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ORIGINAL CONTRIBUTIONS

Experimental ONCOLOGY

LEPTIN RECEPTORS EXPRESSION IN MAMMARY TUMORS AND MAMMARY FAT PAD OF TRANSGENIC MAMMARY CANCER MOUSE MODEL

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***Background*: Leptin is an adipokine encoded by the *Ob* (obese) gene and predominantly produced by adipocytes. The roles of both leptin and leptin receptor (ObR) in numerous pathophysiological conditions including mammary tumor (MT) development have been reported. *Aim*: To examine protein expression levels of leptin and its receptors (ObR) including the long form, ObRb, in MT tissue and mammary fat pad of a transgenic mammary cancer mouse model. Further, we investigated whether the effects of leptin on MT development are systemic or local. *Materials and Methods*: MMTV-TGF-α transgenic female mice were fed *ad libitum* from week 10 up to week 74. Protein expression levels of leptin, ObR, and ObRb were measured in the mammary tissue samples of 74-week old MMTV-TGF-α mice with and without MT (MT-positive/MT-negative) by Western blot analysis. Serum leptin levels were measured by using the mouse adipokine LINCOplex kit 96-well plate assay. *Results*: Protein expression levels of ObRb were significantly lower in MT as compared to control tissue of mammary gland. In addition, protein expression levels of leptin were significantly higher in the MT tissue of MT-positive mice compared to control tissue of MT-negative mice. However, ObR protein expression levels in tissues of mice with and without MT were similar. Serum leptin levels at different ages were not significantly different between the two groups. *Conclusion*: Leptin and ObRb in the mammary tissue may play a critical role in the mammary cancer development, while contribution of short ObR isoform may be less important.**

***Key Words*: leptin, leptin receptor, mammary tumor, mammary fat pad, MMTV-TGF-α mice.**

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Leptin is an adipokine produced and released primarily by the adipocytes correlative to the amount of body fat [1]. A major function of leptin is to adjust food intake as well as energy expenditure [2]. Leptin also has important roles in reproduction, immune system and metabolism [3]. Importantly, leptin is connected with the progression of mammary tumor (MT) development by activating antiapoptotic and metastatic pathways [4–7]. In addition to the breast, leptin and its receptors are produced in many other tissues such as brain [8], liver [9], lung [10], ovary [11] and prostate [12].

Leptin response is mediated by leptin receptors (ObRs). ObR has at least six different isoforms, which have intracellular domains of different lengths. The long leptin receptor isoform (ObRb) has the greatest signaling capacity owing to its long intracellular domain [13]. Binding of leptin to ObRb stimulates cancer development by triggering a number of signaling pathways, including JAK/STAT3, PI3K/AKT, and MAPK/ERK, participating in proliferation, apoptosis, migration, invasion, and angiogenesis [14–16]. Consistently, it has been previously reported that leptin and its receptors are overexpressed in breast cancer [17–20]. It is known that the leptin hormone uses various mechanisms for this task. Leptin initiates cell escape mechanisms from apoptosis, stimulates cell growth, induces matrix degrading enzymes and angiogenic factors that accelerate cancer progression [21] through binding to its receptor (ObRb) and activating cancer related signaling pathways [22]. Also, the overexpression of ObR in cancerous cells has led to its suggestion as a diagnostic marker for ovarian and endometrial cancer [23, 24]. However, the molecular basis for the effects of leptin on cancer progression remains largely unknown [22].

Based on recent studies, increased leptin expression in overweight and obese people plays a role in the development and progression of many types of cancer. It has been reported that high ObRb expression is involved in induction of metastatic cell migration and invasion in obese women [25]. The relationship between leptin and breast cancer risk has been reported to be associated with menopausal status: Pan *et al.* [26] showed that leptin level is associated with breast cancer in the post-menopausal period, but not in the pre-menopausal period. A recent study revealed that inhibiting leptin expression in breast cancer cells reduces the tumor volume and the aggressiveness of breast cancer [27].

Our group previously reported that mice lacking leptin and its receptors do not develop MTs. In this context, we showed that obese MMTV-TGF-α-

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Abbreviations used: BW – body weight; MFP – mammary fat pad; MT – mammary tumor; ObR – leptin receptor; ObRb – long form of leptin receptor.

$Lep^{ob}Lep^{ob}$ mice, produced by cross-breeding of a Lep strain mice with the transgenic MMTV-TGF-α mice, do not develop oncogene-induced MTs, whereas their non-obese littermates do [28]. Similarly, when a Lepr strain mice containing a mutation in its ObR was mated with the MMTV-TGF-α mice, obese MMTV-TGF-α-$Lepr^{db}Lepr^{db}$ mice did not develop MTs [29]. These data can be interpreted as MT development in MMTV-TGF-α mice is not associated with obesity. However, in another study we observed that in diet-induced obese MMTV-TGF-α mice, MT latency is shortened [7]. All these studies suggest that leptin is an important protein for breast cancer development.

Studies from different groups reported controversial results regarding the link between breast cancer and serum leptin levels: a positive correlation between serum leptin levels and the risk of breast cancer development has been reported in a number of studies [26, 30], while others suggested either no correlation or a negative correlation [31, 32]. Notably, there are only a few studies evaluating both the serum leptin levels and protein expression of leptin in breast tissue. However, evaluating the serum leptin levels, expression of leptin and ObRs together in both healthy breast tissue and breast tumor is necessary and important to better understand the role of leptin signaling in breast cancer development.

There are no previous *in vivo* studies investigating both serum leptin levels and ObR protein expression levels in cancer tissues. Besides, a few studies examining the expression levels of ObRs in healthy breast tissue have been conducted mostly in humans. In this study, we investigated the protein levels of both the short (ObR) and long (ObRb) isoforms of leptin receptor in mouse mammary fat pad (MFP) tissues, MT tissue as well as in healthy mammary tissue surrounding tumor tissue as a control to investigate whether their expression levels differ between MT and healthy tissue. We also examined the protein expression levels of leptin and its receptors (short and long isoforms) in the MFP tissue of 74-week-old MMTV-TGF-α mice with and without MT development.

MATERIALS AND METHODS

Study design. MMTV-TGF-α transgenic mice, overexpressing TGF-α (transforming growth factor-α) in mammary epithelial cells [33] used in the present study were originally developed in Dr. Coffey's laboratory [34]. These mice were obtained from a breeding colony maintained at the Hormel Institute using the breeding protocol and genotyping assay previously described [35]. Mice were fed AIN-93M diet [36] (Harlan Teklad, USA) from 8 until 74 weeks of age. Mice were caged individually. Food intake was determined daily, while body weight measurements along with physical examination for any pathologies, including the presence of MTs, were performed weekly. Serum samples were collected when possible during the course of the experiment. Terminal blood samples were collected from all mice at euthanasia. Suspected MT/mammary tissues at the back of the neck, where MTs first develop, along with mammary, parametrial and retroperitoneal fat pads, were removed and weighed. Tissue samples were maintained in formalin and sent to the Department of Pathology and Laboratory Medicine, Mayo Foundation, Rochester, MN for histopathological analyses in a blinded fashion. Based on histopathological parameters, all confirmed MT samples were grade 2 (Fig. 1). Remaining tissues were stored at –80 °C until further analyses. Mice were divided into two groups (n = 8) according to their MT status: those with MT (MT-positive) and without MT (MT-negative). The mice, which had similar tumor weights, fat pad weights, and body weights were selected in both MT-positive and MT-negative groups in order to minimize the effects of these parameters. The mammary tissue of MT-negative mice was used as control. All procedures involving experimental mice were conducted under the guidelines and approval of the University of Minnesota Institutional Animal Care and Use Committee in an AAALAC accredited facility.

Western blot analysis for leptin and its receptors. MFP and tumor tissues collected from individual mice were lysed in an extraction buffer containing protease inhibitors. Total protein was extracted using T-PER Tissue Total Protein Extraction reagent (Pierce, USA), according to the manufacturer's protocol and quantified by the Bio-Rad protein assay kit, using bovine serum albumin as the standard (Bio-Rad Laboratories, USA). Protein extracts were separated by electrophoresis on 4–15% polyacrylamide gradient gels (Bio-Rad Laboratories, USA) and transferred onto a polyvinylidene difluoride membrane (Immobilon-P, Millipore, USA). Membranes, blocked in 1% milk concentrate and 0.1% Tween-20 containing Tris-base solution (Bio-Rad Laboratories, USA) were probed with anti-ObR (Santa Cruz Biotechnology Inc, USA), anti-ObRb (Linco — Millipore, USA), anti-leptin (Santa Cruz Biotechnology Inc, USA), anti-β-actin (Delta Biolabs, USA) primary antibodies. Following incubation with the primary antibody, membranes were incubated with an appropriate alkaline phosphatase-conjugated secondary antibody (Santa Cruz Biotechnology Inc, USA). Blots were stained with Enhanced Chemifluorescence (Amersham Biosciences, USA) and visualized using a Storm 840 Machine Imaging System (Amersham Biosciences, USA). Band intensities were quantified by densitometric analysis using the UN-SCAN-IT gel software (Silk Scientific, USA). Data are represented as the ratio of the intensity of the protein of interest to the intensity of β-actin from the same sample. The same β-actin density values were used for the normalization analyses when ObR and ObRb expression levels in MT and MFP were analyzed since the same gel and membrane were used for both ObR and ObRb analyses. During Western blot analyses membranes were cut into three parts for ObRb 120 kDa, ObR about 90 kDa and β-actin 42 kDa before the incubation of the membranes with primary antibodies. Each protein was measured in one MT per animal

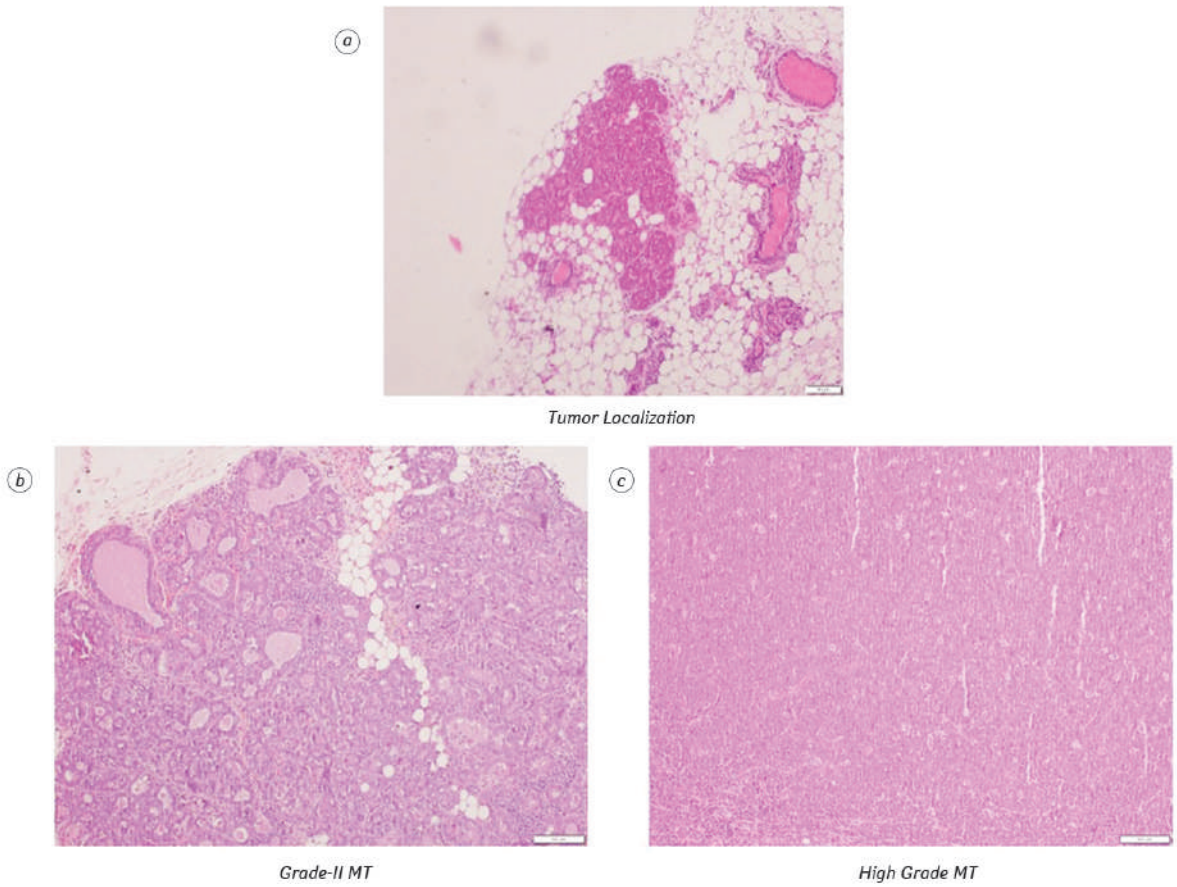

Fig. 1. Histopathological images of MTs of different grades/differentiation: *a* — localization of tumor in mouse mammary tissue; *b* — representative histopathological image of lower degree, grade II MT; *c* — representative histopathological image of higher degree, grade III MT; highly undifferentiated mammary tumor, ×100

and samples from 8 different animals were analyzed by Western blot.

Measurement of serum leptin levels. At each time point, blood samples were taken 5 h after mice were given their daily portion of food. Serum samples were taken at multiple time points in order to investigate whether the MT development was preceded by the changes in serum leptin levels. The "n" value was 8 at week 74, while it was 3–7 at the earlier time points. This was attributable to obtaining small or no samples or some values were out of standard range for the absolute values. Leptin levels were measured using the mouse adipokine LINCOplex kit 96-well plate assay [6].

Statistical analysis. Data were represented as a measure of central tendency (mean) ± standard error of mean. Differences between two groups were analyzed by unpaired Student's *t*-test and considered significant when $p < 0.05$ (two-tailed). In addition, Cohen's d was indicated where statistical significance was achieved as a measure of effect size. Specifically, d = 0.2 is considered as a "small" effect size, 0.5 represents a "medium" effect size and 0.8 a "large" effect size. This means that if the difference between two groups' means is less than 0.2 standard deviations, the difference is negligible, even if it is statistically significant. "n" refers to the number of individual mice in each group. "n" is 8, unless otherwise indicated in figure legends. Data presented in this study were captured in Microsoft Excel (Microsoft Office 2013) and statistical analysis conducted in GraphPad Prism software (Graphpad Prism® version 9.0, GraphPad Software, La Jolla, USA).

RESULTS

Body weights, fat pad weights and MT detection. The final body weights (BWs) of the mice with MT were obtained by subtracting the weight of MT from the total BW. No significant differences in BWs, parametrial, MFP, retroperitoneal, as well as total fat pad weights were detected between MT-positive and MT-negative mice. No MTs were detected by palpation before the terminal age of 74 weeks. The average MT weight was approximately half a gram in MT-positive mice.

Protein levels of short form of ObR and ObRb in MT and MFP. We measured protein levels of short and long leptin receptor isoforms in tissues collected from MT-positive and MT-negative mice. ObR protein levels were similar between MT and the control tissue ($p > 0.05$) (Fig. 2, *a*). Similarly, ObR protein levels in the MFP samples of MT-positive mice were not statistically different compared to that of MT-negative mice ($p > 0.05$) (Fig. 2, *b*). On the other hand, ObRb protein levels were significantly lower in MT compared to the control tissue ($p < 0.001$) (Fig. 3, *a*). However,

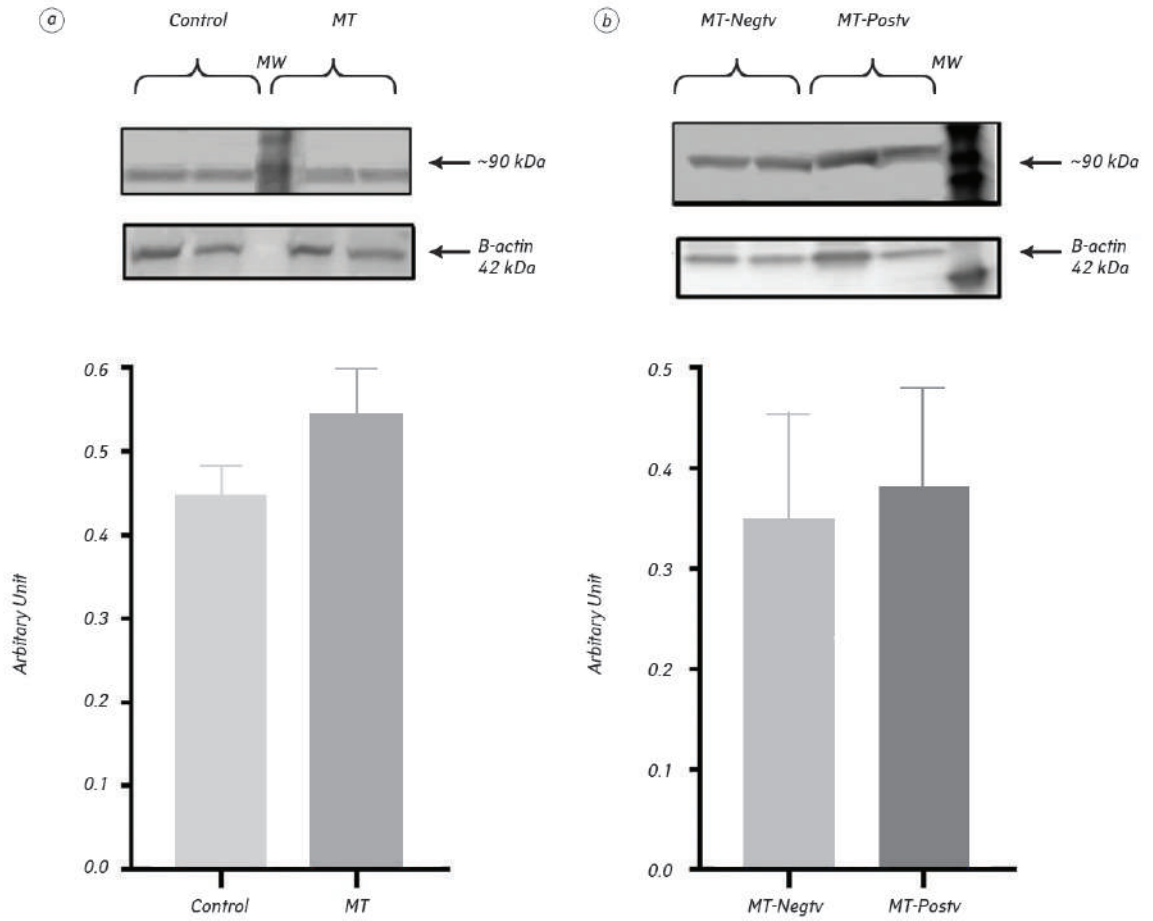

Fig. 2. Protein expression levels of ObR in mice with or without MT at 74 weeks of age: *a* –expression in MT or control tissues; *b* — expression in MFP from MT-positive or MT-negative animals. Control tissues were removed from the same location in MT-negative animals where MT grows in MT-positive animals of the same age. Data are the average density values of eight individual mice in each group (n = 8). MFP and either MT or control tissues were taken from the same animal for each "n" value

the levels of ObRb protein in MFP of MT-positive mice were not significantly different from that of MT-negative mice ($p > 0.05$) (Fig. 3, *b*).

Leptin protein levels in MT and MFP. Levels of leptin protein in MT and MFP samples taken from MT-positive and MT-negative mice were also examined and compared. Leptin levels were significantly higher in MT samples from the MT-positive mice compared to the leptin levels in the control tissue from the MT-negative mice (Fig. 4, *a*) ($p < 0.01$). However, leptin levels in MFP from MT-positive and MT-negative mice were not significantly different (Fig. 4, *b*) ($p > 0.05$).

Serum leptin levels. Serum leptin levels between MT-positive and MT-negative mice at terminal age (74 weeks) were not significantly different (Fig. 5). Average serum leptin levels in MT-positive and MT-negative mice at week 74 were 4.73 ± 0.96 ng/ml and 4.82 ± 1.13 ng/ml, respectively (mean ± standard error of mean) ($p > 0.05$). Serum leptin levels at earlier time points (10, 13, 19, 25, 31, 37, 43, 49, and 58 weeks of age) were also similar between MT-positive and MT-negative mice ($p > 0.05$). However, there was an increase in serum leptin levels over the course of the study both for MT-positive and MT-negative mice after week 13, although this was not statistically significant.

DISCUSSION

In this study, we report that 74-week-old MT-positive mice had significantly higher leptin protein expression levels in the MT tissue compared to the control tissue of the MT-negative mice. However, ObRb protein expression levels decreased dramatically in the MT tissue of MT-positive mice compared to the control tissue. There was no significant difference in ObR expression between tumor and control tissues in these mice. Additionally, no significant difference was measured between MT-positive and MT-negative mice regarding the expression of leptin, ObRb and ObR protein levels in MFP tissues. Moreover, serum leptin levels were also similar in MT-positive and MT-negative mice. These data are consistent with the previous studies reporting the involvement of leptin signaling pathway in breast cancer development.

One of the mechanisms why the expression levels of ObRb is reduced in MT could be explained by negative feedback. It is well known that reduction in ligand receptors (ObRb in the case here) is a common mechanism for the body to sustain its homeostasis to prevent the effects of higher levels of a ligand (leptin in the case here). Literature data including our own results have reported the proliferative effects of leptin presented in excessive amounts on breast

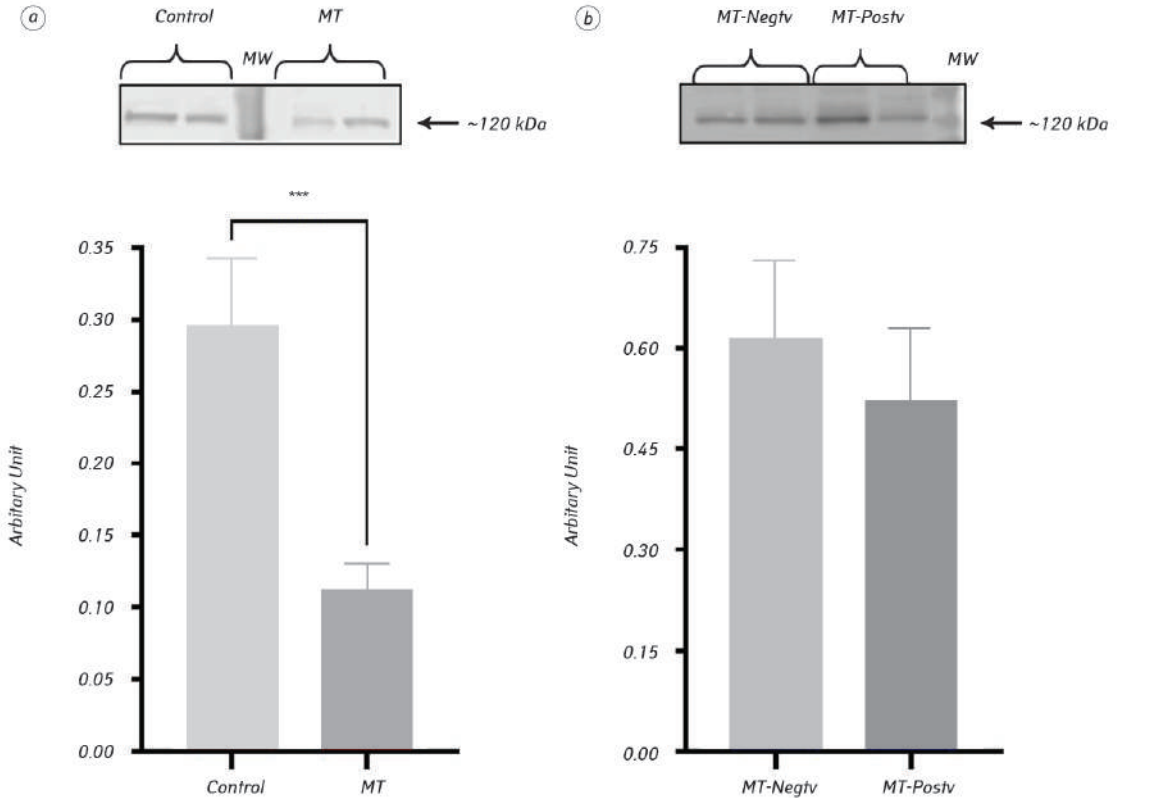

Fig. 3. Protein expression levels of ObRb in mice with or without MT at 74 weeks of age: *a* — expression in MT or control tissues; *b* — expression in MFP tissues from MT-positive or MT-negative animals. Control tissues were removed from the same location in MT-negative animals where MT grows in MT-positive animals at the same age. The same β-actin density values as in Fig. 2 were used for the normalization analyses since ObR and ObRb expression levels in MT and MFP were analyzed using the same gel and membrane for both ObR and ObRb analyses. Data are the average density values of eight individual mice in each group (n = 8). MFP and either MT or control tissues were taken from the same animal for each "n" value. ***Difference between control and MT tissues is significant ($p = 0.0009$)

cancer cells [37, 38]. More importantly, studies have suggested that leptin to adiponectin ratio is more important for cancer cell proliferation than the levels of either leptin or adiponectin alone [39]. ObRb is in the center of most of the leptin and its signaling pathways related studies since leptin functions through activation of ObRb, a transmembrane receptor, extending from extracellular space to intracellular space, while other forms (short form) of leptin receptors stay short to reach intracellular space [27, 40]. Similar to the findings in the present study, both protein and mRNA expression levels of ObRb were lower in MT or MFP tissues of high fat diet fed animals which had higher MT incidence rate compared to that of low fat diet fed animals. On the other hand, expression levels of neither ObR nor ObRb were different in MT of animals applied to different types of caloric restriction, while their expression levels were lower in MFP tissues of animals applied to caloric restriction [41]. Moreover, most studies have reported that ObR and ObRb expression levels are usually affected in the same direction [7, 37] although some studies showed an oppositely directed effect [42]. This possibly depends on the tissue or cell types.

The relationship of leptin and/or ObRs with breast cancer has been presented in a number of *in vivo* and *in vitro* studies [7, 37, 38, 43, 44]. Some of these confirmed the positive correlation between breast cancer development and leptin expression, while others did not find significant differences in this regard. Previously we reported no differences between leptin and ObR protein expression levels in the liver tissue of MT-negative and MT-positive mice [45]. Although in this study, serum leptin amount in MT-positive mice was similar to the control group, in a recently published meta-analysis by Hao *et al.* [46] the serum leptin levels in breast cancer patients were significantly higher compared to the healthy control group. Similarly in another study, Liang *et al.* [47] reported significantly higher leptin mRNA expression levels in MT tissues compared to adjacent healthy tissues of breast cancer patients. Rogozina *et al.* [42] measured leptin protein expression in mammary tissue, MT, and MFP in different types of calorie-restricted mice and reported significantly higher expression in mammary tissue of calorie-restricted mice compared with *ad libitum* group. Also they measured ObR and ObRb mRNA levels in MT but could not find any significant difference between dietary groups [42].

Our main goal was to observe the differences of serum leptin levels and tissue leptin signaling pathways in MT-positive and MT-negative mice to better understand the roles of leptin and its signaling molecules in MT development. In the present study, serum leptin levels increased over the course of the study especially after week 19 compared to week 10 or 13, although this

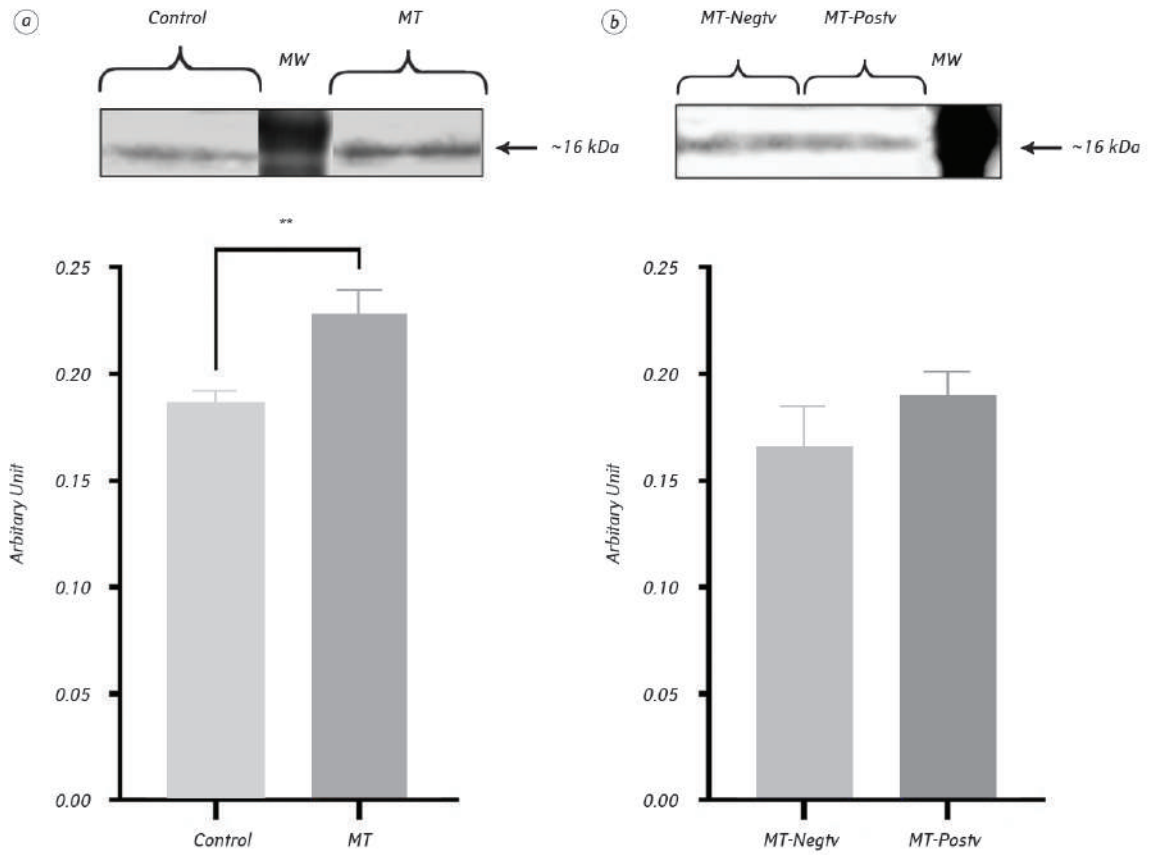

Fig. 4. Protein expression levels of leptin in mice with and without MT at 74 weeks of age: *a* — expression in MT or control tissues; *b* — expression in MFP tissues from MT-positive or MT-negative animals. Control tissues were removed from the same location in MT-negative animals where MT grows in MT-positive animals at the same age. Data are the average density values of eight individual mice in each group (n = 8). MFP and either MT or control tissues were taken from the same animal for each "n" value. **Difference between control and MT tissues is significant ($p = 0.006$)

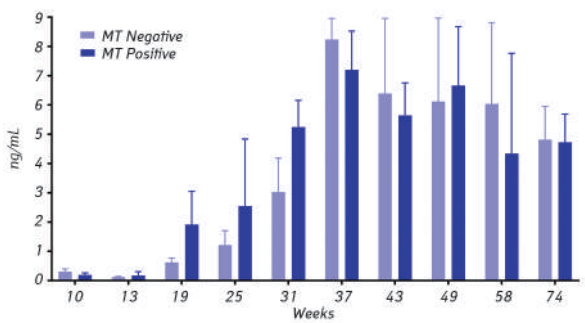

Fig. 5. Serum leptin levels in mice with and without MT. Serum samples were collected at different time points, week 10 through week 74. Serum leptin levels were measured as explained in Materials and Methods. Although serum leptin level is an average of seven to eight individual mice for week 74 (n = 7–8), it is three to seven (n = 3–7) for earlier time points

was not statistically significant. This could be because of the limited number of samples in earlier time points. In addition, the effects of changes in serum adipokines including leptin and adiponectin levels with aging on MT development has already been published in our previous studies [6].

In the present study, we detected a significantly positive correlation between MT development and leptin protein expression levels at the tissue level. Consistent with our data, previously Ishikawa *et al.* [17] showed leptin overexpression in invasive ductal carcinoma cells in comparison to adjacent normal epithelial cells in humans by using immunochemistry technique. Although, in their study, significantly higher ObR expression levels in ductal carcinoma cells compared to normal cells were reported, we did not detect any significant difference in ObR protein expression levels between MT tissue and control mammary tissues of the same animal. There may be several reasons to explain the differences in results amongst the studies. Firstly, our results may be due to the negative feedback effects of high leptin levels in local tissue since it is possible that high leptin levels may downregulate its receptor(s) on cell membranes in order to reduce high level leptin effects. Secondly, other locally secreted factors like insulin-like growth factor 1, tumor necrosis factor alpha, interleukin-6, miRNAs and adiponectin may also reduce ObRb levels *via* their paracrine effects since modulation of leptin signaling by these molecules have been reported in previous studies [48, 49]. For example, the positive correlation between interleukin-6 and leptin and also tumor necrosis factor alpha and leptin was reported in our previous study [48]. Recently, possible modulation of leptin signaling by miRNAs and methylation has also been reported [43, 44]. These modulations may well play roles in the activation of downstream signaling pathways of leptin such as Stat3 and MAPK,

Erk1/2 since activation of these small proteins or transcription factors by leptin is well known. In this context, leptin-induced prostate cancer cell migration is mediated by Stat3. In addition, endometrial cancer cell invasion is modulated by PI3K and Jak/Stat3 signaling pathway. Thirdly, we utilized western blot to quantify ObR and ObRb protein expression levels while other studies measured these protein expression levels by immunohistochemistry staining techniques, which only detects the localization of the receptors, but do not allow their quantification [17]. Finally, previous studies were conducted in clinical samples, while in our current study samples from mice were used. To our knowledge, no study has reported ObR expression levels in tumors of mice.

In conclusion, leptin protein expression levels were significantly higher in MT compared to control mammary tissue of 74-week old mice [18]. On the other hand, ObRb protein expression levels were significantly lower in MT compared to control mammary tissue in MMTV-TGF-α mammary cancer mouse model. Additionally, ObR protein expression levels in MFP were not influenced by the presence of MTs. These results show that two ObR subtypes are differentially regulated in different tissues. Thus, leptin and ObRb in the mammary tissue may play a critical role in the breast cancer development; however, the local expressions of short isoform of ObR may have less contribution to MT development. Further studies are needed to better understand the roles of these two leptin receptor isoforms in the development of breast cancer.

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CONFLICTS OF INTEREST

The authors declare no potential conflicts of interest.

ETHICS APPROVAL

This study was performed according to the guidelines and approval of the University of Minnesota Institutional Animal Care and Use Committee in an AAALAC accredited facility. Approval Number: 0212A37582 Approval Date: December 17th, 2002.

REFERENCES

1. **Izquierdo AG, Crujeiras AB, Casanueva FF, *et al.*** Leptin, obesity, and leptin resistance: where are we 25 years later? Nutrients 2019; **11**: 2704. doi:10.3390/nu11112704

2. **Juárez-Cruz JC, Zuñiga-Eulogio MD, Olea-Flores M, *et al.*** Leptin induces cell migration and invasion in a FAK-Src-dependent manner in breast cancer cells. Endocr Connect 2019; **8**: 1539–52. doi:10.1530/EC-19-0442

3. **Münzberg H, Morrison CD.** Structure, production and signaling of leptin. Metabolism 2015; **64**: 13–23. doi:10.1016/j.metabol.2014.09.010

4. **Everitt AV, Porter BD, Wyndham JR.** Effects of caloric intake and dietary composition on the development of proteinuria, age-associated renal disease and longevity in the male rat. Gerontology 1982; **28**: 168–75. doi:10.1159/000212530

5. **Dogan S, Cicekdal MB, Ozorhan U, *et al.*** Roles of adiponectin and leptin signaling-related micrornas in the preventive effects of calorie restriction in mammary tumor development. Appl Physiol Nutr Metab 2021; **46**: 866–76. doi:10.1139/APNM-2020-1000/SUPPL_FILE/APNM-2020-1000SUPPLA.DOCX

6. **Dogan S, Rogozina OP, Lokshin AE, *et al.*** Effects of chronic vs. intermittent calorie restriction on mammary tumor incidence and serum adiponectin and leptin levels in MMTV-TGF-α mice at different ages. Oncol Lett 2010; **1**: 167–76. doi:10.3892/OL_00000031/HTML

7. **Dogan S, Hu X, Zhang Y, *et al.*** Effects of high-fat diet and/or body weight on mammary tumor leptin and apoptosis signaling pathways in MMTV-TGF-α mice. Breast Cancer Res 2007; **9**: 1–15. doi:10.1186/bcr1840

8. **Malik KF, Young WS.** Localization of binding sites in the central nervous system for leptin (OB protein) in normal, obese (ob/ob), and diabetic (db/db) C57BL/6J mice. Endocrinology 1996; **137**: 1497–500. doi:10.1210/endo.137.4.8625929

9. **Briscoe CP, Hanif S, Arch JRS, *et al.*** Leptin receptor long-form signalling in a human liver cell line. Cytokine 2001; **14**: 225–9. doi:10.1006/cyto.2001.0871

10. **Tsuchiya T, Shimizu H, Horie T, *et al.*** Expression of leptin receptor in lung: Leptin as a growth factor. Eur J Pharmacol 1999; **365**: 273–9. doi:10.1016/S0014-2999(98)00884-X

11. **Choi JH, Park SH, Leung PCK, *et al.*** Expression of leptin receptors and potential effects of leptin on the cell growth and activation of mitogen-activated protein kinases in ovarian cancer cells. J Clin Endocrinol Metab 2005; **90**: 207–10. doi:10.1210/jc.2004-0297

12. **Somasundar P, Frankenberry KA, Skinner H, *et al.*** Prostate cancer cell proliferation is influenced by leptin. J Surg Res 2004; **118**: 71–82. doi:10.1016/j.jss.2004.01.017

13. **Linares RL, Benítez JGS, Reynoso MO, *et al.*** Modulation of the leptin receptors expression in breast cancer cell lines exposed to leptin and tamoxifen. Sci Rep 2019; **9**: 19189. doi:10.1038/s41598-019-55674-x

14. **Haque I, Ghosh A, Acup S, *et al.*** Leptin-induced ER-α-positive breast cancer cell viability and migration is mediated by suppressing CCN5-signaling via activating JAK/AKT/STAT-pathway. BMC Cancer 2018; **18**: 99. doi:10.1186/s12885-018-3993-6

15. **Kumar J, Fang H, McCulloch DR, *et al.*** Leptin receptor signaling via Janus kinase 2/Signal transducer and activator of transcription 3 impacts on ovarian cancer cell phenotypes. Oncotarget 2017; **8**: 93530–40. doi:10.18632/oncotarget.19873

16. **Zahid H, Subbaramaiah K, Iyengar NM, *et al.*** Leptin regulation of the p53-HIF1α/PKM2-Aromatase axis in breast adipose stromal cells: A novel mechanism for the obesity-breast cancer link. Int J Obes 2018; **42:** 711–20. doi:10.1038/ijo.2017.273

17. **Ishikawa M, Kitayama J, Nagawa H.** Enhanced expression of leptin and leptin receptor (OB-R) in human breast cancer. Clin Cancer Res 2004; **10**: 4325–31. doi:10.1158/1078-0432.CCR-03-0749

18. **Sánchez-Jiménez F, Pérez-Pérez A, de la Cruz-Merino L, *et al.*** Obesity and breast cancer: role of leptin. Front Oncol 2019; **9**: 596. doi:10.3389/fonc.2019.00596

19. **Garofalo C, Koda M, Cascio S, *et al.*** Increased expression of leptin and the leptin receptor as a marker of breast cancer progression: Possible role of obesity-related stimuli. Clin Cancer Res 2006; **12**: 1447–53. doi:10.1158/1078-0432.CCR-05-1913

20. **Liu CR, Li Q, Hou C, *et al.*** Changes in body mass index, leptin, and leptin receptor polymorphisms and breast cancer risk. DNA Cell Biol 2018; **37**: 182–8. doi:10.1089/dna.2017.4047

21. **Barone I, Giordano C, Bonofiglio D, *et al.*** Leptin, obesity and breast cancer: progress to understanding the molecular connections. Curr Opin Pharmacol 2016; **31**: 83–9. doi:10.1016/j.coph.2016.10.003

22. **Ghasemi A, Saeidi J, Azimi-Nejad M, *et al.*** Leptin-induced signaling pathways in cancer cell migration and invasion. Cell Oncol 2019; **42**: 243–60. doi:10.1007/s13402-019-00428-0

23. **Zhang Y, Liu L, Li C, *et al.*** Correlation analysis between the expressions of leptin and its receptor (ObR) and clinicopathology in endometrial cancer. Cancer Biomarkers 2014; **14**: 353–9. doi:10.3233/CBM-140415

24. **Uddin S, Bu R, Ahmed M, *et al.*** Overexpression of leptin receptor predicts an unfavorable outcome in Middle Eastern ovarian cancer. Mol Cancer 2009; **8**: 74. doi:10.1186/1476-4598-8-74

25. **Kato S, Abarzua-Catalan L, Trigo C, e*t al.*** Leptin stimulates migration and invasion and maintains cancer stem-like properties in ovarian cancer cells: An explanation for poor outcomes in obese women. Oncotarget 2015; **6**: 21100–19. doi:10.18632/oncotarget.4228

26. **Pan H, Deng LL, Cui JQ, *et al.*** Association between serum leptin levels and breast cancer risk. Medicine 2018; **97**: e11345. doi:10.1097/MD.0000000000011345

27. **Strong AL, Ohlstein JF, Biagas BA, *et al.*** Leptin produced by obese adipose stromal/stem cells enhances proliferation and metastasis of estrogen receptor positive breast cancers. Breast Cancer Res 2015; **17**: 112. doi:10.1186/s13058-015-0622-z

28. **Cleary MP, Phillips FC, Getzin SC, *et al.*** Genetically obese MMTV-TGF-α/Lepob Lepob female mice do not develop mammary tumors. Breast Cancer Res Treat 2003; **77**: 205–15. doi:10.1023/A:1021891825399

29. **Cleary MP, Juneja SC, Phillips FC, *et al.*** Leptin receptor-deficient MMTV-TGF-α/LeprdbLepr db female mice do not develop oncogene-induced mammary tumors. Exp Biol Med 2004; **229**: 182–93. doi:10.1177/153537020422900207

30. **Chen DC, Chung YF, Yeh YT, *et al.*** Serum adiponectin and leptin levels in Taiwanese breast cancer patients. Cancer Lett 2006; **237**: 109–14. doi:10.1016/j.canlet.2005.05.047

31. **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 2006; **118**: 1414–9. doi:10.1002/ijc.21543

32. **Stattin P, Söderberg S, Biessy C, *et al.*** Plasma leptin and breast cancer risk: A prospective study in northern Sweden. Breast Cancer Res Treat 2004; **86**: 191–6. doi:10.1023/B:BREA.0000036782.11945.d7

33. **Panico L, D'Antonio A, Salvatore G, *et al.*** Differential immunohistochemical detection of transforming growth factor α, amphiregulin and CRIPTO in human normal and malignant breast tissues. Int J Cancer 1996; **65**: 51–6. doi:10.1002/(SICI)1097-0215(19960103)65:1<51::AID-IJC9>3.0.CO;2-0

34. **Matsui Y, Halter SA, Holt JT, *et al.*** Development of mammary hyperplasia and neoplasia in MMTV-TGFα transgenic mice. Cell 1990; **61**: 1147–55. doi:10.1016/ 0092-8674(90)90077-R

35. **Cleary MP.** Weight-cycling decreases incidence and increases latency of mammary tumors to a greater extent than does chronic caloric restriction in mouse mammary tumor virus- transforming growth factor-α female mice. CEBP 2002; **11**: 836–43.

36. **Reeves PG, Nielsen FH, Fahey GC.** AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993; **123**: 1939–51. doi:10.1093/jn/123.11.1939

37. **Demirel PB, Dogan S, Ozorhan U, *et al.*** Effects of leptin on the viability of MCF-7 and T47D cells at different glucose concentrations. J Exp Clin Med 2020; **37**: 119–25. PMID: 33408552

38. **Dubois V, Jardé T, Delort L, *et al.*** Leptin induces a proliferative response in breast cancer cells but not in normal breast cells. Nutr Cancer 2014; **66**: 645–55. doi:10.1080/01635581.2014.894104

39. **Grossmann ME, Ray A, Dogan S, *et al.*** Balance of adiponectin and leptin modulates breast cancer cell growth. Cell Res 2008; **18**: 1154–6. doi:10.1038/cr.2008.293

40. **Popko K, Gorska E, Stelmaszczyk-Emmel A, *et al.*** Proinflammatory cytokines IL-6 and TNF-α and the development of inflammation in obese subjects. Eur J Med Res 2010; **15**: 120–2. doi:10.1186/2047-783X-15-S2-120/FIGURES/3

41. **Cleary MP, Hu X, Grossmann ME, *et al.*** Prevention of mammary tumorigenesis by intermittent caloric restriction: does caloric intake during refeeding modulate the response? Exp Biol Med 2007; **232**: 70–80 doi:10.3181/00379727-207-2320070

42. R**ogozina OP, Bonorden MJL, Seppanen CN, *et al.*** Effect of chronic and intermittent calorie restriction on serum adiponectin and leptin and mammary tumorigenesis. Cancer Prev Res 2011; **4**: 568–81. doi:10.1158/1940-6207.CAPR-10-0140

43. **Dogan S, Cicekdal MB, Ozorhan U, *et al.*** Roles of adiponectin and leptin signaling-related microRNAs in the preventive effects of calorie restriction in mammary tumor development. Appl Physiol Nutr Metab 2021; **46**: 866–76. doi:10.1139/APNM-2020-1000

44. **Cicekdal MB, Kazan BT, Tuna BG, *et al.*** Effects of two types of calorie restriction on methylation levels of Adiponectin Receptor 1 (AdipoR1) and Leptin receptor overlapping transcript (Leprot) in a MMTV-TGF-α breast cancer mouse model. Br J Nutr 2020; **123**: 1079. doi:10.1017/S0007114520000471

45. **Guvenc Tuna B, Cleary MP, Demirel PB, *et al.*** Leptin signaling in liver tissue of a transgenic breast cancer mouse model. Cureus 2020; **12**: e6737. doi:10.7759/cureus.6737

46. **Hao JQ, Zhang QK, Zhou YX, *et al.*** Association between circulating leptin concentration and G-2548A gene polymorphism in patients with breast cancer: A meta-analysis. Arch Med Sci 2019; **15**: 275–83. doi:10.5114/aoms.2018.75638

47. **Liang X, Wang S, Wang X, *et al.*** Leptin promotes the growth of breast cancer by upregulating the wnt/β-catenin pathway. Exp Ther Med 2018; **16**: 767–71. doi:10.3892/etm.2018.6212

48. **Dogan S, Ray A, Cleary MP.** The influence of different calorie restriction protocols on serum pro-inflammatory cytokines, adipokines and IGF-I levels in female C57BL6 mice: Short term and long term diet effects. Meta Gene 2017; **12**: 22–32. doi:10.1016/J.MGENE.2016.12.013

49. **Frühbeck G.** Intracellular signalling pathways activated by leptin. Biochem J 2006; **393**: 7–20. doi:10.1042/BJ20051578

ЕКСПРЕСІЯ РЕЦЕПТОРІВ ЛЕПТИНУ В ПУХЛИНАХ ТА ПРОШАРКУ ЖИРОВОЇ ТКАНИНИ МОЛОЧНОЇ ЗАЛОЗИ НА МОДЕЛІ РАКУ МОЛОЧНОЇ ЗАЛОЗИ У ТРАНСГЕННИХ МИШЕЙ

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Стан питання: Лептин є адипокіном, що кодується геном *Ob* та продукується головним чином адипоцитами. Є дані, що свідчать про роль лептину та його рецептора (ObR) за різних патофізіологічних станів, включаючи розвиток раку молочної залози (РМЗ). ***Мета***: Визначити рівень експресії лептину та його рецепторів, включаючи довгу форму ObRb, в тканині РМЗ та прошарку жирової тканини молочної залози на моделі РМЗ у трансгенних мишей, а також з'ясувати, чи вплив лептину на розвиток РМЗ є системним чи місцевим. ***Матеріали та методи***: Трансгенних самок мишей MMTV-TGF-α не обмежували у харчуванні з 10-го до 74-го тижня життя. Експресію лептину, ObR та ObRb на рівні білка визначали методом вестерн-блотингу у зразках тканин та пухлин молочної залози трансгенних мишей MMTV-TGF-α з пухлинами молочної залози або без них. Рівень лептину в сироватці визначали імунофлуоресцентним методом за допомогою набору LINCOplex. ***Результати***: Експресія білка ObRb була вірогідно нижчою в тканині РМЗ, ніж у нормальній тканині молочної залози у мишей без пухлин. Експресія лептину в тканині РМЗ була вищою за таку в нормальній тканині молочної залози у мишей без пухлин. Однак експресія ObR в тканинах мишей з пухлинами і без пухлин не відрізнялася. Рівні лептину в сироватках крові мишей обох груп у віковій динаміці не відрізнялися. ***Висновки***: Лептин та ObRb (на відміну від короткої ізоформи рецептора ObR) в тканині молочної залози мишей може відігравати важливу роль у розвитку РМЗ.

Ключові слова: лептин, рецептор лептину, рак молочної залози, трансгенні миші MMTV-TGF-α, жирова тканина молочної залози.