

MINI REVIEW

Leptin – From Regulation of Fat Metabolism to Stimulation of Breast Cancer Growth

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Leptin restricts intake of calories as a satiety hormone. It probably stimulates neoplastic proliferation in breast cancer, too. Growth of malignant cells could be regulated by various leptin-induced second messengers like STAT3 (signal transducers and activators of transcription 3), AP-1 (transcription activator protein 1), MAPK (mitogen-activated protein kinase) and ERKs (extracellular signal-regulated kinases). They seem to be involved in aromatase expression, generation of estrogens and activation of estrogen receptor α (ER α) in malignant breast epithelium. Leptin may maintain resistance to anti-

estrogen therapy. Namely, it increased activation of estrogen receptors, therefore, it was suspected to reduce or even overcome the inhibitory effect of tamoxifen on breast cell proliferation. Although several valuable reviews have been focused on the role of leptin in breast cancer, the status of knowledge in this field changes quickly and our insight should be continuously revised. In this summary, we provide refreshed interpretation of intensively reported scientific queries of the topic. (Pathology Oncology Research Vol 12, No 2, 69–72)

Key words: leptin, leptin receptor, signal transducer and activator of transcription 3, estrogen receptor α , antiestrogen resistance

Introduction

The term leptin derives from a Greek word meaning thin. The leptin gene encodes a 16-kDa protein.¹ Leptin induces satiety and limits calorie intake. Its action is mediated by leptin receptor OB-R. The long form of the receptor, OB-R_b, mediates most leptin functions. The short form, OB-R_a, is reported to facilitate transport of leptin via brain blood barrier, but its exact role is still to be determined.² Leptin controls metabolism of carbohydrates and lipids,³ therefore, it counteracts manifestation of obesity.⁴ Moreover, leptin is a multifunctional factor in the development of hematopoietic, reproductive and immunologic functions.^{4,5} It derives from adipocytes that constitute a large depository of highly caloric fatty acids. Power for metabolic changes comes from decomposition of these

lipids. Energy is abundantly released from chemical bonds during cell divisions and differentiation. These processes are the most intensified in organogenesis and oncogenesis.³

Not only do caloric diet and obesity favor onset of cardiovascular disorders,⁶ but they are responsible for higher rate of breast cancer incidence as well.⁷ Androstenedione undergoes aromatization to estrone in extragonadal tissues. In addition, adipose tissue emerges as a major source of estrogen in postmenopausal women.⁸ Estrogen receptor α (ER α) regulates survival, migration, differentiation and proliferation of breast cancer cells.^{9,10} Estrogen receptor (ER) and progesterone receptor (PgR) are widely expressed in female sex hormone-sensitive tissues, and are responsible for viability of mammary glandular cells at perimenopausal time. Levels of estradiol, progesterone and leptin also increase in the blood of breast cancer patients.⁴ Secretion of leptin seems to be more associated with regulation of female sex hormone production than with alterations of body weight.^{7,11}

Besides adipose tissue, leptin is expressed in vitro in a normal human breast epithelial cell line (MCF10A), breast

Received: Oct 18, 2005; accepted: March 5, 2006

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cancer cell lines (MCF-7, T47D, MDA-MB-231 and ZR75-1), and in vivo in breast tumors, including ductal cancer and atypical hyperplasia.^{5,12,13} Leptin supports cell proliferation in breast, esophageal and prostate cancer, but counteracts divisions of human Mia-PaCa and PANC-1 pancreatic cancer cells.¹⁰ Although several valuable reviews have been focused on the role of leptin in breast cancer,^{3,14} the current status of knowledge in this field changes quickly, and our insight should be continuously revised.

Obesity and leptin in a perspective of neoplastic growth

Increased supply of lipids resulted in elevation of serum leptin levels in the development of estrogen receptor-negative mammary tumors in MMTV-neu (strain 202) mice (mice mouse mammary tumor virus). However, latency, incidence and metastasis of mammary tumors were not affected by different diet-dependent alterations in leptin level.⁷ Moreover, Cleary *et al.* reported interesting findings concerning the relationship of leptin and obesity in breast cancer. Namely, oncogene-induced rodent mammary tumors did not develop in genetically homozygous obese female mice overexpressing human TGF- α (transforming growth factor- α) but having the leptin gene knocked out. On the other hand, the expected tumorigenesis occurred in lean mice homozygous or heterozygous for the leptin gene.¹⁵

Several studies have attempted to determine the prognostic significance of leptin and its receptor in breast cancer. Leptin receptor (OB-R) is expressed in tissues of human invasive ductal carcinomas. Immunohistochemical staining failed to reveal the presence of this receptor in normal mammary epithelium. Both tumoral and normal mammary glands were leptin-positive with remarkable accumulation in cancer cells. Distant metastases appeared in one third of cancers with strong immunoreactivity for leptin and OB-R. When present in the tumors, leptin and OB-R were ominous signs of poor prognosis. Patients lived shorter in comparison with life spans that were observed in cases of cancers without overexpression of leptin and OB-R.¹⁶

In accordance with insulin regulation and body mass index, circulating blood leptin correlated with overall survival of women with breast cancer.¹⁷ mRNA levels of leptin and its receptors (OB-R_a, OB-R_b) were also studied in invasive and noninvasive ductal and lobular breast cancer tissues. In case of women with high serum leptin or high intratumoral leptin mRNA levels, a poor prognosis was predicted only by high intratumoral OB-R_a and OB-R_b mRNA levels.¹⁸ As high serum levels of leptin are associated with obesity, these studies explain why obesity is a factor related to poor prognosis in breast cancer. Indeed, elevated serum levels of leptin coexisted with obesity, which is a well-known risk factor of postmenopausal breast cancer.¹¹ However, it was reported that loss of body

mass could be accompanied with a decrease in serum leptin level.^{7,11} There is an idea that regulatory properties of leptin depend on its effect on the metabolism of lipids, and as a satiety factor indirectly stimulates proliferation of breast cancer cells. Nevertheless, quantitative changes in serum leptin level did not predict the onset of breast tumors in cases of women whose blood was examined for the presence of leptin two years before incidence of mammary cancer.¹¹ Serum levels of leptin also did not differ significantly between groups of premenopausal breast cancer patients and healthy women. This finding indicates that leptin probably does not affect mammary tumorigenesis before menopause.¹⁹

Nipple aspirate fluid (NAF) also contains leptin, whose concentration increases with body mass index (BMI) only in premenopausal evaluations. At BMI values of <25, there was more leptin in postmenopausal samples of NAF than in premenopausal ones, while such differences were not revealed at higher BMI. On the other hand, serum pool of leptin correlated with quantity of this hormone in premenopausal but not in postmenopausal NAF. According to this study, eventual presence of breast cancer does not seem to influence the level of leptin in NAF, regardless of menopause.²⁰

Molecular background of leptin-associated proliferation

Mammary glandular epithelium interacts with interstitial cells (cross-talk) during development of breast cancer. MCF-7 breast cancer cells produce a high amount of IL-1 α , which activated the expression of leptin in stromal cells in a paracrine manner. This satiety hormone induced transcription of leptin-specific genomic sequences in estrogen-dependent breast tumor in an MCF-7 xenograft model.²¹ Although elevated serum levels of leptin probably contributed to cachexia, which seemed to be independent of other cachexia-cytokine stimulators such as IL-6, tumor necrosis factor- α or interferon- γ , these mice were not anorexic.²¹

Leptin switched on transcription of aromatase, and activated this enzyme with engagement of AP-1 (transcription activator protein 1) promoter, signal transducers and activators of transcription 3 (STAT3) and extracellular signal-regulated kinase-2 (ERK2) in MCF-7 cell line, promoting estradiol synthesis.²² Leptin also boosted stimulation of ER α by another mechanism. It activated ER α via the MAPK (mitogen activated protein kinase) pathway in MCF-7 and HeLa cells, which could be reversed by intracellular introduction of a dominant negative ERK2 or exposition to MAPK inhibitor PD 98059.²³ In CD2F1Cr mice, serum levels of leptin were downregulated after exogenous administration of t10,c12 isomer of antiangiogenic, dietary conjugated linoleic acid (CLA). Simultaneously, VEGF level was severely decreased, and mammary glands showed atrophy of adipocytes and the vasculature.

It might be highly probable for CLA to modify breast structure in a way that counteracts the onset of neoplastic growth or the aggressive course of malignancy.²⁴ Divisions of MCF-7 cells were reduced by a specific inhibitor of STAT3 tyrosine phosphorylation, while there was no significant growth arrest due to inhibition of ERK1/2 by a specific ERK1/2 kinase inhibitor, U0126. This finding indicated a crucial and indispensable role of STAT3 in mediation of leptin-stimulated proliferation.²⁵ Through the long form of its receptor, OB-Rb, leptin caused phosphorylation of STAT3, ERK1, and AP-1, mediating proliferation of normal (HBL100) and malignant (T47D) human breast epithelial cells.²⁶ It was confirmed by the [3H]-thymidine incorporation method that leptin induced MAPK-1/2-dependent proliferation of T47D cells.²⁷ MCF-7 cells grew under stimulation of leptin that activated STAT3 along with p42/p44 MAP kinase. In the light of these findings, leptin is suspected to be a growth factor, which is associated with increased incidence of breast cancer and obesity.²⁸

Leptin-induced resistance to antiestrogens

Leptin was hypothesized to increase aromatase activity *in vivo*, which was shown in cell cultures to derive from breast and abdominal adipose tissue of obese women. Therefore, leptin was a potential stimulator of estrogen synthesis that favored growth of breast glandular epithelium.²⁹ On the other hand, leptin abrogated anticancer function of the antiestrogen ICI 182,780 *in vitro*. This kind of antiestrogen led to fast degradation of membrane ER α , which reduced nuclear expression of the receptor and ER α -dependent transcription. After exposure to leptin, ER α -positive breast cancer cells underwent proliferation, mediated by active STAT3 and ERK1/2. Thereby, it seemed to be possible that elevated serum levels of leptin maintain resistance to antiestrogens during hormonal therapy of breast cancer.³⁰ On the other hand, leptin and IGF-I levels were not affected by antiestrogen treatment of tumors deriving from aromatase gene-transfected (MCF-7CA) cells growing in ovariectomized nude mice.³¹

Serum levels of leptin tend to increase with size of adipose tissue. Obesity is often associated with the incidence of breast cancer. Despite high levels of leptin, malfunction of OB-Rb effectively protected against mammary tumor incidence, and it caused a severe glandular underdevelopment of mammary glands without duct formation and branching in leptin receptor gene-knockout obese mice. Therefore, overproduction of leptin could accelerate tumor growth in the mammary gland.³² On the other hand, serum levels of leptin failed to correlate with lymph node status, so their monitoring role is questioned during progression of the neoplastic disease in the course of breast cancer.³³ Serum content of leptin was higher in breast cancer

patients who were administered antiestrogens, in comparison with a control group of healthy volunteers. Leptin was not related to the stage of malignant mammary disease, however, it increased in response to tamoxifen therapy.^{34,35} Moreover, serum levels of leptin rose significantly in breast cancer patients who developed more advanced hepatic steatosis as a possible side effect of the drug after three months of treatment with tamoxifen.³⁶ This might suggest that leptin could be one of the factors that maintain resistance to antiestrogens during hormonal treatment of breast cancer (Figure 1).

Conclusions

To summarize, leptin appears to be a very important factor in hormonal regulation of breast cancer growth. It has been suspected to affect certain intracellular second mediators that were found to participate in cell proliferation and survival. Antiestrogens seem to compete with leptin for diverse modulation of ER α activity. Therefore, increased serum levels of leptin may be responsible for overcoming antiproliferative effects induced by antiestrogen therapy of breast cancer.

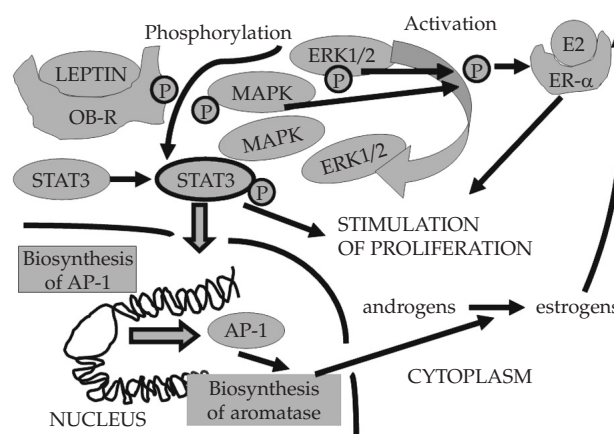


Figure 1. Leptin binds to its receptor OB-R, which becomes phosphorylated by MAPK or ERK. These intracellular kinases probably activate STAT3 by phosphorylation of its tyrosine residue. STAT3 is translocated to the nucleus, where it induces transcription of AP-1 that induces transcription of aromatase. Aromatase changes androgens into estrogens in adipose tissue. Estradiol activates its membrane receptors (ER α) and contributes to proliferation of breast epithelial cells. ER α is sensitized for estrogen stimulation by MAPK and ERK, which can transactivate this receptor independently of estrogens. Apart from induction of aromatase, STAT3 may contribute to cell proliferation by activation of other genes.

OB-R – leptin receptor, P – phosphate residue, AP-1 – transcription activator protein 1, E2 – estradiol, ER α – estrogen receptor α , ERK1/2 – extracellular signal-regulated kinase 1/2, MAPK – mitogen-activated protein kinase

References

1. Zhang Y, Proenca R, Maffei M, et al: Positional cloning of the mouse obese gene and its human homologue. *Nature* 372: 425-432, 1994.
2. Tartaglia LA: The leptin receptor. *J Biol Chem* 272: 6093-6096, 1997.
3. Gaja A and Chury Z: The importance of leptin in oncology—hypothesis or facts? *Vnitr Lek* 47: 245-249, 2001.
4. Tessitore L, Vizio B, Pesola D, et al: Adipocyte expression and circulating levels of leptin increase in both gynaecological and breast cancer patients. *Int J Oncol* 24: 1529-1535, 2004.
5. O'Brien SN, Welter BH and Price TM: Presence of leptin in breast cell lines and breast tumors. *Biochem Biophys Res Commun* 259: 695-698, 1999.
6. Das UN: Is obesity an inflammatory condition? *Nutrition* 17: 953-966, 2001.
7. Cleary MP, Grande JP, Juneja SC and Maihle NJ: Diet-induced obesity and mammary tumor development in MMTV-neu female mice. *Nutr Cancer* 50: 174-180, 2004.
8. Szymczak J, Milewicz A, Thijssen JH, et al: Concentration of sex steroids in adipose tissue after menopause. *Steroids* 63: 319-321, 1998.
9. Surmacz E and Bartucci M: Role of estrogen receptor alpha in modulating IGF-I receptor signaling and function in breast cancer. *J Exp Clin Cancer Res* 23: 385-394, 2004.
10. Koda M, Sulkowski S, Kanczuga-Koda L, et al: Expression of ERalpha, ERbeta and Ki-67 in primary tumors and lymph node metastases in breast cancer. *Oncol Rep* 11: 753-759, 2004.
11. Stattin P, Soderberg S, Biessy C, et al: Plasma leptin and breast cancer risk: a prospective study in northern Sweden. *Breast Cancer Res Treat* 86: 191-196, 2004.
12. Somasundar P, Yu AK, Vona-Davis and McFadden DW: Differential effects of leptin on cancer in vitro. *J Surg Res* 113: 50-55, 2003.
13. Caldefie-Chezet F, Damez M, de Latour M, et al: Leptin: A proliferative factor for breast cancer? Study on human ductal carcinoma. *Biochem Biophys Res Commun* 334: 737-741, 2005.
14. Markowska A, Malendowicz K and Drews K: The role of leptin in breast cancer. *Eur J Gynaecol Oncol* 25: 192-194, 2004.
15. Cleary MP, Phillips FC, Getzin SC, et al: Genetically obese MMTV-TGF-alpha/Lep(ob)Lep(ob) female mice do not develop mammary tumors. *Breast Cancer Res Treat* 77: 205-215, 2003.
16. Ishikawa M, Kitayama J and Nagawa H: Enhanced expression of leptin and leptin receptor (OB-R) in human breast cancer. *Clin Cancer Res* 10: 4325-4331, 2004.
17. Goodwin PJ, Ennis M, Fantus IG, et al: Is leptin a mediator of adverse prognostic effects of obesity in breast cancer? *J Clin Oncol* 23: 6037-6042, 2005.
18. Miyoshi Y, Funahashi T, Tanaka S, et al: High expression of leptin receptor mRNA in breast cancer tissue predicts poor prognosis for patients with high, but not low, serum leptin levels. *Int J Cancer* 118: 1414-1419, 2006.
19. Mantzoros CS, Bolhke K, Moschos S and Cramer DW: Leptin in relation to carcinoma in situ of the breast: a study of premenopausal cases and controls. *Int J Cancer* 80: 523-526, 1999.
20. Sauter ER, Garofalo C, Hewett J, et al: Leptin expression in breast nipple aspirate fluid (NAF) and serum is influenced by body mass index (BMI) but not by the presence of breast cancer. *Horm Metab Res* 36: 336-340, 2004.
21. Kumar S, Kishimoto H, Chua HL, et al: Interleukin-1 alpha promotes tumor growth and cachexia in MCF-7 xenograft model of breast cancer. *Am J Pathol* 163: 2531-2541, 2003.
22. Catalano S, Marsico S, Giordano C, et al: Leptin enhances, via AP-1, expression of aromatase in the MCF-7 cell line. *J Biol Chem* 278: 28668-28676, 2003.
23. Catalano S, Mauro L, Marsico S, et al: Leptin induces, via ERK1/ERK2 signal, functional activation of estrogen receptor alpha in MCF-7 cells. *J Biol Chem* 279: 19908-19915, 2004.
24. Masso-Welch PA, Zangani D, Ip C, et al: Isomers of conjugated linoleic acid differ in their effects on angiogenesis and survival of mouse mammary adipose vasculature. *J Nutr* 134: 299-307, 2004.
25. Yin N, Wang D, Zhang H, et al: Molecular mechanisms involved in the growth stimulation of breast cancer cells by leptin. *Cancer Res* 64: 5870-5875, 2004.
26. Hu X, Juneja SC, Maihle NJ and Cleary MP: Leptin—a growth factor in normal and malignant breast cells and for normal mammary gland development. *J Natl Cancer Inst* 94: 1704-1711, 2002.
27. Laud K, Gourdou I, Pessemesse L, et al: Identification of leptin receptors in human breast cancer: functional activity in the T47-D breast cancer cell line. *Mol Cell Endocrinol* 188: 219-226, 2002.
28. Dieudonne MN, Machinal-Quelin F, Serazin-Leroy V, et al: Leptin mediates a proliferative response in human MCF7 breast cancer cells. *Biochem Biophys Res Commun* 293: 622-628, 2002.
29. Magoffin DA, Weitsman SR, Agarwal SK and Jakimiuk AJ: Leptin regulation of aromatase activity in adipose stromal cells from regularly cycling women. *Ginek Pol* 70: 1-7, 1999.
30. Garofalo C, Sisci D and Surmacz E: Leptin interferes with the effects of the antiestrogen ICI 162,780 in MCF-7 breast cancer cells. *Clin Cancer Res* 10: 6466-6475, 2004.
31. Nunez NP, Jelovac D, Macedo L, et al: Effects of the antiestrogen tamoxifen and the aromatase inhibitor letrozole on serum hormones and bone characteristics in a preclinical tumor model for breast cancer. *Clin Cancer Res* 10: 5375-5380, 2004.
32. Cleary MP, Juneja SC, Phillips FC, et al: Leptin receptor-deficient MMTV-TGF-alpha/Lepr(db)Lepr(db) female mice do not develop oncogene-induced mammary tumors. *Exp Biol Med* 229: 182-193, 2004.
33. Coskun U, Gunel N, Toruner FB, et al: Serum leptin, prolactin and vascular endothelial growth factor (VEGF) levels in patients with breast cancer. *Neoplasma* 50: 41-46, 2003.
34. Ozet A, Arpacı F, Yilmaz MI, et al: Effects of tamoxifen on the serum leptin level in patients with breast cancer. *Jpn J Clin Oncol* 31: 424-427, 2001.
35. Marttunen MB, Andersson S, Hietanen P, et al: Antiestrogenic tamoxifen and toremifene increase serum leptin levels in postmenopausal breast cancer patients. *Maturitas* 35: 175-179, 2000.
36. Gunel N, Coskun U, Toruner FB, et al: Serum leptin levels are associated with tamoxifen-induced hepatic steatosis. *Curr Med Res Opin* 19: 47-50, 2003.