







Article

The Chaperone System in Tumors of the Vocal Cords: Quantity and Distribution Changes of Hsp10, Hsp27, Hsp60, and Hsp90 during Carcinogenesis

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Abstract: Laryngeal squamous cell carcinoma (LSCC) constitutes a noteworthy subset of head and neck cancers, contributing to about 4.5% of all malignancies. Its clinical behavior and characteristics exhibit variations contingent upon the specific anatomical site affected, with the glottis, supraglottis, and subglottis emerging as the most prevalent locations. Notably, squamous cell carcinoma represents a predominant histological type, accounting for 85% to 95% of all laryngeal cancers. The gender disparity is evident, with a higher incidence among males, exhibiting a ratio of 3.9:1. Moreover, disparities among racial groups are observed, as African American patients tend to manifest the condition at a younger age, coupled with lower overall survival rates compared to their Caucasian, Hispanic, and Asian counterparts. The primary etiological factors implicated in the onset of laryngeal cancer are tobacco and alcohol consumption, with a direct correlation to the intensity and duration of usage. Importantly, the risk diminishes gradually following cessation, necessitating a substantial period of at least 15 years for a return to baseline rates. Given the diverse nature of laryngeal SCC, treatment modalities are tailored based on the specific site and stage of the disease. Therapeutic interventions, such as radiotherapy, transoral laser microsurgery, open horizontal partial laryngectomy, or total laryngectomy, are employed with the overarching goal of preserving organ function. This study delves into the intricate realm of laryngeal SCC, specifically exploring the involvement of heat shock proteins (HSPs) in disease progression. This research meticulously examines the expression levels of Hsp10, Hsp27, Hsp60, and Hsp90 in dysplastic and benign tissue samples extracted from the right vocal cord, utilizing immunohistochemistry analysis. The focal point of the investigation revolves around unraveling the intricate role of these molecular chaperones in tissue differentiation mechanisms and cellular homeostasis, particularly within the inflammatory milieu characteristic of the tumor phenotype. The findings from this study serve as a robust histopathological foundation, paving the way for more in-depth analyses of the underlying mechanisms governing the contribution of the four chaperones to the development of squamous cell carcinoma in the larynx. Additionally, the data gleaned from this research hint at the potential of these four chaperones as valuable biomarkers, not only for diagnostic purposes but also for prognostication and ongoing patient monitoring. As our understanding of the molecular intricacies deepens, the prospect of targeted therapeutic interventions and personalized treatment strategies for laryngeal SCC becomes increasingly promising.



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1. Introduction

Laryngeal squamous cell carcinoma (LSCC) represents a significant portion, approximately 4.5%, of all malignancies, making it the most prevalent form of head and neck cancer in males [1,2]. The variability in tumor characteristics, clinical behavior, and prognosis is closely tied to the anatomical site affected, including the glottis, supraglottis, or subglottis regions [3]. Squamous cell carcinoma constitutes a substantial majority, accounting for 85% to 95% of all laryngeal cancers. Alarming statistics from 2018 in the United States alone reported around 13,150 newly diagnosed cases of laryngeal cancer, leading to approximately 3710 deaths [4]. The incidence ratio between males and females is notably high at 3.9:1 [5]. While racial predilection is not overtly evident, race emerges as an independent prognostic factor. African American patients present at a younger age and exhibit lower overall survival rates compared to their Caucasian, Hispanic, and Asian counterparts [6]. This disparity underscores the intricate interplay of genetic and environmental factors in the development and progression of LSCC. Tobacco and alcohol use stand out as primary causative factors for laryngeal cancer, with risk escalating in proportion to the intensity and duration of consumption. Interestingly, after cessation of tobacco or alcohol use, it takes approximately 15 years for the risk levels to normalize, emphasizing the long-term impact of these habits [1].

The management of LSCC involves a nuanced approach, with treatment options contingent on the location and stage of the disease [7]. Organ preservation strategies, including radiotherapy [8,9] and transoral laser microsurgery (TLM) [10,11], as well as open horizontal partial laryngectomy or definitive surgery (total laryngectomy) [12], are employed. Surgical interventions, whether diagnostic or therapeutic, play a pivotal role in providing critical insights into tumor biology and guiding the subsequent course of treatment.

Despite advances in understanding various human tumor types, the precise role and contribution of members of the chaperone system (CS), such as heat shock proteins (HSPs), in the carcinogenic process of the larynx, especially the vocal cords, remain to be firmly established [13–18]. Researchers studying various types of carcinomas have discovered that heat shock proteins can have an additional role. When released outside of cells, these proteins can cause chronic inflammation. They promote cytokines and interact with other proteins, which are crucial in regulating the immune response, inflammation, and tumor microenvironment in cancer. Pro-inflammatory cytokines, including TNF- α , IL-1 β , and IL-6, can exacerbate cancer invasiveness and malignancy. These cytokines facilitate cancer progression by inducing persistent inflammation and continuously activating signaling pathways like NF- κ B. Furthermore, cytokines can affect the activity of tumor-associated macrophages (TAMs), which secrete factors that contribute to tumor growth and metastasis while maintaining an immunosuppressive environment [19–22]. Another possibility that the study and analysis of various HSPs may provide is to use this class of proteins for prognostic purposes. High expression levels of heat shock proteins may be associated with worse prognostic pictures. Thus, having these data available could be crucial for optimizing the individual therapy of individuals with carcinoma [23–26]. Recent studies have revealed the crucial role of HSPs in maintaining cellular stability and averting programmed cell death (apoptosis). This, in turn, poses a considerable risk factor for cancer treatments, potentially causing resistance. Therefore, when devising an effective cancer therapy for a patient, it is imperative to take this factor into consideration [27–29]. This pilot study embarks on an immunomorphological evaluation of the tissue levels and topography of specific HSPs (Hsp10, Hsp27, Hsp60, and Hsp90) utilizing immunohistochemistry. This study meticulously quantifies the presence of these molecules in vocal cord tissue during

stages of dysplasia and malignancy. The primary objective is to establish a robust platform for further investigations into the mechanisms underlying the participation of HSPs in laryngeal carcinogenesis. The study aims to ascertain whether and how the quantitative levels of these CS components influence the progression of tissue lesions.

Anticipating that the results will shed light on the potential role of HSPs as biomarkers, this study seeks to provide valuable insights for diagnosis, prognostication, and patient monitoring. The implications of this research extend beyond the laboratory, holding the promise of more personalized and effective approaches to managing laryngeal squamous cell carcinoma. The quest for biomarkers and a deeper understanding of the molecular intricacies involved paves the way for advancements in the field of head and neck oncology, offering hope for improved outcomes and enhanced patient care.

2. Materials and Methods

2.1. Sample Collection

The vocal cord tissue samples utilized in this study were sourced from the archives of the “Ospedali Riuniti VILLA SOFIA-CERVELLO” hospital in Palermo. These biopsy samples from Italian patients who had been cigarette smokers for at least five years, collected over two years from 2020 to 2022, were meticulously categorized into three distinct groups to facilitate a comprehensive morphological analysis. The stratification of these tissue specimens aimed at capturing the spectrum of conditions within the vocal cords, providing valuable insights into the molecular and cellular changes associated with health, dysplasia, and squamous cell carcinoma (SCC) (Table 1).

Table 1. Description of demographic information, such as gender and age, and clinical characteristics of individual patients in each group (healthy, dysplasia, and squamous cell carcinoma), including TNM staging.

Group	Gender	Age (y.o.)	T	N	M
Healthy	Female	48	-	-	-
Healthy	Female	50	-	-	-
Healthy	Male	53	-	-	-
Healthy	Male	57	-	-	-
Healthy	Male	60	-	-	-
Healthy	Male	62	-	-	-
Healthy	Female	65	-	-	-
Healthy	Male	68	-	-	-
Healthy	Female	69	-	-	-
Healthy	Male	70	-	-	-
Dysplasia	Female	45	-	-	-
Dysplasia	Female	49	-	-	-
Dysplasia	Male	53	-	-	-
Dysplasia	Female	55	-	-	-
Dysplasia	Male	59	-	-	-
Dysplasia	Male	62	-	-	-
Dysplasia	Male	65	-	-	-
Dysplasia	Female	71	-	-	-
Dysplasia	Male	76	-	-	-
Dysplasia	Male	78	-	-	-

Table 1. *Cont.*

Group	Gender	Age (y.o.)	T	N	M
Squamous Cell Carcinoma	Male	68	2	0	0
Squamous Cell Carcinoma	Male	69	3	0	0
Squamous Cell Carcinoma	Female	70	3	0	0
Squamous Cell Carcinoma	Male	73	2	0	0
Squamous Cell Carcinoma	Female	74	2	0	0
Squamous Cell Carcinoma	Male	77	2	0	0
Squamous Cell Carcinoma	Female	78	2	0	0
Squamous Cell Carcinoma	Female	80	2	0	0
Squamous Cell Carcinoma	Female	81	2	0	0
Squamous Cell Carcinoma	Female	82	3	0	0

The first group, denoted as the Healthy group, comprised ten tissue specimens sourced from both male and female individuals with ages ranging from 48 to 70 years. These specimens were characterized by their normal physiological status, serving as a baseline for comparison against the other groups. The inclusion of both genders and a varied age range within this group ensured a representative sample reflecting the diversity of the general population.

The second group, labeled Dysplasia, encompassed ten tissue samples collected from individuals exhibiting dysplastic changes in their vocal cord tissue. Within this group, six males and four females, aged between 45 and 78 years, were represented. Dysplasia, a condition marked by abnormal cellular growth and differentiation, provides a critical intermediary stage in the progression toward more severe pathologies such as cancer. Analyzing this group allowed for a nuanced understanding of the morphological alterations associated with the precancerous state.

The third group, Squamous Cell Carcinoma (SCC), comprised ten tissue specimens from individuals diagnosed with squamous cell carcinoma of the vocal cords. This group featured an equal distribution of five males and five females, aged between 68 and 82 years, and exhibited a state of moderate differentiation.

The comprehensive collection of vocal cord tissue samples over the specified time frame and their meticulous categorization into Healthy, Dysplasia, and SCC groups provided a robust foundation for the morphological study. This stratified approach ensured that the analysis could capture the dynamic changes occurring at different stages of vocal cord pathology. The utilization of archived samples from a reputable hospital further adds credibility to the study, as the samples are likely to be well preserved and accurately documented.

In summary, the vocal cord tissue samples collected from the archives of “Ospedali Riuniti VILLA SOFIA-CERVELLO” hospital in Palermo form a diverse and representative dataset, allowing for a comprehensive investigation into the morphological variations associated with health, dysplasia, and squamous cell carcinoma. The categorization into distinct groups based on health status and the inclusion of age and gender diversity within each group (Table 1) enhances the capacity of the study to uncover meaningful insights into the intricate processes underlying vocal cord pathologies.

2.2. Immunohistochemical Analysis

The immunohistochemical investigations (IHC) were performed on 5-micron thick tissue sections, obtained with a microtome from paraffin blocks. The resulting sections were subjected to deparaffinization, which consisted in immersing the sections in xylene for 30 min at 60 °C. Subsequently, the sections were rehydrated in a descending scale of alcohol at 23 °C (steps: in alcohol 100% for 10 min, alcohol 95% for 5 min, alcohol 80% for 5 min,

alcohol 50% for 5 min), and lastly rinsed with distilled water for 5 min. Then, the sections were incubated for 8 min in 350 mL of saline solution, Sodium Citrate (Sodium Citrate Buffer pH 6), at 80 °C to allow for the unmasking of antigens that may have been masked by fixation and paraffin embedding. Subsequently, to avoid the detachment of the sections from the slide, the sections were immersed in acetone at −20 °C for 5 min. All subsequent reactions were conducted at room temperature (23 °C), using the Immunoperoxidase Secondary Detection System (Millipore, Burlington, MA, USA & Canada, cat. N DAB-500.). In the next step, the sections were rinsed with PBS (Phosphate Buffered Saline pH 7.4) for 5 min, followed by the incubation of the sections with 3% hydrogen peroxide for the inhibition of endogenous peroxidase for 5 min. After rinsing with PBS for 5 min, the non-specific sites were blocked by using a protein-blocker reagent (blue-colored reagent) for 5 min. Subsequently, the sections were incubated with the primary antibodies anti-Hsp10 (rabbit anti-Hsp10 polyclonal antibody, D-8, Santa Cruz Biotechnology, Dallas, TX, USA, cat. N: sc-20958, dilution 1:200), anti-Hsp27 (mouse anti-Hsp27 monoclonal antibody, F-4; Santa Cruz Biotechnology, cat N: sc-13132, dilution 1:200), anti-Hsp60 (rabbit polyclonal anti-Hsp60 antibody, Abcam, Cambridge, CB2 0AX, UK, Cat. No. ab46798, dilution 1:400), and anti-Hsp90 (mouse anti-Hsp90 monoclonal antibody, clone F-8, Santa Cruz Biotechnology, Cat. No. sc-13119, dilution 1:200), overnight at 4 °C. For all reactions, the appropriate negative controls were also carried out by adding only PBS to the sections instead of the primary antibody. After the incubation with the primary antibody, the samples were rinsed with PBS for 30 s with Rinse Buffer 1× and incubated with secondary antibody (biotinylated goat anti-mouse IgG, Cat. No. 20775, Millipore Burlington, MA, USA & Canada, cat. N DAB-500) drops for 10 min at room temperature. After subsequent rinsing with PBS for 5 min, the reaction continued with incubation with Streptavidin HRP (Streptavidin HRP: Diluted in Tris Buffered Saline, Cat. No. 2774, Millipore Burlington, MA, USA & Canada, cat. N DAB-500) drops for 10 min and, after further washing in PBS for 5 min, the samples were incubated with an adequate volume of Chromogen Reagent, and for the nuclear blue counterstaining, after another buffer rinse, stained with the Hematoxylin Counter Stain solution for 1 min at room temperature. Finally, the slides were mounted with coverslips using the permanent mount (Vecta Mount, H-5000, Vector Laboratories, Inc., Burlingame, CA, USA). Sections were observed with an optical microscope (Microscope Axioscope 5/7 KMAT, Carl Zeiss, Oberkochen, Germany) connected to a digital camera (Microscopy Camera AxioCam 208 color, Carl Zeiss). All evaluations were performed at 400× magnification and repeated for 10 High-Power Fields (HPF).

2.3. Statistical Analysis

The statistical analysis was performed using GraphPad Prism 4.0 software (GraphPad Inc., San Diego, CA, USA) employing one-way ANOVA analysis to determine the presence of statistically significant differences. All data were reported as mean ± standard deviation, and the threshold for statistical significance was set at $p \leq 0.05$.

3. Results

3.1. Hsp10

The results of our immunohistochemical assessment of Hsp10 experiments have shown that healthy tissue has an average tissue level of $(26.5 \pm 6.5)\%$, with a cytoplasmic granular immunolocalization. However, the dysplastic tissue group exhibited higher tissue levels, with a percentage of $(69.5 \pm 7)\%$ and a cytoplasmic and sometimes nuclear localization (Figure 1A). The tumor tissue group showed even higher tissue levels, consisting of $(93.5 \pm 3)\%$, with cytoplasmic and nuclear localization (also seen in Figure 1A). From our data, we can conclude that there is a gradual increase in Hsp10 tissue expression levels, progressing from healthy tissue to dysplastic tissue and tumor tissue (Figure 1B). Through statistical analysis, we have discovered a significant difference ($p < 0.01$) between the healthy tissue group (Healthy) and the dysplastic (Dysplasia) and squamous cell carcinoma group (SCC), as well as between the dysplastic group and squamous carcinoma group (Figure 1B).

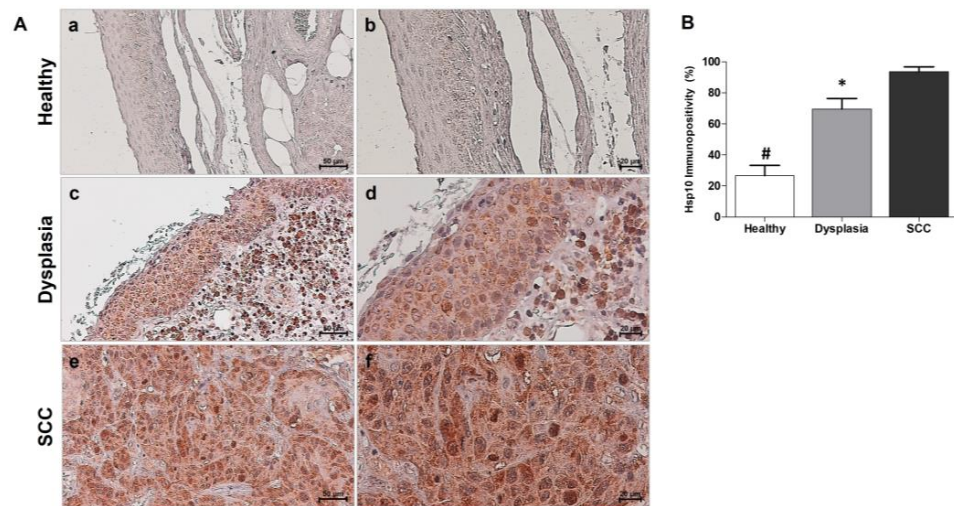


Figure 1. (A) Hsp10 immunohistochemistry. Representative images of immunohistochemical results for Healthy (a,b), Dysplasia (c,d), and SCC (squamous cell carcinoma); (e,f). (a,c,e): magnification 200 \times , scale bar 50 μ m; (b,d,f): magnification 400 \times ; scale bar 20 μ m. (B) Statistical analysis of Hsp10 results. The histogram revealed a significant difference between the Healthy group and the Dysplasia and SCC groups (# $p \leq 0.01$), as well as between the Dysplasia group and the SCC group (* $p \leq 0.01$).

3.2. Hsp27

The immunopositivity of Hsp27 appeared granular in the cytoplasm in all samples of the healthy tissue group with an average percentage of (30.5 \pm 4)% , whereas in the dysplastic tissue group, it was observed in the cytoplasm diffusely with an average percentage of (66 \pm 5.5)%. Finally, in the squamous carcinoma tissue group, the Hsp27 immunopositivity exhibited a significantly higher value of (92.5 \pm 4)% and was localized in the cytoplasm and occasionally in the nucleus (Figure 2A). The statistical analysis conducted on the normal tissue, dysplastic tissue, and squamous carcinoma tissue (Figure 2B) revealed a progressive elevation in the tissue levels of Hsp27 with a significant difference ($p < 0.01$) between the healthy tissue group and the dysplastic and squamous carcinoma group, and between the dysplastic group and squamous carcinoma group (Figure 2B).

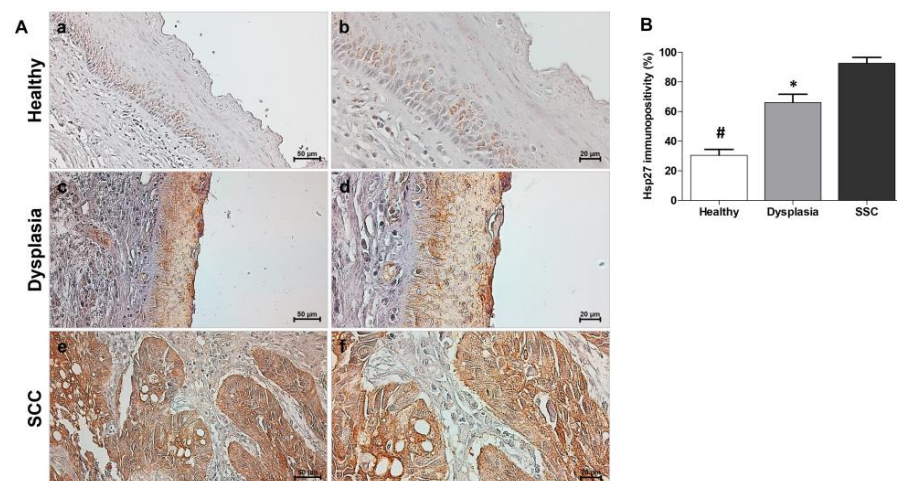


Figure 2. (A) Hsp27 immunohistochemistry. Representative images of immunohistochemical results for Healthy (a,b), Dysplasia (c,d), and squamous cell carcinoma (SCC; (e,f)). (a,c,e): magnification 200 \times , scale bar 50 μ m; (b,d,f): magnification 400 \times ; scale bar 20 μ m. (B) Statistical analysis of Hsp27 results. The histogram revealed a significant difference between the Healthy group and the Dysplasia and SCC groups (# $p \leq 0.01$), as well as between the Dysplasia group and the SCC group (* $p \leq 0.01$).

3.3. Hsp60

In the healthy tissue group, Hsp60 immunopositivity was observed to be cytoplasmic and granular, with an average percentage of $(22 \pm 4.5)\%$. However, in the dysplastic tissue group, the Hsp60 percentage immunopositivity was $(78 \pm 2.5)\%$, while in the squamous carcinoma group, it was $(92 \pm 3)\%$. The immunolocalization was predominantly diffuse and cytoplasmic (Figure 3A). The statistical analysis revealed a significant increase in the tissue expression levels of Hsp60 observed during the transition from normal to dysplastic tissue and neoplastic tissue groups. A statistically significant difference ($p < 0.01$) was detected between the healthy tissue group and the dysplasia and carcinoma groups (Figure 3B).

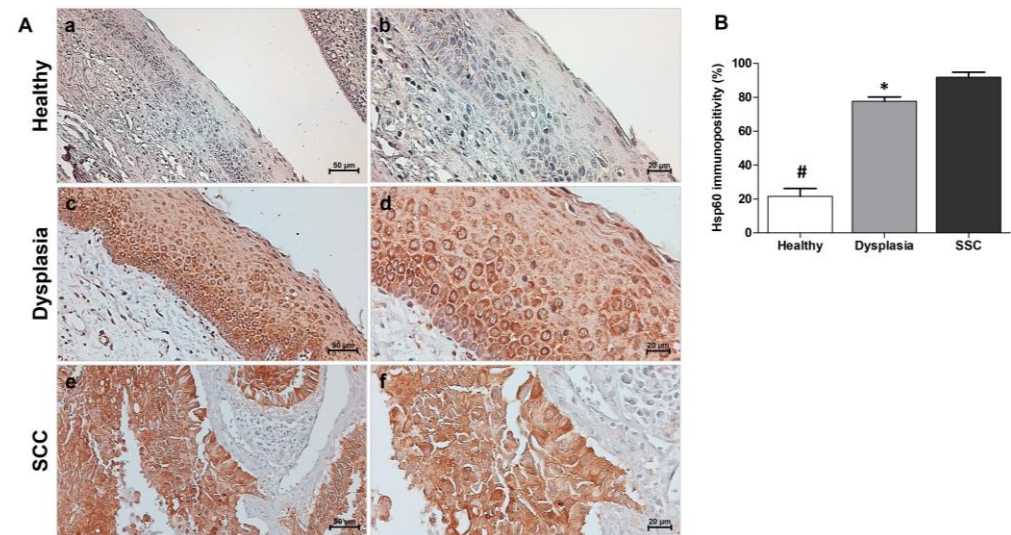


Figure 3. (A) Hsp60 immunohistochemistry. Representative images of immunohistochemical results for Healthy (a,b), Dysplasia (c,d), and SCC (squamous cell carcinoma); (e,f). (a,c,e): magnification 200 \times , scale bar 50 μm ; (b,d,f): magnification 400 \times ; scale bar 20 μm . (B) Statistical analysis of Hsp60 results. The histogram revealed a significant difference between the Healthy group and the Dysplasia and SCC groups ($\# p \leq 0.01$), as well as between the Dysplasia group and the SCC group ($* p \leq 0.01$).

3.4. Hsp90

The evaluation of tissue expression levels of Hsp90 in the examined samples exhibited an average percentage of $(22 \pm 5)\%$ in the healthy tissue group, $(60 \pm 7)\%$ in the dysplastic tissue group, and $(90 \pm 4)\%$ in the squamous carcinoma tissue group. Immunopositivity was predominantly localized within the cytoplasm across all samples, except for healthy tissues, where it was primarily observed in the basal layers of the epithelium (Figure 4A). Through a comprehensive statistical analysis encompassing healthy tissues, dysplastic tissues, and squamous carcinoma tissues (Figure 4B), a progressive increase in Hsp90 expression values was observed. Notably, a statistically significant difference ($p < 0.01$) was discerned between the healthy group and the dysplasia and squamous carcinoma groups.

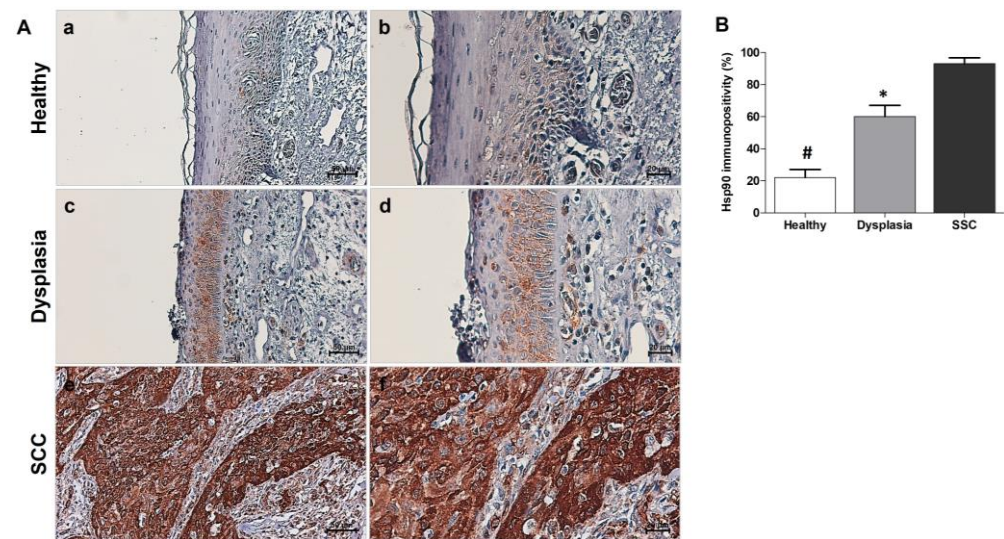


Figure 4. (A) Hsp90 immunohistochemistry. Representative images of immunohistochemical results for Healthy (a,b), Dysplasia (c,d), and SCC (squamous cell carcinoma; e,f). (a,c,e): magnification 200 \times , scale bar 50 μ m; (b,d,f): magnification 400 \times ; scale bar 20 μ m. (B) Statistical analysis of Hsp90 results. The histogram revealed a significant difference between the Healthy group and the Dysplasia and SCC groups (# $p \leq 0.01$), as well as between the Dysplasia group and the SCC group (* $p \leq 0.01$).

4. Discussion

In our morphological study, we sought to substantiate or refute the hypotheses we had posited by assessing the tissue levels of four heat shock proteins, namely, Hsp10, Hsp27, Hsp60, and Hsp90, as well as scrutinizing the immunopositivity localization for each protein across various stages of differentiation within the carcinogenic vocal cord mucosa, juxtaposed with healthy tissue samples.

Cells heavily rely on heat shock proteins for critical tasks essential to maintaining cellular homeostasis. Consequently, these proteins are ubiquitously present and fulfill indispensable roles, encompassing the facilitation of proper protein folding and the translocation, degradation, and elimination of aberrant protein aggregates [30]. These multifaceted functions render them chaperonins. Notably, their involvement transcends these primary duties and extends into other domains, including DNA replication [31], gene expression regulation [30,31], the modulation of apoptotic and autophagic processes, participation in cellular senescence [32], and the induction of an inflammatory state. Remarkably, our study unveiled an unexpected association between these proteins and the intricate processes underpinning vocal cord carcinogenesis [33]. The findings of this analyzed protein pool align with existing scientific evidence that supports HSPs playing a role in the oncogenic process and its progression [34].

In several recent studies, the implication of heat shock proteins in various mechanisms underlying the onset of different types of tumors has been hypothesized and, at times, confirmed [35–37]. Gastric, hepatic, breast, ovarian, lung, pancreatic, colorectal, squamous cell of tongue, glioma, and osteosarcoma cancers are among the many tumor variants in which the involvement of heat shock proteins has been highlighted. This implication of heat shock proteins has also been found in laryngeal squamous cell carcinoma [38–40].

Heat shock proteins seem to be involved in the carcinogenesis process either directly or indirectly. Due to the hostile tumor microenvironment, cells overexpress hsp genes in response to altered oxygen levels, nutrient and metabolite deficiencies, and significant pH shifts [31]. One of the indirect mechanisms that are commonly recognized involves cancer cells releasing extracellular vesicles that contain Hsp27 and Hsp90 into the tumor environment. These heat shock proteins interact with plasma receptors on cancer cells, triggering internal signaling pathways that promote the epithelial–mesenchymal transition (EMT) process. EMT is a significant characteristic of cancer [41]. In addition, HSPs

play a pivotal role in the regulation of extracellular matrix (ECM) remodeling and the modulation of its stiffness through their interactions with and control over a multitude of extracellular client proteins. Notably, Hsp90a, and in certain instances, Hsp60, have been identified as indispensable factors in driving the invasiveness and fostering the development of metastases in conditions such as fibrosarcoma, esophageal squamous cell carcinoma, and breast cancer cells [32]. As mentioned before, molecular chaperones can also act on a tumor level by being released into the extracellular tumor environment. This is essential to play their pro-inflammatory role, which contributes to the modulation of the tumor microenvironment. Extracellular HSPs can act as danger signals, triggering immune responses and activating various inflammatory pathways. The extracellular HSPs can activate inflammatory pathways, fostering a microenvironment conducive to tumor progression [42].

Additionally, HSPs can indirectly contribute to carcinogenesis through epigenetic interactions involving HSP receptors and translation factors such as hsf-1. Moreover, a noteworthy category of cellular pathways in which these molecules participate in the carcinogenesis process involves a set of post-translational modifications, including sumoylation, methylation, and phosphorylation, which their domains, such as the AKT domain or C-terminal domain, and their receptors undergo [33]. Moreover, another avenue through which HSPs promote tumor growth and impede therapeutic intervention lies in their role in fostering resistance to chemotherapy and radiation. Indeed, empirical observations have indicated that when tumor cells are subjected to treatments such as ionizing radiation or alkylating agents, the HSP family actively undermines the efficacy of these therapies by deploying defense mechanisms designed to safeguard the structural integrity of the cell and the integrity of its DNA [39,43].

The Hsp10–Hsp60 complex is crucial in regulating intracellular pathways related to cellular proliferation and apoptosis, and it is known that the malfunctioning of this complex can result in malignant cell transformation [38,44,45]. Furthermore, Hsp60 has been found to exhibit pro-angiogenic and pro-metastatic properties [37,46]. Hsp27 also seems to be implicated in cellular apoptosis, and its involvement in this process plays a pivotal role in cancer regulation development, progression, and metastasis [47]. Additionally, Hsp27 has been identified as a contributor to the suppression of cellular senescence, enabling tumor cells to acquire an immortal phenotype [48]. Therefore, the dysregulation of Hsp90 can have significant oncogenic implications, as it is involved in numerous processes that drive cell proliferation. It is crucial to recognize that Hsp90 has a fundamental function in tumor transformation. It regulates cell adhesion processes that are critical for the development of metastatic capabilities. Additionally, it promotes neo-angiogenesis, which is the foundation of tumor growth and increases the potential for metastasis [49–51].

The examined molecules in this study are all involved in metabolic processes that enable atypical cells to deviate from normal physiological processes. The findings of this study, combined with previous research, confirm a connection between heightened levels of heat shock proteins and disorders marked by cellular imbalance and a loss of differentiation. The immunohistochemical analysis of the study detected HSPs in the vocal cord epithelium across all three morphological states (healthy, dysplastic, and squamous carcinoma tissue), with a gradual rise in tissue levels from healthy to dysplastic and cancerous forms. The gradual and consistent increase in HSP levels is most likely a result of changes in both intra- and extracellular conditions. In healthy tissues, HSPs are found at normal levels. However, in dysplasia, there is an increase in HSPs, which suggests changes in the structure of the cell and a greater need for protein expression. Cancerous tissues exhibit even higher levels of HSPs, indicating a complete disruption of cellular homeostasis and altered cellular differentiation. Our analysis also revealed a shift in the distribution of HSPs within cells, which is consistent with previous research showing elevated levels of HSPs in many cancer pathologies. Furthermore, research has demonstrated that HSPs change their localization, moving from mitochondria to the cytoplasm and sometimes even the cellular membrane [34,35,52]. Our study revealed that Hsp10 and Hsp27 were localized

in the cytoplasm, whereas Hsp60 and Hsp90 were present in both the cytoplasm and nucleus, with Hsp10 showing nuclear localization. There exists a difference in the age range between the dysplasia and carcinoma study groups which is also normal as the onset of the cancerous state develops later than the dysplastic state. To ensure statistical significance, each group, including the control group, had ten patients to form homogeneous groups.

These findings, in line with the literature, support the role of HSPs in the development of vocal cord tissue cancer. Thus, their involvement in the processes of tissue remodeling, altered cell differentiation, angiogenesis, and apoptosis was confirmed.

5. Conclusions

The pilot study expounded upon in this report lays the groundwork by furnishing essential histopathological data, serving as a pivotal springboard for an exhaustive exploration into the intricate mechanisms governing the involvement of heat shock proteins (HSPs), specifically Hsp10, Hsp27, Hsp60, and Hsp90, in the progression of squamous cell carcinoma affecting the vocal cords. A discernible correlation has been identified between the levels of these four chaperones and the sequential advancement of malignancy, tracing its trajectory from dysplasia to fully fledged cancer.

The potential significance of these biomolecular assessments goes beyond mere observation, presenting a promising avenue for their expeditious application as indispensable biomarkers in the realms of diagnostic procedures, prognostic evaluations, and continuous patient monitoring. This reservoir of scientific evidence concurrently establishes a robust foundation, paving the way for subsequent analyses poised to validate the earlier reported outcomes. The envisaged substantiation not only augurs well for the credibility of the initial findings but also instills confidence in the prospective classification of the heat shock protein family as pivotal biomarkers, significantly contributing to the nuanced landscape of pathological evaluations and clinical diagnoses.

The data gleaned from this study propel our understanding of the dynamic interplay between heat shock proteins and the progression of vocal cord squamous cell carcinoma. The observed correlation provides a tangible link between the levels of these proteins and the evolving stages of malignancy. This nuanced insight positions heat shock proteins as potential indicators of disease severity and progression.

Furthermore, the implications extend to the clinical arena, where the identified biomarkers hold promise for refining diagnostic accuracy, prognostic assessments, and the development of targeted therapeutic strategies. As these findings are integrated into the broader scope of cancer research, the scientific community is poised to unlock new dimensions in the comprehension and management of squamous cell carcinoma, potentially paving the way for more personalized and effective treatment modalities.

In conclusion, this pilot study serves as a crucial cornerstone in unraveling the intricacies of heat shock protein involvement in vocal cord squamous cell carcinoma. The correlation established here sets the stage for a more comprehensive exploration and application of these biomarkers in clinical settings, fostering advancements that may revolutionize the landscape of cancer diagnostics and treatment.

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Institutional Review Board Statement: All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the “Comitato di bioetica”, University of Palermo; approval code: 122/2023; date of approval: 26-01-2023.

Informed Consent Statement: The cases chosen and analysed within this study were selected from the archives of the “Ospedali Riuniti VILLA SOFIA-CERVELLO” hospital in Palermo. Therefore, each individual patient had already provided informed consent at the time of surgery.

Data Availability Statement: The data presented in this study are available in article.

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