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Leptin expression is substantially correlated with prognosis of urinary bladder carcinoma

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ABSTRACT

This study examined leptin expression in cases of bladder cancer and its diagnostic and prognostic usefulness in bladder malignancies.

A set of 128 urinary bladder cancer cases and 24 normal specimens of bladders were employed for an immunohistochemical investigation of leptin expression in tissue microarrays.

Leptin was up-regulated during transformation and was identified as brown cytoplasmic granules in the malignant urothelium of 123 (96%) bladder neoplasms, of which 68 (53.1%) cases showed high levels (moderate to strong) of staining. Strong staining was found to be associated with high stages ($P = 0.001$), muscularis propria infiltration ($P < 0.001$), vascular invasion ($P < 0.03$), lymph node involvement ($P < 0.02$), metastases ($P < 0.05$), and mortality ($P < 0.03$). Furthermore, various important survival distributions were detected with leptin expression in the malignant urothelium ($P < 0.03$).

These pilot results suggest that leptin might be a valid marker for predicting the stage and bad prognoses in bladder carcinoma.

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Leptin; urinary bladder cancer; immunohistochemistry

1. Introduction

Malignant tumors of the urinary bladder are destructive diseases and are important causes of mortality worldwide [1]. Recently, Siegel et al. [2] reported that urinary bladder cancer is ranked six on the list of the most common neoplasms excluding skin cancer, with around 81,400 newly registered cancer patients and more than 17,980 associated mortalities in the USA. In spite of significant improvements in tumor therapy, bladder cancer is still a challenge for physicians as a consequence of the great relapse frequency. For example, up to 70% of newly recorded bladder neoplasms relapse in five years, and there is an enormous chance of them developing into invasive, muscle infiltrative, and metastatic types [3,4].

The treatment of urinary bladder carcinoma highly depends on the patients' clinical factors as signs of good or bad prognosis. However, available clinical data are not sufficient to estimate the disease consequences as there are great inconsistencies within similar stages or grades, which are mostly due to tumor-cell heterogeneity [5]. Thus, it is important to acquire novel investigative modalities that can fulfill clinicians' demands for bladder cancer management.

Great attempts are being made to find original biochemical markers to help in the diagnosis and predict tumor prognosis, enrich the stratification of high risk patients, and enhance tumor management [6,7]. However, a good number of the available biomarkers are inadequately specific or sensitive, which makes it necessary to discover other markers that would be more accurate and more predictive of clinical outcomes.

The obese (Ob) gene encodes a protein (167 amino acids) called leptin, which was initially reported to be made by adipose tissue. Later, other studies stated that tissues of different organs produce leptin, such as the stomach, liver, ovaries, skeletal muscles, pituitary gland, and placenta [8–10]. Leptin is an adipokine that has a substantial role in several biological processes, such as lipogenesis, metabolism, inflammation, glucose homeostasis, reproduction, tissue remodeling, bone formation, immune response, and neoangiogenesis [11–15]. Several scientific reports have stated that leptin stimulates the proliferation of malignant cells, invasion, and metastasis. It also prevents apoptosis by many ways [14,16–21].

Other studies have reported that leptin plays a role in the development of tumor risk, but the results are still debatable [22–25]. Thus, confirmation is necessary to clear up leptin's specific function in the initiation and progression of urinary bladder cancer. Identification of the relationship between leptin and bladder cancer could improve people's awareness of bladder carcinogenesis and assist in improving

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medication and preventative strategies. Thus, our study defined leptin phenotype in cases of urinary bladder cancer and assesses its relationship with clinical factors and follow-up data.

2. Materials and methods

Paraffin-embedded tissues from urinary bladder carcinomas (128 cases) and a control group (24 non-cancerous bladder tissue samples) were collected from the archives of the pathology department. The clinical reports of all recruited cases indicated that the tissue samples were obtained by transurethral surgery. All cases that received intravesical pharmacotherapy or neoadjuvant chemotherapy were excluded.

Paraffin-embedded tissues were sliced into 4-µm sections, stained with hematoxylin and eosin, and reevaluated. Neoplasm features and patient clinicopathological information were gathered from the medical records of King Abdulaziz University Hospital (patient's sex and age and tumor's histotype, stage, and metastasis). All control-group tissues were collected from patients who had been sampled for non-cancer syndromes. The World Health Organization's recommendations were adopted in the bladder carcinoma staging processes.

2.1. Tissue microarray construction

A Tissue Microarray (TMA) was constructed as reported in an earlier paper [26]. All 128 bladder carcinoma cases and 24 control specimens were used in this process. TMA Blocks were cut into 4-µm slices and placed on aminosilane-coated slides.

2.2. Immunohistochemistry staining method

Immunohistochemistry staining was done using a BenchMark ULTRA autostainer (Ventana, Arizona, USA) as reported previously [27]. Leptin polyclonal antibody was diluted to a ratio of 1:100 (catalog code: sc-842, Santa Cruz Biotechnology, USA) and used in the immunohistochemical staining protocol, followed by visualization using by an ULTRAVIEW TM DAB system. A negative control slide treated with Tris buffer in place of leptin antibody was used in every staining run.

A 4-µm section of placenta specimen was used as a positive control. All cases that showed cytoplasmic brown color in more than 5% of malignant cells were considered positive. The immunohistochemistry staining was analyzed by 2 pathologists.

The rate of positively stained cells was estimated using a semiquantitative technique (3 fields of 40x lenses). Our laboratory scoring system for IHC was applied for the leptin staining intensity using scores 0 to 3 with 0 used for negative results. The grades of staining strength were divided into two groups: low scores (0 and 1) and high scores (2 and 3).

2.3. Statistical analysis

All information was evaluated using IBM-SPSS software (version 21). All results are exhibited as incidences and percentages. The relationship between clinical data of bladder carcinoma and leptin expression was investigated statistically by Fisher and chi-squared tests. Assessment of survival distributions for different leptin staining intensity scores was conducted using the Breslow Generalized Wilcoxon test. The significance level was considered as $P < 0.05$.

3. Results

There were 128 cases of urinary bladder cancer that were reexamined (104 males and 24 females). Both genders showed similar distribution patterns of immunohistochemical staining. Clinicopathological parameters of these cases are displayed in Table 1. The most common type was urothelial carcinoma (78.9%), followed by squamous differentiation variant (13.3%), squamous cell carcinoma (4.7%), and urothelial carcinoma in situ (3.1%) (Table 1). The median age

Table 1. Distribution of various clinicopathological variables with leptin immunostaining in urinary bladder cancer.

		Leptin staining in epithelial cells				
		High		Low		
		n	%	n	%	P-Value
Gender (Male/Female)	Female	15	62.5%	9	37.5%	= 0.31
	Male	53	51.0%	51	49.0%	
Histotype of Cancer (Urothelial or Squamous)	Squamous	3	50.0%	3	50.0%	= 0.71
	Urothelial	55	54.5%	46	45.5%	
	Urothelial/CIS	1	25.0%	3	75.0%	
	Urothelial/Squamous	9	52.9%	8	47.1%	
Stage	0a	4	23.5%	13	76.5%	= 0.001
	0is	1	20.0%	4	80.0%	
	I	18	58.1%	13	41.9%	
	II	25	65.8%	13	34.2%	
	III	4	57.1%	3	42.9%	
	IV	15	75.0%	5	25.0%	
Undecided	1		10.0%	9	90.0%	
Grade	High Grade	37	56.9%	28	43.1%	= 0.53
	Low Grade	24	47.1%	27	52.9%	
	NA	7	58.3%	5	41.7%	
Muscular Invasion (MIBC or NMIBC)	MIBC	45	71.4%	18	28.6%	< 0.001
	NMIBC	19	40.4%	28	59.6%	
Undecided	4		22.2%	14	77.8%	
Vascular Invasion (Y/N)	NON	54	49.1%	56	50.9%	< 0.03
	Positive	14	77.8%	4	22.2%	
Lymph Node (Y/N)	NON	53	48.6%	56	51.4%	< 0.02
	Positive	15	78.9%	4	21.1%	
Metastasis	NON	52	49.1%	54	50.9%	< 0.05
	Positive	16	72.7%	6	27.3%	
Alive/deceased status	Alive	42	46.7%	48	53.3%	< 0.03
	Deceased	26	68.4%	12	31.6%	

CIS: Carcinoma in situ; MIBC: Muscle-Invasive Bladder Cancer; NMIBC: Non-Muscle-Invasive Bladder Cancer.

was 62.4 years (range 31–93 years). There were 118 staged cases of bladder cancer, and 116 cases were graded (Table 1). In total, there were 38 (29.7%) deaths from bladder cancer. There were 63 cases showing muscularis propria invasion, 18 cases with vascular invasion, 19 cases with lymph node involvement, and 22 cases with distant metastases (Table 1).

Leptin was found to be up-regulated during transformation, which was found in 123 (96%) urinary bladder neoplasms, of which 68 (53.1%) cases showed high levels (moderate to strong) of immunostaining (Figures 1a–c). More than 89% of tumor cases showed immunostaining in more than 60% of the malignant cells. Only 12 (50%) control cases showed positive leptin immunostaining.

Several tumors showed apical cytoplasmic staining. In other cases, a number of tumor cells exhibited brown color in all parts of the cytoplasm, and a few cases presented perinuclear staining. Only one tumor case showed both cytoplasmic and nuclear expression. Thus, these observations indicate substantial heterogeneity in the leptin immunohistochemical staining patterns between urinary bladder tumors.

Leptin immunohistochemical staining was found to be significantly correlated with the stage of urinary bladder tumor, muscularis propria invasion, vascular invasion, lymph node invasion, remote metastasis, and patient mortality. Advance stages of urinary bladder cancer were significantly linked with leptin staining intensity ($P = 0.001$). A considerable fraction of low-stage tumors had low leptin immunostaining scores, whereas strong immunostaining of leptin was found in 75% of stage IV tumors (Table 1).

Muscularis propria, vascular and lymph node invasion, and remote metastasis occurred more frequently in bladder cancers where cells exhibited a high score for leptin immunostaining ($P < 0.001$, $P < 0.03$, $P < 0.02$, $P < 0.05$, respectively). Furthermore, most bladder tumors that did not invade the muscularis propria, blood vessels, and lymph nodes showed a low level of leptin immunoreactivity. Patient mortality was also significantly associated with leptin staining, with a higher proportion of deaths being observed in cases with a high score of leptin staining. No significant associations were observed with gender or tumor histotype.

The Breslow Generalized Wilcoxon test was performed to examine the equality of survival distributions for the high and low scores of leptin immunostaining in the malignant urothelium. Significantly diverse survival distributions were detected with leptin immunostaining ($P < 0.03$). Significantly poor survival behavior was found in cases with a high score of leptin staining (Table 2),

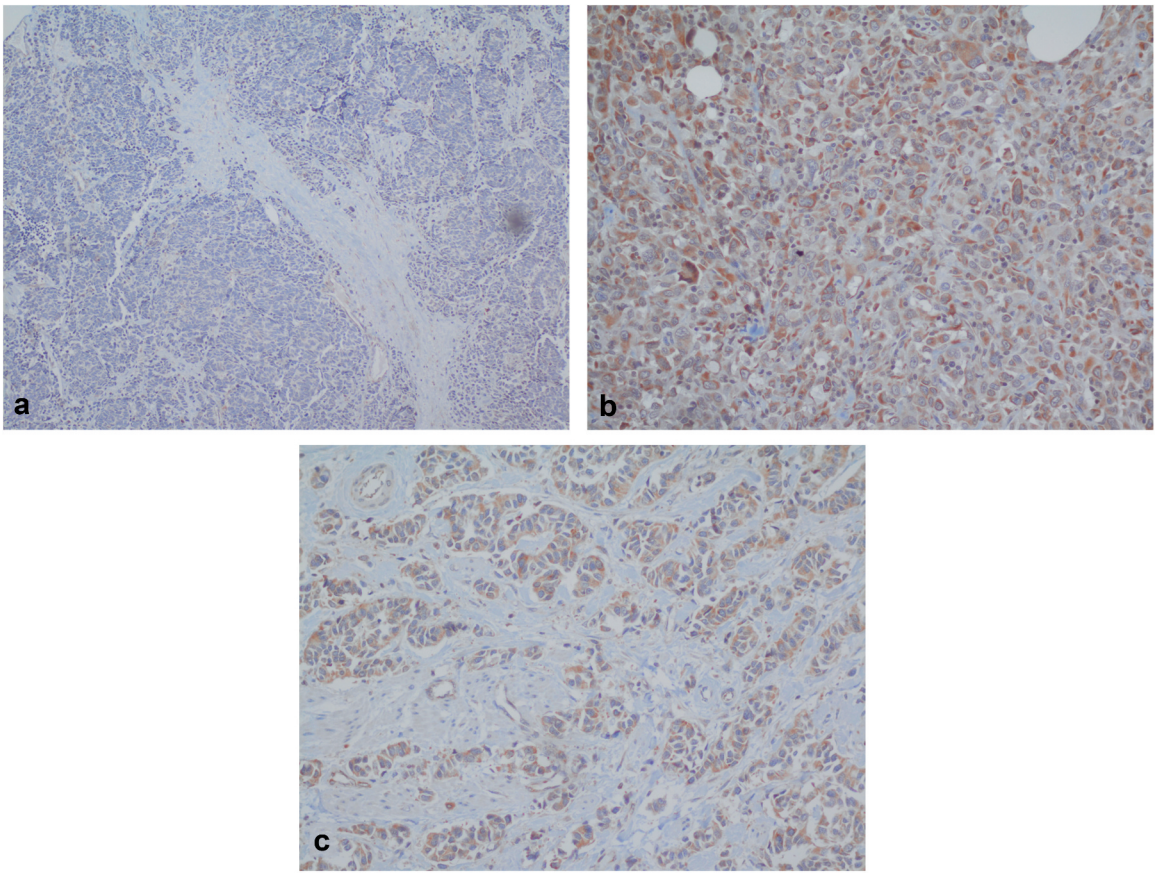

Figure 1. Leptin immunohistochemistry staining patterns in bladder cancer. (a), negative leptin staining in bladder cancer (10 X); (b), strong leptin staining in malignant urothelium of bladder cancer (40 X); (c), moderate leptin staining in malignant urothelium of bladder cancer (40 X).

Table 2. Survival table, summary of cases censored in each group.

LSEC	Total N	N of Events	Censored	
			N	Percent
High	66	26	40	60.6%
Low	56	12	44	78.6%
Overall	122	38	84	68.9%

LSEC: Leptin staining in epithelial cells.

There were 66 cases of high leptin staining scores, of which 26 showed the event of interest (death), and 40 cases were censored. Similarly, there were 56 cases with low leptin staining scores, of which 12 showed the event of interest, and 44 were censored.

The Kaplan Meier survival curve showed higher survival among low-urothelial tumor cases until 45 months (Figure 2). However, the reverse was observed after 100 months. Since the curves are intersecting, the log-rank test most likely showed an insignificant difference. Therefore, we can say that a high score of leptin staining in the malignant urothelium is positively correlated with poor survival.

4. Discussion

Bladder tumors still represent a big task for physicians because of the poor prognosis and high recurrence rate [3,4], irrespective of advances in tumor diagnosis and therapy. At present, there is no single tissue biomarker that can predict the clinical outcomes of bladder cancer, and there are no markers of the pathogenesis of bladder cancer that can be used to recognize individuals at risk for tumor progression. In the last decade, more attention has been given to obesity and its association with higher risk of certain tumors. Because of the significant function of leptin in the pathophysiology of obesity, the investigation of leptin's relationship with the risk of cancer is of substantial value. Thus, this study examined leptin expression in bladder cancer and its diagnostic and prognostic usefulness in bladder malignancies.

Many studies have defined the involved role of multifunctional intracellular and extracellular leptin, its expression pattern, and its regulation in the process of tumor development, progression, and metastasis [14,16–21]. Leptin has been found to be upregulated during the transformation process in different human malignancies, such as in liver, thyroid, colon, and brain tumors [17,28–30]. Therefore, the expression of leptin was suggested as a possible marker for the diagnosis or prognosis of tumors located in different organs. Nevertheless, there is conflict between the outcomes obtained from several neoplasms [22–25].

To the best of our knowledge, only two reports have studied leptin immunoexpression in a small number of bladder cancer cases [31,32]. The first study [31] included only three cases, and the second study employed 23 cases [32]. Nevertheless, neither study detected leptin expression in malignant bladder tissues. The current research is the first to state that leptin immunoexpression patterns are considerably

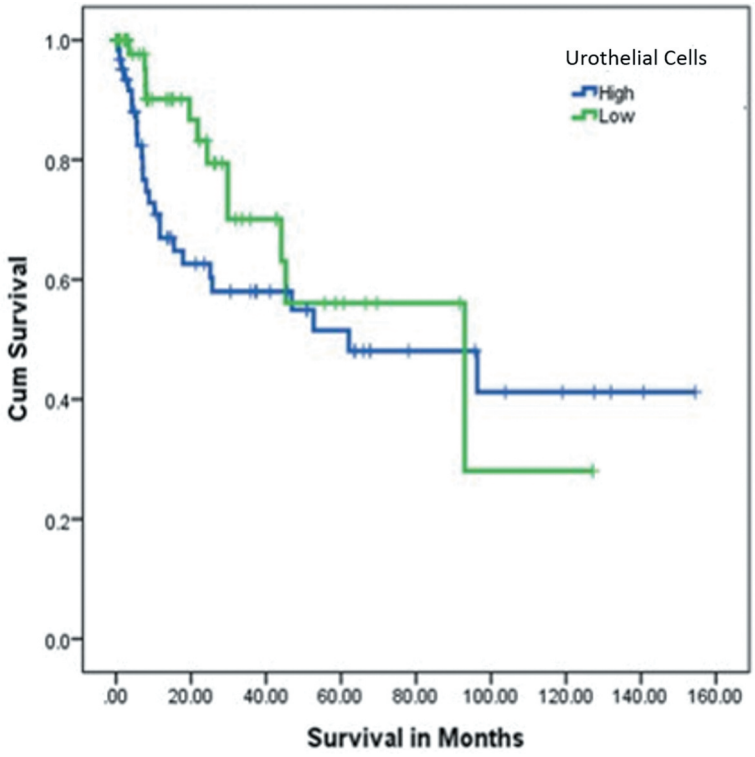

Figure 2. Kaplan Meier survival curves by patterns of leptin immunostaining show significantly poor survival behavior associated with leptin immunostaining in bladder cancer.

correlated with stage, muscularis propria invasion, vascular invasion, lymph node involvement, and poor outcomes (death) of bladder carcinomas.

Our outcomes are in agreement with various studies that have linked the leptin immunophenotype with different phases of the transformation process, such as cell proliferation, cell migration, infiltration, metastasis, and recurrence in many organs. These studies have stated that leptin expression is associated with at least one of the clinicopathological parameters, including high stage, invasion, metastasis, recurrence, and poor prognostic signs in endometrial cancer [27], colorectal cancer [28], hepatocellular carcinoma [17], thyroid carcinoma [29], lung cancer [33], gastric cancer [34], breast cancer [19], laryngeal carcinoma [35], and esophageal carcinoma [36]. In this study, the expression of leptin was linked with the degree of cancer progression and invasiveness. A high score of leptin immunoexpression in bladder neoplasms was found to be related to a higher tumor stage with muscularis propria, vascular invasion, and lymph node invasion. Increased expression of leptin may lead to changes in the biological behavior of malignant cells, such as growth, proliferation, invasion, and survival [37]. Thus, our findings indicate that leptin is a possible biomarker for the stratification of bladder tumor subtypes for personalized therapy.

The variations between the earlier reports and the present one might be explained by the sensitivity of the methods, difference among patients, and differences in sample size. However, there are some weak points in the current study and the earlier ones that measured the importance of leptin expression in the diagnoses and prognoses of bladder neoplasms. For instance, a comparatively small sample size was used in these reports and semiquantitative immunostaining analyses. Thus, more comprehensive studies would be unquestionably valuable for assessing the capacities of this molecule in the diagnosis and prognosis of bladder tumors.

5. Conclusion

Leptin can be a useful marker for predicting the stage and bad prognoses in bladder cancer, as well as the stratification of bladder tumor subtypes for personalized therapy. The relationship of leptin with some clinicopathological factors suggests the involvement of this molecule in the progression of bladder tumors.

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