effects of this compound on the expression of ERα and on ERα-dependent transcription.

LEPTIN AND ANGIOGENESIS

Vascular endothelial growth factor (VEGF) is the main mediator of tumor-associated angiogenesis, and, therefore, plays an important role in the growth of primary tumors and metastasis. Leptin has been recently suggested to regulate angiogenesis by modulating VEGF activity [Gonzalez et al., 2006]. In fact, leptin can increase the expression of VEGF and its receptor VEGFR-2 in 4T1 mouse mammary cancer cells; furthermore, blockade of leptin receptor signaling slows breast cancer growth and results in reduced expression of VEGF, VEGFR-2 and cyclin D1 in vivo [Gonzalez et al., 2006]. Activation of JAK is a critical component of the leptin-induced increase in VEGF and VEGFR-2, since treatment with a JAK inhibitor blocked the induction of VEGF and VEGFR-2 by leptin [Gonzalez et al., 2006]. In contrast, inhibition of STAT3 with siRNAs did not negatively affect the induction of leptin by VEGFR-2 and VEGF. Finally, leptin-induced increase of VEGF and VEGFR-2 also involves PI3K and MEK-1/MAPK signaling. In fact, the inhibition of PI3K in 4T1 cells prevented the leptin induced VEGF secretion and VEGFR-2 expression. In contrast, the inhibition of the MAPK pathway completely inhibited the leptin-induced increase of VEGFR-2 but had partial effects on the ability of leptin to induce VEGF [Gonzalez et al., 2006].

CROSS-TALK BETWEEN LEPTIN AND GROWTH FACTORS

Peptide growth factors play a fundamental role in the development, differentiation and homeostasis of normal tissues. These proteins function through autocrine, paracrine and/or juxtacrine pathways by binding to growth factor receptors expressed on the cell membrane of target cells. The role of growth factor driven signaling in the pathogenesis and progression of human carcinoma has been revised in several articles from our group [Normanno et al., 2005; Normanno et al., 2006a,b; De Luca et al., 2008].

We have above described how leptin is able to induce proliferation, survival and anchorage-independent growth of breast cancer cells through activation of the Jak/STAT3, MAPK and PI3K pathways. However, in addition to its own effects, leptin interacts with other signaling systems in breast cancer cells. In particular, interactions between leptin and receptors of the ErbB tyrosine kinase receptors have been demonstrated. This family comprehends four distinct members: the epidermal growth factor receptor (EGFR–ErbB-1), ErbB-2 (HER2/Neu), ErbB-3 (HER3), and ErbB-4 (HER4). Activation of these receptors is triggered by binding to specific ligands that belong to the epidermal growth factor (EGF) family of peptides. Following ligand binding, the ErbB receptors form homo- or hetero-dimers that are able to activate several different intracellular signaling pathways, including the PI3K/AKT and the RAS/RAF/MEK/MAPK pathways. Activation of ErbB receptors has been shown to induce proliferation and survival in breast cancer cells [Normanno et al., 2005, 2006a,b].

Eisemberg et al., [2004] demonstrated that both obRa and obRb leptin receptor isoforms trans-phosphorylate ErbB-2 and successively promote MAPK activation. Despite the fact that obRa has a lesser ability to directly activate MAPK, its capacity to enhance phosphorylation of ErbB-2 and activation of MAPK through transactivation of ErbB-2 was stronger as compared with obRb in human embryonic kidney HEK 293T cells [Eisemberg et al., 2004]. Although this phenomenon could be partially attributed to its higher expression, this finding may be of physiological or pathological significance as obRa is more abundantly expressed in peripheral tissues. The mechanism by which the obRs and ErbB-2 interact has not been completely elucidated. Two alternative hypotheses have been suggested: the first, that obR and ErbB-2 have no direct interaction but rather leptin activates JAK2 that translocates to activate ErbB-2; the second, that obR and ErbB-2 do form a trimeric complex through binding to JAK2 [Eisemberg et al., 2004].

Soma et al. [2008] found that exogenous leptin induces tyrosine phosphorylation of ErbB-2 in SK-BR-3, cells which show marked overexpression of this receptor. Exogenous leptin was also able to induce phosphorylation of ERK/MAPK in SK-Br-3 cells, a phenomenon that was significantly inhibited by AG825, a specific inhibitor of ErbB-2 [Soma et al., 2008]. Moreover, AG825 totally abrogated the growth stimulatory effect of leptin in ErbB-2-expressing breast cancer cells [Soma et al., 2008]. Exogenous leptin also induced the phosphorylation of ErbB-2 in T-47D ER-positive breast cancer cells that moderately express this receptor. These results indicate that leptin promotes the transactivation of ErbB-2 in ER-positive cells, even if the treatment with AG825 did not significantly reduce the growth-stimulatory effect of leptin in these cells [Soma et al., 2008].

The stimulation of class I cytokine receptors by IL-6, growth hormone or prolactin has been reported to induce tyrosine phosphorylation of EGFR family members. These receptors have structural similarity to leptin receptors, suggesting the possible transactivation of EGFR by leptin. In fact, it has already been shown that leptin can induce phosphorylation of EGFR in esophageal [Ogunwobi et al., 2006] and gastric cancer cells [Shida et al., 2005]. Recently, Saxena et al. [2008] demonstrated a bidirectional crosstalk between leptin and IGF-1 signaling that leads to transactivation of the EGFR and promotes invasion and migration of triple negative breast cancer cells. In fact, leptin induced in breast cancer cells tyrosine phosphorylation of IGF-1 receptor (IGF-1R), and IGF-1 itself produced a remarkable tyrosine phosphorylation of leptin receptor. The contemporary administration of leptin and IGF-1 determined a synergistic activation of ob-Rb and IGF-1 together with activation of Akt and MAPK. Moreover, leptin and IGF-1 synergistically transactivated EGFR, via obRb and IGF1-R, thus inducing proliferation of triple negative breast cancer cells. The phosphorylation of EGFR was blocked not only by the EGFR-tyrosine kinase inhibitor AG1478, but also by a broad-spectrum matrix metalloproteinase inhibitor, GM6001, suggesting

that leptin- and IGF-1-induced EGFR transactivation is dependent on proteolytic release of EGFR ligands. The combined treatment of triple negative breast cancer cells with leptin and IGF-1 promoted invasion, and the inhibition of EGFR activation by using erlotinib (a reversible inhibitor of HER1/EGFR/tyrosine kinase) or lapatinib (an EGFR/ErbB-2 inhibitor) effectively inhibited leptin- and IGF-1-induced invasion and migration [Saxena et al., 2008].

CONCLUSION

The data summarized in this review demonstrate that the leptin system might play an important role in breast cancer pathogenesis and progression. The high levels of expression of leptin in obese women; the ability of leptin to increase estrogen stimulation in postmenopausal women; the high levels of expression of leptin and its receptors in breast cancer cells; the proliferative response of these cells to exogenous leptin; the ability of this peptide to activate different intracellular signaling pathways; the interactions of the leptin system with several, different growth promoting pathways that have been demonstrated to play a role in breast cancer progression; all these features make of leptin an important candidate as a novel growth factor driving breast cancer progression.

Interestingly, preclinical findings suggest that leptin might be involved in the growth of different subtypes of breast cancer. Leptin has been shown to promote the growth of ER-positive breast cancer cells. However, the ability of leptin to directly or indirectly transactivate ErbB-2 and EGFR suggest a role of this growth factor in the ErbB-2 overexpressing- and in the triple negative-breast cancer subtypes. In this regard, different preclinical studies have confirmed that leptin can enhance the growth of both ErbB-2 overexpressing and triple negative breast cancer cell lines [Ray et al., 2007; Soma et al., 2008].

Although these findings are suggestive of the role of leptin in the growth of breast cancer, the lack of drugs specifically targeting the leptin system has not allowed up to now to assess the potential clinical impact of leptin inhibition in breast carcinoma. However, the evidence reported in this article strongly suggest that leptin might be a novel target for therapeutic intervention in breast cancer.

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