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# Prognosis and predictive value of heat-shock proteins expression in oral cancer

## A PRISMA-compliant meta-analysis

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**Abstract**

**Background:** Heat-shock proteins (HSP) is a key chaperone protein which maintains intracellular proteostasis and is expressed on the surface of solid and hematological malignancies. Several studies have reported paradoxical evidence of the association between HSP expression and prognosis of oral cancer. To address the discrepancy, we carried out the meta-analysis to assess the role of HSP such as: HSP70, HSP90, HSP27, HSP60, and HSP105 in susceptibility, progression, and prognosis of oral cancer.

**Materials and methods:** We retrieved the PubMed, Embase, Web of science, China National Knowledge Infrastructure (CNKI), and Wanfang databases to acquire the eligible studies which were associated with HSP70, HSP90, HSP27, HSP60, and HSP105 protein expression and oral cancer. We applied hazard ratio (HR) and its 95% confidence interval (95% CI) to assess the value of HSP protein expression in overall survival of oral cancer; odds ratio (OR) and its 95% CI were used to evaluate the association of risk and clinical features of oral cancer. Funnel plot, Begg test, and Egger line regression test were utilized to observe publication bias among studies. All statistical analysis was performed with Stata 14.0 software (Stata Corporation, College Station, TX).

**Results:** A total of 26 studies were included in the present meta-analysis. On based of the results, HSP70 and HSP27 had no significant association with progression of oral cancer. However, the pooled HR and 95% CI revealed a significant well effects of HSP70 and HSP27 expression on survival of oral cancer. Moreover, the susceptibility of oral cancer was significantly associated with HSP70 and HSP60 overexpression.

**Conclusion:** HSP70 and HSP27 protein overexpression might be valuable biomarkers for the prognosis of oral cancer. And HSP70 and HSP60 might have potential predictive effects on the risk of oral cancer.

**Abbreviations:** EGFR = epidermal growth factor receptor, HER2 = erb-b2 receptor tyrosine kinase 2, HER3 = erb-b2 receptor tyrosine kinase 3, HER4 = erb-b2 receptor tyrosine kinase 4, HR = hazard ratio, HSP = heat-shock proteins, IHC = immunohistochemistry, NR = not reported, OLC = oral leukoplakia cancer, OS = overall survival, OSCC = oral squamous cell carcinoma, SCCT = squamous cell carcinoma of the tongue.

**Keywords:** heat-shock proteins, meta-analysis, oral cancer, prognosis

## 1. Introduction

Oral cancer is the most common cancer of head and neck malignancies worldwide, in which oral squamous cell carcinoma accounts for 90% of oral cancers. And the 5 years survival rate of oral cancer patients is approximately 63%.[1] In the past few decades, the overall survival rate was not significantly increased, which mainly attributed to recurrence and distant metastasis of oral tumor.[2] Oral cancer is not very commonly in western countries, but it is very prevalent in India which accounts for nearly 45% of all cancers, and the mainly cause is betel nut chewing.[3] Other environmental factors are also important in the incidence of oral cancer such as: sun exposure, alcohol and tobacco, and human papilloma virus in which approximately 14% oral cancer cases presented the infection of human papilloma virus (HPV-16 and HPV-18).[4] Several germline gene mutations were significantly associated with higher incidence of oral cancer. For instance, Li-Fraumeni syndrome patients with germline TP53 mutation often developed into early-onset oral cancer.[5] In addition, dyskeratosis congenita patients had a higher tendency to get oral cancer due to defective telomerase maintenance.[6] Lots of genetics or epigenetics alterations were found in cancerous cells which included gene mutations, deletions, translocations, gene copy number variation, microsatellite instability, loss of heterozygosity, DNA methylations, and acetylation of protein.[7] These variations leaded to many disorders of cell function including deregulation of growth, genomic instability, increased oxidative stress, increased proteotoxic stress, evading immune surveillance, ineffective xenobiotic metabolism, evading cell death and senescence, limitless replicative potential, sustained angiogenesis, tissue invasion, and metastasis.[8] The study of signal

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WL and YW contributed equally to this work.

The authors have no conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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transduction pathway was a pivotal way to investigate interaction between molecules which derived cancers. Epidermal growth factor receptor (EGFR) could sustain normal cell growth which the expression was often changed in cancer cells, thus it was widely studied in the growth of tumor cells. These members of EGF family included EGFR, Her-2, Her-3, and Her-4 which were homodimers or heterodimers, and their ligands included the molecules of MAPK, PI3K/AKT, mTOR, JAK, and STAT signal pathways.[9] Moreover, RAS gene including H-RAS, K-RAS and N-RAS, PI3K/NF-kB, Cyclin D1, Bcl-2, Bax, Survivin, p53/p21/p27, vascular endothelial growth factor (VEGF), Cadherins, Matrix metalloproteinases, and COX-2 were all involved in the occurrence and development of oral cancer.[8] Although the possible biomarkers were growing, there was still few transfer of these molecules into clinical application. Therefore, we should develop more biomarkers for the early diagnosis and treatment of oral cancer. And we may have a good understanding of the development of oral cancer by these molecules.

Heat shock protein (HSP) is one of the most abundant intracellular substances, which are conserved proteins throughout evolution.[10] This class of proteins maintain the essential functions of the cell under physiological conditions and protect cells from high temperature or different stresses including oxygen free radicals, infections, inflammation, hyperthermia, heavy metals, and ischemia.[11] HSPs could assists in protein folding to form a normal spatial structure and carry these proteins toward organelles to make it function properly.[12] Other studies also demonstrated that HSPs activated the immune system to suppress tumors progression.[13] In recent years, the associations of HSPs including HSP70, HSP27, HSP90, HSP60, and HSP105 with risk, progression, and prognosis were widely studied in clinical samples of oral cancer. However, we could not acquire a consistent conclusion after literature retrieval because of contradictory results. Therefore, we performed the meta-analysis to investigate the correlation between HSP protein expression in tissues and oral cancer.

## 2. Materials and methods

### 2.1. Search strategy

According to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, we carried out this meta-analysis.[14] The searching of eligible articles was updated to May 29, 2020 from the PubMed, Embase, Web of science, NCBI, and Wanfang databases, which was performed by 2 researchers independently. To acquire the relevant studies, the following search terms were used: "oral cancer," "oral carcinoma," "oral squamous cell carcinoma," "carcinoma of gingiva," "tongue cancer," "cancer of tongue," "squamous cell carcinoma of the tongue," "cancer of lip," "heat shock protein," "HSP70," "HSP27," "HSP60," "HSP90," "HSP105," and "prognosis." Additional eligible articles in the references list were obtained by manual searching.

### 2.2. Inclusion and exclusion criteria

All eligible studies had to meet the following inclusion criteria: case-control studies or cohort; articles assessing the association between HSP protein expression and oral cancer; articles providing enough information for calculating OR, HR, and 95% CI; studies which classified HSP expression as "high" and "low" or "positive" and "negative". The exclusion criteria were as follows: meta-analysis, review, case-report, letter, review, comments, editorials, and conference abstracts; non-human research or cell studies; studies not focused on the independent role of HSP protein expression in oral cancer.

### 2.3. Data extraction and quality assessment

Two investigators independently read the titles and abstract to select eligible studies and extract acquired information. These information included first author information, the date of publication, country, ethnicity of subjects, subtype of oral tumors, positive or negative of COX-2 protein expression, clinical information of oral cancer such as: clinical stage, grade, prognosis of oral cancer, lymph node metastasis, and differentiation of oral tumor. Hazard ratio (HR) and 95% confidence interval (CI) were extracted from relevant studies. If the eligible studies did not provide HR and 95% CI, we extracted the value from Kaplan–Meier curve with Engauge Digitizer version 4.1.[15] The quality of eligible studies were assessed by 2 researchers on based of the Newcastle-Ottawa Scale (NOS) criteria 100.[16] The range of quality score was 0 to 9, and the quality score of $\geq 6$ was considered as a high quality. In addition, approval of an ethics committee or institutional review board is not needed since this is a meta-analysis.

### 2.4. Statistical analysis

We carried out Cochran $Q$ and $I^2$ tests to find the heterogeneity among studies.[17,18] When $I^2 > 50\%$ or the corresponding $P$ value $<.05$, HR, odds ratio (OR), and their 95% CI were calculated with a random effect model; otherwise, a fixed effect model was applied to estimate the pooled results.[19] In addition, subgroup analysis based on race was further performed to find the source of heterogeneity. In order to observe the effect of each signal study on the pooled results, sensitivity analysis was conducted. Publication bias among eligible studies was also evaluated by Egger test and Begg funnel plot.[20] All the statistical analysis was carried out by using Stata 14.0 software, while $P$ value $<.05$ was considered as a statistical significance.

## 3. Results

### 3.1. Literature searching and characteristics of included studies

In the present study, a total of 26 studies involving HSP70, HSP27, HSP60, HSP90, HSP105 protein expression and oral cancer were included to assess clinical predictive and prognostic value of HSP expression (Tables 1 and 2). Most studies were focused on the association of HSP70 and HSP27 with oral cancer. Of 26 eligible studies, there were 13 studies were on HSP70 protein expression and oral cancer[21–27,33,34,37,41–43] (315 patients), 9 on HSP27[12,23,28,29,35,36,38–40] (111 patients), 4 on HSP90[23,25,32,44] (90 patients), 3 on HSP60[23,30,31] (60 patients), and 2 on HSP105[12,45] (126 patients). Two published studies were conducted for the prognosis value of HSP70[24,43] and 3 for HSP27[35,39,40] protein expression in oral cancer. The eligible studies retrieval process was summarized in Fig. 1. The study contained 3 oral cancer subtypes: oral squamous cell carcinoma (OSCC), squamous cell carcinoma of the tongue (SCCT), and oral leukoplakia cancer (OLC). The detection method of HSP protein was IHC in oral cancer tissue or normal oral mucosa tissue.

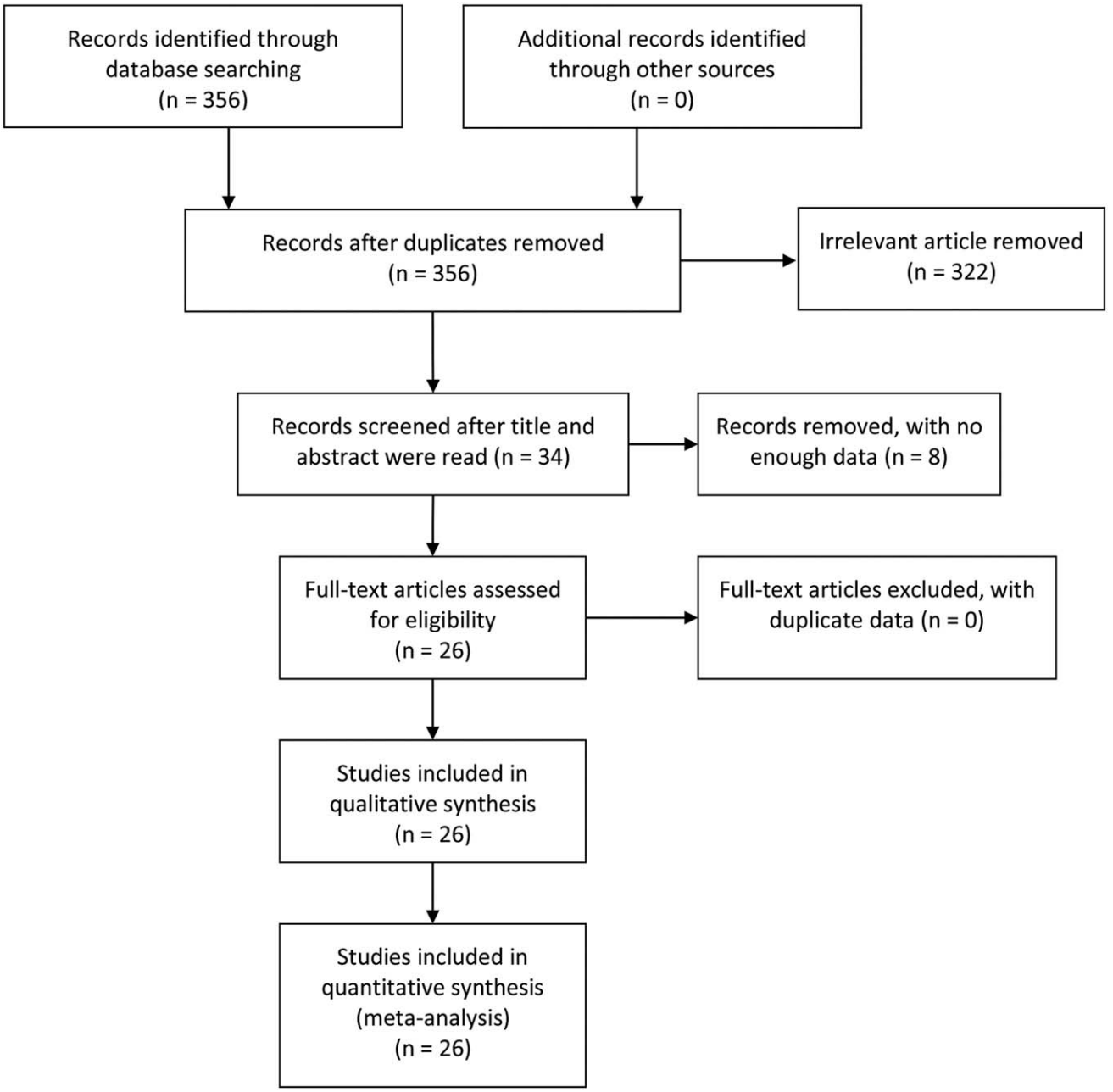


**Figure 1.** Literature searching progress.

### *3.2. Prognostic value of HSP expression for OS*

According to the published data of 2 included studies,[12,21–35,36–45] we found that HSP70 protein overexpression was significantly associated with the well prognosis of oral cancer (HR = 0.15, 95% CI = 0.15–0.99, $P < .05$) (Fig. 2A). Similarly, there was a significant association of HSP27 overexpression with well prognosis of oral cancer (HR = 0.38, 95% CI = 0.21–0.56, $P < .05$)[21–27] (Fig. 2B). Therefore, the results suggested that HSP27 and HSP70 overexpression significantly increased the survival rate of oral cancer patients which was consistent with their function of maintaining cell homeostasis. However, one included studies found that HSP105 positive expression was an unfavorable factor for oral cancer patients survival ($P = .042$, n = 103),[45] while similar result was also found for HSP90 ($P = .013$, n = 30).[44] Only little heterogeneity was found for HSP70 and prognosis of oral cancer ($I^2 = 77\%$, $P = .037$), so the random effects model was applied to calculate the pooled HR (Table 3).

### *3.3. Correlation of HSP with clinicopathological features of oral cancer patients*

Firstly, we observed the effects of HSP70, HSP27, HSP90, and HSP60 protein expression on the oral cancer risk, in which HSP70 (OR = 10.85, 95% CI = 1.37–86.13, $P < .05$) and HSP60 (OR = 23.91, 95% CI = 5.39–10.616, $P < .05$) were significantly correlated with susceptibility of oral cancer. And no significant correlations were found between HSP27, HSP90, and risk of oral cancer (Fig. 3). To further discuss the role of HSP in progression

**Table 1**

**Eligible studies for association of HSP protein expression with prognosis of oral cancer.**

| | Author | Reference | Time | Country | Ethnicity | Cancer type | Num. | Follow-up, mo | Method | Survival analysis | Source of HR | OS | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | HR | LL | UL | *P* |
| HSP70 | Kaur | [24] | 1998 | India | Asians | OSCC | 125 | 0–60 | IHC | OS | Curve | 2.13 | 1.57 | 4.62 | <.05 |
| | Tavassol | [43] | 2011 | Germany | Caucasians | OSCC | 28 | 0–144 | IHC | OS | Curve | 0.44 | 0.07 | 0.95 | <.05 |
| HSP27 | Mese | [35] | 2002 | Japan | Asians | OSCC | 40 | 0–48 | IHC | OS | Curve | 0.247 | 0.12 | 0.686 | <.01 |
| | Wang | [40] | 2009 | USA | Caucasians | SCCT | 42 | 0–100 | IHC | OS | HR | 0.375 | 0.19 | 0.741 | .048 |
| | Chen | [39] | 2018 | China | Asians | SACC | 67 | 0–120 | IHC | OS | Curve | 0.64 | 0.24 | 0.982 | .049 |
| HSP90 | Chang | [44] | 2017 | China | Asians | OSCC | 36 | 0–60 | IHC | OS | Curve | 2.36 | 1.85 | 5.39 | .013 |
| HSP105 | Arvanitidou | [45] | 2019 | Switzerland | Caucasians | OSCC | 70 | 0–130 | IHC | OS | Curve | 2.79 | 1.18 | 7.44 | .042 |

HR = hazard ratio; IHC = immunohistochemistry; OS = overall survival; OSCC = oral squamous cell carcinoma; SACC = salivary adenoid cystic carcinoma; SCCT = squamous cell carcinoma of the tongue.

of oral cancer, we assessed the association of HSP with clinical features of oral cancer. The pooled ORs indicated that no positive results were found. In a single study, HSP105 overexpression was significantly associated advanced stage of oral cancer ($P = .01$).[12] However, there were no correlation between HSP105 overexpression and grade of oral cancer.[12] Although many studies found that HSP had a significantly association with progression of oral cancer, no positive results were found in the pooled overall results (Table 3).

### 3.4. Publication bias and sensitivity analysis

The results of Begg and Egger test indicated no significant publication bias was detected. Further, sensitivity analysis also confirmed the stability of the pooled HR and OR, and no individual study affected the overall result (Fig. 2C, D).

## 4. Discussion

Studies have found HSPs on the surface of malignant cells could function as antigens or carriers of antigens such as peptides and it could signal to effector molecules to activate the immune system.[46] In addition, HSP family proteins also played important roles in regulating cellular responses and functions during carcinogenesis. Most studies were focused on the expression of HSP27 and HSP70 protein in oral cancer. HSP27 could regulate cancer development, diagnosis, and treatment, in which phosphorylation at Ser-15, Ser-78, and Ser-82 of HSP27 protein could enhance its activity which were induced by some stresses.[47] p38 mitogen-activated protein kinase of MAPK signal pathway activated mitogen-activated protein kinase-activated protein kinases (MAPKAPK) 2 and 3 and further catalyzed phosphorylation of HSP27. Activated HSP27 could interact with β-catenin, histone deacetylases 6 (HDAC6), signal transducer and activator of transcription 2 (STAT2), and procaspase-3 and regulate the development and metastasis of cancer, cell apoptosis and drug resistance.[48] Published studies suggested that HSP27 enchanced cells invasion of bladder tumor cells and was significantly associated with peritoneal metastasis of epithelial ovarian cancer.[49,50] Furthermore, inhibition of HSP27 expression increased colon cancer sensitivity to 5-fluorouracil (5-FU) in a mouse model.[51] Therefore, HSP27 might be a predictor, prognostic factor or potential therapeutic targets of cancers. HSP70 was a very

**Table 2**

**Eligible studies for the HSP70, HSP27, HSP60, HSP90 protein expression and risk of oral cancer.**

| | Author | Reference | Time | Country | Ethnicity | Method | Histology | Control | | Case | | Cut-off value | NOS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | HSP70– | HSP70+ | HSP70– | HSP70+ | | |
| HSP70 | Sugerman | [21] | 1995 | Australia | Caucasians | IHC | OSCC | 7 | 8 | 14 | 15 | NR | 6 |
| | Kaur | [22] | 1995 | India | Asians | IHC | OSCC | 25 | 0 | 8 | 30 | NR | 6 |
| | Ito | [23] | 1998 | Japan | Asians | IHC | SCCT | 3 | 21 | 0 | 24 | NR | 6 |
| | Kaur | [24] | 1998 | India | Asians | IHC | OSCC | 91 | 5 | 33 | 92 | NR | 6 |
| | Yin | [25] | 2006 | China | Asians | IHC | OSCC | 0 | 10 | 2 | 40 | 5% | 6 |
| | Markopoulos | [26] | 2009 | Greece | Caucasians | IHC | OSCC | 10 | 0 | 0 | 30 | 0% | 6 |
| | Nair | [27] | 2013 | India | Asians | IHC | OSCC | 0 | 5 | 3 | 24 | 10% | 6 |
| | | | | | | | | HSP27– | HSP27+ | HSP27– | HSP27+ | | |
| HSP27 | Ito | [23] | 1998 | Japan | Asians | IHC | SCCT | 0 | 24 | 6 | 18 | NR | 6 |
| | Suzuki | [28] | 2007 | Japan | Asians | IHC | OSCC | 0 | 5 | 6 | 31 | 5% | 6 |
| | Li | [29] | 2013 | China | Asians | IHC | OSCC | 12 | 8 | 10 | 40 | 10% | 6 |
| | | | | | | | | HSP60– | HSP60+ | HSP60– | HSP60+ | | |
| HSP60 | Ito | [23] | 1998 | Japan | Asians | IHC | SCCT | 4 | 20 | 0 | 24 | NR | 6 |
| | Chen | [30] | 2005 | China | Asians | IHC | OLC | 8 | 3 | 2 | 16 | 5% | 6 |
| | Li | [31] | 2016 | China | Asians | IHC | OSCC | 7 | 3 | 0 | 18 | NR | 6 |
| | | | | | | | | HSP90– | HSP90 + | HSP90– | HSP90+ | | |
| HSP90 | Yin | [25] | 2006 | China | Asians | IHC | OSCC | 7 | 3 | 13 | 29 | 5% | 6 |
| | Feng | [32] | 2009 | China | Asians | IHC | OSCC | 17 | 9 | 11 | 37 | 10% | 8 |
| | Ito | [23] | 1998 | China | Asians | IHC | SCCT | 1 | 24 | 17 | 7 | NR | 6 |

IHC = immunohistochemistry; NR = not reported; OLC = oral leukoplakia cancer; OSCC = oral squamous cell carcinoma; SCCT = squamous cell carcinoma of the tongue.

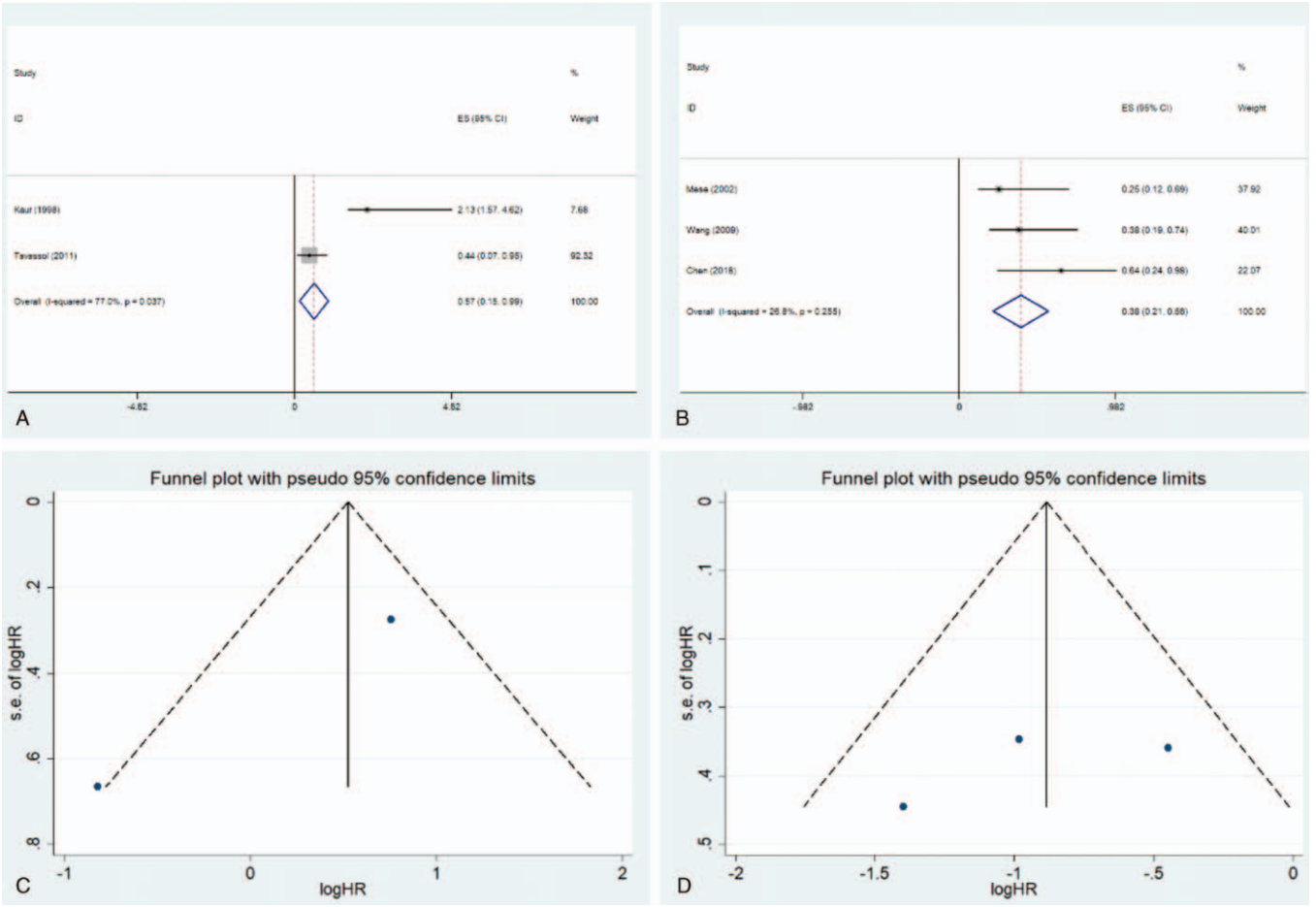


**Figure 2.** Meta-analysis for the association of HSP27 and HSP70 with overall survival of oral cancer. A: Forest plot for overall survival in HSP70; B: Forest plot for overall survival in HSP27; C: Funnel plot for overall survival in HSP70; D: Funnel plot for overall survial in HSP27.

conserved protein which had 3 domain structure: ATPase domain, substrate-binding domain, and the C-terminal domain.[52] HSP70 could promote normal folding of proteins and enhance cell survival under stresses. It has been reported that HSP70 resisted TNF-induced cytotoxicity in cancer cells and even promoted tumor growth by immunological escape mechanism, thus HSP70 could promote tumorigenesis.[53] Five proteins interacted with HSP70 and functioned together including: HSP72, HSPA6, HSPA9, GRP78, and HSC70.[54] These 5 proteins were also significant associated with progression and prognosis of oral cancer. Although HSP90, HSP60, and HSP105 were well studied in other cancers, few studies were conducted in oral cancer.

In the present study, we included 3 studies to calculate the pooled HR to assess the association between HSP27 protein expression and overall survival of oral cancer,[35,39,40] of which one study provided directly HR and other 2 studies only drawed survival curve. The pooled HR revealed that HSP 27 protein overexpression significantly increased the survival rate of oral cancer patients, which was consistent with the results of 3 published studies. No obvious heterogeneity among was found, so the results were robust. However, there were no significant association of HSP27 expression with risk and clinical characteristics of oral cancer. In 3 included studies,[23,28,29] only one study indicated HSP27 overexpression significantly increased the risk of oral cancer.[29] Interestingly, 4 studies were carried out to discuss the role of HSP27 in differentiation of oral cancer,[12,28,35,36] one study[28] got high risk result in OSCC and one study[12] obtained opposite result in tongue cancer while 2 other studies did not find significant results in OSCC. HSP27 might have different roles in different subtype of oral cancers, and tumor stage might affect the HSP27 expression. So, the included samples should have in detailed information in future studies. For the grade and lymph node metastasis of oral cancer, no positive results were detected in the eligible studies and the pooled OR indicated same results. Finally, Li's study suggested that HSP27 overexpression had a protective role in the higher grade,[29] but the pooled results did not got meaningful results.

The pooled results of included studies revealed that HSP70 overexpression was a protective factor for overall survival of oral cancer,[24,43] of which study of Kaur et al indicated poor survival patients had a higher HSP70 protein expression. Thus, if we want to get a more accurate result, more samples need to be included and more relevant studies should be carried out. In the analysis for the susceptibility of oral cancer, HSP70 overexpression significantly increased the risk of oral cancer in Asians, which was consistent with the function of promoting the survival of tumor cells. However, the pooled OR indicated that there were no significant associations of clinical features of oral cancer with HSP70 expression. In fact, many conflicting results were acquired

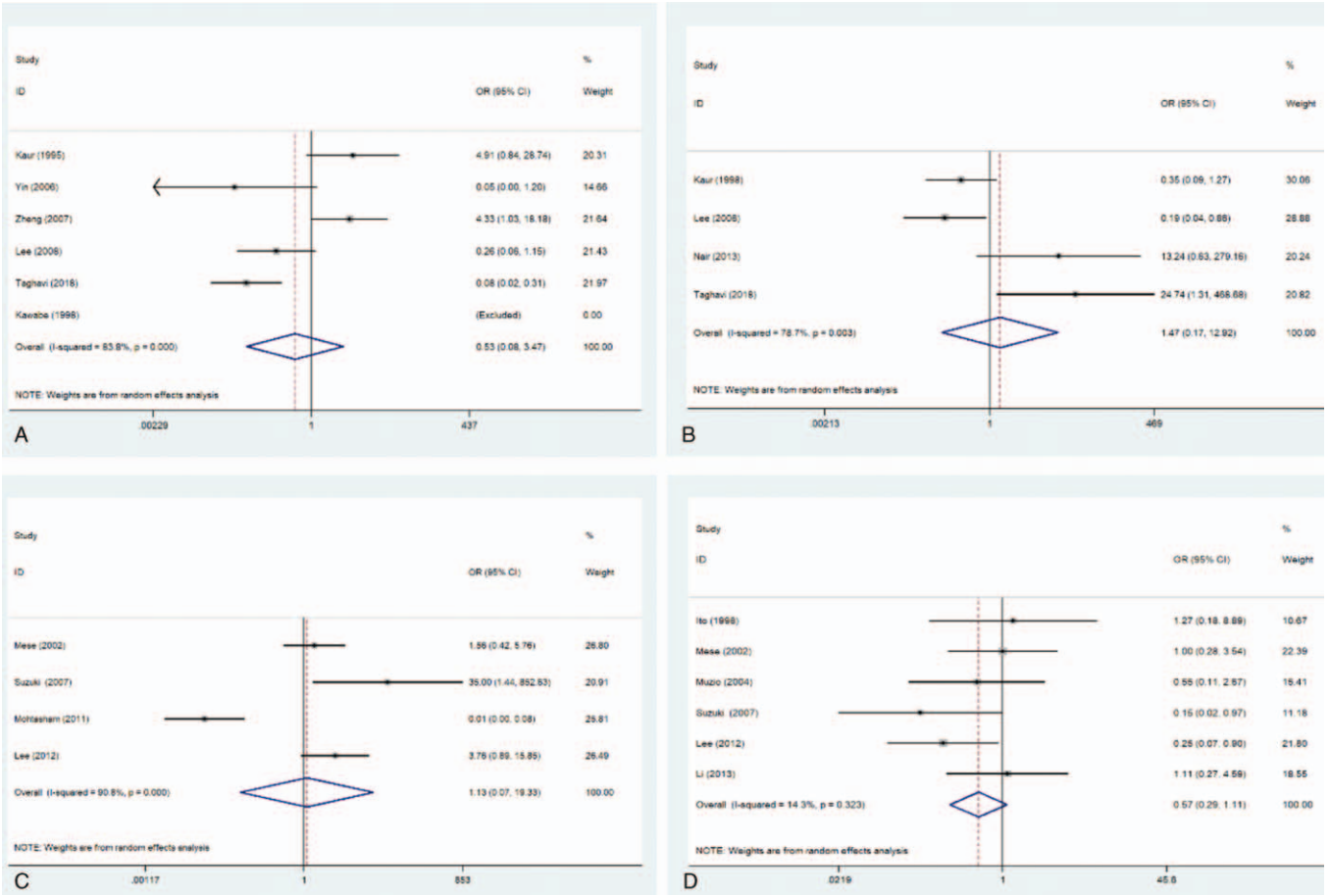


**Figure 3.** Meta-analysis for the association of HSP27 and HSP70 with clinical features of oral cancer. A: Stage for oral cancer in HSP70; B: Differentiation for oral cancer in HSP70; C: Differentiation for oral cancer in HSP27; D: lymph node metastasis for oral cancer in HSP27.

**Table 3**

**Meta-analysis for association of HSP protein expression with risk, progression, and prognosis of oral cancer.**

| | | | | | Heterogeneity | | Begg test | | Egger test | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Genes** | **Characteristics** | **Studies** | **Pooled OR/HR (95% CI)** | ***P*** | $I^2$ **(%)** | ***P*** | **Z** | ***P*** | **T** | ***P*** |
| HSP70 | Risk | 7 | 10.85 (1.37,86.13) | <.05 | 0.849 | .000 | 0.000 | 1.000 | –0.090 | .929 |
| | Caucasian | 2 | 27.67 (0.01–52937.48) | >.05 | 0.924 | .000 | – | – | – | – |
| | Asian | 5 | 10.85 (1.37, 86.13) | <.05 | 0.724 | .006 | 1.710 | .086 | –1.490 | .233 |
| | Tumor grade | 2 | 0.10 (0.01, 1.89) | >.05 | – | – | – | – | – | – |
| | Tumor stage | 6 | 0.53 (0.08–3.47) | >.05 | 0.838 | .000 | –0.240 | 1.000 | –0.140 | .898 |
| | Lymph node metastasis | 4 | 0.13 (0.01, 1.20) | >.05 | 0.836 | .002 | 1.040 | .296 | –2.350 | .257 |
| | Differentiation | 4 | 1.47 (0.17, 12.92) | >.05 | 0.787 | .003 | 0.340 | .734 | 3.870 | .061 |
| | T2 | 2 | 1.11 (0.50, 2.47) | >.05 | 0.000 | .964 | – | – | – | – |
| | Overall survival | 2 | 0.57 (0.15, 0.99) | <.05 | 0.770 | .037 | 0.000 | 1.000 | –0.500 | .704 |
| HSP27 | Risk | 3 | 0.66 (0.03, 16.12) | >.05 | 0.823 | .004 | 0.000 | 1.000 | –2.930 | .209 |
| | Tumor grade | 5 | 0.62 (0.32, 1.23) | >.05 | 0.557 | .060 | 0.730 | .462 | 0.860 | .451 |
| | Caucasians | 2 | 0.55 (0.21, 1.43) | >.05 | 0.000 | .988 | – | – | – | – |
| | Asians | 3 | 0.71 (0.27, 1.85) | >.05 | 0.775 | .012 | 1.040 | .296 | 10.670 | .059 |
| | Tumor stage | 6 | 1.07 (0.35, 3.30) | >.05 | 0.682 | .008 | 0.000 | 1.000 | –0.710 | .519 |
| | Lymph node metastasis | 6 | 0.57 (0.29, 1.11) | >.05 | 0.143 | .323 | 0.380 | .707 | –0.240 | .820 |
| | Differentiation | 4 | 1.13 (0.07, 19.33) | >.05 | 0.908 | .000 | –0.340 | 1.000 | 0.200 | .859 |
| | Overall survival | 3 | 0.38 (0.21, 0.56) | <.05 | 0.268 | .255 | 0.000 | 1.000 | –0.940 | .519 |
| HSP90 | Risk | 3 | 0.94 (0.04, 24.57) | >.05 | 0.928 | .000 | 1.040 | .296 | –1.890 | .310 |
| HSP60 | Risk | 3 | 23.91 (5.39, 10.6.16) | <.05 | 0.000 | .647 | 0.000 | 1.000 | 0.280 | .828 |

in the included studies for progression of oral cancer. For example, study of Zheng et al[37] demonstrated patients with HSP70 overexpression had a more advanced stage of oral cancer, but Taghavi' study found HSP70 overexpression was a protective factor for advanced stage.[34] At the same time, opposite results were also detected for the differentiation or oral cancer,[33,34] in which the 2 studies were all performed in OSCC patients. So we might confirm that HSP70 had a closely association with risk or prognosis of oral cancer, but the published studies could not provide definite results because of fewer studies and opposite results. It was worth noting that HSP70 protein expression levels might be different along with the development of oral cancer, which might explain the inconsistency of results.

Few studies were conducted for the association between HSP90 (4 studies),[23,25,32,44] HSP60 (3 studies),[23,30,31] and HSP105 (2 studies)[12,45] protein expression and oral cancer. As mentioned above, many studies were carried to explore the function of HSP90 in cancer cells. HSP90 protein possesses alternative ATP-binding sites which are the binding site for nucleotides and for small molecules drugs such as nucleotides, novobiocin, and cisplatin.[55] Due to intrinsic ATPase activity in HSP90 protein, it assists in protein folding, client protein, maturation, and protein trafficking and further is involved in PI3K/AKT pathway, JAK/STAT pathway, RAS/ERK pathway, and NF-kB pathway.[56,57] So, HSP90 is associated with tumor growth, adhesion, metastasis, invasion, and angiogenesis.[58] The results of meta-analysis did not found any significant association of HSP90 overexpression with risk of oral cancer patients. Ito' study found HSP90 was a protective factor for risk of oral cancer.[23] Only one study was conducted for the role of HSP90 overexpression for clinical stage of oral cancer in Asians, so we did not conduct pooled analysis for HSP90 expression and progression of oral caner. HSP60, locating in the mitochondria, cytosol, cell surface and we could detected it in peripheral blood.[59] HSP60 could protect cancer cells from chemotherapeutic agents and promote cell survival which revealed HSP60 overexpression was a risk factor for oral cancer.[60] Our pooled results were similar to previous findings, of which people with HSP60 protein overexpression was 23.91 times more likely to suffer from oral cancer. This was an encouraging result and no significant heterogeneity among studies were detected, so this result was stable and credible. In the literature retrieval, 2 studies which talked about HSP105 and oral cancer including OSCC and squamous cell carcinoma of the tongue were included.[12,45] Arvanitidou et al[45] found the patients with increased HSP105 expression presented a poor prognosis for malignancy ($P = .042$). And study conducted by Mohtasham et al[12] demonstrated that HSP105 overexpression promoted the progression of oral tumor, in which patients with advanced stage had a higher HSP105 protein expression in epithelial cells. There was no obvious association between HSP105 protein expression and grade of oral tumor. In the latest study, HSP105 peptide vaccine induced immunological effects colorectal cancer and esophageal cancer patients and obciously improved their prognosis.[61] The vaccine against the HSP protein might be used to treat oral cancer in the future. But before that, we still had to carry out numerous studies to investigate the role of HSP in progression and prognosis of oral cancer.

Several factors still limited statistical power of data. First, few studies were conducted for HSP60, HSP90, and HSP106. Although some studies were performed for HSP70 and HSP27, the number of included studies in subgroup analysis still was too small. Second, we found the HSP protein expression in eligible studies had a big difference, in which stage of patients or tumor subtype might affect the HSP expression. So, when we carried out the correlation analysis, clinical information and stratification analysis based on patients' clinical stage would be very important. Third, the overall results revealed each HSP had special function for tumor cells, thus we could not put all the data together to calculate an overall OR and analyze the function of HSP, which leaded to complexity in understanding the pooled results. But, on the other hand, it might explain the importance of HSP family protein in cancer cells.

In general, HSP70 and HSP27 might be potential biomarkers for assessing the occurrence and prognosis of oral cancer. HSP60 overexpression was significantly associated with increased risk of oral cancer. However, given the small sample size, studies with more samples, detailed clinical information, especially clinicopathological data, should be carried out to observe the effects HSP family protein in risk, progression, and prognosis of oral cancer.

## Author contributions

**Data curation:** Yongwu Wang, Min Gan.
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**Writing – original draft:** Wei Lu.
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## References

[1] Miller KD, Siegel RL, Lin CC, et al. Cancer treatment and survivorship statistics, 2016. CA Cancer J Clin 2016;66:271–89.
[2] Dzioba A, Aalto D, Papadopoulos-Nydam G, et al. Functional and quality of life outcomes after partial glossectomy: a multi-institutional longitudinal study of the head and neck research network. J Otolaryngol Head Neck Surg 2017;46:56.
[3] Tytor M, Olofsson J. Prognostic factors in oral cavity carcinomas. Acta Otolaryngol Suppl 1992;492:75–8.
[4] Neville BW, Day TA. Oral cancer and precancerous lesions. CA Cancer J Clin 2002;52:195–215.
[5] McBride KA, Ballinger ML, Killick E, et al. Li-Fraumeni syndrome: cancer risk assessment and clinical management. Nat Rev Clin Oncol 2014;11:260–71.
[6] Scully C, Langdon J, Evans J. Marathon of eponyms: 26 Zinsser-Engman-Cole syndrome (Dyskeratosis congenita). Oral Dis 2012;18: 522–3.
[7] Kutler DI, Auerbach AD, Satagopan J, et al. High incidence of head and neck squamous cell carcinoma in patients with Fanconi anemia. Arch Otolaryngol Head Neck Surg 2003;129:106–12.
[8] Sinevici N, O'sullivan J. Oral cancer: deregulated molecular events and their use as biomarkers. Oral Oncol 2016;61:12–8.
[9] Sigismund S, Avanzato D, Letizia L, et al. Emerging functions of the EGFR in cancer. Mol Oncol 2018;12:3–20.
[10] Lindquist S, Craig EA. The heat-shock proteins. Annu Rev Genet 1988;22:631–77.
[11] Trautinger F, Kindas-Mügge I, Dekrout B, et al. Expression of the 27-kDa heat shock protein in human epidermis and in epidermal neoplasms: an immunohistological study. Br J Dermatol 1995;133: 194–202.
[12] Mohtasham N, Babakoohi S, Montaser-Kouhsari L, et al. The expression of heat shock proteins 27 and 105 in squamous cell carcinoma of the tongue and relationship with clinicopathological index. Med Oral Patol Oral Cir Bucal 2011;16:e730–5.
[13] Multhoff G, Botzler C, Jennen L, et al. Heat shock protein 72 on tumor cells: a recognition structure for natural killer cells. J Immunol 1997;158:4341–50.

[14] Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and metaanalyses:the PRISMA statement. PLoS Med 2009;62:1006–12.
[15] Tierney JF, Stewart LA, Ghersi D, et al. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials 2007;8:16.
[16] Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010;25:603–5.
[17] DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177–88.
[18] Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 1959;22:719–48.
[19] Lau J, Ioannidis JP, Schmid CH. Quantitative synthesis in systematic reviews. Ann Intern Med 1997;127:820–6.
[20] Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629–34.
[21] Sugerman PB, Savage NW, Xu LJ, et al. Heat shock protein expression in oral epithelial dysplasia and squamous cell carcinoma. Eur J Cancer B Oral Oncol 1995;31:63–7.
[22] Kaur J, Ralha R. Differential expression of 70-kDa heat shock-protein in human oral tumorigenesis. Int J Cancer 1995;63:774–9.
[23] Ito T, Kawabe R, Kurasono Y, et al. Expression of heat shock proteins in squamous cell carcinoma of the tongue: an immuno histochemical study. J Oral Pathol Med 1998;27:18–22.
[24] Kaur J, Srivastava A, Ralhan R, et al. Expression of 70-kDa heat shock protein in oral lesions: marker of biological stress or pathogenicity. Oral Oncol 1998;34:496–501.
[25] Yin C, Shen L, Xie S, et al. Expression and significance of Heat Shock Protein 70, 90 in orla squamous cell carcinoma and oral precancerous lesions. J Fourth Mil Med Univ 2006;27:1792–4.
[26] Markopoulos AK, Deligianni E, Antoniades DZ. Heat Shock Protein 70 membrane expression in oral cancer: a possible new target in antineoplastic therapy? Chemotherapy 2009;55:211–4.
[27] Nair S, Kotrashetti VS, Nayak R, et al. HSP70 induces TLR4 signaling in oral squamous cell carcinoma: an immunohistochemical study. J Cancer Res Therap 2013;9:624–9.
[28] Suzuki H, Sugimura H, Hashimoto K, et al. Overexpression of heat shock protein 27 is associated with good prognosis in the patient with oral squamous cell carcinoma. Br J Oral Maxillofacial Surg 2007;45:123–9.
[29] Li S, Li Y, Liu Z, et al. Study on the expression and correlation of PKA1, HSP27 and PTEN in oral squamous cell carcinoma. J Oral Sci Res 2013;29:742–7.
[30] Chen J, Ping F, Yanhua G. Expression and significance of HSP60 in oral mucosal white spot and squamous cell carcinoma. Journal of Practical Oncology 2005;20:510–2.
[31] Li F, Yang L, Liu Y, et al. Expression of heat shock protein 60 in oral squamous cell carcinoma and its clinical significance. Heilongjiang Med J 2016;40:1119–20.
[32] Feng Q, Gao Y, Jiang R, et al. Expression and significance of HSP90. Shandong medicine 2009;49:83–4.
[33] Lee S-S, Tsai C-H, Ho Y-C, et al. The upregulation of heat shock protein 70 expression in areca quid chewing-associated oral squamous cell carcinomas. Oral Oncol 2008;44:884–90.
[34] Taghavi N, Mohsenifar Z, Baghban AA, et al. CD20+ Tumor infiltrating B lymphocyte in oral squamous cell carcinoma: correlation with clinicopathologic characteristics and heat shock protein 70 expression. Patholog Res Int 2018;4:4810751.
[35] Mese H, Sasaki A, Nakayama S, et al. Prognostic significance of heat shock protein 27 (HSP27) in patients with oral squamous cell carcinoma. Oncol Rep 2002;9:341–4.
[36] Lee S-S, Tsai C-H, Ho Y-C, et al. Heat shock protein 27 expression in areca quid chewing-associated oral squamous cell carcinomas. Oral Dis 2012;18:713–9.
[37] Zheng S, Zhu Y, Ding X, et al. Relationship between HSP-70 expression and clinic. Zhejiang Clin Med 2007;9:731–2.
[38] Chen W, Ren X, Wu J, et al. HSP27 associates with epithelial–mesenchymal transition, stemness and radioresistance of salivary adenoid cystic carcinoma. J Cell Mol 2018;22:2283–98.
[39] Lo Muzio L, Leonardi R, Mariggiò MA, et al. HSP 27 as possible prognostic factor in patients with oral squamous cell carcinoma. Histol Histopathol 2004;19:119–28.
[40] Wang A, Liu X, Sheng S, et al. Dysregulation of heat shock protein 27 expression in oral tongue squamous cell carcinoma. BMC Cancer 2009;9:167.
[41] Fourati A, Véronique El May M, Ben Abdallah M, et al. Prognostic evaluation of p53, heat shock protein 70, Ki67, and CD34 expression in cancer of the tongue in Tunisia. J Otolaryngol Head Neck Surg 2009;38:191–6.
[42] Pranay A, Shukla S, Kannan S, et al. Prognostic utility of autoantibodies to -enolase and Hsp70 for cancer of the gingivo-buccal complex using immunoproteomics. Proteomics Clin 2013;7:392–402.
[43] Tavasso F, Starke OF, Kokemüller H, et al. Prognostic significance of heat shock protein 70 (HSP70) in patients with oral cancer. Head Neck Oncol 2011;3:1–0.
[44] Chang W-C, Tsai P-T, Lin C-K, et al. Expression pattern of heat shock protein 90 in patients with oral squamous cell carcinoma in Northern Taiwan. Br J Oral Maxillofac Surg 2017;55:281–6.
[45] Arvanitidou S, Martinelli-Kläy CP, Samson J, et al. HSP105 expression in oral squamous cell carcinoma: correlation with clinicopathological features and outcomes. J Oral Pathol Med 2020;49:665–71.
[46] Murshid A, Gong J, Calderwood SK. The role of heat shock proteins in antigen cross presentation. Front Immunol 2012;3:63.
[47] Aparna V, Nancy AW, Arjang D. Heat shock protein 27 (HSP27): biomarker of disease and therapeutic target. Fibrogenesis Tissue Repair 2012;5:7.
[48] Cayado-Gutiérrez N, Moncalero VL, Rosales EM, et al. Downregulation of Hsp27 (HSPB1) in MCF-7 human breast cancer cells induces upregulation of PTEN. Cell Stress Chaperones 2013;18:243–9.
[49] Yu L, Yuan X, Wang D, et al. Selective regulation of p38b protein and signaling by integrin-linked kinase mediates bladder cancer cell migration. Oncogene 2014;33:690–701.
[50] Zhao M, Shen F, Yin YX, et al. Increased expression of heat shock protein 27 correlates with peritoneal metastasis in epithelial ovarian cancer. Reprod Sci 2012;19:748–53.
[51] Hayashi R, Ishii Y, Ochiai H, et al. Suppression of heat shock protein 27 expression promotes 5-fluorouracil sensitivity in colon cancer cells in a xenograft model. Oncol Rep 2012;28:1269–74.
[52] Tuoen L, Christopher KD, Shousong C, et al. Comprehensive review on the HSC70 functions, interactions with related molecules and involvement in clinical diseases and therapeutic potential. Pharmacol Ther 2012;136:354–74.
[53] Jäättelä M, Wissing D, Bauer PA, et al. Major heat shock protein hsp70 protects tumor cells from tumor necrosis factor cytotoxicity. EMBO J 1992;11:3507–12.
[54] Harm H, Kampinga JHET-AL>. Guidelines for the nomenclature of the human heat shock proteins. Cell Stress Chaperones 2009;14:105–11.
[55] Söti C, Péter AR, et al. A nucleotide-dependent molecular switch controls ATP binding at the C-terminal domain of Hsp90. N-terminal nucleotide binding unmasks a C-terminal binding pocket. J Biol Chem 2002;277:7066–75.
[56] Suzuki R, Hideshima T, Mimura N, et al. Anti-tumor activities of selective HSP90//b inhibitor, TAS-116, in combination with bortezomib in multiple myeloma. Leukemia 2015;29:510–4.
[57] Kamal A, Boehm MF, Burrows FJ. Therapeutic and diagnostic implications of Hsp90 activation. Trends Mol Med 2004;10:283–90.
[58] Aoyagi Y, Fujita N, Takashi T. Stabilization of integrin-linked kinase by binding to Hsp90. Biochem Biophys Res Commun 2005;331:1061–8.
[59] Wadhwa R, Takano S, Kaur K, et al. Identification and characterization of molecular interactions between mortalin/mtHsp70 and HSP60. Biochem J 2005;391(pt 2):185–90.
[60] Pace A, Barone G, Lauria A, et al. Hsp60, a novel target for antitumor therapy: structure-function features and prospective drugs design. Curr Pharm Des 2013;19:2757–64.
[61] Shimizu Y, Yoshikawa T, Kojima T, et al. Heat shock protein 105 peptide vaccine could induce antitumor immune reactions in a phase I clinical trial. Cancer Sci 2019;110:3049–60.