Original Article

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Evaluation of Tissue Expression of HMBG1 protein in Patients With Breast Cancer

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ABSTRACT

Objective: High mobility group box 1 (HMGB1) is a nonhistone chromatin-associated protein involved in chromatin remodeling, transcription, DNA replication, and repair. The purpose of this study was to assess the relationship between tissue expression of HMGB1, clinical outcomes, and histopathological characteristics in patients with breast cancer.

Materials and Methods: The study included 282 patients with breast cancer. An *in vitro* diagnostic HMGB1 antibody was applied to the slides of tumor specimens.

Results: Overexpression of HMGB1 was found in tumor cells of 123 (43.6%) patients. HMGB1 was only expressed in the nucleus in most tumors (88.7%), while in 32 (11.3%) tumors HMBG1 expression was cytoplasmic and/or extracellular. Severe inflammatory infiltration of the peritumoral stroma was observed in 76 (27%) patients. There was a correlation between remarkable inflammatory cell infiltration in the tumor microenvironment and HMGB1 overexpression, regardless of the molecular subtype, as well as the extranuclear location of HMGB1 expression (p = 0.023). HMGB1 expression was not found to be associated with overall or disease-free survival. However, axillary lymph node metastasis was significantly more common in tumors with intense inflammation (p = 0.024).

Conclusion: The proportion of breast cancer patients with HMGB1 expression was lower in the present study than that reported previously. Furthermore, we did not detect a relationship between HMGB1 expression and prognosis. However, the relationship between HMGB1 expression and prognosis had been previously reported only in aggressive breast cancers. It is suggested that understanding the significance of HMGB1 expression in breast cancer may open new treatment opportunities, especially in aggressive and/or triple negative tumors.

Keywords: Breast cancer; HMGB1 protein; high mobility group box-1; tissue expression

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Key Points

- High mobility group box 1 (HMGB1) expression was mostly associated with prognosis in the most aggressive triple-negative breast cancer group.
- Since the number of triple-negative cases in our series was quite low, we may not have detected a close relationship between HMGB1 expression and prognosis.
- Understanding HMGB1 expression in breast cancers may lead to new treatment opportunities, particularly in aggressive carcinomas.

Introduction

Non-histone nuclear proteins, known as high mobility group box 1 (HMGB) proteins, perform several important biological tasks in cells (1). Members of the HMGB protein family are HMGB1, HMGB2, and HMGB3. Only HMGB1 is widely expressed in the nuclei of almost all eukaryotic cells, but HMGB2 is mainly expressed in the thymus, and testes, and HMGB3 in hematopoietic stem cells. In contrast to its limited expression in the testes and lymphoid organs of adults, HMGB2 is also highly expressed during embryogenesis (1, 2). HMGB1 is predominantly expressed in nuclei as it is involved in chromatin remodeling, DNA replication, repair, and transcription. In immunohistochemical (IHC) studies, the nuclear expression rate of HMGB1 was generally reported to be very high. Investigations also revealed that following posttranslational changes, such as acetylation,

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phosphorylation, and methylation, HMGB1 may migrate from the nucleus to the cytoplasm. HMGB1 can also be released to the extracellular environment in response to hypoxia or chemoradiotherapy, primarily through active secretion from immunocompetent cells or passive release from necrotic or apoptotic cells. When HMGB1 is released into the extracellular space, it acts as a cytokine-like molecule, signaling tissue damage and contributing to inflammation (1-3).

Overexpression of HMGB1 has been reported in various types of cancers, including breast carcinoma (4). HMGB1 may promote tumor progression through various mechanisms. By facilitating DNA repair and replication, HMGB1 may facilitate tumor cell survival and proliferation. HMGB1 may promote neoangiogenesis, which supply the tumor with nutrients and oxygen. In addition, HMBG1 may modulate the immune response, helping cancer cells evade detection and destruction by the immune system. HMGB1 may even be involved in tumor cell migration and, therefore, metastasis (1-5). High levels of HMGB1 expression in tumors have been associated with poor prognosis, higher tumor grade, and increased metastatic potential. Given these roles in cancer progression, HMGB1 is being explored as a potential therapeutic target. Strategies include blocking its extracellular signaling pathways or reducing its expression to inhibit tumor growth and spread (6-9).

Breast cancer continues to be a major global health concern for women with its high incidence rate. Even while improvements in early detection and treatment of breast cancer have somewhat reduced its mortality rate, many individuals still die from a range of intricate malignant morphologies. Abnormal HMGB1 levels have also been previously reported in breast cancer. The clinical use of HMGB1 in the detection and treatment of breast cancer has also been demonstrated by numerous investigations (5, 6). However, a deeper comprehension of dual role of HMGB1 in cancer growth is necessary due to its pro- and anti-tumoral properties. More significantly, HMGB1 has a role in controlling patients' response to radiation and chemotherapy for breast cancer. The complexity of the association between HMBG1 and the development of breast cancer has led to the continuous development of novel therapeutic approaches that target HMGB1, including the detection of putative inducers of immunogenic cell death and combination treatments with immune checkpoint inhibitors (7-10). The purpose of this study was to assess the expression of HMGB1 by IHC staining in breast cancer tissues and to investigate any correlation between HMBG1 expression and clinicopathological traits in breast cancer patients.

Materials and Methods

The expression of HMBG1 protein was investigated in tissue samples taken from primary breast carcinoma patients who had undergone mastectomy or excisional breast biopsy between 2011 and 2018 and whose diagnoses were confirmed by IHC analysis of stained slides in our hospital's pathology laboratory. The local Ethics Committee of Buca Seyfi Demirsoy Training and Research Hospital approved this project (reference number: 293, date: 29.05.2024). Informed consent forms were signed preoperatively by all participating patients. Archival slides stained with hematoxylin and eosin (H&E) were reassessed using the World Health Organization's 2012 categorization of breast tumors criteria. H&E stained slides were used for IHC analysis in order to detect the viable tumor regions and choose suitable paraffin blocks for study-specific IHC analysis. The 2-micron diameter paraffined cylindrical tissue samples were taken from donor blocks best suited for IHC analysis and identified first on the slide and subsequently in the block. IHC analysis was then carried out using diluted monoclonal rabbit antibodies against HMGB1 (Atlas, ATL-HPA049521, USA) at a dilution of 1:500 after several blocks had been created using mapping and addressing procedures. Histopathologists, blinded to the patients' clinical characteristics, examined the slides, and classified staining patterns based on their staining intensities. Diffuse nuclear and/or cytoplasmic staining of the tumor cells (Figure 1) was considered HMGB1 positivity, and the number of positive cells was recorded. Furthermore, invasion of the tumor tissue by inflammatory cells that were HMBG1-positive was assessed and evidence of any extracellular expression of HMGB1 was also investigated (Figure 2).

Statistical Analysis

SPSS, version 25.0, was used for the statistical analysis (IBM Inc., Armonk, NY, USA). The chi-square test was employed to compare quantitative data. Non-parametric data were compared using the Mann-Whitney U test. The non-parametric Kruskal-Wallis test was used to compare the measurements in more than two groups. The difference in survival across groups was compared using Kaplan-Meier survival analysis. The threshold for statistical significance was set at $p<0.05$.

Results

There were 282 patients' samples included in the study with a median (range) age of 54 (27–85) years and a mean age of 55.5±12.2 years. The mean follow-up period was 48.3±24.1 months. Two hundred and

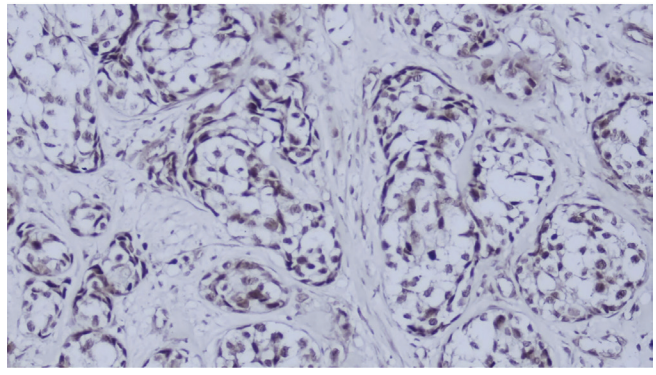

Figure 1. Strong nuclear expression of HMGB1 in tumor cells (DAB x 200)

HMGB1: High mobility group box 1; DAB: diaminobenzidine

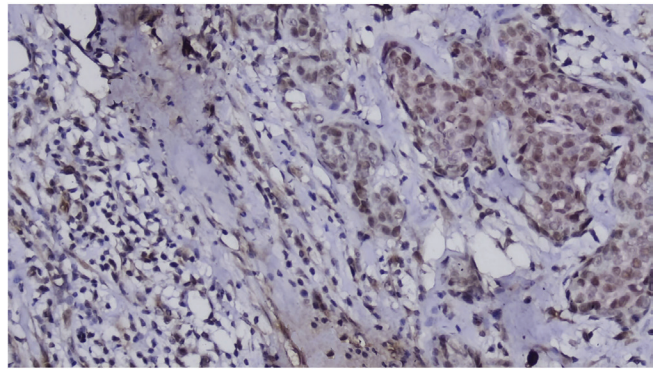

Figure 2. Note the weaker cytoplasmic HMGB1 expression in tumor cells compared to inflammatory cells (DAB x 200)

HMGB1: High mobility group box 1; DAB: diaminobenzidine

forty-eight (87.9%) patients were alive, but 34 (12.1%) patients had died at the time of data analysis. The mean overall survival time was 21 months, ranging from 1.5 to 79.9 months. The mean tumor diameter was 3.4±2.9 cm (0.4–18 cm). Clinical and histopathologic findings of the patient are shown in Table 1.

Estrogen receptor-positivity was found in 229 (81.2%) and progesterone receptor-positivity in 204 (72.4%) of the 282 patients who were part of the study. C-erbB2, which was used to assess human epidermal growth factor receptor 2 (HER2)/neu amplification, was 1+ or negative in 192 patients (68.1%), and both these groups were regarded as HER2-negative. Thirty-three cases (11.7%) were HER2-positive by combined IHC-fluorescent *in situ* hybridization examination, and each of them was treated specifically. Every case was examined using the Ki-67 proliferation index, with a mean value of 31.1±24%, ranging from 2–80%. The number of cases with luminal A (n = 114; 40.4%), luminal B (n = 112; 39.7%), HER2-positive (n = 33: 11.7%), and triple-negative (n = 23; 8.2%) were identified, based on the molecular classification. Patients in the various molecular groups had mean ages that were relatively similar to one another (p = 0.603). The survival time in this series did not differ significantly between the molecular subtypes of the malignancies (p = 0.178). A few inflammatory cells were identified in the peritumoral stroma of almost all tumors. However, there was severe inflammatory infiltration in the peritumoral stroma of 76 (27%) tumors. HMBG1 expression was confined to the nucleus in 250 (88.7%) tumors. However, in 32 (11.3%) tumors, there was nuclear and cytoplasmic and/or extracellular expression of HMGB1 (Table 2).

Table 1. Clinical and histopathologic data

		n	%
Survival	Survived	248	87.9
	Exited	34	12.1
Tumor location	Right breast	134	47.6
	Left breast	148	52.4
	Bilateral	-	-
Diagnosis	IDC	200	70.9
	ILC	18	6.4
	IPC	7	2.5
	IDC with dominant *in situ* component	33	11.7
	Other histologic variants	24	8.5
Grade	Grade 1	20	7.1
	Grade 2	141	50
	Grade 3	121	42.9
Pathologic T-stage	pT1	115	41
	pT2	120	42.4
	pT3	34	12.1
	pT4	13	4.5
***In situ* component**	Yes	182	64.5
Type of *in situ* component (if any)	Comedo	26	14.2
	Non-comedo	76	41.8
	Mixed	80	44.0
Lymph node metastasis	Yes	112	39.7
Capsular invasion in the lymph node	Yes	79	70.5
Multifocality	Multifocal	22	7.8
Nipple involvement	Yes	20	7.1
Dermal/epidermal invasion	Yes	22	7.8
Lymphovascular invasion	Yes	102	36.2
Perineural invasion	Yes	71	25.2

IDC: Invasive ductal carcinoma; ILC: Invasive lobular carcinoma; IPC: Invasive papillary carcinoma

In all cases, there were HMGB1 expressions in the nuclei of tumor cells, with a mean proportion 8.84% staining positive, but the intensity ranged widely from 1% to 90% of cells. There was no significant difference in survival rates and the presence of cytoplasmic/extracellular HMBG1 or isolated nuclear staining (p = 0.295). The mean survival time was 51.1±23.2 months for the patients with a low expression rate of HMGB1 (<5%) while the mean survival time was 44.7±24.7 months for the patients with high HMGB1 expression. There was a statistically significant difference in survival time of the patients according to the HMGB1 expression rate in tumor cells (p = 0.035).

Discussion and Conclusion

Breast cancers, like many other cancers, may exhibit IHC expression of HMGB1 in both the cytoplasm and the nuclei of breast cancer cells. While cytoplasmic and extracellular expressions of HMGB1 has been associated with its role in inflammation and the progression of cancer, its nuclear localization is related to its role in DNA-related functions. Moreover, research has demonstrated that, in contrast to normal breast tissue, HMGB1 is overexpressed in breast cancer tissues (11). Expression level of HMGB1 varies according to subtype, and stage of breast cancer. Aggressive phenotypes of breast cancer have been linked to higher levels of HMGB1 expression. For instance, HMGB1 is frequently overexpressed in cases of triple-negative breast cancers (TNBC), which are notorious for their poor prognosis and lack of targeted treatment modalities (11). Furthermore, it has been found that in individuals with breast cancer, HMGB1 overexpression is linked to a lower overall and disease-free survival rates. Thus, HMGB1 is thought to be a possible prognostic indicator, particularly for patients with more aggressive types of breast cancer. Since HMGB1 protein is secreted extracellularly or translocated to the cytoplasm, nuclear HMGB1 expression may be significantly reduced in more aggressive tumors, such as some types of colorectal cancer, gastric cancer, and advanced breast cancer, particularly TNBC. Although 40–70% of tumor cells may demonstrate nuclear expression of HMGB1, the other cells may exhibit extracellular or cytoplasmic HMGB1 expression. In contrast to earlier studies, in the present study, the highest mean nuclear HMGB1 expression rate was noted in patients with luminal A-subtype tumors, while the mean HMGB1 expression rates in the other three subtypes were similar. Furthermore, no significant correlation was found between the molecular subtype and the presence of cytoplasmic or extracellular HMGB1 expression (2, 11-13).

It was previously demonstrated that HMGB1 also plays an important role in mediating immunoregulatory functions. For instance,

Table 2. Immunohistochemical and molecular findings

Parameters		n	%
ER status	Positive	229	81.2
PR status	Positive	204	72.4
c-erbB-2 expression (according to ASCO/CAP 2013 criteria)	Negative or 1+	192	68.1
	2+	51	18.1
	3+	39	13.8
HER2 amplification (FISH method)	Positive	29	10.3
	Negative	23	8.2
Molecular subtypes	Luminal A	114	40.4
	Luminal B	112	39.7
	HER2-positive	33	11.7
	Triple-negative (basal-like)	23	8.2
HMBG1 expression	Nuclear	250	88.7
	Cytoplasmic/extracellular	32	11.3
Severe inflammation	Yes	76	27
	No	206	73

ER: Estrogen receptor; PR: Progesterone receptor; FISH: Fluorescent *in situ* hybridization; ASCO/CAP: American Society of Clinical Oncology/College of American Pathologists; HER2: Human epidermal growth factor receptor 2

extracellular HMGB1 modulates the immune response by acting as a damage-associated molecular pattern molecule. It can produce an immunosuppressive tumor microenvironment, which helps cancer cells evade the immune system. In the present study, there was a significant relationship between prominent inflammatory cell infiltration in the tumor microenvironment, regardless of the molecular subtype, and extranuclear location of HMGB1 expression (p = 0.023). This appears to be further evidence of the hypothesis that extracellular or cytoplasmic expressions of HMGB1 is associated with the immune response (2, 13).

One of molecular functions HMGB1 in breast cancer is to enhance tumor cell survival by encouraging DNA repair, shielding cancer cells from the genotoxic stress that is often brought on by treatments like radiotherapy and chemotherapy. HMGB1 also promotes cell invasion and migration, which facilitates the metastatic process. In addition, it contributes to the epithelial-mesenchymal transition, a crucial step in the spread of cancer (14-19). In the present study, no relationship was found between HMGB1 protein expression and metastasis according to different cut-off values. However, axillary lymph node metastasis was seen at a significantly higher rate in tumors with intense inflammation (p = 0.024). Similarly, higher extranuclear HMGB1 expression rates were found in tumors with intense inflammation, but without any difference when compared with other breast tumor subtypes (p = 0.194).

HMGB1 has emerged as a possible therapeutic target because of its roles in cancer biology. Therapies could target HMBG1 directly or indirectly through signaling pathways, like the receptor for advanced glycation end products and toll-like receptor 4. Inhibiting HMGB1 may slow the growth of tumors and improve the efficacy of current therapies. By weakening HMGB1-mediated resistance mechanisms, HMGB1 inhibitors may be used in conjunction with traditional treatments such as immunotherapy, radiotherapy, or chemotherapy so as to increase their effectiveness. A growing amount of data points to a connection between the emergence of several cancers and HMGB1 overexpression. For example, *in vitro* research using gastric cancer cell lines showed that elevated HMGB1 levels were associated with cell metastasis (11). In addition, when HMGB1 was inhibited *in vivo* using short hairpin RNA, the NF-κB pathway was used to inhibit the proliferation and invasion of gastric cancer cells, indicating that HMGB1 may be a therapeutic biomarker for the disease. Similarly, HMGB1 expression was inhibited by downregulating the PI3k/Akt signaling pathway, which stopped xenograft tumor development and the proliferation and spread of hepatocellular carcinoma cells. Furthermore, inhibition of HMGB1 expression led to downstream activation of AKT signaling and a notable decrease in the growth of VCP-mediated hepatocellular carcinoma, indicating that it is a useful therapeutic target for focused intervention and enhanced cancer patient survival. Similarly, HMGB1 downregulation was related with the suppression of *in vivo* and *in vitro* development and metastasis of lung, hepatocellular, and prostate cancer cells (11, 12, 16-19).

In summary, among 282 cases of breast cancer lower overall HMGB1 expression rates were found, compared to previous studies. We suggest that this may have been due to the primary antibody we used in our study. Similarly, we did not detect a relationship between isolated nuclear or extranuclear expression of HMGB1 and prognosis. However, in previous studies, the HMGB1 expression was mostly associated with prognosis in the most aggressive TNBC group. Since the number of TNBC cases in our series was quite low, we may not have been able to detect any relationship between HMGB1 expression and prognosis. The role of HMGB1 expression in breast cancer should be fully elucidated through further larger studies. Given its apparent role in particularly aggressive breast cancer and the possible therapeutic target role for HMGB1, this research is required as quickly as possible as it may improve outcomes for some of the most severe forms of breast cancer.

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Ethics

Ethics Committee Approval: The local Ethics Committee of Buca Seyfi Demirsoy Training and Research Hospital approved this project (reference number: 293, date: 29.05.2024).

Informed Consent: Informed consent forms were signed preoperatively by all participating patients.

Footnotes

Authorship Contributions

Surgical and Medical Practices: G.D., D.S.K., D.A., U.V., M.D.; Concept: G.D., İ.G.; Design: G.D., İ.G., D.S.K., D.A.; Data Collection or Processing: G.D., İ.G., D.S.K., D.A.; Analysis or Interpretation: G.D., D.S.K.; Literature Search: G.D., İ.G., D.S.K., D.A., U.V., M.D.; Writing: G.D., İ.G., D.S.K., D.A., U.V., M.D.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Wang S, Zhang Y. HMGB1 in inflammation and cancer. J Hematol Oncol. 2020; 13: 116. (PMID: 32831115) [Crossref]
2. Tang D, Kang R, Zeh HJ, Lotze MT. The multifunctional protein HMGB1: 50 years of discovery. Nat Rev Immunol. 2023; 23: 824-841. (PMID: 37322174) [Crossref]
3. Shao LH, Zhu L, Wang M, Ning Y, Chen FQ, Gao XQ, et al. Mechanisms involved in the HMGB1 modulation of tumor multidrug resistance (Review). Int J Mol Med. 2023; 52: 69. (PMID: 37387415) [Crossref]
4. Idoudi S, Bedhiafi T, Pedersen S, Elahtem M, Alremawi I, Akhtar S, et al. Role of HMGB1 and its associated signaling pathways in human malignancies. Cell Signal. 2023; 112: 110904. (PMID: 37757902) [Crossref]
5. Jiao Y, Wang HC, Fan SJ. Growth suppression and radiosensitivity increase by HMGB1 in breast cancer. Acta Pharmacol Sin. 2007; 28: 1957-1967. (PMID: 18031610) [Crossref]
6. Dong H, Zhang L, Liu S. Targeting HMGB1: an available therapeutic strategy for breast cancer therapy. Int J Biol Sci. 2022; 18: 3421-3434. (PMID: 35637945) [Crossref]
7. Liu B, Qi X, Zhang X, Gao D, Fang K, Guo Z, et al. Med19 is involved in chemoresistance by mediating autophagy through HMGB1 in breast cancer. J Cell Biochem. 2019; 120: 507-518. (PMID: 30161287) [Crossref]
8. Amornsupak K, Thongchot S, Thinyakul C, Box C, Hedayat S, Thuwajit P, et al. HMGB1 mediates invasion and PD-L1 expression through RAGE-PI3K/AKT signaling pathway in MDA-MB-231 breast cancer cells. BMC Cancer. 2022; 22: 578. (PMID: 35610613) [Crossref]
9. Akoz G, Diniz G, Ekmekci S, Ekin ZY, Uncel M. Evaluation of human epididymal secretory protein 4 expression according to the molecular subtypes (luminal A, luminal B, human epidermal growth factor receptor 2-positive, triple-negative) of breast cancer. Indian J Pathol Microbiol. 2018; 61: 323-329. (PMID: 30004048) [Crossref]
10. Degirmenci M, Diniz G, Kahraman DS, Sahbazlar M, Koral L, Varol U, et al. Investigating the correlation between long-term response in patients with metastatic HER2+ breast cancer and the activity of regulatory T cells: a retrospective study. Breast Cancer (Dove Med Press). 2024; 16: 645-655. (PMID: 39355199) [Crossref]
11. Zhang QY, Wu LQ, Zhang T, Han YF, Lin X. Autophagy-mediated HMGB1 release promotes gastric cancer cell survival via RAGE activation of extracellular signal-regulated kinases 1/2. Oncol Rep. 2015; 33: 1630-1638. (PMID: 25652880) [Crossref]
12. Zhang J, Kou YB, Zhu JS, Chen WX, Li S. Knockdown of HMGB1 inhibits growth and invasion of gastric cancer cells through the NF-κB pathway *in vitro* and *in vivo*. Int J Oncol. 2014; 44: 1268-1276. (PMID: 24481712) [Crossref]
13. Lv G, Yang M, Gai K, Jia Q, Wang Z, Wang B, et al. Multiple functions of HMGB1 in cancer. Front Oncol. 2024; 14: 1384109. (PMID: 38725632) [Crossref]
14. Pu Z, Duda DG, Zhu Y, Pei S, Wang X, Huang Y, et al. VCP interaction with HMGB1 promotes hepatocellular carcinoma progression by activating the PI3K/AKT/mTOR pathway. J Transl Med. 2022; 20: 212. (PMID: 35562734) [Crossref]
15. Wang XH, Zhang SY, Shi M, Xu XP. HMGB1 promotes the proliferation and metastasis of lung cancer by activating the Wnt/β-catenin pathway. Technol Cancer Res Treat. 2020; 19: 1533033820948054. (PMID: 32815451) [Crossref]
16. Zhao CB, Bao JM, Lu YJ, Zhao T, Zhou XH, Zheng DY, et al. Co-expression of RAGE and HMGB1 is associated with cancer progression and poor patient outcome of prostate cancer. Am J Cancer Res. 2014; 4: 369-377. (PMID: 25057439) [Crossref]
17. Dong YD, Cui L, Peng CH, Cheng DF, Han BS, Huang F. Expression and clinical significance of HMGB1 in human liver cancer: knockdown inhibits tumor growth and metastasis in vitro and in vivo. Oncol Rep. 2013; 29: 87-94. (PMID: 23042506) [Crossref]
18. Shrivastava S, Mansure JJ, Almajed W, Cury F, Ferbeyre G, Popovic M, et al. The role of HMGB1 in radioresistance of bladder cancer. Mol Cancer Ther. 2016; 15: 471-479. (PMID: 26719575) [Crossref]
19. Ma H, Zheng S, Zhang X, Gong T, Lv X, Fu S, et al. High mobility group box 1 promotes radioresistance in esophageal squamous cell carcinoma cell lines by modulating autophagy. Cell Death Dis. 2019; 10: 136. (PMID: 30755598) [Crossref]