Leptin Expression in High-Grade Serous Ovarian Carcinoma: The Controversy of Leptin Paradox in Ovarian Cancer

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Abstract

Objectives: To analyse the characteristics and expression patterns of Leptin in High-grade serous epithelial ovarian carcinoma (HGSC), the most prevalent subtype of ovarian carcinoma, and to compare them with overall serous ovarian carcinoma population.

Methods: A cross-sectional study was performed on a total of 77 paraffin-embedded HGSC tissue samples from patients over a period of 3 years. Immunohistochemical analysis was performed using a polyclonal Leptin antibody to samples. Data were analyzed using SPSS version 22.0.

Results: Among HGSC patients, the majority (64.3%) were over 50 years old, and a significant portion (39.3%) were obese. Leptin showed a strong cytoplasmic expression in 69.6% of HGSC tumor cells and in 100% of LGSC tumor cells (p-value = 0.004). There was no correlation between lymphovascular space invasion and leptin expression. Interestingly, leptin expression in overall serous ovarian carcinoma patients exhibited a protective effect against metastasis (p-value = 0.047), suggesting a leptin paradox exists in this type of cancer**.** However, this association was no longer significant when the analysis excluded the LGSC group (p-value = 0.193).

Conclusion: This study suggest that leptin expression is not a significant prognostic factor in HGSC. Comparison of HGSC with the overall serous ovarian carcinoma population reveals that the results of several previous studies were likely confounded by the inclusion of heterogeneous tumor morphologies within their samples. The presence of low-grade serous carcinoma within the population may have inadvertently led to the observation of a seemingly protective effect of leptin, a phenomenon sometimes referred to as the 'leptin paradox'.

Keywords: high-grade serous carcinoma, leptin paradox, immunohistochemistry, leptin, obesity

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INTRODUCTION

Ovarian cancer is still the most cancer among all gynecological malignancies.[1] Ovarian cancer is a heterogeneous group of tumors. These tumors can originate from epithelial cells, germ cells, or stromal/sex cord cells. Around 90% of ovarian cancers originate from ovarian surface epithelium.[2-4] The American Cancer Society ranks ovarian cancer as the 5th leading cause of death in women due to malignancy. Data from 2018 in Indonesia also shows a high mortality rate from ovarian cancer: 3.8% of all diagnosed cases.[3,5,6]

Serous type ovarian neoplasm is the most common type of ovarian epithelial cancer. Based on their histopathological characteristics, serous ovarian cancers are further divided into low-grade type (LGSC) and high-grade type (HGSC). This division also reflects the differences in the carcinogenesis process, mutation patterns, and prognosis between the two types of serous ovarian cancer. Thus, high-grade serous carcinoma does not develop from low-grade serous carcinoma. Instead, both types arise through their own distinct developmental pathways.[2,7,8]

Previous research focused on conventional risk factors for ovarian cancer, such as family history, genetic predisposition, and reproductive factors including nulliparity, early menarche, and late menopause.[9] However, despite this focus, ovarian cancer incidence has remained high in recent decades. Obesity, a growing global health concern, has emerged as a significant metabolic risk factor linked to ovarian cancer.[10]

There are many studies linking body mass index (BMI) parameters to an increased risk of cancer.[11-13] The results of these studies remain mixed. Therefore, there is a need for more specific variables that better represent the obese population and can reveal more meaningful correlations than clinical parameters alone.

There are some efforts to identify correlations between the pathophysiological pathways of obesity and ovarian cancer.[14] Adipose tissue in obese conditions influences the microenvironment of cancer cells by providing fatty acids as an energy source, activating pro-inflammatory cytokines and protease enzymes, creating an imbalance in the overproduction of the pro-inflammatory adipokine leptin compared to the reduced production of the anti-inflammatory adiponectin.[14,15] Interleukin-6, TNF-α, and leptin are a group of cytokines produced by adipocytes. Their levels increase as body fat mass increases.

In individuals with obesity, leptin often acts as a pro-inflammatory adipokine and can even be carcinogenic, promoting the invasion and migration of tumor cells (metastasis). Thus the combined presence of high leptin concentration and its receptor (Ob-R) expression in certain tumors is associated with a poor prognosis.[1,13,14,16-23] However, some studies suggest that high leptin expression in ovarian cancer patients can be associated with improved outcomes, such as increased disease-specific survival (DSS), disease-free survival (DFS), and reduced recurrence rates, as well as a lower incidence of lymphovascular space invasion (LVSI).[24-25]

This contrasting effect of leptin on cancer is known as the "obesity/leptin paradox".[25,26] This phenomenon has been observed in several types of cancer, including colorectal adenocarcinoma.[25,27] The exact mechanism behind this paradox remains unclear, but one hypothesis suggests that overweight and obese individuals may have larger energy reserves, allowing them to survive cancer for a longer period.

Considering leptin dualism which could be a poor prognostic factor in one study and a protective prognostic factor in another study, we investigate its effects in a more targeted population – women with serous type ovarian carcinoma. Previous studies have employed ovarian cancer samples without considering their heterogeneity, leading us to hypothesize that a more specific investigation focusing on samples with distinct morphologies, serous subtype, would yield more reliable results.

METHODS

Patients and specimens

This cross-sectional study was performed from January to June 2023 at Pathology Anatomy Laboratory, Faculty of Medicine, Hasanuddin University. This study analyzed resected ovarian tissue samples obtained from hysterectomy oophorectomy procedure at Wahidin Sudirohusodo General Hospital Makassar over the past 3 years. Samples were histopathologically diagnosed with High-Grade Serous Carcinoma or Low-Grade Serous Carcinoma using hematoxylin-eosin staining. Seventy-seven patients were included in this study, of whom 56 had high-grade serous carcinoma.

All accessible subjects meeting the inclusion criteria were consecutively enrolled from the Department of Anatomic Pathology, Wahidin Sudirohusodo General Hospital, Makassar, until the sample size of 49 subjects was reached. 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 mass index, lymphovascular space invasion (LVSI), and metastasis. Exclusion criterion: Samples damaged during reprocessing for Leptin immunohistochemistry examination.

BMI was measured using the Asia-Pacific Body Mass Index Classification. A person is considered obese if their BMI is ≥25 kg/m² (table 2). This value is slightly lower than the World Health Organization (WHO)

standards due to adjustments for the posture of Asian populations.[10,28]

H&E 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 reagen, and dehydrated according to a standard protocol and sealed with deck glass. Negative controls were prepared by omitting the leptin antibody during the primary antibody incubation. Positive controls consisted of liver tissues which showed 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 (24,27). The intensity scale ranged from 0 (no expression) to 3 (strong expression). 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, Pearson's chi-square test was used to examine the relationships between body mass index (BMI) and both leptin expression and tumor morphology. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The study included a total of 77 samples collected over the past 3 years. Among these samples, 56 were diagnosed with the high-grade serous ovarian carcinoma (HGSC) and 21 with the low-grade type (LGSC). The patients' ages ranged from 25 to 70 years old, with a median age of 51 and an average age of 50.43 years. Two age groups were identified: less than 50 years old (41.6%) and greater than 50 years old (58.4%). The HGSC group tended to have a higher proportion of patients in the older age group (>50 years).

Body mass index (BMI) were divided into three groups, the underweight group was 12 (15.5%), the optimal group was 31 (40.3%), the overweight/obesity group was 31 (40.3%). The results of the analysis did not show a correlation between serous carcinoma morphology and BMI (p-value 0.961).

There were 27 samples (35.1%) that showed lymphovascular space invasion, while 50 samples (64.9%) did not show lymphovascular space invasion (LVSI). The results of the analysis did not reveal a correlation between the morphology of serous carcinoma and LVSI (p-value 1.000).

The number of samples that experienced metastases was 46 (60%) while

the group that did not have metastases was 31 (40%). A total of 71.4% of the HGSC group experienced metastasis. On the other hand, in the LGSC group, the majority did not experience metastasis, 76.2%. Thus, the results of the analysis showed a correlation between the morphology of serous carcinoma and metastasis (p-value 0.000).

Among the samples, 44 (57.1%) showed necrosis, while 33 (42.9%) did not. The HGSC group had a higher prevalence of necrosis, with 71.4% exhibiting large areas. Conversely, the LGSC group showed a much lower prevalence, with most samples (81%) lacking necrosis. This finding suggests a correlation between serous carcinoma morphology and tumor necrosis (p-value = 0.000).

Strong leptin expression was observed in 60 samples (77.9%), while only 17 (22.1%) showed weak staining intensity and proportion. The HGSC group had a high prevalence of strong leptin expression (69.6%). Interestingly, all LGSC samples (100%) appeared to express strong leptin. This suggests a correlation between serous carcinoma morphology and leptin expression. Leptin expression was significantly higher in the LGSC group (p-value = 0.004).

DISCUSSION

While morphologically similar, low-grade and high-grade serous ovarian carcinomas are distinct entities with differing pathogenesis and molecular profiles.[8] Our sample population's incidence aligns with epidemiological studies, which show that LGSC has a lower incidence and typically presents at an earlier age compared to HGSC (7). (Table 1)

Classified as a type I ovarian carcinoma with indolent behavior, low-grade serous carcinoma exhibits a significantly lower frequency of tumor invasion and metastasis.[29] LGSC arises from a spectrum of benign lesions, progressing from benign adenofibroma or serous cystadenoma to precursor serous borderline tumors, and finally to low-grade serous carcinoma. Mutations in KRAS, BRAF, and/or ERBB2 oncogenes activate the MAPK pathway, driving LGSC carcinogenesis. This process may be further enhanced by leptin binding to its receptor on tumor cells, which also activates the MAPK pathway.[1,2,7,8,25]

HGSC, a type II ovarian carcinoma, is an aggressive and invasive tumor. It can arise from the fimbria epithelium of the fallopian tube. Notably, HGSC develops from a non-cancerous (non-neoplastic) lesion in the tubal epithelium called secretory cell outgrowth (SCOUT), which lacks TP53 mutations and has low Ki67 proliferation rates. In contrast, the neoplastic lesions arise due to a TP53 mutation. This mutation gives rise to the first cancerous (neoplastic) entity, known as serous tubal intraepithelial lesions (STILs) or tubal dysplasia. STILs are characterized by positive p53 immunohistochemistry (IHC) or a Ki67 proliferation rate exceeding 10%. STILs can then progress to serous tubal intraepithelial carcinomas (STICs), a more severe form of dysplasia with positive for p53 immunohistochemistry and a Ki67 proliferation rate exceeding 10%.[2,7,8,30] Leptin is known to be a regulator of p53 expression. The anti-apoptotic effect of leptin comes from leptin's ability to suppress the p53 pathway (p53 downregulation). However, regardless of the p53 status in the tumor, leptin stimulation has the potential to trigger tumor cell proliferation.[21,31]

Leptin is a protein hormone composed of 167 amino acids (16 kDa) encoded by the Lep gene on chromosome 7. It is primarily synthesized by white adipose tissue alongside other hormones like resistin, adiponectin, visfatin, omentin, and vaspin. Interestingly, leptin expression is not limited to adipose tissue; it's also found in the gastrointestinal system, brain, and muscle. Under normal physiological conditions, leptin expression is regulated by cortisol and insulin. However, in inflammatory states, interleukin-1β (IL-1β) can influence leptin expression. [25,32-34]

A recent study found that 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 through the Central Anorexigenic Pathway.1,10,32,35

It is known that free leptin and serum leptin levels are consistently higher in people with a high BMI compared to the normal weight/BMI population.[33,35] This study also found a tendency for leptin expression in tumor cells to increase with increasing body mass index (p-value 0.059) (Table 2). Even though it

is not yet significant, the results of our research are quite representative compared to previous studies. The previous study found no significant correlation between BMI and leptin expression in tumors (p-value = 0.20).[24] These results could be caused by the sample size being insufficient or the sample population being too heterogeneous.[24,26,36]

Furthermore, our study demonstrated significantly higher rates of metastasis and tumor necrosis in high-grade serous carcinoma (p-values = 0.000 and 0.000, respectively) (Table 1). These findings suggest that our sample population reflects the characteristics of the actual tumor population, potentially leading to more representative results for leptin analysis.

We further attempted to formulate the role of leptin in the process of ovarian carcinogenesis by reviewing previous studies (see Figure 2). Leptin exerts anti-apoptotic effects in ovarian carcinoma cells by inhibiting components like TNFR1, Bad, caspase-6, and caspase-3. Additionally, activation of the PI3K/AKT pathway promotes cell survival and proliferation, alongside the JAK/STAT and MEK/ERK pathways. Leptin also binding to its receptor (Ob-R) on immune cells modifies the tumor microenvironment. This inhibits IFN-γ production in Natural Killer (NK) cells, reducing their cytotoxicity. Leptin also activates tumor-associated macrophages (TAMs), which secrete: MMPs (promoting invasion and migration) and growth factors (EGF, FGF, VEGF) for remodeling and angiogenesis. Furthermore, TAMs modulate T cells by recruiting regulatory T cells (Tregs). CCL22 secretion by TAMs attracts Tregs, which then secrete immunosuppressive factors like TGF-β and IL-10 (14). TAMs, stromal adipose tissue, and tumor cells themselves also secrete IL-6. This, along with TNF-α, induces aromatase expression. The resulting hyperestrogenic environment acts as a mitogenic agent on ovarian epithelial cells, and promoting their growth.[37,38] Additionally, elevated IL-6 levels are associated with increased chemotherapy resistance[14,23]

Leptin facilitates cell migration through several mechanisms. The first involves inducing the secretion of MMP-9, MMP-2, and MMP-14 by TAMs (tumor-associated macrophages). Matrix metalloproteinases (MMPs) are enzymes known to degrade extracellular matrix components, which is crucial for cancer cell invasion and metastasis.[14] Leptin also promotes cell migration by inducing increased expression of uPA (urokinase plasminogen activator) in cancer cells. This activation occurs through the RhoA/ROCK intracellular signaling pathway.[39] uPA is an extracellular proteolytic enzyme that activates a cascade of proteases upon binding to its receptor (uPAR).[40] This cascade ultimately leads to the degradation of the extracellular matrix, facilitating tumor metastasis.[39,40] Leptin induces invasion/migration of cancer cells by activating intracellular signaling such as RhoA/ROCK, PI3K/AKT and JAK/STAT3 pathways (see Figure 2).[9,39]

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, lncRNAs, 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.[41]

Our study found that leptin antibodies stained positive in all low-grade serous ovarian carcinomas, while only 69.6% of high-grade tumors showed strong expression (p-value = 0.004) (Table 1). This is surprising because leptin is typically thought to be more abundant in the less aggressive form of serous cancer. Interestingly, leptin antibody expression was localized to tumor nests with well-differentiated tumor cell architecture and cytology. No previous studies have been able to explain this phenomenon. The authors hypothesize a role for estrogen in this case. It is known that LGSC tumor cells are more immunoreactive to hormonal receptors, particularly estrogen receptors (ER), compared to HGSC. Our hypothesis is that estrogen stimulates an increase in the number of leptin receptors on tumor cells. However, further studies are needed to investigate the relationship between

estrogen and leptin expression in ovarian tumor cells.

While no studies have directly investigated leptin's tendency towards specific EOC subtypes, research using body mass index (BMI) suggests a correlation between obesity and low-grade serous ovarian carcinoma. This type of cancer is generally considered indolent and well-differentiated, but also more resistant to chemotherapy.[11,12,42-44] In cases of endometrial carcinoma, the influence of obesity also predominates in type I carcinoma which has good tumor differentiation and a better prognosis.[20,26]

In summary, overexpression of leptin is linked to a poor prognosis in ovarian cancer. This is because leptin promotes several processes that enable cancer progression, including migration and metastasis of cancer cells, angiogenesis (the formation of new blood vessels that nourish tumors), epithelial-to-mesenchymal transition (a cellular shift that enhances invasiveness) (44), and degradation of the extracellular matrix (the network that provides structural support to tissues). However, several studies have found the leptin paradox phenomenon among various cancers. The obesity/leptin paradox is a phenomenon in which a cancer population has a significantly better prognosis when the body mass index is more than normal, when the opposite would be expected.[25,26] This phenomenon applies to several types of cancer, including ovarian cancer.[24]

The analysis of the entire serous carcinoma population revealed a significant correlation (p-value = 0.047) between leptin expression and metastasis incidence. Interestingly, 90.63% of ovarian carcinoma patients without metastases had strong leptin expression on tumor cells, compared to only 68.89% of those with metastases who had strong leptin expression (Table 4). This suggests a potential protective role of leptin, with an odds ratio (OR) of 0.23 for reduced metastasis risk. However, it's important to consider the heterogeneity of epithelial ovarian cancer (EOC) (7). High-grade and low-grade serous carcinomas are distinct entities with different biology and molecular characteristics. When analyzing the high-grade population specifically, no significant association was found between leptin expression and either protection from metastasis (p-value = 0.193) or lymphovascular space invasion (LVSI) (p-value = 0.341). This suggests a potential "pseudo-leptin paradox" effect in this study, likely influenced by the inclusion of low-grade serous carcinoma samples, which rarely metastasize.

Overexpression of leptin has been associated with a better prognosis in various cancers, including colorectal carcinoma,[27]hepatocellular carcinoma, pancreatic cancer,[25] and ovarian cancer.[24] Studies on nonmucinous colorectal adenocarcinoma have revealed that strong leptin expression in tumor cells is associated with a more favorable prognosis, characterized by lower depth of invasion, less frequent nodal metastasis, better tumor differentiation according to the American Joint Committee on Cancer (AJCC) and Dukes' staging systems, and significantly improved overall and disease-free survival rates.[27] Conversely, studies on ovarian carcinoma have yielded conflicting results. One previous study found that high leptin expression is associated with good prognosis, characterized by a lower chance of LVSI and significantly improved disease-specific survival (DSS) and disease-free survival (DFS) in ovarian cancer patients.[24] While other studies have demonstrated that leptin can activate intratumoral proliferation pathways such as the Janus kinase 2 (JAK2)[19] and RHOA/ROCK pathways,[39] stimulate migration and invasion,[18] and most critically, drive epithelial-mesenchymal transition (EMT).[44]

The results of the colorectal adenocarcinoma study are considered more reliable due to the more homogeneous characteristics of the sample population. In contrast, many ovarian cancer studies have overlooked the significant heterogeneity of epithelial ovarian cancer. For example, the study by other researchers included all types of ovarian cancer, encompassing both epithelial ovarian cancer (EOC) and non-epithelial ovarian cancer. Previous research has shown that only EOC is linked to obesity.[11,12,45] Furthermore, EOC itself has six different tumor types, each with unique molecular characteristics and behavior.[2,7,8] Notably, even serous carcinoma includes two distinct entities: HGSC and LGSC, which have opposite clinical courses. This reflects the heterogeneity of

ovarian cancers. Combining these entities, or including other ovarian tumor types, can introduce bias and potentially lead to misleading results. The results of leptin analysis in previous ovarian cancer studies may be confounded by the presence of several ovarian carcinoma subtypes with indolent characteristics that tend to be confined to the ovary.

Despite the heterogeneity of the sample, the underlying mechanism for this "leptin paradox" remains unclear. However, one hypothesis suggests that overweight or obese individuals might have larger energy reserves, leading to a longer survival even with cancer. Additionally, leptin might activate tumor-infiltrating lymphocytes (TILs), promoting anti-tumor immunity.[46] Our findings do not support the existence of a "leptin paradox" within the high-grade serous carcinoma population. Conversely, low-grade serous carcinomas exhibited unexpectedly strong leptin immunostaining. Based on these observations, we conclude that leptin expression correlates with well-differentiated serous ovarian carcinoma. Therefore, further studies are necessary to specifically investigate the role of leptin expression in low-grade serous carcinoma in order to evaluate the potential metastasis, invasion, prognosis and its impact on chemoresistancy .

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CONFLICT of INTEREST

The authors declare no conflicts of interest, financial or otherwise.

CONCLUSION

High-grade serous carcinoma shows no association between leptin expression and the occurrence of metastasis or lymphovascular space invasion. The conflicting findings on leptin in previous ovarian carcinoma studies (leptin paradox) might be due to the inclusion of samples with heterogeneous characteristics. Notably, leptin expression is higher in ovarian carcinomas with less aggressive features, such as low-grade serous carcinoma. Thus, including these two distinct tumor types (HGSC and LGSC) within the same study group can bias the results. Future research on leptin in ovarian carcinoma should utilize homogeneous samples to ensure reliable outcomes.

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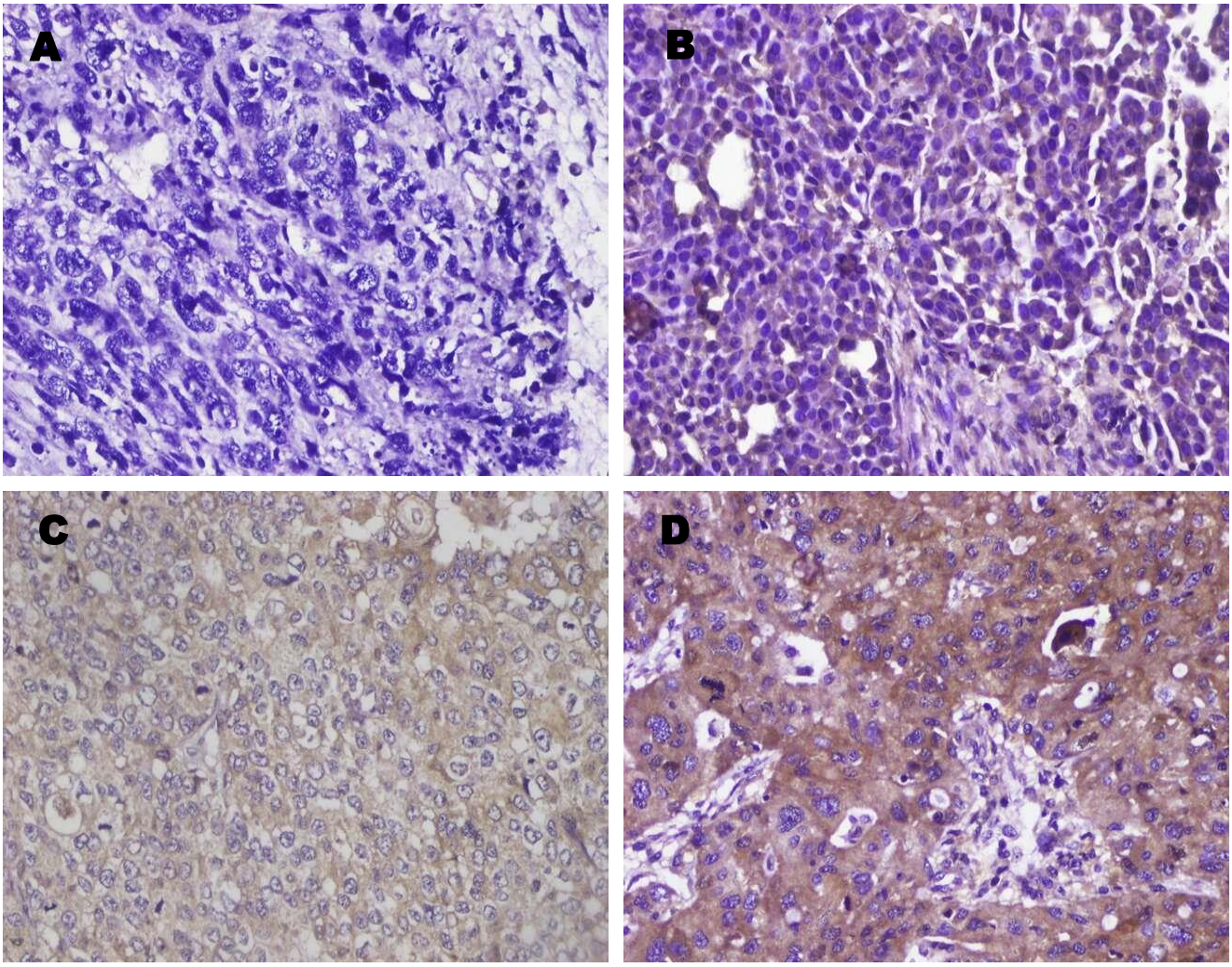

Figure 1. Leptin immunohistochemical staining in high-grade serous carcinoma. Leptin protein was expressed in the cytoplasm of tumor cells with varying intensity ranging from A Negative, B Weak, C Moderate, D Strong.

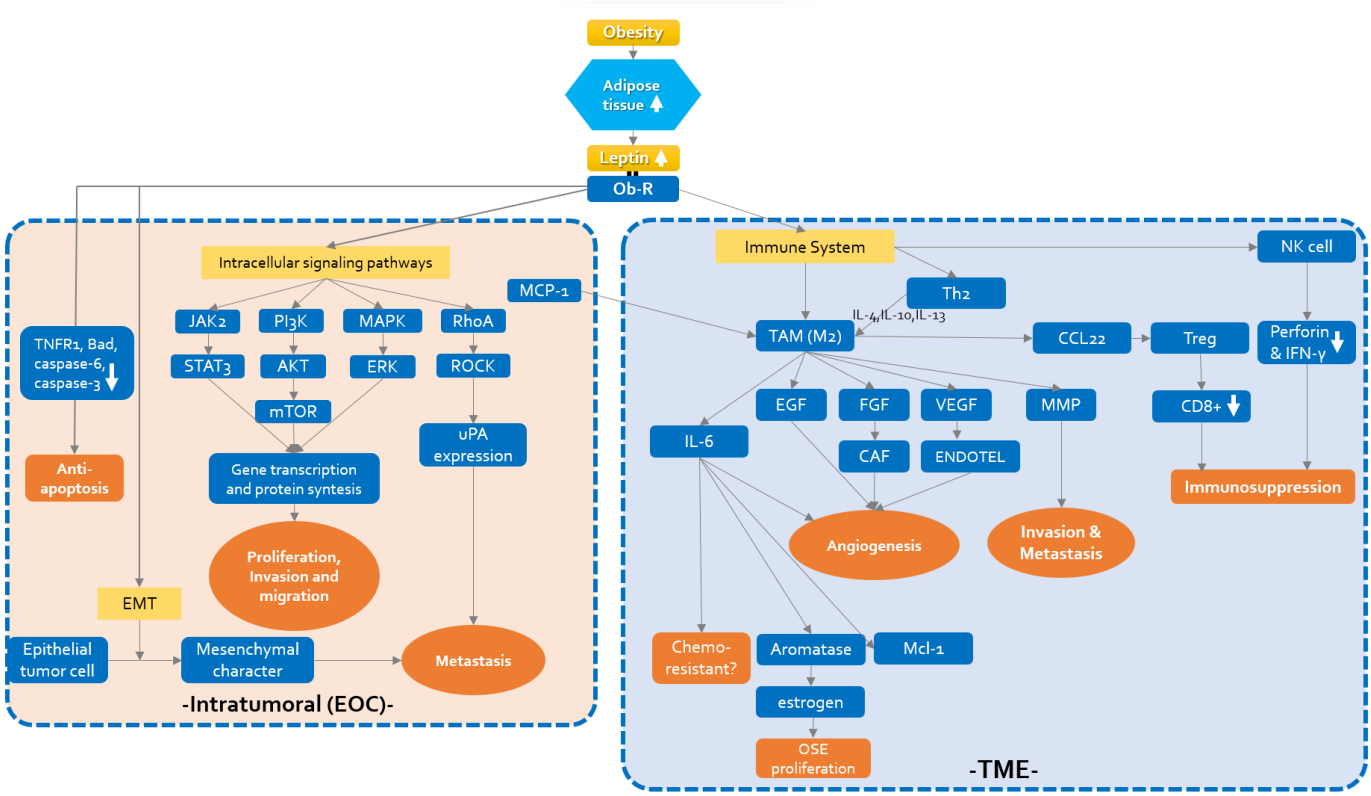

Figure 2. Role of leptin in the carcinogenesis of epithelial ovarian cancer.
CAF: Cancer-associated fibroblast, CCL22 : Chemokine (C-C Motif) Ligand 2, EGF: Epidermal growth factor, EMT: Epithelial–mesenchymal transition, IFN-γ : Interferon - Gamma MAPK/ERK: Mitogen Activated Protein Kinase/ Extracellular-Signal-Regulated Kinase Pathway, JAK2: Janus kinase 2, MCP1: Monocyte Chemoattractant Protein 1, MMP: Matrix metalloproteinase, NK cell: Natural Killer cell, Ob-R: Leptin receptor, PI3K: Phosphatidylinositol 3-kinase, Rhoa/ROCK : Ras Homolog Family Member A/ Rho-Associated, Coiled-Coil Containing Protein Kinase Pathway , STAT3: Signal transducer and activator of transcription 3, TME: Tumor microenvironment, TAM: Tumor-associated macrophages, uPA: Urokinase Plasminogen Activator , VEGF: Vascular endothelial growth factor

Table 1. Subject Characteristics

Variables	n(%)	HGSC (N=56)	LGSC (N=21)	P-value*
Age (Year)				
<50	32 (41.6)	20 (35.7)	**12 (57.1)**	0.150
>50	45 (58.4)	**36 (64.3)**	9 (42.9)	
IMT				
Underweight	12 (15.6)	9 (16.1)	3 (14.3)	0.961
Optimal	31 (40.3)	22 (39.3)	9 (42.8)	
Overweight/Obesity	31 (40.3)	**22 (39.3)**	**9 (42.8)**	
Missing	3 (3.9)	3 (5.4)		
LVSI				
Yes	27(35.1)	20 (35.7)	7 (33.3)	1.000
No	50 (64.9)	**36 (64.3)**	**14 (66.7)**	
Metastasis				
Yes	46(60)	**40 (71.4)**	5 (23.8)	**0.000**
No	31(40)	16 (28.6)	**16 (76.2)**	
Necrosis				
Yes	44 (57.1)	**40 (71.4)**	4 (19)	**0.000**
No	33 (42.9)	16 (28.6)	**17 (81)**	
Leptin Expression				
High	60 (77.9)	**39 (69.6)**	**21 (100)**	**0.004**
Low	17 (22.1)	17 (30.4)	0 (0)	

Chi-square test

Table 2. Analysis of the Relationship Between Body Mass Index and Leptin Protein Expression in Serous Ovarian Carcinoma

Leptin Expression	BMI			Total	P value*
	Underweight	Optimal	Overweight/ obesity		
	(%)	(%)	(%)		
Low	5 (41.7)	7 (24.1)	3 (9.7)	15	***0.059***
High	7 (58.3)	24 (75.9)	28 (90.3)	59	
Total (%)	12 (100.0)	31 (100.0)	31 (100.0)	74	

**** Pearson Chi-square***

Table 3. Leptin Expression and LVSI in Serous Ovarian Carcinoma: A Comparison Between Overall and High-Grade Serous Tumors

Leptin expression	Serous Ovarian Carcinoma				OR 95% CI	P-value*	High-grade Serous Carcinoma				OR 95% CI	P-value*
	No LVSI		LVSI				No LVSI		LVSI			
	n	%	n	%			n	%	n	%		
Low	13	26.00	4	14.81	2.020 (0.587-6.951)	0.4	13	36.11	4	20	2.261 (0.623-8.21)	0.341
High	37	74.00	23	85.19			23	63.89	16	80		
Total	50	100	27	100			36	100	20	100		

Chi-square test

Table 4. Leptin Expression and Metastasis in Serous Ovarian Carcinoma: A Comparison Between Overall and High-Grade SerousTumors

Leptin expression	Serous Ovarian Carcinoma				OR 95% CI	P-value *	High-grade Serous Carcinoma				OR 95% CI	P-value *
	No Metastasis		Metastasis				No Metastasis		Metastasis			
	n	%	n	%			n	%	n	%		
Low	3	9.38	14	31.11	0.229 (0.060-0.880)	**0.047**	3	18.75	14	35	0.429 (0.104-1.762)	0.193
High	29	90.63	31	68.89			13	81.25	26	65		
Total	32	100	45	100			16	100	40	100		

Chi-square test