© Wydawnictwo UR 2019
ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.1.3

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (1): 16–21

ORIGINAL PAPER

Aleksandra Barańska [1(BCDEG)], Aleksandra Maziarz [2(DEFG)], Jacek Roliński [1(AH)], Janusz Klatka [3(BH)], Katarzyna Dudzińska-Ćwiek [3(B)], Jacek Tabarkiewicz [2,4(ADEFG)]

Expression of heat shock protein 70 in the tissue of patients with laryngeal squamous cell carcinoma

[1] Chair and Department of Clinical Immunology, Medical University of Lublin, Lublin, Poland
[2] Institute of Experimental and Clinical Medicine, Department of Immunology, Faculty of Medicine, University of Rzeszow, Rzeszow, Poland
[3] Chair and Department of Otolaryngology and Laryngological Oncology, II Faculty of Medicine with English Language Division, Medical University of Lublin, Lublin, Poland
[4] Centre for Innovative Research in Medical and Natural Sciences, Faculty of Medicine, University of Rzeszow, Rzeszow, Poland

ABSTRACT

Introduction. Laryngeal squamous cell carcinoma (LSCC) is a common type of head and neck malignancy. Because of unsatisfactory results of therapy, development of new strategies for LSCC treatment is needed. It is believed that heat shock protein 70 (HSP70) is involved in pathogenesis of LSCC. Thus, targeting HSP70 seems to be promising strategy for laryngeal cancer treatment.

Aim. The aim of the study was to assess the HSP70 concentration in laryngeal squamous cell carcinoma specimens and its correlation with tumor volume and TNM staging.

Material and methods. An ELISA method and a Bradford protein assay were used to evaluate the HSP70 concentration in peripheral blood cells, tumor tissue and lymph nodes from the patients suffering from LSCC.

Results. We demonstrated that the HSP70 concentration is significantly different between examined compartments. The highest level was observed in peripheral blood, while the lowest was in the lymph nodes. The HSP70 expression was correlated to tumor volume.

Conclusion. Our results showed varied expression of HSP70 in tissue from patients with LSCC, but there was no association between HSP70 concentration and TNM staging. Currently, application of HSP70 inhibition as a LSCC treatment could be rather associated with systemic blocking of this molecule than target inhibition in tumor tissue. However, further analysis on a larger group of patients is needed.

Keywords. HSP70, LSCC, treatment

Corresponding author: Aleksandra Maziarz, Warzywna, e-mail: a.maziarz01@gmail.com

Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 11.12.2018 | Accepted: 5.02.2019
Publication date: March 2019

Barańska A, Maziarz A, Roliński J, Klatka J, Dudzińska-Ćwiek K, Tabarkiewicz J. *Expression of heat shock protein 70 in the tissue of patients with laryngeal squamous cell carcinoma.* Eur J Clin Exp Med. 2019;17(1):16–21. doi: 10.15584/ejcem.2019.1.3

The list of abbreviations:

CD – cluster of differentiation, **DCs** – dendritic cells, **HSPs** – heat shock proteins, **HSP27** – heat shock protein 27, **HSP70** – heat shock protein 70, **HSP72** – heat shock protein 72, **HSP90α** – heat shock protein 90α, **HSP90β** – heat shock protein 90β, **LSCC** – laryngeal squamous cell carcinoma, **PBMC** – peripheral blood mononuclear cells, **SCC** – squamous cell carcinoma

Introduction

Laryngeal squamous cell carcinoma (LSCC) is one of the most common types of head and neck malignant tumors. The incidence of LSCC remains high, with high rates of metastasis and recurrence. Laryngeal cancer occurrence is related to age, most frequently affecting individuals in sixth or seventh decades of life. It affects male more often than females.[1–3] There are several risk factors for laryngeal cancer; two of the major risk factors are tobacco smoking and alcohol consumption. Other risk factors for LSCC include occupational agents, such as asbestos, polycyclic aromatic hydrocarbons, solvents and dust. The role of other factors, like viral infections, genetics and environmental influence remains unclear.[1]

LSCC treatment includes several methods, such as laryngectomy, radiotherapy and chemotherapy or combination of these. Treatment modality is determined by tumor location and staging. However, despite of application of different treatment methods, the 5-year survival rate in advanced LSCC is still unsatisfactory.[1] Therefore, a search for new possibilities for laryngeal cancer therapy is needed.

Resistance of tumor cells to therapy may be caused by overexpression of heat shock proteins (HSPs).[4] Many previous studies are focused on the role of HSPs in carcinogenesis. Due to the fact that HSPs are able to inhibit apoptosis of cancer cells, the inhibition of HSPs activity is considered as a cancer treatment.[5]

It was shown that different types of HSPs were overexpressed in different types of tumors. Moreover, increased expression of these proteins was associated with histological grade, recurrence and metastasis of malignant tumors.[6] Overexpression of heat shock protein 70 (HSP70) was observed in LSCC among head and neck malignant tumors.[7] However, the role of HSP70 in LSCC is not fully understood.

HSP70 as a molecular chaperone is involved in process of folding of newly synthesized proteins, assembly of protein complexes and transmembrane transport of proteins. HSP70 enhanced the cell tolerance to effects of stress condition, such as increased concentration of unfolded and denatured proteins.[5] It has long been known that HSP70 is able to inhibit apoptosis and increase an oncogenic potential of tumor cells.[5,8]

Aim

The aim of the study was to determine the concentration of HSP70 in blood cells, tumor tissue and lymph nodes of patients suffered from laryngeal squamous cell carcinoma and to determine the correlation between expression of HSP70 and type of tissue, tumor volume and TNM staging.

Material and methods

Peripheral vein blood samples, fragments of tumor and lymph nodes were obtained from 16 patients suffering from laryngeal squamous cell carcinoma who underwent tumor resection in Department of Otolaryngology and Laryngological Oncology, Medical University in Lublin. The material was collected from patients with tumor in Grade 2 or Grade 1 (only one case). The patient characteristics are summarized in Table 1.

Table 1. Characteristics of the patient group

Patient	Tumor grade	TNM staging
1	G-2	T2N3M0
2	G-2	T3N1M0
3	G-2	T3N2M0
4	G-2	T3N0M0
5	G-2	T3N0M0
6	G-2	T3N3M0
7	G-2	T3N0M0
8	G-1	T3N2M0
9	G-2	T3N1M0
10	G-2	T3N0M0
11	G-2	T3N0M0
12	G-2	T4N0M0
13	G-2	T4N2M0
14	G-2	T3N1M0
15	G-2	T4N2M0
16	G-2	T3N2M0

Peripheral blood mononuclear cells (PBMC) were isolated by gradient centrifugation using Gradisol L (Aqua Medica, Poland). Cells were washed twice with phosphate buffer saline (PBS) w/o Mg^{2+} and Ca^{2+} (PAA Laboratories GmbH, Austria). Lymph node and tumor tissue fragments were homogenized using tissue knives (Medicon, Dako, Denmark) and MediMachine whipper (Dako, Denmark). Cells were suspended in cryomedium containing 70% of RPMI 1640 (PAA, Austria), 20% of human albumin (Baxter, Austria) and 10% of dimethyl sulfoxide (DMSO) (ICN Polfa Rzeszów, Poland) and cryopreserved in liquid nitrogen vapor. Cells were thawed using CTL-Test Medium (C.T.L Ltd, USA) and supplemented with 10% of CTL-Wash Supplement (C.T.L Ltd, USA) and after subsequent PBS wash they were suspended in 1 mL of PBS. Cells lysates were prepared by freezing cells in -80°C

Table 2. The standardized HSP70 concentration in examined tissue

	Mean ± standard deviation [pg/ml]	Median [pg/ml]	Minimum [pg/ml]	Maximum [pg/ml]
HSP70 PBMC	2535.37±816.23	2658.65	572.27	4017.23
HSP70 lymph node's cells	1435.34±1246.28	1218.56	31.60	4107.66
HSP70 tumor's cells	1822.09±1394.04	2088.43	121.64	3603.29

Table 3. Correlation between concentration of HSP70 in different tissue and tumor volume (*p < 0.05)

Pair of variables	Spearman's R	p
HSP70 PBMC [pg/ml] & tumor volume [cm^3]	-0.195	0.523
HSP70 lymph node's cells [pg/ml] & tumor volume [cm^3]	0.826	0.011*
HSP70 tumor's cells [pg/ml] & tumor volume [cm^3]	0.226	0.559

and thawing in water bath at 37°C five times. Samples were centrifuged and the supernatant was collected.

Total protein concentration was determined by Bradford protein assay. The absorbance was measured using Spectrophotometer SmartSpecTM 3000 UV/VIS (Bio-Rad) at a wavelength of 595 nm. HSP70 expression was examined using commercial kit for Sandwich ELISA Human/Mouse/Rat Total HSP70 Immunoassay (R&D Systems, USA). The procedure was performed according to the protocol. The plate was analyzed using a Multilabel Plate Reader Victor 3^{TM} (Perkin-Elmer) at a wavelength of 450 nm. HSP70 concentration was standardized in reference to 1 mg of total protein.

Statistical analysis of the data was performed using Statistica 9.0 PL. Wilcoxon signed-rank test, Spearman's rank correlation coefficient, median's test for multiple group comparison and Friedman ANOVA test were used for the analysis. Data are presented as mean ± standard deviation, median, minimum and maximum result. Results were considered as statistically significant at the significance level $p < 0.05$.

Results

The expression of HSP70 was determined using an ELISA assay and then HSP70 concentration was standardized in reference to 1 mg of total protein. The highest concentration of HSP70 was observed in PBMC, while the lowest concentration was observed in the lymph node cells (Table 2 and Figure 1). With the use of Friedman's ANOVA test, we did not find any statistically significant differences between examined tissue (χ^2 ANOVA=2.8; p=0.25). The post-hoc Wilcoxon signed-rank test showed statistically significant differences between HSP70 concentrations in PBMC and lymph node cells (p=0.050). There were no significant differences between HSP concentrations in other tissue (Figure 1).

Statistical analysis of correlation between concentration of HSP70 in different tissues and tumor volume was performed using Spearman's rank correlation test. We observed statistically significant positive correlation between HSP70 concentration in lymph node cells and tumor volume (R=0.826, p=0.011). Correlations between HSP70 concentrations in white blood cells and tumor cells were nonsignificant (Table 3).

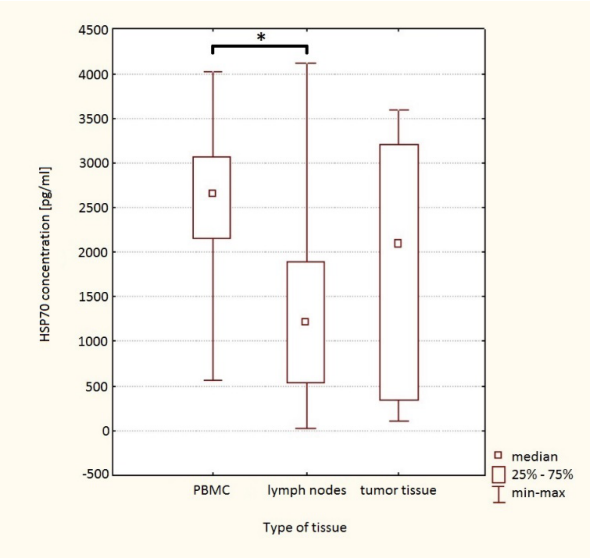

Fig. 1. HSP70 concentration [pg/ml] in PBMC, tumor tissue and lymph node's cells (*p < 0.05, Wilcoxon signed-rank test)

Comparison between HSP70 concentration and TNM staging performed using the median's test for multiple group comparison did not show any significant associations. However, because the study group was relatively small, it can be assumed that a study performed on a larger group of patients may reveal a different result.

Discussion

Heat shock proteins play a protective role in organisms and support adaptation to extreme conditions. HSPs represent the evolutionary old defensive system in all living organisms. However, higher expression of HSPs occurs also in cancer tissue. Due to HSPs overexpression and their possible role in cancer pathogenesis, inhibition of these proteins has been considered as a promising strategy in cancer treatment.[9]

HSP70 has the most conservative structure among the heat shock protein family. It plays a pivotal role in protein folding process and maintenance of genome stability under stress conditions. Moreover, HSP70 indicates cytoprotective features and it has capability to inhibit apoptosis. As a highly protective protein, HSP70 may lead to survive of abnormal and defective cells.[10,11] Enhanced expression of HSP70 is observed in cancer cells as well. It is believed that its overexpression is related to carcinogenesis and cancer progression.[11,12] Furthermore, high expression of HSP70 in cancer tissue is also associated with metastasis and therapeutic resistance, and consequently shorter patient survival prognosis.[11,13,14]

Several studies have shown that increased level of HSP70 expression was associated with a poor clinical outcome and poor response to therapy in colorectal cancer, breast cancer and head and neck squamous cell carcinoma (SCC).[14–18] It has been suggested that inhibition or knockdown of HSP70 applied as a cancer treatment may contribute to tumor regression.[11] Chen et al. demonstrated that viral proteins may suppress tumor growth via interaction with HSP70. Overexpressed viral protein formed a complex with HSP70 causing inhibition of its activity, which consequently led to inhibition of cancer cell viability and induction of cancer cell death.[12]

In contrast, it has been demonstrated by Enomoto et al. and Gong et al. that HSP70 is able to induce antitumor immune response and prevent the tumor growth. They used HSP70 isolated from tumor-dendritic cell fusion to vaccinate mice and showed that HSP70 stimulated dendritic cells (DCs) maturation and T cell proliferation. HSP70 induced T cell-mediated immune response. It significantly increased the proliferation of CD8+ lymphocytes and induced the effector and memory T cells.[19,20] Similarly, a study performed by Chen et al. showed that HSP70 from tumor cells is involved in initiation of antitumor immunity by activation of DCs and monocytes/macrophages, and it is involved in enhancement presentation of tumor antigens to T lymphocyte. Moreover, they observed that stress conditions, such as hyperthermia, accelerated release of HSP70 by tumor cells and enhanced stimulation of antitumor immune response.[21] On the basis of these studies, it is believed that HSP70 and other HSPs may be a promising tool in the development of anticancer vaccines.

The expression of HSP70 in laryngeal cancer tissue has been studied previously. Xu X et al. evaluated the expression level of heat shock proteins (HSP90α, HSP90β, HSP70 and HSP27) in laryngeal carcinoma and normal laryngeal mucosa. They perceived fivefold overexpression of HSP90α and HSP70 in carcinoma tissue, suggesting that HSP90α and HSP70 can be involved in the pathogenesis of laryngeal cancer.[22] Yang et al. also analyzed expression of HSP70 in specimens of human laryngeal squamous cell carcinoma and specimens of para-carcinoma. They observed increased expression of HSP70 in carcinoma tissue compared with para-carcinoma tissue, which was significantly correlated with the differentiation of LSCC.[23] Xu J et al. showed that expression of HSP70 is correlated with histological grade of LSCC. Using immunohistochemistry methods, they observed HSP70 presence in 96% of samples of tumor tissue. The level of HSP70 expression was significantly lower in early stages of LSCC than in late stages.[6]

According to mentioned studies, the concentration of HSP70 was increased in LSCC tissue compared with samples of healthy tissue. Therefore, we can suppose that HSP70, such as HSP90 can be involved in pathogenesis of laryngeal squamous cell carcinoma.

Results obtained in our study revealed that the level of expression of HSP70 in patients with LSCC is various in different types of tissue. The highest expression of HSP70 was observed in white blood cells, while the lowest was observed in lymph node's cells. Nevertheless, HSP70 expression did not show cancer tissue tropism, which would be desirable for potential application of HSP70 inhibition in cancer therapy.

Additionally, in present study we surprisingly observed a strong positive correlation between HSP70 concentration in lymph node's cells, which was the lowest among examined compartments, and tumor volume. Because HSP70 is involved in delivery of antigens to antigen-presenting cells and therefore stimulate an adaptive response,[24] the low concentration of HSP70 in lymph nodes draining tumor could be associated with decreased activation of tumor specific lymphocytes and subsequently increased tumor progression. On the other hand, we found a positive correlation between concentration of HSP70 in lymph nodes and tumor volume, what could be associated with its protective role and anti-apoptotic functions, HSP70 can increase oncogenic potential of cells and it can contribute to tumor progression.[5] The positive correlation between concentration of HSP70 in lymph nodes and tumor volume could be theoretically explained by the fact, that HSP70 is transferred from tumor to lymph nodes via lymph and represent higher concentration of protective HSP70 in malignant tissue.

Previous studies have demonstrated that HSP70 expression correlates with the clinical stage of oral SCC. Tavassol et al. observed that survival of patients with T2 tumors and positive expression of HSP70 was eightfold higher than in the case of patients with T2 tumors and the lack of expression of HSP70. However, the expression of HSP70 affects survival only in the early stages of the disease. The level of expression of HSP70 had no prognostic significance for T3 and T4 tumors.[25] Furthermore, Taghavi et al. noticed a significant correlation

between expression of HSP70 and clinical stage, lymph node metastasis and tumor volume, whereas no association with histological grade was found.[26] In contrast, a study performed on esophageal SCC showed greater expression of HSP70 and HSP27 in tumor tissue, which was related to follow-up of patients. HSP70 and HSP27 expression exhibited a significantly better prognosis. While there was no correlation with other clinical parameters such as gender, age, lymph node status and tumor differentiation.[27] In the present study, the analysis of the correlation between HSP70 concentration and TNM staging did not show any statistically significant associations. However, it is worth noting that the limiting factor of the analysis was a small study group. For this reason we can suppose that a study conducted on a larger group of patients may reveal a different result.

Conclusions

to summarize, in the present study we observed a strong positive correlation between HSP70 concentration in lymph node cells and tumor volume. The level of expression of HSP70 in different tissue from LSCC patients was varied, and did not show cancer tissue tropism. The HSP70 expression did not correlate with TNM staging, however, due to the fact that in our study group was only one case with T2 tumor, it can be assumed that the result of study performed on a larger group of patient would be different. Nevertheless, on the basis of current results, the application of HSP70 inhibition as a LSCC treatment could be rather associated with systemic blocking of this molecule than target inhibition in tumor tissue. Moreover, previous studies have shown that the expression of HSP70 is higher in patients with LSCC compared to healthy ones. Because of that, it seems necessary to compare results obtained in our study with the control group. Therefore, future studies should focus on the influence of other HSPs on LSCC.

Acknowledgement

The study was performed within the project "Center for Innovative Research in Medical and Natural Sciences" realized by University of Rzeszow, and co-financed within Regional Operational Program for the Podkarpackie Province over the years 2007-2013, contract number UDA-RPPK.01.03.00-18-004/12-00.

References

1. Rudolph E, Dyckhoff G, Becher H, Dietz A, Ramroth H. Effects of tumour stage, comorbidity and therapy on survival of laryngeal cancer patients: a systematic review and a meta-analysis. *Eur Arch Otorhinolaryngol*. 2011;268(2):165-179. doi:10.1007/s00405-010-1395-8
2. Ludwig ML, Birkeland AC, Hoesli R, Swiecicki P, Spector ME, Brenner JC. Changing the paradigm: the potential for targeted therapy in laryngeal squamous cell carcinoma. *Cancer Biol Med*. 2016;13(1):87-100. doi:10.28092/j.issn.2095-3941.2016.0010
3. Giotakis AI, Kontos CK, Manolopoulos LD, Sismanis A, Konstadoulakis MM, Scorilas A. High BAX/BCL2 mRNA ratio predicts favorable prognosis in laryngeal squamous cell carcinoma, particularly in patients with negative lymph nodes at the time of diagnosis. Clin Biochem. Clin Biochem. 2016;49(12):890-6. doi:10.1016/j.clinbiochem.2016.04.010
4. Dempsey NC, Ireland HE, Smith CM, Hoyle CF, Williams JHH. Heat Shock Protein translocation induced by membrane fluidization increases tumor-cell sensitivity to chemotherapeutic drugs. *Cancer Letters*. 2010;296(2):257-267. doi:10.1016/j.canlet.2010.04.016
5. Garrido C, Gurbuxani S, Ravagnan L, Kroemer G. Heat Shock Proteins: Endogenous Modulators of Apoptotic Cell Death. *Biochemical and Biophysical Research Communications*. 2001;286(3):433-442. doi:10.1006/bbrc.2001.5427
6. Xu J, Wang K, Zhang X, et al. HSP70: a promising target for laryngeal carcinoma radiaotherapy by inhibiting cleavage and degradation of nucleolin. *J Exp Clin Cancer Res*. 2010;29:106. doi:10.1186/1756-9966-29-106
7. Weber A, Hengge UR, Stricker I, et al. Protein microarrays for the detection of biomarkers in head and neck squamous cell carcinomas. *Hum Pathol*. 2007;38(2):228-238. doi:10.1016/j.humpath.2006.07.012
8. Jäättelä M, Wissing D, Bauer PA, Li GC. Major heat shock protein hsp70 protects tumor cells from tumor necrosis factor cytotoxicity. *EMBO J*. 1992;11(10):3507-3512.
9. Bao X-Q, Liu G-T. Heat shock proteins: new target in cytoprotective and tumor therapy. *Yao Xue Xue Bao*. 2008;43(3):234-240.
10. Hunt CR, Dix DJ, Sharma GG, et al. Genomic instability and enhanced radiosensitivity in Hsp70.1- and Hsp70.3-deficient mice. *Mol Cell Biol*. 2004;24(2):899-911.
11. Kumar S, Stokes J, Singh UP, et al. Targeting Hsp70: A possible therapy for cancer. *Cancer Lett*. 2016;374(1):156-166. doi:10.1016/j.canlet.2016.01.056
12. Chen W, Lin K, Zhang L, et al. The cytomegalovirus protein UL138 induces apoptosis of gastric cancer cells by binding to heat shock protein 70. *Oncotarget*. 2016;7(5):5630-5645. doi:10.18632/oncotarget.6800
13. Wang X, Shi X, Tong Y, Cao X. The Prognostic Impact of Heat Shock Proteins Expression in Patients with Esophageal Cancer: A Meta-Analysis. *Yonsei Med J*. 2015;56(6):1497-1502. doi:10.3349/ymj.2015.56.6.1497
14. Kim LS, Kim JH. Heat Shock Protein as Molecular Targets for Breast Cancer Therapeutics. *J Breast Cancer*. 2011;14(3):167-174. doi:10.4048/jbc.2011.14.3.167
15. Gunaldi M, Kocoglu H, Okuturlar Y, et al. Heat shock protein 70 is a useful marker for predicting colorectal cancer. *J BUON*. 2015;20(6):1464-1470.
16. Davidson B, Reinertsen KV, Trinh D, Reed W, Bøhler PJ. BAG-1/SODD, HSP70 and HSP90 are potential prognostic markers of poor survival in node-negative breast car-

cinoma. *Hum Pathol*. 2016;54:64-73. doi:10.1016/j.humpath.2016.02.023

17. Calderwood SK. Heat shock proteins in breast cancer progression-a suitable case for treatment? *Int J Hyperthermia*. 2010;26(7):681-685. doi:10.3109/02656736.2010.490254
18. Stangl S, Tontcheva N, Sievert W, et al. Heat shock protein 70 and tumor-infiltrating NK cells as prognostic indicators for patients with squamous cell carcinoma of the head and neck after radiochemotherapy: A multicentre retrospective study of the German Cancer Consortium Radiation Oncology Group (DKTK-ROG). *Int J Cancer*. 2018;142(9):1911-1925. doi:10.1002/ijc.31213
19. Enomoto Y, Bharti A, Khaleque AA, et al. Enhanced Immunogenicity of Heat Shock Protein 70 Peptide Complexes from Dendritic Cell-Tumor Fusion Cells. *J Immunol*. 2006;177(9):5946-5955. doi:10.4049/jimmunol.177.9.5946
20. Gong J, Zhu B, Murshid A, et al. T cell activation by HSP70 vaccine requires TLR signaling and scavenger receptor SREC-1. *J Immunol*. 2009;183(5):3092-3098. doi:10.4049/jimmunol.0901235
21. Chen T, Guo J, Han C, Yang M, Cao X. Heat shock protein 70, released from heat-stressed tumor cells, initiates antitumor immunity by inducing tumor cell chemokine production and activating dendritic cells via TLR4 pathway. *J Immunol*. 2009;182(3):1449-1459.
22. Xu X, Zhao M, Shi Y. Expression of heat shock proteins in laryngeal carcinoma. *Zhonghua Er Bi Yan Hou Ke Za Zhi*. 1998;33(4):232-234.
23. Yang S, Chen H, Zhang W, Liu D, Fan E, Li Y. Expression and significance of TGF-beta1 and HSP70 in human laryngeal squamous cell carcinoma. *Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi*. 2007;21(12):531-534.
24. Malyshev I. HSP70 in the Immune Responses. In: Malyshev I, ed. *Immunity, Tumors and Aging: The Role of HSP70*. SpringerBriefs in Biochemistry and Molecular Biology. Dordrecht: Springer Netherlands; 2013:63-82. doi:10.1007/978-94-007-5943-5_5
25. Tavassol F, Starke OF, Kokemüller H, et al. Prognostic significance of heat shock protein 70 (HSP70) in patients with oral cancer. *Head Neck Oncol*. 2011;3:10. doi:10.1186/1758-3284-3-10
26. Taghavi N, Mohsenifar Z, Baghban AA, Arjomandkhah A. CD20+ Tumor Infiltrating B Lymphocyte in Oral Squamous Cell Carcinoma: Correlation with Clinicopathologic Characteristics and Heat Shock Protein 70 Expression. *Patholog Res Int*. 2018;2018:4810751. doi:10.1155/2018/4810751
27. Luz CCF, Noguti J, de Araújo LB, Santos GMSD, Gomes TS, Neto RA. Hsp27 and Hsp70 Expression in Esophageal Squamous. *Asian Pac J Cancer Prev*. 2017;18(3):789-794. doi:10.22034/APJCP.2017.18.3.789