https://doi.org/10.48047/AFJBS.7.1.2025.411-428

African Journal of Biological Sciences
Journal homepage: http://www.afjbs.com

Research Paper Open Access

Leptin Tumoral Expression Correlates with Favorable Differentiation of Serous Ovarian Carcinoma

Jonathan HAM [1*], Andi S.H.D. NASSER [2], Rina MASADAH [1], Syahrul RAUF [2], Berti J. NELWAN [1], Upik A. MISKAD [1], Muhammad H. CANGARA[1], Suryani TAWALI [3], Mardiati [4]

[1] Department of Pathology Anatomy , Faculty of Medicine , Hasanuddin University, Makassar, Indonesia
[2] Department of Obstetric and Gynecology, Faculty of Medicine , Hasanuddin University, Makassar, Indonesia
[3] Department of Public Health, Faculty of Medicine , Hasanuddin University, Makassar, Indonesia
[4] Pathology Anatomy Laboratory, Hasanuddin University Hospital, Makassar, Indonesia

Corresponding author: Jonathan HAM
Department of Pathology Anatomy, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia, 90245
Address: Jalan Perintis Kemerdekaan KM 11, Makassar, Indonesia
Email: jonathanhampatologi@gmail.com
Contact: +6282193858220

Volume 7, Issue 1, Jan 2025

Received: 15 Nov 2024

Accepted: 25 Dec 2024

Published: 05 Jan 2025

doi:10.48047/AFJBS.7.1.2025.411-428

Abstract

Background: The dualistic nature of leptin, potentially serving as both a favorable and unfavorable prognostic factor, remains a subject of debate. This study investigates leptin expression in serous ovarian carcinomas and assesses its impact on metastasis and lymphovascular invasion in high-grade serous carcinoma.

Method: This study employed a cross-sectional design. Leptin expression in tissue microarrays from 77 patients with serous ovarian cancer was evaluated immunohistochemically. Paraffin-embedded tissue samples were obtained from the Wahidin Sudirohusodo Hospital, Indonesia, over the past three years. Data were analyzed using chi-square and Mann-Whitney U tests.

Result : The majority of patients (58.4%) were over 50 years old, and another 40.3% of patients were obese. Sixty percent of patients had metastasis, 35.1% showing lymphovascular invasion and 57.1% showing tumor necrosis. We found that leptin was consistently expressed in tumor cells of low-grade tumor, while high-grade serous carcinoma showed variable expression (p-value 0.004). Leptin analysis in high-grade tumor did not reveal a significant association with the incidence of metastasis and invasion (p-value <0.05).

Conclusion: A correlation exists between tumoral leptin expression and low-grade serous carcinoma. Nevertheless, additional research on the leptin paradox within this tumor subtype is required to assess its impact on metastasis, invasion, and prognosis.

Keywords: Leptin Expression, High-Grade Serous Carcinoma, Low-Grade Serous Carcinoma, Immunohistochemistry

Introduction

Ovarian epithelial cancer is the most common group of ovarian tumors, arising from the ovarian surface epithelium (OSE) and exhibiting highly heterogeneous differentiation (Kumar et al., 2020 and IARC, 2022). Based on the differentiation of the epithelial neoplasm, the World Health Organization (WHO) of tumor classification has established eight groups of epithelial ovarian neoplasms, including: high-grade and low-grade serous type, mucinous type, endometrioid type, clear cell type, transitional/brenner tumor type, carcinosarcoma, mixed epithelial tumor, and undifferentiated carcinoma (Kumar et al.,2020 and Board WHOCWC, 2020).

Epidemiological data on ovarian cancer in Indonesia, obtained from the Ministry of Health of the Republic of Indonesia (Kemenkes RI) in 2019, revealed that the incidence of ovarian cancer in 2018 ranked third (3.8% of total gynecological cancer cases) and eighth in terms of the proportion of cross-gender cancer cases (Budijanto et al.,2019). This is noteworthy as the incidence of ovarian cancer remains relatively high even when combined with the male population, which is inherently devoid of ovaries (Budijanto et al.,2019). One of the reasons for the high mortality rate observed in ovarian cancer is that most women with ovarian cancer are diagnosed at an advanced stage of the disease. Serous ovarian carcinoma is the most common type and is most often diagnosed at an advanced stage (Facg et al.,2017b).

In the past decade, the high incidence of ovarian cancer has once again received attention and has finally been linked to obesity (Ross et al.,2020 and Tworoger and Huang, 2016). It is known that adipose tissue affects the tumor microenvironment by providing fatty acids as the main energy source for cancer cells, activating pro-inflammatory cytokines, and protease enzymes (Craig et al.,2016).

Leptin, a newly discovered pro-inflammatory cytokine, is a hormone composed of 167 amino acids (16 kDa) synthesized from the Lep gene strand on chromosome 7 by white adipose tissue. In addition to adipose tissue, leptin is also expressed by other tissues such as the gastrointestinal system, brain, and muscle (Catteau et al., 2015). Recent research by Ray et al. (2017) has found that the leptin protein behaves differently in obese populations compared to those with ideal body weight. Physiologically, leptin plays a role in regulating energy homeostasis and body weight by acting on its receptors in the arcuate nucleus (ARC) in the hypothalamus through a negative feedback mechanism, also known as the central anorexigenic pathway (Ross et al.,2020; Sherwood, 2015; Mantzoros et al., 2011; Ray and Cleary, 2017).

The role of the leptin protein in cancer is being debated due to the dual role played by the leptin protein itself. Overexpression of leptin and its receptor (Ob-R or LepR) in ovarian carcinoma tissue indicates the occurrence of a severe malignant process (Ray and Cleary, 2017; Kato et al., 2015; Gharehbaghian et al., 2017; Ray et al., 2018). Meanwhile, one study has found a positive effect of leptin on ovarian epithelial cancer (Jimenez-Cortegana et al., 2021).

Previous studies that considered the ovarian cancer population as a homogeneous entity tended to produce variable and unreliable conclusions. In this study, we investigated leptin expression in ovarian carcinoma tissues with spesific differentiation. Serous carcinoma represents a unique differentiation of ovarian tumors. Based on their biological and developmental characteristics, serous carcinomas are classified into two types: indolent type 1 tumors (low-grade serous carcinoma/LGSC) and more aggressive type 2 tumors (high-grade serous carcinoma/HGSC) (Kumar et al.,2020 and Koshiyama et al., 2014). Combining both types in a single study sample tends to lead to erroneous conclusions. We also evaluated the clinicopathological significance of leptin expression in high-grade serous carcinoma.

Materials And Methods

Patients and specimens

This cross sectional study was performed from January to June 2023 at Anatomical Pathology Laboratory, Faculty of Medicine, Hasanuddin University, Indonesia. This study analyzed resected ovarian tissue samples obtained from hysterectomy oophorectomy procedures at Wahidin Sudirohusodo General Hospital Makassar, Indonesia over 2019-2022. The samples were diagnosed with high-grade serous carcinoma or low-grade serous carcinoma using hematoxylin-eosin staining. A total of 77 patients were included, of whom 56 had high-grade serous carcinoma.

All samples met the following pre-determined inclusion and exclusion criteria. Inclusion criteria: Paraffin blocks with a pathology report diagnosing high-grade serous carcinoma or low-grade serous carcinoma; Availability of other medical records to confirm clinicopathological parameters such as age, body weight and height, lymphovascular space invasion (LVSI), and metastasis. Exclusion criteria: Samples damaged during reprocessing for leptin immunohistochemistry staining.

Body mass index (BMI), calculated as weight in kilograms divided by the square of height in metres (kg/m2). Interpretation of BMI scores requires adjustment for the body composition of Asian populations. Therefore, BMI was classified using the Asia-Pacific Body

Mass Index Classification definitions of obesity (<18.5 'underweight'; 18.5–22.9 'normal weight'; 23–24.9 'overweight'; ≥25 'obesity') (Lim et al.,2017).

Hematoxylin Eosin and Immunohistochemical Staining

The collected tissue blocks were cooled again in the refrigerator before being cut into 3 μm sections using a microtome. These sections were then transferred to a 60°C water bath. A polysilane glass object was used to retrieve sections from the water bath, which were then dried and placed on an adhesive-coated slide warmer at 60°C for 15 minutes. A single tissue slide was deparaffinized with xylol three times, hydrated through a series of graded ethanols in water, stained with hematoxylin and eosin (H&E), dehydrated through graded ethanols, cleared, and finally mounted with a coverslip.

For immunohistochemical staining, sections were cut to 3 μm thickness and transferred to a water bath. They were then retrieved using a glass slide. TMA slides were deparaffinized with xylene and rehydrated through a series of graded ethanol washes (100%, 96%, and 70%) for 5 minutes each. The slides then underwent pre-treatment with Tris-EDTA solution at 95°C for 10 minutes. This was followed by sequential incubations with: peroxidase block containing 3% hydrogen peroxide for 10 minutes, Super Block for 5-10 minutes, primary antibody (primary polyclonal rabbit anti-leptin antibody, DF8583, Affinity Biosciences, Cincinnati, OH, USA) diluted 1:200 in goat serum for 45 minutes, UltraTek Anti-polyvalent for 10 minutes, UltraTek HRP for 10 minutes, and DAB solution for 1-5 minutes. After each incubation step, the slides were washed with PBS (pH 7.4) before proceeding to the next incubation.

The slides were then conterstained with hematoxyline, soaked in bluing bluing reagen, and dehydrated according to a standard protocol and sealed with coverslips. Negative controls were prepared by omitting the leptin antibody during the primary antibody incubation. Positive controls consisted of liver tissues previously classified as positive for leptin expression.

Interpretation of Leptin Expression

Two pathologists and a researcher semiquantitatively evaluated the results of immunohistochemical staining using a light microscope. The IHC staining score was determined based on both color intensity and the proportion of positively stained areas within a visual field (Assidi et al., 2021 and Paik et al., 2008). Leptin staining intensity was scored on a scale of 0 (negative), 1 (weak), 2 (moderate), and 3 (strong), with 0 being negative and 3 being strong. Staining extent was similarly scored on a scale of 0 (10%), 1 (10-39%), 2 (40-90%), and 3 (>90%) based on the percentage of positively stained cells. The final staining score was obtained by multiplying the intensity and extent scores. All cases were then classified into two expression groups based on their final score: low expression (0-3) and high expression (4-9).

Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics (version 22.0). The chi-square test was employed to assess the association between ordinal variables in two or more unpaired groups. Specifically, Mann-Whitney test was used to examine the relationships between body mass index (BMI) and leptin expression. A p-value of less than 0.05 was considered statistically significant.

Results

Table 1. Clinicopathologic and demographic Characteristics (N=77)

Variables	n (%)
Ages (years)	
<50	32 (41,6)
>50	45 (58,4)
mean ± SD= 50,43 ± 8,59	
Education	
Less than 12 years	39 (50,65)
≥ 12 years	38 (49,35)
Health Insurance	
Uncovered	0 (0)
Government insurance	77 (100)
Other insurance	0 (0)
BMI	
Underweight	12 (15,6)
Optimal	31 (40,3)
Overweight/Obesity	31 (40,3)
Missing	3 (3,9)
Differentiation	
Low-grade	21 (27,3)
High-grade	56 (72,7)
Lymphovascular space invasion (LVSI)	
Yes	27(35,1)
No	50 (64,9)
Metastasis	
Yes	46(60)
No	31(40)
Necrosis	
Yes	44 (57,1)
No	33 (42,9)
Leptin Expression	

Positive (High expression)	60 (77,9)
Negative (Low expression)	17 (22,1)
Ca-125 level	
<35	5 (6,5)
35-500	29 (37,7)
>500	43 (55,8)

A total of 77 serous carcinoma patients were evaluated, and 58.4% of them were over 50 years old. There was no significant difference in the level of education among the patients. The entire treatment process, including surgery and post-operative care, was covered by the Indonesian government's health insurance program, JKN. Since its launch in 2014, JKN (Jaminan Kesehatan Nasional) has proven to be one of the successful government programs. Currently, JKN can be accessed online and has covered and supported the healthcare needs of almost the entire Indonesian population (Agustina et al., 2019).

A total of 31 (40.3%) of the 74 samples were classified as overweight/obese, with the majority (57.1%) exhibiting high-grade serous carcinoma as the histopathological type. The majority of samples (60%) showed evidence of metastasis to the omentum and other organs based on radiological and/or histopathological examination. Microscopically, LVSI was detected in 27 (35.1%) cases, and tumor necrosis was present in 57.1% of tumor masses. Serum Ca-125 levels were >500 U/mL in 55.8% of the population. The baseline characteristics of the women included in the final analysis are presented in Table I.

Although not yet significant, body mass index (BMI) is reported to have a positive correlation with leptin protein expression in tumor cells (p-value 0,07) (Figure 1). The leptin-positive group had a mean BMI of 23.21 (median 25.1), which was higher than the leptin-negative group (mean: 20.73; median 19.9).

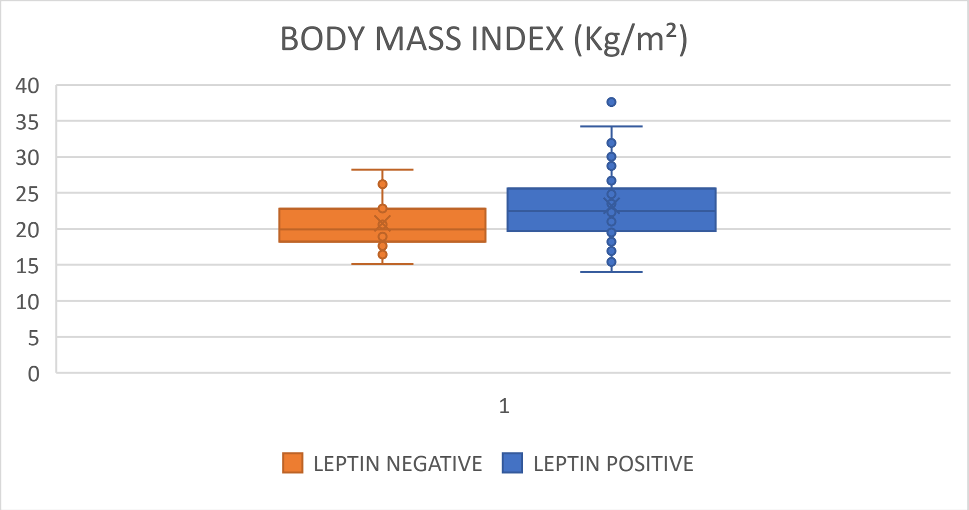

Figure 1. Association between body mass index and tumoral expression of leptin (p-value 0,07, ns)

Immunohistochemical staining of leptin in serous ovarian carcinoma tumor can be seen in Figure 2. Of the 21 total low-grade carcinoma samples, all samples (100%) strongly expressed leptin. Of the 56 total high-grade carcinoma samples, 39 (69%) samples strongly expressed leptin, while 17 (31%) samples weakly expressed leptin. Based on the Fisher test, a p-value of 0.004 ($p < 0.05$) was obtained (Table 2), indicating a significant association between leptin tumoral expression frequency and the morphological type of serous ovarian carcinoma.

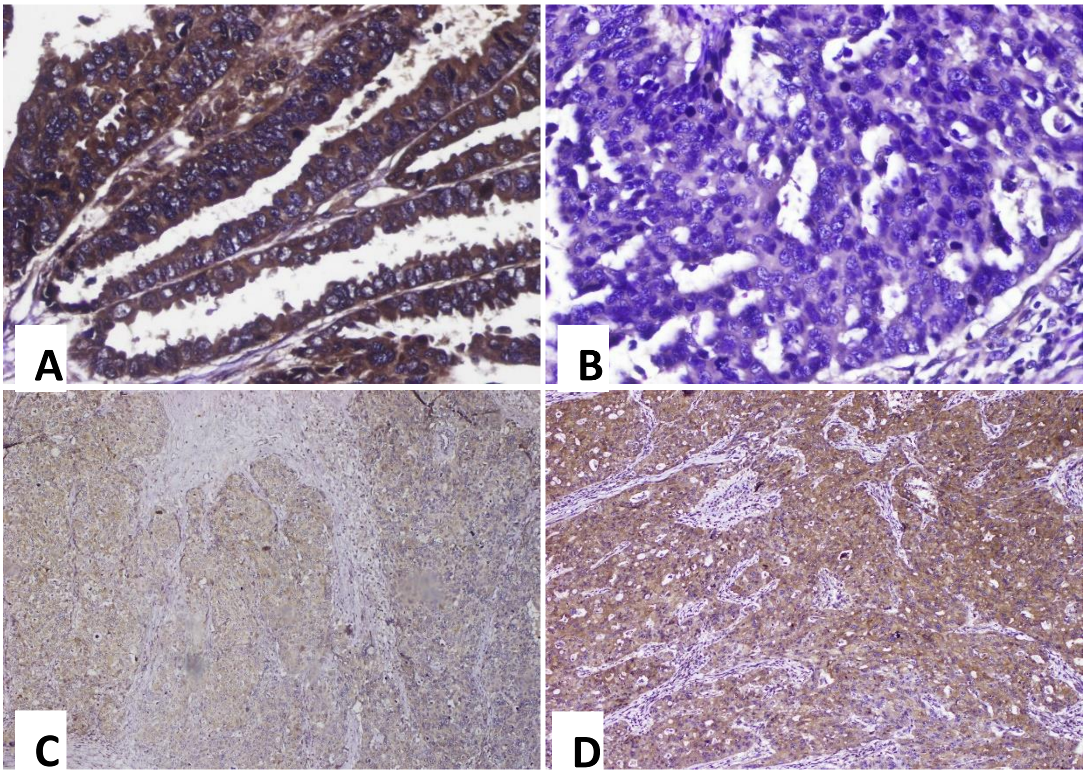

Figure 2. A) Strong leptin cytoplasmic expression in low-grade serous carcinoma; Variable intensity of leptin tumoral expression in high-grade serous carcinoma: +1 (B), +2 (C), +3(D).

Table 2. Leptin Expression in Serous Ovarian carcinoma

Leptin Expression	**n(%)**	**HGSC (N=56)**	**LGSC (N=21)**	**P-value**
High	60 (77,9)	**39 (69,6)**	**21 (100)**	**0,004**
Low	17 (22,1)	17 (30,4)	0 (0)	

Chi-square test

Next, the low-grade serous carcinoma population was excluded. Samples were then analyzed to determine whether metastasis and lymphovascular space invasion in HGSC was influenced by leptin expression in tumor cells. The results of the Pearson chi-square test showed no significant relationship between leptin expression and the occurrence of metastasis (p-value 0.193) or lymphovascular space invasion (p-value 0.908) (Table 3).

Table 3. Leptin Expression and its Correlation with Metastasis and Lymphovascular Space Invasion in HGSC Population

Leptin expression	High-grade Serous Carcinoma				OR 95% CI	P-value	High-grade Serous Carcinoma				OR 95% CI	P-value
	No Metastasis		**Metastasis**				**No LVSI**		**LVSI**			
	n	%	n	%			n	%	n	%		
Low	3	18.75	14	35	0.429 (0.104-1.762)	0.193	13	36.11	4	20	2.261 (0.623-8.21)	0.908
High	13	81.25	26	65			23	63.89	16	80		
Total	16	100	40	100			36	100	20	100		

Chi-square test

Discussion

Numerous studies have linked body mass index (BMI) parameter to an increased risk of ovarian cancer (Olsen et al., 2013 and Dixon et al., 2016). These studies have shown a trend of increased ovarian cancer risk in population with higher BMI. However, the results of these studies appear to be mixed. Therefore, there is a need for more specific variables in the obese population that can show a more meaningful correlation compared to clinical parameters alone.

Leptin, an adipokine secreted by adipose tissue, has emerged as a novel parameter linking obesity to cancer. Obesity leads to an imbalance in adipokine production, where leptin secretion increases with increasing body fat mass, while adiponectin, an anti-inflammatory adipokine, experiences a decrease in secretion. In the boxplot (see Figure 1), the mean body mass index tends to be higher in the group with strong leptin expression compared to the BMI of the weak leptin expression group (23.21 vs 20.73). Although not significant, these results are in line with previous theories and studies that have also found significantly higher leptin expression in the obese group compared to the overweight and control groups in serous ovarian cancer population (Hosney et al., 2017). Another study concluded that there is no significant correlation between Body Mass Index (BMI) and leptin expression (p-value 0.20) (Assidi et al., 2021). This could be due to several factors, such as a small sample size, a heterogeneous sample population, and the limitations of BMI in distinguishing between body fat mass and non-fat mass, as claimed by some studies (Assidi et al., 2021; Nuttall FQ, 2015; Lennon et al., 2016).

Based on the leptin expression, we can conclude that high-grade and low-grade serous carcinomas have different expression patterns. (Table 2) Leptin expression was consistently strongly stained in the cytoplasm of low-grade carcinoma tumor cells. Meanwhile, in high-grade serous carcinoma, leptin was expressed variably (Figure 2). This is surprising because leptin is typically thought to be more abundant in the less aggressive form of serous cancer. These results are in line with previous studies that have found that increased BMI increases the risk of developing low-grade serous carcinoma and its precursor, serous borderline ovarian tumor (SBOT) (Olsen et al., 2013 and Dixon et al., 2016). Several experimental studies have demonstrated that elevated 17β-estradiol levels can block the leptin-activated PI3K/AKT signaling pathway in tumor cells, thereby reducing ovarian cancer cell proliferation and migration rates (Achkar et al., 2019 and Hoffmann et al.,2016). This finding is intriguing given that HGSCs are generally considered less estrogen-responsive than LGSCs (Fernandez et al., 2020). Consequently, the blockade of the PI3K/AKT pathway by 17β-estradiol is likely more pronounced in LGSCs. This could explain the strong leptin staining observed in LGSCs, despite their relatively slower proliferation and invasion rates compared to HGSCs. However, the impact of leptin on lymphovascular space invasion, metastasis, and survival/prognosis in low-grade serous carcinoma warrants further investigation to determine the existence of a leptin paradox in this cancer type.

Indeed, HGSC and LGSC are distinct entities. LGSC is classified as type I ovarian tumor, which has relatively good genetic stability, making it more indolent and develops in a stepwise manner (Koshiyama et al. 2014). Therefore, it is still common to find LGSC precursor tumors, such as serous borderline ovarian tumor (SBOT) or serous cystadenoma. While HGSC is classified as type II ovarian tumor, which is genetically unstable and more aggressive. HGSC originates from a malignancy in the fallopian tube (Serous Tubal Intraepithelial Carcinoma/ STIC) so there is no benign precursor lesion and is often found in advanced stages (III/IV) (Kumar et al.,2020 ; Koshiyama et al. 2014; Charkhchi et al., 2020).

The biological and developmental differences between HGSC and LGSC also ultimately affect their metastatic behavior. HGSC tends to metastasize more often (Board WHOCWC, 2020). If the sample population of type 1 ovarian tumors is mixed with type 2 tumors, or there are more type 1 tumor samples, then the leptin expression analysis may be erroneous and ultimately lead to the statement that leptin expression has a good prognosis in ovarian cancer because the incidence of metastasis tends to decrease. However, in fact, type 1 tumors rarely metastasize (Board WHOCWC, 2020). Errors like this are what have emerged in previous studies.

In this study, we only analyzed HGSC samples in investigating the correlation between leptin and the metastatic ability of ovarian serous cancer cells to maintain homogeneity, making the results more reliable. Analysis of the relationship between leptin expression and metastasis and LVSI in LGSC could not be performed because leptin was consistently strongly expressed in the entire population, and a larger sample size of LGSC may be needed to do so.

Analysis of leptin expression in 56 HGSC samples showed no significant correlation with metastasis (p-value > 0.05). Similarly, there was no strong association between leptin expression and lymphovascular space invasion (LVSI) in the HGSC group (p-value > 0.05).

Our study is the first to evaluate leptin expression in ovarian carcinoma based on tumor differentiation, and even follows the classification of ovarian tumors based on their carcinogenesis, type 1 tumors for LGSC and type 2 tumors for HGSC.

Oncology studies on obesity and leptin expression face challenges due to the "obesity/leptin paradox" phenomenon. The obesity paradox is a phenomenon where the cancer population has a significantly better prognosis when their body mass index (BMI) is above normal, where the opposite is expected (Jimenez-Cortegana et al., 2021 and Lennon et al., 2016). Studies of colorectal adenocarcinoma have found that while leptin plays a role in its carcinogenesis, it also has a good impact on depth of invasion, lymph node metastasis, LVSI, and patients' overall survival and disease-free survival (Paik et al., 2008). A study by Assidi et al. 2021 found that strong leptin expression was associated with low LVSI rates, improved patient survival (DSS/ disease-specific survival and DFS/ disease-free survival) and even reduced recurrence rates in ovarian cancer patients (Assidi et al., 2021). The exact mechanism of the leptin paradox is still unclear, but it is believed that people who are overweight and obese have more energy reserves to survive for longer periods of time in cancer conditions (Craig et al.,2016).

However, other studies have shown that leptin has protumorigenic effects through intracellular signaling and modification of the tumor microenvironment, even supporting the migration and metastasis of cancer cells, resulting in a worse prognosis (Craig et al.,2016) (Ray and Cleary, 2017; Kato et al., 2015. Gharehbaghian et al., 2017; Ray et al., 2018; (Zhang et al., 2014; Uddin et al., 2009; Zwezdaryk et al., 2018; Ray et al., 2021; Brown and Scherer, 2023; Pati et al., 2023; Kumar et al., 2017). The binding of leptin to its receptor on tumor cells activates intracellular signaling pathways, including the PI3K/AKT pathway, JAK/STAT pathway, MEK/ERK pathway, and RhoA/ROCK pathway, which stimulates tumor cell proliferation and migration) (Ray et al., 2021; Brown and Scherer, 2023; Kumar et al., 2017; Olea-Flores et al., 2020). Leptin also exerts anti-apoptotic effects on ovarian cancer cells by inhibiting various apoptotic components such as TNFR1, Bad, caspase-6, and caspase-3. In addition, leptin modifies the tumor microenvironment by binding to its receptor (Ob-R) on immune cells and suppressing the production of IFN-γ by natural killer cells, thereby reducing their cytotoxic activity. Leptin also activates tumor-associated macrophages (TAMs), which induce the secretion of MMPs (matrix metaloproteinases), CCL22 (Chemokine (C-C Motif) Ligand 2), and growth factors such as Epidermal Growth Factor (EGF), Fibroblast Growth Factor (FGF), and Vascular Endothelial Growth Factor (VEGF), which trigger remodeling and angiogenesis. Leptin also modulates T cells through the role of TAMs in recruiting Tregs (regulatory T cells). The secretion of CCL22 by TAMs attracts Tregs, which then secrete immunosuppressive TGF-β and IL-10 (Craig et al.,2016; Ray and Cleary, 2017). Leptin induces the secretion of MMP-9, MMP-2, and MMP-14 by TAMs, which can degrade extracellular matrix components during cancer cell invasion and metastasis (Craig et al.,2016; Ray and Cleary, 2017; Kato et al., 2015). Recent studies have re-emphasized the potential role of leptin in promoting epithelial mesenchymal transition (EMT) in cancer cells, including epithelial ovarian cancer. EMT is a process of reprogramming epithelial carcinoma cells to acquire mesenchymal characteristics, which can be identified by changes in phenotype, transcription factors, miRNAs, IncRNAs, cell junctions, cytoskeletal proteins, and secreted factors. EMT enhances the malignancy of tumor cells because with mesenchymal phenotype, cancer cells can easily invade, migrate, evade the immune system, and ultimately lead to successful metastasis (Olea-Flores et al., 2020).

While EMT, facilitated by leptin, significantly contributes to the aggressive behavior of cancer cells, the complex nature of ovarian cancer has hindered a clear understanding of leptin's exact role in this specific malignancy. The variation in previous research findings on leptin in ovarian cancer may be due to the morphological heterogeneity and differential characteristics of type 1 and type 2 ovarian tumors. Further research is still needed and it is hoped that the homogeneity of ovarian cancer samples will be maintained to obtain reliable outcomes.

Limitation of the study

Leptin expression was stained in all LGSCs, necessitating a larger sample size for the evaluation of metastasis and lymphovascular invasion. The sample tended to be homogeneous, thus the conclusions drawn may not be representative of other ovarian carcinoma subtypes.

Conclusions

The results of the study demonstrate a clear association between leptin expression and favorable differentiation of serous ovarian carcinoma. Leptin is more highly expressed in low-grade serous carcinoma than in high-grade serous carcinoma. Leptin tumoral expression in high-grade serous carcinoma was not found to correlate with both metastasis and lymphovascular space invasion. These findings suggest that the leptin paradox phenomenon in high-grade serous carcinoma is not evident. Future research on leptin in ovarian carcinoma should focus on low-grade serous ovarian carcinomas (LGSCs) to investigate the impact of leptin on metastasis, invasion, migration, and prognosis, with a particular emphasis on estrogen receptor (ER)-positivity.

References

1. Kumar, v., Abbas, A. and Aster, J., 2021. Robbins & Cotran Pathologic Basic of Disease. 10th ed. New York: Elsevier.
2. The International Agency for Research on Cancer (IARC). (2021, March). Global Cancer Observatory- GLOBOCAN Indonesia 2020. Https://Gco.Iarc.Fr/. Retrieved March 3, 2022, from https://gco.iarc.fr/
3. Budijanto, D., Pangribowo, S. and et al, 2019. Infodatin Beban Kanker Di Indonesia. Jakarta: Kementerian Kesehatan RI.
4. Facg, F. F. M. J. G. R., Md, L. L. W., Md, M. J., & Md, J. M. L. (2017b). Rosai and Ackerman's Surgical Pathology - 2 Volume Set (11th ed.). Elsevier
5. Ross A.C., Caballero B., Cousins R.J., Tucker K.L., Ziegler T.R. 2014. Modern nutrition in health and disease: Eleventh edition. Lippincott Williams & Wilkins Modern Nutrition in Health and Disease: Eleventh Edition, , pp. 1-1616. Schulte,M et al. Atlas of Anatomy-Latin Nomenclature. Thieme Medical Publishers, Inc.2009
6. Craig ER, Londoño AI, Norian LA et al. Metabolic risk factors and mechanisms of disease in epithelial ovarian cancer: A review. Gynecol Oncol. 2016;143:674-83.
7. Catteau, A.; Caillon, H.; Barrière, P.; Denis, M.G.; Masson, D.; Fréour, T. (2015). Leptin and its potential interest in assisted reproduction cycles. Human Reproduction Update, (), dmv057–. doi:10.1093/humupd/dmv057
8. Sherwood, L. (2015). Human Physiology: From Cells to Systems (9th ed.). Cengage Learning.
9. Mantzoros, C. S.; Magkos, F.; Brinkoetter, M.; Sienkiewicz, E.; Dardeno, T. A.; Kim, S.-Y.; Hamnvik, O.-P. R.; Koniaris, A. (2011). Leptin in human physiology and pathophysiology. AJP: Endocrinology and Metabolism, 301(4), E567–E584. doi:10.1152/ajpendo.00315.2011
10. Ray, A., & Cleary, M. P. (2017). The potential role of leptin in tumor invasion and metastasis. Cytokine & Growth Factor Reviews, 38, 80–97. https://doi.org/10.1016/j.cytogfr.2017.11.002

11. Kato S, Abarzua-Catalan L, Trigo C et 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.
12. Gharehbaghian, Ahmad; Hashemy, Seyed Isaac; Aghaei, Mahmoud; Panjehpour, Mojtaba (2017). RhoA/ROCK pathway mediates leptin-induced uPA expression to promote cell invasion in ovarian cancer cells. Cellular Signalling, 32(), 104–114. doi:10.1016/j.cellsig.2017.01.020
13. Ray A, Fornsaglio J, Dogan S, Hedau S, Naik D, De A. Gynaecological cancers and leptin: A focus on the endometrium and ovary. Facts Views Vis Obgyn. 2018 Mar;10(1):5-18. PMID: 30510663; PMCID: PMC6260667.
14. Jiménez-Cortegana, C., López-Saavedra, A., Sánchez-Jiménez, F., Pérez-Pérez, A., Castiñeiras, J., Virizuela-Echaburu, J. A., de la Cruz-Merino, L. D. L., & Sánchez-Margalet, V. (2021). Leptin, Both Bad and Good Actor in Cancer. Biomolecules, 11(6), 913. https://doi.org/10.3390/biom11060913 (Jiménez-Cortegana et al., 2021)
15. Koshiyama, M., Matsumura, N., & Konishi, I. (2014). Recent Concepts of Ovarian Carcinogenesis: Type I and Type II. BioMed Research International, 2014, 1–11. https://doi.org/10.1155/2014/934261
16. Lim JU, Lee JH, Kim JS, Hwang YI, Kim TH, Lim SY, Yoo KH, Jung KS, Kim YK, Rhee CK. Comparison of World Health Organization and Asia-Pacific body mass index classifications in COPD patients. Int J Chron Obstruct Pulmon Dis. 2017 Aug 21;12:2465-2475. doi: 10.2147/COPD.S141295. PMID: 28860741; PMCID: PMC5571887.
17. Agustina R, Dartanto T, Sitompul R, Susiloretni KA, Suparmi N, Achadi EL, et al. (2019). Universal health coverage in Indonesia: concept, progress, and challenges. Lancet [Internet]. 1;393(10166):75–102. Available from: https://doi.org/10.1016/s0140-6736(18)31647-7
18. Olsen CM, Nagle CM, Whiteman DC et al. Obesity and risk of ovarian cancer subtypes: evidence from the Ovarian Cancer Association Consortium. Endocr Relat Cancer. 2013;20:251- 62.
19. Dixon SC, Nagle CM, Thrift AP et al. Adult body mass index and risk of ovarian cancer by subtype: a Mendelian randomization study. Int J Epidemiol. 2016;45:884-95.

20. Hosney M, Sabet S, El-Shinawi M, Gaafar KM, Mohamed MM. (2014) Leptin is overexpressed in the tumor microenvironment of obese patients with estrogen receptor positive breast cancer. Experimental and Therapeutic Medicine [Internet]. Mar 30;13(5):2235–46. Available from: https://doi.org/10.3892/etm.2017.4291

21. Assidi, M., Yahya, F. M., Al-Zahrani, M. H., Elkhatib, R., Zari, A., Elaimi, A., Al-Maghrabi, J., Dallol, A., Buhmeida, A., & Abu-Elmagd, M. (2021, November 28). Leptin Protein Expression and Promoter Methylation in Ovarian Cancer: A Strong Prognostic Value with Theranostic Promises. International Journal of Molecular Sciences, 22(23), 12872. https://doi.org/10.3390/ijms222312872

22. Nuttall FQ. Body Mass Index: Obesity, BMI, and Health: A Critical Review. Nutr Today. 2015 May;50(3):117-128. doi: 10.1097/NT.0000000000000092. Epub 2015 Apr 7. PMID: 27340299; PMCID: PMC4890841.

23. Lennon, H., Sperrin, M., Badrick, E., & Renehan, A. G. (2016). The Obesity Paradox in Cancer: a Review. *Current oncology reports*, *18*(9), 56. https://doi.org/10.1007/s11912-016-0539-4

24. Charkhchi P, Cybulski C, Gronwald J, Wong FO, Narod SA, Akbari MR. (2020 Dec). CA125 and ovarian Cancer: A Comprehensive review. Cancers [Internet]. 11;12(12):3730. Available from: https://doi.org/10.3390/cancers12123730

25. Board, W. H. O. C. W. C. O. T. E., & Editorial, W. C. O. T. (2020). *Female genital tumours: WhO classification of tumours*.

26. Paik, S. S., Jang, S. O., Jang, K., Lee, K. H., Choi, D., & Jang, S. J. (2008). Leptin Expression Correlates with Favorable Clinicopathologic Phenotype and Better Prognosis in Colorectal Adenocarcinoma. *Annals of Surgical Oncology*, *16*(2), 297–303. https://doi.org/10.1245/s10434-008-0221-7

27. Zhang Y, Liu L, Li C, Ai H. Correlation analysis between the expressions of leptin and its receptor (ObR) and clinicopathology in endometrial cancer. Cancer Biomark. (2014); 14:353–359. DOI: 10.3233/CBM-140415 [PubMed: 25171477]

28. 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

29. Zwezdaryk, K. J., Sullivan, D. E., & Saifudeen, Z. (2018). The p53/Adipose-Tissue/Cancer Nexus. *Frontiers in Endocrinology*, *9*. https://doi.org/10.3389/fendo.2018.00457
30. Ray, I., Meira, L. B., Michael, A., & Ellis, P. E. (2022). Adipocytokines and disease progression in endometrial cancer: a systematic review. *Cancer metastasis reviews*, *41*(1), 211–242. https://doi.org/10.1007/s10555-021-10002-6
31. Brown, K. A., & Scherer, P. E. (2023). Update on adipose tissue and cancer. *Endocrine Reviews*. https://doi.org/10.1210/endrev/bnad015
32. Pati, S., Irfan, W., Jameel, A. T., Ahmed, S., & Shahid, R. (2023). Obesity and Cancer: A Current overview of epidemiology, pathogenesis, outcomes, and management. *Cancers*, *15*(2), 485. https://doi.org/10.3390/cancers15020485
33. 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.
34. Olea-Flores M, Juárez-Cruz JC, Zuñiga-Eulogio MD, et al. (2020). New Actors Driving the Epithelial-Mesenchymal Transition in Cancer: The Role of Leptin. *Biomolecules,* 10(12):1676. Published 2020 Dec 15. doi:10.3390/biom10121676
35. Tworoger SS, Huang T. (2016). Obesity and ovarian cancer. In: Recent results in cancer research [Internet]. p. 155–76. Available from: https://doi.org/10.1007/978-3-319-42542-9_9
36. Achkar IW, Bhat AA, Zafar M, et al. (2019). Oncogenic role of dysregulated leptin signaling in the pathogenesis of ovarian cancer. *Translational Medicine Communications*, 4(1). doi:10.1186/s41231-018-0031-2
37. Hoffman, B., Schorge, J., Bradshaw, K., Halvorson, L., Schaffer, J., & Corton, M. (2016). Williams Gynecology, Third Edition (3rd ed.). McGraw Hill / Medical.
38. Fernandez ML, Dawson A, Kim H, et al. (2020). Hormone receptor expression and outcomes in low-grade serous ovarian carcinoma. *Gynecologic Oncology,* 157(1):12-20. doi:10.1016/j.ygyno.2019.11.029

CONFLICTS OF INTEREST

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

FUNDING

The authors report no involvement in the research by the sponsor that could have influenced the outcome of this work.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the manuscript and read and approved the final version of the manuscript.