Università degli Studi di Palermo

AREA RICERCA E INNOVAZIONE
SETTORE DOTTORATI E CONTRATTI PER LA RICERCA
U. O. DOTTORATI DI RICERCA

PhD School of BIOMEDICINE, NEUROSCIENCE AND ADVANCED DIAGNOSTICS
BiND Department

SSD BIOS-07/A Biochemistry

IDENTIFICATION OF BIOMARKER IN LARYNGEAL SQUAMOUS CELL CARCINOMA: IN SEARCH OF NEW THERAPEUTIC STRATEGIES

PhD Student
Dr. Barbara Verro
0765379

Coordinator
Prof. Fabio Bucchieri

Tutor
Prof. Marianna Lauricella

XXXVIII COURSE
2025-2026

INDEX

ABSTRACT

Laryngeal squamous cell carcinoma (LSCC) is one of the most common malignancies among head and neck cancers, and is associated with a poor prognosis, although advances in diagnosis and treatment. Oxidative stress is progressively acknowledged as a key factor in carcinogenesis, causing DNA damage, epigenetic alterations, and dysregulation of cellular signaling pathways. In parallel, metabolic reprogramming has developed as a hallmark of cancer, supporting tumour growth and survival.

The aim of this study was to investigate the expression of proteins involved in oxidative stress and metabolic regulation in LSCC. Specifically, the analysis focused on superoxide dismutase (SOD), catalase (CAT), nuclear factor erythroid 2-related factor 2 (NRF2), heme oxygenase-1 (HO-1), metallothionein (MT), and vimentin (VIM), together with key markers of glucose and amino acid metabolism, such as glutamine fructose-6-phosphate amidotransferase (GFAT), glutaminase (GLS), asparagine synthetase (ASNS), and alanine-serine-cysteine transporter 2 (ASCT2).

Protein expression levels were evaluated in LSCC tissue samples using Western blot analysis in paired tumour and adjacent healthy tissues, and the results were correlated with clinico-pathological parameters. The study showed alterations in proteins involved in antioxidant defense and redox regulation, suggesting the presence of an adaptive response to oxygen stress within the tumour microenvironment. Furthermore, the upregulation of metabolic markers supports the presence of metabolic reprogramming, particularly involving glutamine metabolism and amino acid biosynthesis pathways, which may contribute to tumour progression.

Several proteins were also associated with specific clinical and pathological features, indicating a possible link between molecular alterations and tumour behaviour. Overall, these results suggest that oxidative stress and metabolic reprogramming are closely interconnected in LSCC and may contribuite to its development and progression.

In conclusion, the proteins investigated in this study could represent useful biomarkers for a better understanding of the biology of LSCC and could provide a basis for the identification of novel therapeutic targets. However, further studies involving larger patient cohorts are needed to confirm these observations and clarify their potential clinical implications.

INTRODUCTION

a. Laryngeal Squamous Cell Carcinoma

i. Epidemiology

Head and neck cancers (HNCs) are among the most common malignancies in the world and represent the seventh most common type of cancer. HNCs include several anatomical sites, including oral cavity, oropharynx, hypopharynx, larynx, salivary glands, nasopharynx, nasal cavity, sinus cavities, and ear [1]. Most of these tumours are squamous cell carcinoma (SCC) [2]. Currently, HNCs account 660,000 new cases diagnosed and 325,000 deaths annually [3]. Carcinoma of the larynx is the second most common cancer among HNCs with 177,422 new cases per year and 94,771 deaths per year worldwide [4]. In particular, LSCC accounts for one-third of all HNCs worldwide, with 12,380 new cases in the United States in 2023 [5-6-7]. Laryngeal cancer mainly affects the adult population aged between 55 and 65 years old, but cases have also been described in young adults (under 40 years of age) [8]. The LSCC occurs more frequently in men than in women, with an approximate male-to-female ratio of 4:1, a difference that is commonly linked to the higher incidence of tobacco use among males, the main recognized risk factor [9-10]. However, an increasing incidence has been reported in females, due to the increased widespread of smoking habit, being smoking habit and alcohol abuse represent the leading and main risk factors [1]. Smoking represents the principal etiological factor for LSCC: smokers have a 10- to 15-fold higher risk of developing this cancer compared with non-smokers, and heavy smokers may reach an increased risk up to 30-fold [11-12]. Alcohol consumption is also a well-established risk factor in the development of LSCC. In particular, the risk appears to increase in a dose-dependent manner, increasing by approximately 9% for every additional 10 g of alcohol consumed daily, with a marked effect observed among moderate and heavy drinkers [13-14-15]. Occupational and environmental exposures, including asbestos, textile dust, and polycyclic aromatic hydrocarbons, have been implicated as potential contributors to the development of LSCC [16-17-18]. The role of laryngopharyngeal reflux (LPR) in LSCC carcinogenesis remains under investigation, with ongoing debate over its clinical significance [19-20]. Dietary factors may also influence cancer risk. Indeed, higher fruit and vegetable consumption has been associated with a protective effect, reducing the risk of LSCC by approximately 60% compared to lower consumption levels. Conversely, increased meat consumption has been linked to a higher likelihood of LSCC, with risk increases reported of up to 67% [21]. Furthermore, some studies suggest a possible involvement of Human PapillomaVirus (HPV) infection in the development of LSCC. Several studies have

identified HPV-16 as the most frequently detected and internalized viral genotype in LSCC tissues. Its ability to encode oncoproteins that promote cell cycle progression has been well characterized [22]. In particular, HPV-16 positivity has been independently associated with the onset of LSCC, and the risk of developing LSCC appears to be higher in "never-smokers" than in "ever-smokers" [23].

The glottis is the most commonly affected laryngeal region (approximately 65-70% of cases), followed by the supraglottis and subglottis [24]. Clinically, LSCC can present with symptoms that vary depending on the anatomical site: dysphonia in the glottic tumours, dysphagia or swallowing pain in the supraglottic tumour, and dyspnea in the subglottic ones. Glottic tumours tend to be diagnosed at an early stage due to the early onset of voice disorders, while other sites often quietly progress to advanced stages.

ii. Histopathology, Treatment & Prognosis

The prognosis depends on several factors, including the tumour stage (according to the tumour-node-metastasis TNM stage system), histology, as well as the patient's health status, considering age, risk factors, performance status and socioeconomic conditions [25-26]. Regarding histology, squamous cell carcinoma (SCC) represents approximately 95% of laryngeal tumours, with a prevalence of low and moderate degrees of differentiation [27].
Biopsy with histological examination of the sample continue to be the gold standard for diagnosis. The identification of LSCC is mainly based on histopathological analysis by haematoxylin-eosin staining (H&E), considered the diagnostic gold standard. In selected cases, additional immunohistochemical analyses may be performed to better characterize the lesion or for research purposes, including markers such as p53, CD44 and EGFR (Epidermal Growth Factor Receptor) [27]. However, these markers are not routinely required for the pathological diagnosis of LSCC. The degree of tumour differentiation, evaluated histologically, remains an essential parameter for estimating tumour aggressiveness and is commonly classified as well differentiated (G1), moderately differentiated (G2) or poorly differentiated (G3). Histological confirmation of metastatic extension to the neck lymph nodes plays a key role in planning appropriate adjuvant treatment [28]. LSCC can result from several premalignant alterations, including epithelial hyperplasia, squamous metaplasia, acanthosis, keratosis, dyskeratosis, pachydermia, and varying degrees of dysplasia. Among these, dysplastic lesions are particularly relevant, as persistent dysplasia has been strongly associated with a higher likelihood of malignant progression, with a markedly higher risk of transformation in case of high-grade dysplasia. Several studies have

described in detail the histopathological progression of this neoplasm: from epithelial hyperplasia to dysplasia, to in situ carcinoma and finally to invasive form [1].

There are several surgical and non-surgical strategies to manage LSCC [29]. The choice of therapy, surgical or non-surgical, depends not only on the characteristics of the carcinoma but also on the patient in terms of age, comorbidities, preference and socio-family background. Early diagnosis provides access to a broader spectrum of potentially successful therapeutic strategies. In recent years, significant advances have transformed the therapeutic landscape of LSCC, improving patient outcomes and quality of life. Until the 1980s, the standard approach for advanced tumours consisted of total laryngectomy (TL) followed by radiotherapy (RT). Nowadays, the therapeutic strategy is guided by a multidisciplinary team (MDT) and a thorough pre-operative evaluation to ensure the most appropriate treatment plan. For early-stage (T1–T2) LSCC, organ- and function-preserving treatments, including transoral CO_2 laser surgery or RT, are preferred [30]. In cases of locally advanced disease (T3–T4), the current approach favors conservative surgical techniques (e.g., open partial laryngectomy OPHL) or their combination with chemotherapy (CT). The most commonly used chemotherapeutic agents are cisplatin and 5-fluorouracil, with newer agents such as taxanes and monoclonal antibodies like cetuximab [31]. Total laryngectomy, once routinely performed, is now reserved for patients with extensive T3–T4 tumours unresponsive to conservative strategies. Actually, TL provides excellent local disease control and favourable survival outcomes in selected patients. However, the procedure entails permanent anatomical and functional changes, including loss of natural voice, creation of a permanent tracheostoma, and potential swallowing impairment, all of which may substantially impact patients' quality of life and psychosocial well-being.

Despite public health policies against smoking and alcohol consumption, which have contributed to a reduction in the incidence of LSCC, and despite the introduction of more sensitive diagnostic techniques [32] as well as more targeted therapies, the prognosis of LSCC remains unsatisfactory. Approximately 60% of patients are diagnosed with advanced-stage disease, and the 5-year overall survival (OS) rate remains around 60%, with survival falling below 50% in advanced cases [29, 33-34]. Thus, LSCC is a major public health challenge, not only because of its relatively high incidence, but also because of the functional, psychological and social consequences associated with its treatment. Although organ preservation strategies, such as transoral CO_2 laser microsurgery and OPHL, generally provide better short-term functional outcomes, they are not without complications or limitations. In particular, OPHLs may be associated with prolonged swallowing impairment, aspiration, and delayed recovery of voice function, often requiring intensive post-operative

rehabilitation before decannulation and restoration of satisfactory oral feeding. Furthermore, conservative approaches may not be suitable for all tumour and patients, and careful patient selection is essential to ensure adequate oncological control while preserving laryngeal function. Therefore, in addition to survival, comprehensive care must include rehabilitation, psychosocial support, and personalized treatment planning to improve long-term patient-reported outcomes.

b. Oxidative Stress In Lscc

Recent evidence indicates that several pathological conditions, including metabolic disorders, neurodegenerative diseases, and cancers, are strongly associated with daily habits for example inadequate nutrition, lowered physical activity, and chronic exposure to digital technologies. A key underlying mechanism common to these conditions is oxidative stress, triggered by excessive production of reactive oxygen species (ROS) – particularly superoxide anions – that can induce molecular damage to DNA, RNA, and proteins, eventually promoting cellular dysfunction and disease progression [35].

Evidence indicates that cancer patients often exhibit reduced antioxidant capacity and elevated levels of oxidative stress prior to therapeutic treatment [36]. This redox imbalance has been shown to have significant prognostic value and may influence the selection of the most appropriate therapeutic strategy. Indeed, many conventional anticancer therapies rely on agents that stimulate ROS production to induce selective apoptotic damage in tumour cells. However, this therapeutic approach is limited by the potential substantial toxicity that also affects healthy tissues.

Normal cells, unlike tumour cells, maintain relatively stable basal ROS levels through tight regulation of reducing equivalents and antioxidant systems [37]. ROS can come from both endogenous and exogenous sources. Endogenously, they are generated as physiological by-products of cellular metabolism and, under pathological conditions, may accumulate to levels capable of damaging nucleic acids, lipids, and proteins, thus compromising cellular function. Major endogenous sources include mitochondria, peroxisomes, and inflammatory cells [38]. In inflammatory cells, ROS are generated mainly through the reduction of molecular oxygen, whereas during mitochondrial respiration most oxygen is reduced to water, with a small fraction (up to about 5%) converted into superoxide anion [38]. Additional intracellular sources include enzyme reactions catalysed by NADPH (Nicotinamide Adenine Dinucleotide Phosphate) oxidase, xanthine oxidase, lipoxygenase and cyclooxygenase. Peroxisomes contribute significantly to intracellular redox balance, as they generate substantial amounts of hydrogen peroxide and superoxide anion while

simultaneously hosting key antioxidant enzymes, such as catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD). Immune cells, including neutrophils, eosinophils, and macrophages, are also able to produce ROS through activation of NADPH oxidase as part of the inflammatory response [39]. In addition to endogenous sources, several exogenous factors can stimulate ROS production, including ionising radiation, chemical exposure, alcohol consumption, cigarette smoke, certain dietary components, infectious agents, and chemotherapeutic drugs [40]. Ionising radiation contributes to carcinogenesis by inducing DNA damage and triggering apoptotic pathways. Cigarette smoking is well known to cause genetic lesions, particularly in lung tissue, whereas alcohol consumption has been strongly associated with the development of HNC.

Under physiological conditions, low concentrations of ROS play important signalling roles by regulating cellular processes such as proliferation, migration and differentiation [41]. However, when ROS levels exceed a critical threshold, they can activate apoptotic pathways and directly contribute to neoplastic transformation through tumour initiation and promotion, proto-oncogenes activation, and inactivation of tumour suppressor or genomic stability genes [42].

Chronic inflammation represents a potential predisposing factor for the development of pre-neoplastic conditions [43]. In the setting of persistent inflammatory stimuli, excessive production of ROS and reactive nitrogen species (RNS) promotes the recruitment and activation of additional immune cells, establishing a self-amplifying cycle that improves tissue damage and promotes a pre-neoplastic state. When the burden of ROS and RNS exceeds endogenous antioxidant capacity, oxidative damage becomes irreversible, affecting nucleic acids, lipids, and proteins, and leading to genetic and epigenetic alterations that may compromise the oncogene and tumour suppressor gene function.

Oxidative stress and chronic inflammation are therefore closely interconnected processes that, if uncontrolled, can drive molecular changes that promote carcinogenesis [44]. Several studies have shown that oxidative stress interferes with multiple intracellular signalling pathways involved in cell proliferation and survival [42]. Among these, the EGFR pathway is particularly relevant. Key signalling molecules, including nuclear factor erythroid 2-related factor 2 (NRF2), the RAS/RAF cascade, MAP kinases ERK1/2 and MEK, phosphatidylinositol 3-kinase (PI3K), phospholipase C, and protein kinase C (PKC) are all modulated by ROS activity [45-46]. In addition, oxidative stress can influence the expression and function of the p53 tumour suppressor gene, which plays a central role in the induction of apoptosis.

In summary, oxidative stress not only alters gene expression and cell cycle regulation but also actively contributes to both the initiation and progression of the tumour development by modulating cellular proliferation, survival, and apoptotic balance [47-48].

Proteins are essentials biomolecules required for the proper execution of cellular functions; however, under conditions of oxidative stress, they may undergo structural modifications that impairs their activity and disrupt cellular homeostasis. Among ROS, the hydroxyl radical is considered the most damaging to DNA. ROS-induced DNA damage is a key factor in mutagenesis, genetic instability, and epigenetic alterations, ultimately contributing to tumour initiation and progression.

Antioxidants, both endogenous and exogenous, play a fundamental role in prevention and repair of ROS-induced damage. For this reason, they are often referred to as "free radical scavengers", as they neutralize these reactive species, support immune function, and reduce the risk of disease, including cancer. Major enzymatic antioxidants include SOD, GPx and CAT, which detoxify superoxide radicals and peroxides. Together, these enzymes constitute the major endogenous antioxidant defence network, essential for maintaining cellular redox homeostasis [49].

SOD represents the first line of defence against free radicals, catalysing the dismutation of the superoxide radical ($O_2^{\bullet -}$) into hydrogen peroxide (H_2O_2) [50]. The hydrogen peroxide thus generated is subsequently converted to water and molecular oxygen (O_2) by CAT or GPx. In particular, glutathione peroxidase (GPx), a selenium-dependent enzyme, catalyzes the reduction of hydrogen peroxide through the oxidation of reduced glutathione (GSH) to its oxidized form (GSSG). The latter is subsequently converted back to GSH by glutathione reductase in a reaction that requires NADPH. In addition to H_2O_2, GPx also reduces lipid and non-lipid hydroperoxides, thereby protecting cellular structures from oxidative damage [45].

In addition to enzymatic systems, non-enzymatic antioxidants – including vitamins E and C, coenzyme Q, carotenoids and glutathione – provide an additional protective barrier against ROS-induced oxidative injury [51].

i. Tobacco smoke, oxidative stress and cancer

Tobacco smoke consists of a complex mixture of chemicals, including numerous ROS and RNS, capable of damaging biological macromolecules such as lipids, proteins and nucleic acids. This creates a vicious circle in which the oxidative stress induced by smoking stimulates an inflammatory response, which in turn promotes the production of further ROS, intensifying the oxidative damage and potentially triggering the carcinogenic process.

It is important to distinguish between the oxidation mechanisms caused by ROS present directly in smoke and those induced indirectly by toxic substances contained in the smoke itself. In fact, the direct oxidative damage caused by free radicals present in the gaseous phase of smoke may be less significant than the indirect effects mediated by compounds such as aldehydes, particulates or their secondary metabolites, which are able to activate complex pro-oxidant cellular responses. Furthermore, cigarette smoke contains several carcinogens that can promote neoplastic transformation by mechanisms not necessarily related to oxidative damage, demonstrating the multiplicity of pathogenic pathways involved. Cellulose acetate is currently the most widely used material in cigarette filters [52]. This type of filter can effectively remove a significant amount of tar from the smoke [53]. However, while permitting a substantial reduction in the levels of tar and inhaled carcinogenic compounds, filtered cigarettes don't eliminate exposure to toxic and carcinogenic substances present in tobacco smoke. In daily practice, reducing the nicotine content in cigarettes can push smokers to increase their consumption to reach the same amount of nicotine on which they depend [54-55]. Additionally, filter ventilation – designed to increase airflow through the cigarette and reduce inhalation resistance – often prompts smoker to take longer, deeper puffs, thereby increasing the volume of smoke inhaled [55]. This increased amount of smoke, combined with the reduction in combustion temperature due to filter ventilation, facilitates deep inhalation and the deposition of higher concentrations of toxic and carcinogenic substances into the smoker's lungs [52,55]. The overall result is increased exposure of lung tissues to these compounds, with a possible increased risk of developing smoking-related diseases [54-55].

The classification of carcinogens by the International Agency for Research on Cancer (IARC) is divided into four groups, based on the strength of the available scientific evidence. The first group includes substances whose ability to cause cancer in humans is scientifically proven (e.g., tobacco smoke, asbestos, and alcoholic beverages). The second group is divided into 2A, which includes agents considered potentially carcinogenic (e.g., consumption of red meat), and 2B, reserved for those for which the risk can't be ruled out but trials are limited (e.g., radiofrequency electromagnetic fields). Finally, Group 3 includes compounds for which currently available information doesn't allow definitive conclusions about their carcinogenicity (e.g., caffeine).

Of the thousands of chemicals in tobacco smoke – over 7,000 – about 70 have been identified as carcinogenic or suspicious. Examples include benzo[a]pyrene, a polycyclic aromatic hydrocarbon associated with the onset of lung cancer, and 4-aminobiphenyl, known for its role in the development of bladder cancer. In addition to these molecules, free radicals

generated during tobacco combustion also have an important role in oncogenic processes, particularly in the oral cavity. These reactive compounds promote a chronic inflammatory state that, over time, may contribute to cellular transformation and the initiation of the neoplastic process in the oral mucosa.

Tobacco smoke consists mainly of two fractions: the main smoke (mainstream smoke, MS) and the side smoke (sidestream smoke, SS), both characterized by the presence of high amounts of free radicals [56-57]. The main smoke is generated directly by the act of inhalation by the smoker, passing through the filter and entering the respiratory tract [58]. The side smoke, on the other hand, is emitted by the continuous combustion of the lit cigarette between one shot and another [58]. Both types of smoke can be divided into two distinct components according to the particle size contained: a particulate phase (tar) and a gaseous phase [58-59]. The tar phase is composed of particles with a diameter between 0.1 and 1 micrometer (with an average of about 0.2 μm), while the gaseous phase comprises molecules smaller than 0.1 μm [58]. Both the gaseous and particulate components of cigarette smoke contain huge amounts of free radicals. It has been estimated that each shot can carry up to 10^{15} free radicals through the gas phase alone, while a single gram of tar can produce up to 10^{17} [60]. Among the present radicals are species centered on carbon atoms, nitrogen and oxygen, such as the radicals semiquinone, hydroxyl and superoxide [54]. The small radicals present in the gaseous phase, especially those based on oxygen and carbon, are characterized by a much higher reactivity than those contained in the particulate phase [54]. Both the gaseous and particulate components of tobacco smoke are responsible for the massive production of ROS [56,61]. The ROS present in the gaseous phase are formed directly during the combustion of tobacco and are inhaled together with the main smoke [62]. The particulate phase, on the other hand, contains relatively stable radicals such as quinone/hydroquinone complexes (Q/QH2) which bind to the tar matrix of the smoke [60]. These complexes act as real active redox systems: in the lung of the smoker, they can reduce molecular oxygen generating $O_2^{\bullet -}$, which in turn can give rise to other reactive species, such as H_2O_2 and hydroxyl radicals (•OH) [60].

It is essential to distinguish between ROS generated directly from tobacco combustion and those formed indirectly, for example from toxic or carcinogenic substances contained in smoke and their metabolites. These two sources may have different biological effects, both in terms of intensity of oxidative damage and cellular response [41]. Oxidative stress damage may be further aggravated if endogenous antioxidant systems are saturated or compromised, or when other reactive species are produced through the biotransformation of inhaled compounds.

With the accumulation of tar particles in the lungs a layer is formed which, encountering the aqueous lung environment, can trigger redox cycles capable of generating different reactive species. Phenolic compounds, quinones, heavy metals and free radicals [54] are among the main contributors to this process.

In particular, heavy metals present in cigarette smoke represent a significant source of oxidative stress in smokers [63]. Tobacco leaves absorb these metals – including chromium, cadmium, arsenic, beryllium and nickel – from the soil as plants grow [64]. Many of them are classified as group 1 carcinogens by the IARC [65]. Some metals, such as chromium, nickel, iron and copper, are active in redox reactions and generate ROS through mechanisms similar to the Fenton reaction, favouring the formation of the hydroxyl radical •OH [54]. The latter is highly reactive and able to interact directly with DNA, causing oxidative lesions such as single-strand breaks. If not properly repaired, such ruptures may be mutagenic [63]. Single-strand breaks are often associated with a nucleotide gap in the damaged area [66]; during the repair process, incorrect insertion of a base can introduce permanent mutations into the genome [67].

In our body there are numerous mechanisms of antioxidant defence that regulate the levels of ROS, ensuring cellular redox balance. These systems include both enzymatic and non-enzymatic components. The most important antioxidant enzymes include SOD, GPx and glutathione S-transferases (GST) [68-69].

A study by Orhan et al. [70] showed a significant reduction in the activity of SOD and GPx in the red blood cells of smokers compared to non-smokers. This suggests that the high production of ROS associated with smoking may overload these enzymes, compromising their functional availability. In addition, the expression of GPx in smokers varies according to the type of tissue: in patients with smoking-related Chronic Obstructive Pulmonary Disease (COPD), an overregulation at the level of the pulmonary epithelium has been observed, but a reduction in blood compartments such as plasma and erythrocytes [71].

In addition to directly generating ROS, some metals found in tobacco smoke – including chromium, nickel, iron and copper – can interfere with the body's antioxidant defences. These metals, through redox cycles in the presence of H_2O_2, contribute to the formation of additional ROS, intensifying the oxidative load [72-73].

When antioxidant systems are compromised or saturated, levels of ROS are no longer adequately controlled, increasing the risk that key molecules such as proteins, lipids and nucleic acids will be damaged. This type of oxidative stress can alter vital cellular functions and contribute to the onset of chronic diseases [72-73].

When cells undergo oxidative damage, one of the first biological events that occurs is the release of arachidonic acid from the cell membrane [74]. This step is important for activating the inflammatory response, as arachidonic acid is converted by specific enzymes – such as cyclooxygenases (COX-1 and COX-2) and lipoxygenase (LPO) – into pro-inflammatory mediators, including prostaglandins and leukotrienes [75]. These signalling molecules attract neutrophils and subsequently macrophages to the site of tissue damage. Once activated, the macrophages release inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α), interleukin-1 (IL-1) and interleukin-8 (IL-8), which help recruit additional immune cells, feeding an amplification cycle of the inflammatory response and progressively increasing levels of cytokines and chemokines [74].

This mechanism of activation underlies a self-amplifying loop linking tobacco-induced oxidative stress to the inflammatory response, both of which play a central role in the process of carcinogenesis. The substances contained in the smoke can damage cellular and subcellular components [76], activating transcription factors such as NF-κ B and other molecules involved in the regulation of inflammation [76]. In turn, inflammatory processes stimulate the production of ROS, RNS and other reactive compounds, which intensify oxidative stress and cause ever-increasing molecular damage. This vicious cycle, in which inflammation and oxidative damage feed on each other, can contribute decisively to the beginning and progression of the neoplastic process [76].

ii. Alcohol consumption, oxidative stress and cancer

As early as 1988, the IARC classified alcoholic beverages as group 1 carcinogens, which means agents whose ability to cause cancer in humans is proven. Initially, the causal correlation was established for cancers of the oral cavity, pharynx, larynx, oesophagus and liver [76-78]. The World Cancer Research Fund (WCRF), through its continuous update project (Continuous Update Project), also assesses the association between dietary factors, physical activity and cancer risk, conducting meta-analyses based on the quality of epidemiological evidence. The latest report on diet, nutrition, physical activity and cancer strongly confirmed the association between alcohol consumption and increased risk for numerous cancers including oral, pharyngeal, laryngeal, esophageal (SCC), liver, colon-rectal and breast (postmenopausal), possibly also correlating with pre-menopausal tumours of the stomach and breast [79]. Once ingested, alcohol is metabolised by several enzymes including alcohol dehydrogenase (ADH), cytochrome P-450 2E1 (CYP2E1) and bacterial catalase leading to the production of acetaldehyde [80], a highly reactive compound known for its carcinogenic and genotoxic properties. Acetaldehyde can bind to DNA forming

adducts that alter its structure and hinder the synthesis and repair of genetic material [81]. These adducts are particularly dangerous because they can cause point mutations, double-strand breaks, exchanges between sister chromatids and chromosomal structural alterations [81]. Both ethanol and acetaldehyde also interfere with DNA methylation processes, with potential effects on the expression of oncogenes and tumour suppressor genes [81]. Another mechanism by which alcohol contributes to carcinogenicity is the induction of oxidative stress, considered a key factor in the onset of disease [82]. The increased activity of CYP2E1 during ethanol metabolism is a major source of ROS, which are produced in large quantities during the conversion of ethanol to acetaldehyde. This enzyme is particularly overexpressed in the esophagus of heavy drinkers [83]. Other intracellular sources of ROS associated with alcohol metabolism include the mitochondrial respiratory chain and some cytosolic enzymes [84]. ROS not only cause direct damage to DNA but can also alter cell cycle processes. They act as messengers in different intracellular signalling pathways, activating transcription factors such as NF-κ B and promoting cell proliferation and metastatic progression through modulation of MAP kinases, in addition to increasing the expression of Vascular Endothelial Growth Factor (VEGF) and Monocyte Chemoattractant Protein-1 (MCP-1), both involved in angiogenesis [85]. Inflammation, closely associated with tumour progression, is further amplified by chronic alcohol consumption. Alcohol promotes the recruitment of monocytes and macrophages in the tumour microenvironment, where these cells release pro-inflammatory cytokines – such as Tumor Necrosis Factor alpha (TNF-α), Interleukin-1 (IL-1), IL-6 and IL-8 – which activate ROS-producing enzymes [85]. The same cytokines activate Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB), generating a self-feeding cycle of oxidative stress and chronic inflammation.

c. Pathogenesis & Epigenetic Changes

LSCC pathogenesis is not yet fully understood and requires further investigation. One of the main hypotheses involves the role of oxidative stress in causing DNA damage by exceeding the cellular antioxidant capacity [86]. Numerous studies have highlighted the pivotal role of ROS in several processes underlying malignancy, such as tumorigenesis, dysregulated cell growth, metastatic spread, and angiogenesis. [87-88].

Under physiological conditions, intracellular redox homeostasis is maintained through a dynamic balance between the generation of ROS and the activity of antioxidant defence systems. When ROS production exceeds cellular antioxidant capacity, oxidative stress occurs. This disequilibrium may impair the function of antioxidant enzyme and cause DNA damage, contributing to genomic instability. In this context, ROS-induced damage to cellular

macromolecules has been strongly implicated in carcinogenesis [86,89]. Although antioxidant enzymes typically act to neutralize free radicals and protect cell health, their function may be impaired under conditions of persistent oxidative stress [89-90-91-92-93].

Recent findings regarding the mutational profile of LSCC have highlighted the involvement of multiple key molecular pathways in its tumorigenesis. Among the most significantly affected processes are those regulating cell survival and proliferation (e.g., TP53 and EGFR), cell cycle control (e.g., CDKN1A), and cell differentiation (e.g., NOTCH1) [94].

Among the several molecular elements studied for their diagnostic and therapeutic potential, non-coding RNAs (ncRNAs) have emerged as central regulators, attracting increasing scientific attention due to their wide range of biomedical applications. These molecules are closely linked to epigenetic mechanisms that can influence gene expression without altering the DNA sequence itself [95]. In the context of HNC, including LSCC, tumour development is driven by an intricate interaction of genetic and epigenetic alterations. In particular, DNA methylation has been identified as an important epigenetic mechanism involved in tumour initiation and progression, due to its tissue specificity, reversibility, and susceptibility to environmental factors [96].

As observed in many solid malignancies, the progression and invasiveness of LSCC are strongly influenced by a pro-inflammatory tumour microenvironment (TME) [97]. A tight interplay exists between inflammation, innate immune responses, and tumour development, driven by physiological and pathological mechanisms that are activated in response to tissue injury. These processes involve numerous chemical mediators that stimulate mast cell activation and promote the migration of leukocytes – such as neutrophils, monocytes and eosinophils – from the bloodstream to injured tissues. It is now widely recognised that cell proliferation, when supported by a microenvironment rich in inflammatory cells, pro-inflammatory mediators, growth factors, activated stroma and agents promoting DNA damage, can facilitate tumour onset and progression. These mediators include cytokines, chemokines, COX-2, prostaglandins, fibroblast growth factor (FGF), metalloproteases (MMP) and VEGF [97]. Among the pro-inflammatory cytokines, IL-6, together with IL-1 and TNF-α, plays a central role in the acute inflammatory response and is also involved in the maintenance of chronic inflammation, thereby contributing to the establishment of tumour-promoting microenvironment. IL-6 is secreted by different cell types, including B and T lymphocytes, macrophages, fibroblasts, keratinocytes and cancer cells, and regulates both proliferative and apoptosis processes.

Finally, it should be emphasised that the immune system, particularly in the early stages of tumour development, can exert a protective role through the recognition and elimination of

immunogenic cancer cells by cytotoxic effector cells such as CD8+ T lymphocytes and natural killer (NK) cells [98].

d. Oxidative Stress Biomarkers

i. Superoxide dismutase

SOD represents a primary defence system against ROS-induced cellular damage. Superoxide anions, generated through enzymatic signalling pathways or as a metabolic by-product of mitochondrial respiration, are converted by SOD into molecular oxygen and hydrogen peroxide. The resulting hydrogen peroxide can then participate in various downstream cellular processes [35]. Three forms of superoxide dismutase have been identified in humans: Cu/Zn-SOD (SOD1), distributed mainly in the cytoplasm and nucleus [99]; Mn-SOD (SOD2), located within the mitochondrial matrix [100]; and extracellular SOD (SOD3), present outside the cell [101]. Among these, the Cu/Zn and Mn isoforms play the most relevant role in antioxidant protection [102]. Although numerous studies have investigated the role of the antioxidant enzyme SOD in tumour processes, its function in carcinogenesis remains controversial and appears to vary depending on the anatomical district considered.

Regarding squamous cell carcinoma of the oral cavity (OSCC), a recent systematic review that analyzed 1,147 patients with OSCC and 1,058 healthy subjects highlighted a significant reduction in mean SOD activity levels in several samples of cancer patients compared to controls ($p < 0.05$) [103]. This decrease could be explained by multiple factors: the excessive oxidative stress due to the accumulation of ROS, an inadequate production of antioxidant enzymes, the accelerated consumption or degradation of SOD caused by the ROS themselves and, more generally, the reduced antioxidant capacity typical of the tumour microenvironment.

However, some studies have reported opposite results, noting an increase in SOD activity in OSCC patients [104-105]. Such increment has been interpreted as an adaptive or compensatory response of the cell, aimed at counteracting the excessive production of $O_2^{\bullet -}$ generated by the intense oxidative stress and lipid peroxidation associated with the tumour.

In this context, Fu et al. [106] observed that high MnSOD expression was positively related to better disease-specific survival compared to patients with low expression levels ($p = 0.009$) in buccal mucosal squamous cell carcinomas. In particular, overexpression of MnSOD was associated with more favorable prognoses in patients with moderately or poorly differentiated tumours ($p = 0.045$), in clinical stage I ($p = 0.002$) and in those undergoing post-operative adjuvant RT ($p = 0.048$).

The study by Strycharz-Dudziak et al. evaluates the activity of the antioxidant enzymes GPx and SOD in oropharyngeal carcinoma associated with Epstein-Barr Virus (EBV) and HPV coinfection, finding a significant reduction in enzyme activity in patients with coinfection compared to those with single EBV or HPV infection [107]. These alterations suggest increased oxidative stress, potentially implicated in tumour initiation and progression. Furthermore, the work emphasizes how oncogenic virus infection is associated with a state of chronic inflammation, during which epithelial and inflammatory cells produce ROS and RNS, as well as cytokines and growth factors. These biochemical events can induce DNA damage and dysfunction in critical cell signaling pathways, thus facilitating carcinogenesis and tumour progression.

The study by Liu et al. [108] evaluates the role of MnSOD (SOD2) in the progression of tongue squamous cell carcinoma (TSCC), focusing on the molecular mechanisms regulating cell migration and invasion. In their previous work, the authors demonstrated that SOD2 expression is markedly elevated in cases of TSCC presenting neck node metastasis [109]. This increase suggests a potential link between elevated SOD2 levels and metastatic behavior in TSCC, denoting its usefulness as a prognostic indicator of metastatic involvement. Based on this assumption, in the new paper, the authors highlight that MnSOD overexpression significantly increases H_2O_2 production in tumour cells, a reactive metabolite produced by the dismutation of superoxide ions. The intracellular increase in H2O2 acts as a redox signal that induces the expression of the transcription factor Snail, which, in turn, reduces the expression of epithelial markers (such as E-cadherin) and increases the expression of mesenchymal markers (such as vimentin) [110]. Functionally, TSCC cells with overexpressed MnSOD show a marked increase in migratory and invasive capacity in in-vitro assays, confirming the contribution of this redox pathway to tumour progression. Inhibition of H_2O_2 production or Snail silencing significantly reduced these effects, underscoring the key role of the H_2O_2-Snail signal in the pro-tumour action of MnSOD.

The paper by Parascandolo et al. [111] highlights the crucial role of EcSOD or SOD3 in the microenvironment of papillary thyroid carcinoma (PTC), particularly in tumour-associated mesenchymal/stromal stem cells (MSCs). SOD3 expression in these stromal cells influences extracellular redox signals, modulating tumour cell proliferation and migration. The authors propose a dynamic model in which SOD3 performs different functions during the different phases of tumorigenesis. At an early stage, with moderate levels of RAS pathway activation (less than a tenfold increase compared to normal), SOD3 appears to promote tumour growth by promoting a regulated oxidative environment [112]. However, when SOD3 expression

reaches non-physiological and toxic levels, cancer cells reduce autocrine SOD3 production to avoid direct harmful effects. Simultaneously, paracrine secretion of SOD3 by surrounding stromal cells is stimulated [113]. This paracrine secretion of SOD3 continues to support tumour cell growth, but also alters interactions with the tumour stroma. Indeed, reducing the expression of chemotactic cytokines reduces the affinity of tumour cells for stroma, promoting their local migration within the tumour. This mechanism may facilitate the spread and progression of thyroid cancer. In summary, SOD3 acts as a key modulator of the TME with a biphasic role: it promotes growth in the early stages and, subsequently, through the regulation of paracrine secretion and interactions with the stroma, contributes to tumour migration and progression [114-115].

The study by Li et al. [116] analyzes the role of MnSOD or SOD2 in nasopharyngeal carcinoma (NPC), focusing on its ability to mediate resistance to anoikis, a mechanism of apoptosis induced by loss of cell adhesion to the substrate, that is critical for the metastatic process. The authors observed that MnSOD overexpression in NPC cells significantly increases their survival under detachement conditions, as demonstrated by suspended culture cell viability assays, in which cells with upregulated MnSOD show a marked reduction in anoikis compared to controls ($p < 0.01$). This protection is attributable to MnSOD's ability to reduce intracellular oxidative stress by dismutating superoxide to hydrogen peroxide, thereby maintaining mitochondrial integrity and preventing the release of apoptotic signals. Consequently, tumour cells with high MnSOD expression show increased migratory and invasive capacity in migration and invasion assays (40-60% increase compared to cells with normal or silenced MnSOD, $p < 0.05$). Thus, MnSOD acts as a key regulator of tumour aggressiveness in NPC and represents a possible therapeutic target for strategies intended to counteracting anoikis resistance and reducing metastasis.

Only a limited number of studies have specifically evaluated SOD activity in LSCC [117-118-119]. Overall, evidence suggests a modulation of SOD, particularly cytosolic isoforms, in tumour tissues compared to adjacent non-neoplastic mucosa. This variation is generally interpreted as part of a complex cellular response to oxidative stress, in which SOD supports maintaining redox balance and survival of neoplastic cells, potentially reflecting an adaptive response of tumour cells to increased oxidative stress. However, the results aren’t entirely consistent, and the precise role of SOD in the process of carcinogenesis and LSCC progression isn’t fully and clearly defined.

ii. Catalase

CAT is an antioxidant enzyme that converts hydrogen peroxide into water and oxygen, serving a crucial role in defence against oxidative stress [120]. In solid tumours, its expression shows wide variability: it is often reduced compared to healthy tissues, as reported in hepatocellular and bladder cancer, promoting a pro-oxidant microenvironment that supports proliferation and invasiveness [121-122]. Conversely, catalase overexpression, associated with resistance to pro-oxidant treatments, has been described in some tumours such as breast cancer [123-124-125]. This heterogeneity suggests that catalase may have a dual role in tumorigenesis and represents a possible therapeutic target [126]. Wang et al. [127] analysed 53 thyroid tumour samples (both benign and malignant) against matched healthy thyroid tissue, evaluating a range of antioxidant molecules via immunohistochemistry and western blots, including MnSOD, thioredoxin reductase, glutathione peroxidase, and CAT. While markers such as MnSOD, thioredoxin reductase, and glutathione peroxidase were generally overexpressed in neoplastic tissues compared to normal thyroid parenchyma, CAT showed a significant reduction ($p = 0.0398$). Such an opposite behaviour, not yet fully elucidated, could reflect the function of catalase as the main enzyme responsible for the detoxification of hydrogen peroxide: the excessive production of H_2O_2, typical of the neoplastic thyroid, would cause functional overload, resulting in enzymatic consumption or inactivation. This hypothesis was supported by in vitro experiments, in which the exposure of thyroid cells to H_2O_2 reproduced the same pattern of CAT reduction and increase as the other antioxidants.

The only study to date available in the literature on CAT expression in LSCC tissue is that of Durak et al [117]. The authors showed that CAT activity is significantly increased in cancerous laryngeal tissues compared to neighbouring non-cancerous ones ($p < 0.025$). This increase could represent an adaptive response of tumour cells aimed at counteracting high oxidative stress, given that catalase is involved in the degradation of hydrogen peroxide, thus helping to reduce free radical-induced damage.

iii. Nuclear factor erythroid 2-related factor 2

NRF2 represents a central regulator of the cellular antioxidant defence system, modulating the transcription of a wide range of genes involved in protection against oxidative damage. Under conditions of oxidative stress, NRF2 dissociates from its cytoplasmic inhibitor Keap1 (Kelch-like ECH-associated protein 1) and translocates into the nucleus. There, it interacts with antioxidant response elements (AREs) located in the

promoter regions of target genes, such as HO-1, thus inducing their expression. In cancer, its overexpression has been linked to increased resistance to CT and an overall worse clinical outcome [128]. Menegon et al. reported the dual role of NRF2 in cancer, acting as a tumour suppressor under physiological conditions by transiently activating antioxidant and detoxifying genes that prevent DNA damage and malignant transformation, but functioning as an oncogene when persistently activated in tumour cells, where it enhances antioxidant capacity, supports anabolic metabolism, and promotes resistance to oxidative stress and CT [129]. Consistent with this oncogenic role, Alakuş et al. demonstrated that high NRF2 expression was an independent predictor of poor OS in patients with gastric adenocarcinoma [128]. Similarly, Cheraghi et al. demonstrated that deletion of NRF2 in colorectal cancer cells reduced the expression of detoxifying and mitochondrial proteins, thereby increasing oxidative stress and sensitizing the cells to chemotherapeutic agents, supporting the notion that sustained NRF2 activity can protect tumour cells from oxidative damage and therapy-induced cell death [130]. Jana et al. demonstrated that in A549 lung cancer cells, characterized by high basal NRF2 activity, this transcriptional factor directly activates the p21 gene. Contrary to its canonical cell cycle arrest role, p21 acts here as a protective factor that promotes cell survival under H_2O_2 - induced oxidative stress. This mechanism is independent of the p53 protein and suggests that the Nrf2-p21 axis contributes to tumour resistance to oxidative stress-based therapies [131].

Some studies have explored the involvement of the NRF2/KEAP1 pathway in LSCC, highlighting its alteration in tumour tissues compared to normal ones [7, 132-133]. Overall, NRF2 activation appears associated with the oxidative stress response and possible adaptation mechanisms of neoplastic cells. This modulation suggests a possible role for the pathway in supporting tumour cell survival and contributing to disease progression processes, as well as response to treatments.

iv. Heme oxygenase-1

The heme oxygenase (HO) acts as a cellular stress sensor and key regulator of redox balance [1]. This enzyme degrades heme producing carbon monoxide, ferrous iron, and biliverdin, later converted to bilirubin. In humans there are two isoforms: HO-1, inducible by numerous physiological and pathological stimuli, and HO-2, expressed constitutively [134]. HO-1 has protective effects on cells, thanks to its antioxidant, antiapoptotic and anti-inflammatory functions. However, its overexpression in tumours appears to provide a selective advantage to neoplastic cells, allowing them to cope with the high oxidative stress typical of carcinogenesis and cancer therapies [135]. Numerous studies have documented an

increment of HO-1 expression in most solid tumours, including head and neck carcinomas. In particular, in OSCC the enzyme is almost absent in healthy tissues, slightly increased in adjacent tissues and strongly expressed in tumour samples [136-137]. However, the evidence remains controversial: some studies correlate low HO-1 levels with the presence of lymph node metastases and poorly differentiated histotypes [136], while others report an association between high expression of the enzyme and metastases, without significant variations with respect to the degree of differentiation [138]. Gandini et al. [139] reported a distinct pattern of HO-1 localization in head and neck squamous cell carcinoma (HNSCC). Nuclear HO-1 expression was absent in normal tissues, weak in tumour-adjacent epithelium, and markedly increased in malignant cells, indicating that altered intracellular localization of HO-1 may represent a feature of tumour transformation. This localization appears to increase with tumour progression and is more marked in tumours with aggressive biological behaviour. In parallel, experimental evidence suggests that HO-1 inhibition may represent a promising therapeutic strategy. In particular, Yang et al. [140] demonstrated that HO-1 inhibitors induce cell cycle arrest and reduce tumour growth in thyroid carcinoma models, suggesting potential interest in other HNCs as well.

Only a limited number of studies have investigated the role of HO-1 in LSCC [141-142]. Overall, evidence suggests that HO-1 may be involved in modulating cellular responses to oxidative stress and in influencing tumour cell behaviour, including sensitivity to therapy. Some findings indicate a potential association between HO-1 expression levels and tumour characteristics, although results are not entirely consistent. So, these findings suggest a possible involvement of HO-1 in the biological and clinical features of laryngeal carcinoma, but its exact role remains to be fully clarified.

v. Metallothionein

In addition to classical antioxidant enzymes, other redox-responsive proteins have emerged as important modulators of oxidative stress and tumour progression. Among these, MTs play a key role in metal homeostasis, redox regulation, and cellular protection against oxidative damage. MTs are low molecular weight, cysteine-rich, ubiquitously expressed proteins. The MT1 and MT2 isoforms, present in all eukaryotes, are induced by metals, oxidative stress, cytokines and glucocorticoid hormones. MTs play an important role in carcinogenesis, inhibiting apoptosis through modulation of proteins such as p53, B-cell lymphoma 2 (Bcl-2) and c-Myc, as well as through caspase inhibition. They regulate cell proliferation through the control of zinc homeostasis, which activates specific transcription factors [143]. In addition, they influence angiogenesis by modulating factors such as FGF,

Transforming Growth Factor-beta (TGF-β), and VEGF, and contribute to tumour resistance by protecting cells from oxidative stress and sequestering chemotherapy drugs. The expression of MTs varies according to the degree of tumour differentiation, metastatic potential, and prognosis [144]. The study by Brazão-Silva et al. highlights that metallothionein gene expression is markedly altered in oral carcinoma compared to normal tissues. These changes appear to be related to the tumour's ability to metastasize, denoting a role for MT in disease progression and aggression [143]. Bezerra et al. analyse MT expression by paying attention to its nuclear localization in OSCC cells. The authors highlight how high nuclear expression of MT is associated with more aggressive features of the neoplasm, such as larger tumour size and presence of neck node metastases. Furthermore, elevated nuclear MT expression has been reported to correlate with decreased survival in patients with OSCC. These results indicate that nuclear MT may play an active role in tumour progression and that its presence may represent a potential negative prognostic marker in this type of carcinoma [145]. The article by Li et al. explores the specific role of MT1M isoform of metallothionein in esophageal carcinoma. According to the study, MT1M acts as a suppressor of carcinogenesis in these tumour cells. In particular, MT1M inhibits the epithelial-mesenchymal transition (EMT) process, a key mechanism that promotes tumour migration and invasion. Furthermore, MT1M negatively modulates the SOD1/PI3 K axis, a signalling pathway involved in cell proliferation and survival. These combined impacts lead to a reduced in the growth and metastatic capacity of esophageal carcinoma cells [146].

Some studies have investigated the role of MTs in LSCC, finding that their overall expression is higher in tumour tissues than in healthy ones [40, 147-148]. Overall, evidence suggests a possible involvement of MTs in cell proliferation processes and tumour progression, as well as their association with characteristics of increased aggressiveness. Furthermore, some data indicate that genetic variants of MT-encoding genes may also be related to personal susceptibility to disease development, pointing to a potential role both as biological markers and as factors implicated in laryngeal carcinogenesis [149].

vi. Vimentin

In addition to antioxidant and stress-sensitive enzymes, oxidative stress also influences cytoskeletal remodelling and EMT, processes critical for tumour invasion and metastasis. In this context, VIM serves as a key downstream marker of the functional consequences of redox imbalance. VIM is a type III intermediate filament protein expressed predominantly in fibroblasts, endothelial cells, and lymphocytes. It is a key structural component of the cytoskeleton, playing a role in cell shape maintenance and providing

anchoring sites for the nucleus and intracellular organelles. In addition to its architectural role, VIM is also involved in essential cellular processes, including migration, differentiation, proliferation, adhesion, and invasion. Its expression is closely associated with EMT, a key event in tumour invasion and metastasis. Indeed, in cancer, VIM not just marks the mesenchymal phenotype but can also be found in the extracellular space, where it modulates angiogenesis and suppresses immune responses within the TME, thus limiting lymphocyte infiltration [150]. Elevated VIM expression has been associated with tumour aggressiveness, therapeutic resistance, and poor clinical outcomes in various malignancies, including HNSCC [151], breast cancer [152], and colorectal cancer [153]. In gastric cancer, a meta-analysis confirmed that elevated VIM expression correlates with advanced tumour stage, lymph node metastasis, and reduced OS, highlighting its role as a negative prognostic marker [154]. Furthermore, studies on polyploid giant tumour cells have shown that vimentin filaments drive persistent cell migration, a key factor in tumour invasiveness and metastasis, and that VIM inhibition substantially reduces this migratory capacity [155].

The expression of VIM in LSCC has been the subject of few studies [156-157], which showed an increase in tumour tissues compared to normal ones. Overall, this finding suggests an association between VIM and more aggressive clinical features, indicating a possible role for the protein as a prognostic indicator of worst tumour behaviour in LSCC (Figure 1).

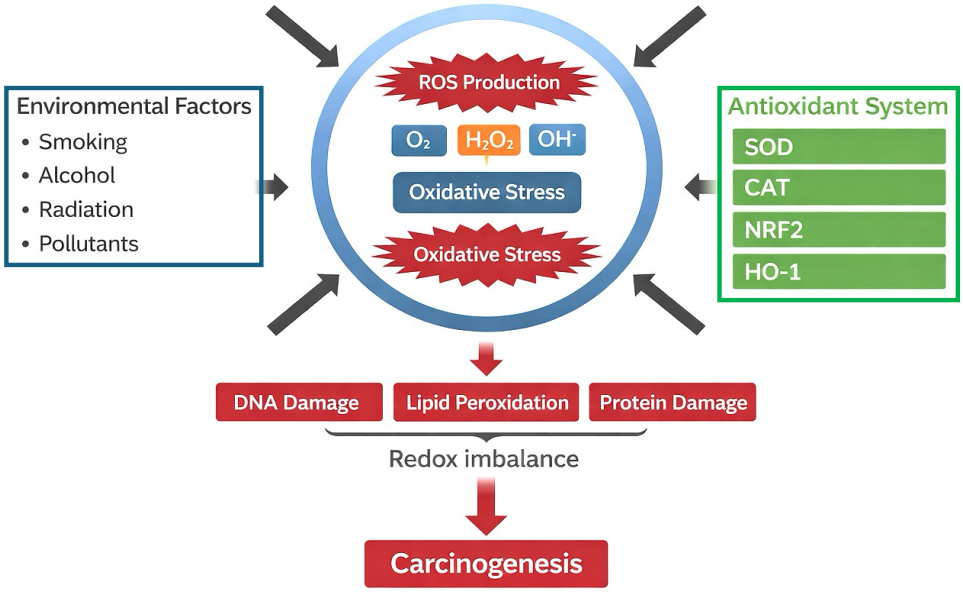


Figure 1 Schematic representation of the mechanisms of oxidative stress in laryngeal carcinogenesis. *Environmental factors promote the production of reactive oxygen species (ROS), leading to oxidative damage to DNA, lipids, and proteins. The imbalance between ROS and antioxidant defence systems contributes to tumour initiation and progression.*

e. Metabolism And Metabolic Reprogramming

The concept that cancer cells undergo profound metabolic adaptations is not new. As early as 2008, Kroemer and Pouysségur had proposed that tumour cell metabolism represents "cancer's Achilles' heel", noting that metabolic remodelling is not a passive consequence of transformation yet an active process that supports tumour growth and survival [158]. This pioneering study established the basis for the contemporary concept of metabolic reprogramming, now widely recognized as a hallmark of cancer. Metabolic reprogramming reflects the ability of malignant cells to alter nutrient uptake and intracellular metabolic fluxes to sustain proliferation, redox homeostasis, and survival under difficult microenvironmental conditions [159]. Indeed, through genetic and epigenetic alterations that impact oncogenes and tumour suppressors, tumour cells acquire metabolic plasticity that allows them to engage in alternative energy and biosynthetic pathways in response to cellular demand. In HNSCC, metabolomic profiling revealed extensive remodelling of central metabolic pathways, including glycolysis, the pentose phosphate pathway (PPP), the tricarboxylic acid cycle, and particularly amino acid metabolism [160]. These alterations are accompanied by accumulation of metabolites such as lactate, succinate, and glutathione, which promote tumour growth, epigenetic plasticity, and microenvironmental interaction.

In LSCC, recent evidence suggests that mitochondrial metabolism is not simply complementary to glycolysis but plays an active role in the tumour metabolic strategy: LSCC cells appear capable of integrating oxidative and glycolytic metabolism to provide energy, biosynthetic intermediates, and maintain redox balance [161]. Furthermore, Zheng et al. [162] identified two metabolic subtypes of laryngeal cancer, each associated with divergent prognoses and distinct molecular features (e.g., differences in methylation, mutational landscape, and immune infiltration), underscoring the intrinsic metabolic heterogeneity of LSCC.

At the same time, metabolic reprogramming extends its influence on the immune microenvironment, molding the activation and function of infiltrating immune cells and promoting an immunosuppressive environment [163]. The interaction between tumour metabolism and antitumour immunity suggests that manipulation of metabolic pathways may enhance immunotherapeutic efficacy.

Taken together, current evidence supports a model in which LSCC is characterized by metabolic plasticity, molecular heterogeneity, and strong interactions with the immune microenvironment. Analysis of these mechanisms could detect novel prognostic biomarkers and metabolic vulnerabilities for precision therapeutic interventions.

i. Carbohydrate metabolism

PPP is a key branch of glucose metabolism that supplies NADPH and ribose-5-phosphate (R5P) to cells. While NADPH provides reducing power for biosynthetic reactions and protection against oxidative stress, R5P act as a key precursor for nucleotide and nucleic acid synthesis. Therefore, PPP plays a dual role: it supports both anabolic metabolism and redox homeostasis, processes that are particularly vital for rapidly proliferating cells, such as tumours.

The rate-limiting enzyme of this pathway, glucose-6-phosphate dehydrogenase (G6PD), catalyses the oxidation of glucose-6-phosphate to 6-phosphogluconolactone, generating NADPH in the process.

Beyond its classical metabolic role, G6PD has surfaced as a vital regulator of cell growth and stress response. In tumour cells, which are often exposed to high levels of ROS and have increased biosynthetic demands, upregulation of G6PD secures a continuous supply of NADPH and anabolic precursors, allowing survival under metabolic stress. Zhang et al. [164] highlighted that G6PD is consistently overexpressed in a wide variety of tumours, including those of the liver, breast, lung, colon and skin [165-166-167-168]. This overexpression is related to tumour growth, invasiveness, resistance to apoptosis, and chemoresistance. The authors proposed that G6PD not only acts as a biomarker of malignancy but also function as a possible therapeutic target, as its inhibition can limit NADPH production, compromise antioxidant defences, and limit nucleotide synthesis – ultimately suppressing tumour proliferation. In agreement, Li et al. [169] provided an updated and comprehensive review, demonstrating that G6PD expression is tightly controlled by multiple key oncogenic and tumour suppressor pathways, including PI3K/Akt, p53, and NRF2, which modulate both metabolic reprogramming and redox balance. The review also demonstrated that G6PD inhibition sensitizes tumour cells to oxidative stress and CT, underscoring its value as a therapeutic target in redox- and metabolism-based cancer therapy. Interestingly, Cheng et al. [170] reported a context-dependent aspect of G6PD activity in NPC: patients with tumours showing low G6PD activity had a significantly worse prognosis, suggesting that insufficient NADPH production may impair DNA repair and antioxidant defences, leading to genomic instability and more aggressive tumour behaviour. These finding highlight that the clinical significance of G6PD depends on tissue and context, and that both positive and negative regulation may impact tumour progression. Overall, these studies' results emphasize that G6PD is a central metabolic element in cancer, integrating anabolic metabolism, redox balance, and proliferation, and influencing clinical outcomes.

Its dual role as a metabolic driver and potential prognostic marker makes it an attractive target for cancer therapy, particularly for strategies aimed at disrupting metabolic adaptations of tumour cells (Figure 2).

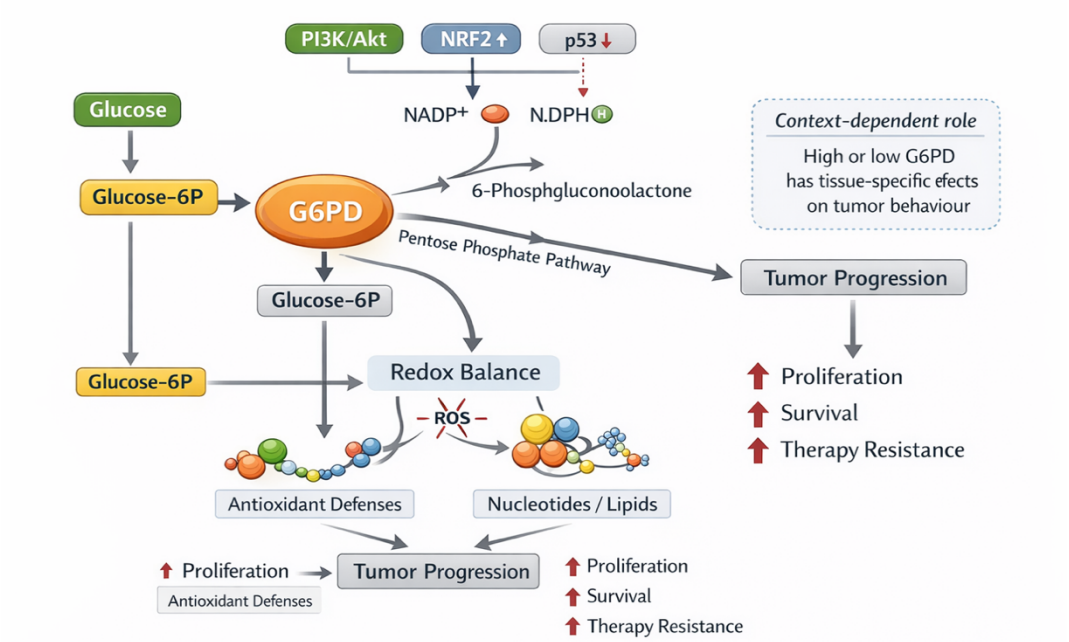


Figure 2 G6PD-driven metabolic reprogramming in tumour cells.
Glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the oxidative branch of the pentose phosphate pathway (PPP), catalyes the conversion of glucose-6-phosphate into 6-phosphogluconolactone, generating NADPH. This reducing equivalent is essential for maintaining redox homeostasis, limiting reactive oxygen species (ROS), and supporting antioxidant defenses, while supporting anabolic processes such as nucleotide and lipid biosynthesis. G6PD activity is tightly regulated by major oncogenic and tumour suppressor pathways, including PI3K/Akt, NRF2, and p53, which link metabolic flux to cell signaling. In tumour cells, upregulation of G6PD promotes proliferation, survival, and resistance to therapy by improving both biosynthetic capacity and tolerance to oxidative stress. In particular, the role of G6PD may be context-dependent, as variations in its activity may differentially influence tumour behavior depending on tissue type and metabolic status. Overall, G6PD acts as a central metabolic hub integrating redox balance, anabolic metabolism, and tumour progression.

The study by Dessì et al., published in 1990, represents the only available evidence evaluating G6PD activity in LSCC [171]. The authors evaluated enzymatic activity in both tumour and adjacent normal tissues from patients with and without G6PD deficiency using a spectrophotometric assay. G6PD activity was significantly higher in tumour tissues than in normal tissues in both groups ($p < 0.05$), including G6PD-deficient subjects. These results suggest increased enzymatic activity in LSCC, regardless of the underlying deficiency. This pioneering study provided fundamental evidence of the potential involvement of G6PD in the pathophysiology of LSCC and pointed out the need for additional research to explore its role in tumour development and as a possible therapeutic target.

ii. Amino acid metabolism

In recent years, amino acid metabolism has become a central element in tumour biology. In addition to providing the precursors for protein synthesis, amino acids support multiple processes critical to the survival of neoplastic cells, including energy production, biomolecule synthesis, and maintenance of redox homeostasis [172-173]. In particular, glutamine represents a key energy and biosynthetic source for tumour cells, as it provides carbon and nitrogen to produce nucleotides, lipids and other amino acids. Many tumours exhibit "glutamine dependence", a phenomenon that promotes growth and resistance to conditions of metabolic stress. Furthermore, glutamine contributes to the regulation of the TME, influencing immune cells function and inflammatory response [172]. In HNCs, recent studies have presented a marked reprogramming of amino acid metabolism, with increased expression of transporters and enzymes involved in glutamine uptake and use [174-175]. These metabolic alterations have been associated with increased tumour aggressiveness, increased invasive capacity, and a worse prognosis. Furthermore, it has been hypothesized that amino acid metabolism contributes to maintaining an immunosuppressive microenvironment, thereby promoting disease progression [174].

In the large solute carrier (SLC) family, the alanine-serine-cysteine transporter 2 (ASCT2) represents an important mediator of glutamine uptake into tumour cells [176]. In this context, glutaminase 1 (GLS1), the enzyme that catalyzes the conversion of glutamine to glutamate, is often upregulated in HNSCC and has been associated with worse disease-free survival [177]. A recent study by Zhang et al. [178] found that ASCT2 and GLS mRNA expression levels were significantly upregulated in HNSCC tissues compared to adjacent normal counterparts. This transcriptional upregulation was reflected at the protein level, as demonstrated by immunohistochemical analyses evaluating ASCT2 and GLS expression in tumour samples. Furthermore, in a cohort of 124 HNSCC patients undergoing surgical resection, Kaplan–Meier analysis showed that higher ASCT2 and GLS expression were significantly associated with reduced OS. These findings show that increased glutamine uptake and metabolism are not only hallmark metabolic traits of HNSCC but also have prognostic significance. Additional exploration highlighted that ASCT2 levels were significantly associated with patient sex ($p = 0.006$) and tumour anatomical subsite ($p < 0.001$) and that higher ASCT2 expression was related to more aggressive disease characteristics, including a poorer degree of differentiation ($p = 0.002$) and a higher incidence of lymphovascular invasion ($p = 0.017$). Similar results in terms of GLS overexpression in HNSCC samples compared to normal tissues and its positive correlation

with tumour grade were reported by Basheer et al. [175]. Although glutamine metabolism has been recognized as a key hallmark of cancer, studies precisely addressing the role of GLS and glutamine transporters such as ASCT2 in HNSCC are still limited.

Glutamine:fructose-6-phosphate amidotransferase (GFAT) acts as a rate-limiting enzyme of the hexosamine biosynthetic pathway (HBP), a metabolic pathway that links glucose and glutamine metabolism to produce UDP-N-acetylglucosamine, an essential substrate for protein glycosylation. Through this pathway, GFAT plays a central role in regulating cell signaling, transcriptional activity, and metabolic adaptation [179]. Accumulating evidence suggests that dysregulation of HBP and GFAT activity contributes to pathological processes, including cancer progression, by modulating metabolic and inflammatory responses [180]. In particular, GFAT has been implicated in the fine regulation of cellular responses to metabolic stress, linking nutrient availability to downstream regulatory mechanisms.

Asparagine synthetase (ASNS) is a key enzyme in amino acid metabolism, responsible for synthesizing asparagine from aspartate and glutamine. Its expression is upregulated under conditions of cellular stress, particularly in response to nutrient deprivation, through activation of the integrated stress response (ISR) and the transcription factor ATF4. By maintaining intracellular asparagine levels, ASNS supports protein synthesis and contributes to cellular adaptation to metabolically stressful environments [181] (Figure 3).

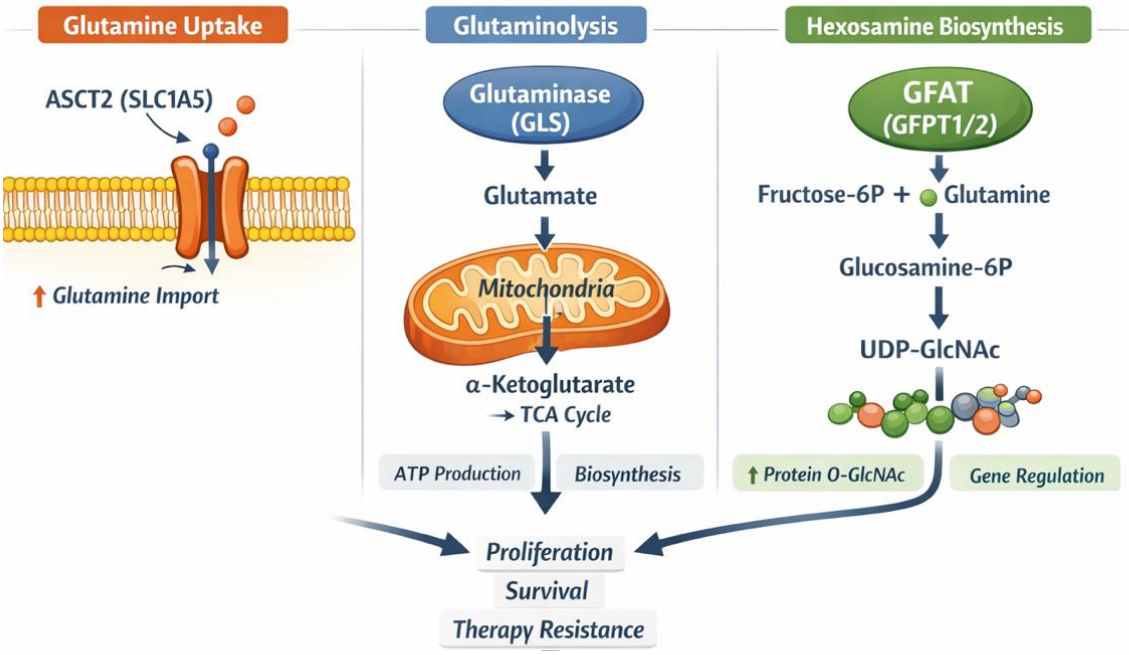


Figure 3 Glutamine metabolism in cancer cells and its role in metabolic reprogramming.
The glutamine transporter ASCT2 (SLC1A5) mediates increased glutamine uptake in cancer cells. Intracellular glutamine is then directed toward two major pathways: glutaminolysis, driven by glutaminase (GLS), which converts glutamine into glutamate to fuel the tricarboxylic acid (TCA) cycle and support energy production and biosynthesis; and the hexosamine biosynthetic pathway (HBP), initiated by GFAT (GFPT1/2), leading to UDP-GlcNAc production and enhanced protein O-GlcNAcylation and gene regulation. Together, these pathways contribute to metabolic reprogramming, promoting tumour cell proliferation, survival, and therapy resistance.

RESEARCH PROJECT TARGETS

An effective diagnostic pathway and precision therapeutic strategy for LSCC require a complete understanding of the disease's natural history and the main clinical and molecular factors that influence its progression. Despite advances in diagnostics and treatment, the pathogenesis of LSCC remains incompletely understood, underscoring the need for further investigation. Among the proposed mechanisms, oxidative stress has appeared as a key factor in tumour development. Oxidative stress occurs when the production of ROS – generated by mitochondria and by external factors such as ionizing radiation – exceeds the cell's antioxidant capacity. This imbalance can damage DNA and other cellular macromolecules, causing genomic instability and promoting carcinogenesis. Conversely, excess ROS can also exert cytotoxic effects, potentially inducing tumour cell death. In parallel, growing evidence indicates that metabolic reprogramming represents a key hallmark of LSCC, involving alterations in glucose metabolism (e.g., G6PD and the PPP), amino acid metabolism (particularly glutamine), lipid metabolism, and ferroptosis-related pathways. These metabolic adaptations are closely interconnected with redox homeostasis, supporting tumour growth, survival, and resistance to therapy.

Over the past 40 years, the 5-year survival rate for LSCC has remained largely unchanged, decreasing only slightly from 66% to 61%, despite a decline in overall incidence. Factors contributing to this stagnation include local tumour recurrence and the development of distant metastases, which often resist conventional therapies. These challenges underscore the urgent need for novel prognostic biomarkers to improve disease management and guide the design of more effective therapeutic strategies. In line with this objective, the present research project focused on the analysis of oxidative stress-related proteins in LSCC tissue compared to adjacent healthy tissue. Specifically, we studied six key proteins involved in redox homeostasis and cellular defence mechanisms: SOD, CAT, HO-1, NRF2, MT and VIM. SOD and CAT are primary antioxidant enzymes that detoxify superoxide radicals and hydrogen peroxide, respectively. HO-1 and NRF2 mediate cytoprotective responses to oxidative stress, while MT contributes to metal ion homeostasis and free radicals scavenging. Furthermore, this study is framed in the wider context of tumour metabolic reprogramming. In particular, G6PD, the rate-limiting enzyme of the PPP, plays a key role in NADPH production, thus helping to redox balance and supporting anabolic processes such as lipogenesis. Moreover, the availability of NADPH is able to enhance antioxidant defences, counteracting oxidative damage and possibly limiting cell death mechanisms such as ferroptosis. In parallel, glutamine metabolism supports tumour bioenergetics by fueling the

tricarboxylic acid (TCA) cycle and supports biosynthetic pathways, including nucleotide synthesis, thus promoting cell proliferation and survival.

By characterizing the expression of antioxidant and redox-related proteins (including SOD, CAT, HO-1, NRF2, and MT), together with key metabolic enzymes such as G6PD and proteins related to glutamine metabolism, in LSCC, within the wider framework of redox regulation and metabolic reprogramming, this study aims to provide information on the molecular mechanisms underlying oxidative stress-driven carcinogenesis and tumour progression, as well as their interaction with cellular metabolism. Furthermore, it seeks to identify potential prognostic biomarkers that may improve clinical management and guide future therapeutic strategies. These molecular insights may help not only improve diagnostic accuracy as well as advance targeted therapeutic approaches.

MATERIAL AND METHODS

The study was designed as a prospective, observational, paired case–control investigation involving patients diagnosed with LSCC and treated at the Otolaryngology Unit of the University Hospital "Paolo Giaccone" in Palermo starting from November 1, 2023. Ethical approval was obtained from the Institutional Ethics Committee (protocol no. 05/2023), and all participants provided written informed consent in accordance with the principles outlined in the Declaration of Helsinki.

Patients were included if they were 18 years of age or older and had a histologically confirmed diagnosis of LSCC. Both male and female patients were eligible for enrolment. No restrictions were applied regarding tumour stage, and patients with any TNM classification were considered eligible for inclusion.

Patients were excluded from the study if they had a history of RT, CT, or immunotherapy involving the head and neck region. Additional exclusion criteria included contraindications to surgery under general anaesthesia, histological diagnoses other than squamous cell carcinoma, the presence of multiple primary malignancies, impaired decision-making ability precluding informed consent, and pregnancy.

a. Clinical Evaluation

i. Patients' data collection

After patients' enrolment, demographic data (sex, age, occupation), behavioural habits (smoking and alcohol consumption), and comorbidities (e.g., diabetes mellitus, LPR, previous malignancies) were collected.

Regarding tobacco use, patients were classified as non-smokers, light smokers (<20 cigarettes/day), or heavy smokers (≥20 cigarettes per day). This limit reflects the typical content of a standard cigarette pack and provides a practical and clinically relevant distinction.

Alcohol consumption was classified as non-drinkers, <4 drinks/day, or ≥4 drinks/day. Each standard beverage was considered equivalent to approximately 14 g of ethanol [182]. This approach minimizes variability related to differing definitions of standard alcohol units across studies, ensuring consistency and comparability.

Tumour characteristics were also recorded, including anatomical site (supraglottic, glottic, subglottic, transglottic, pharyngo-laryngeal), histological grade (G1, well-differentiated; G2, moderately differentiated; G3, poorly-differentiated), and clinical and/or pathological TNM

stage (cTNM/pTNM) according to the Union for International Cancer Control (UICC) TNM Classification of Malignant Tumours, 9th edition [183]. In addition, HPV and Programmed Death-1 Ligand 1 (PD-L1) expression level in laryngeal sample, and serum C-reactive protein (CRP) levels (cut-off <5 mg/L) were evaluated.

CRP levels were measured once in the pre-operative setting, in the absence of active inflammatory or infectious conditions, to reflect baseline inflammatory status and avoid confounding effects related to surgical stress.

HPV status was assessed by using p16 immunohistochemistry as a surrogate marker for transcriptionally active high-risk HPV infection: negative (<50% diffuse moderate-to-strong nuclear and cytoplasmic staining), equivocal (50-69% diffuse and moderate-to-strong nuclear and cytoplasmic staining), and positive (≥70% diffuse and moderate-to-strong nuclear and cytoplasmic staining) [184].

PD-L1 expression was assessed by immunohistochemistry using the Dako PD-L1 IHC 73-10 assay and quantified according to the Combined Positive Score (CPS). Patients were stratified into the following categories: CPS <1; CPS ≥1, which qualifies for first-line treatment with pembrolizumab; and CPS ≥20, a threshold that, although indicative of higher expression, does not independently determine eligibility for first-line pembrolizumab monotherapy [185].

Treatment modalities were documented, including surgical approach – such as transoral laser microsurgery, partial or total laryngectomy with or without neck dissection – as well as RT and CT, administered either as standalone treatments or in the adjuvant setting.

ii. Sample harvesting

Following patient enrolment, laryngeal specimens were obtained during surgery performed under general anaesthesia, either via transoral or open approaches. For each patient, both tumour tissue (LSCC, case) and adjacent macroscopically normal laryngeal mucosa (control) were collected, allowing for a paired analysis and minimizing interindividual variability. All collected tissue specimens were bisected under sterile conditions; one portion was fixed in formalin and processed for histopathological evaluation to confirm that control samples were histologically normal and free from dysplastic or neoplastic changes. To reduce potential bias related to anatomical heterogeneity of the laryngeal epithelium, both tumour and control samples were obtained from the same anatomical region of the larynx. Dry samples were initially stored at -20 °C for short-term preservation and subsequently transferred to -80 °C for long-term storage until further analysis.

b. Biochemical Evaluation

i. Protein extraction from tissue samples

Aliquots of frozen tissue samples (0.02 – 0.10 g) were repeatedly washed with phosphate-buffered saline (PBS) and homogenized on ice in radioimmunoprecipitation assay (RIPA) buffer containing 1× PBS, 1% NP-40, 0.1% sodium dodecyl sulfate (SDS), 5 mmol/L EDTA, and 0.5% sodium deoxycholate. The buffer was supplemented with protease and phosphatase inhibitors, including 0.01% aprotinin, 10 mM sodium pyrophosphate, 2 mM sodium orthovanadate, and 1 mM phenylmethylsulfonyl fluoride (PMSF). The homogenates were then centrifuged to remove cellular debris, and the resulting supernatants were collected and stored at -80 °C until further analysis.

ii. Western blot

After sonication (Soniprep 150, Cellai Srl, Milan, Italy), protein concentrations were determined using the Bradford Protein Assay (BioRad, Hercules, CA, USA). Equal amounts of protein (30 µg) were denaturated at 95 °C for 5 minutes, separated by SDS-PAGE, and transferred onto nitrocellulose membranes by electroblotting.

Membranes were incubated overnight at 4 °C with primary antibodies against superoxide dismutase (SOD, 25 kDa; Santa Cruz Biotechnology, Dallas, TX, USA), catalase (CAT, 60 kDa; Sigma-Aldrich, St. Louis, MO, USA), heme-oxygenase 1 (HO-1, 33 kDa; Biorbyt, Cambridge, UK), metallothionein (MT, 14 kDa; GeneTex, Alton Pkwy Irvine, CA, USA), vimentin (VIM, 50 kDa; Santa Cruz Biotechnology, Dallas, TX, USA), and nuclear factor erythroid 2-related factor 2 (NRF2, 61 kDa; Novus Biologicals, Centennial, CO, USA), glucose-6-phosphate dehydrogenase (G6PD, 58 kDa; Cell Signaling Technology, Danvers, MA, USA), alanine-serine-cysteine transporter 2 (ASCT2, 49 kDa; Cell Signaling Technology, Danvers, MA, USA), glutaminase (GLS, 55-65 kDa; Cell Signaling Technology, Danvers, MA, USA), glutamine:fructose-6-phosphate amidotransferase (GFAT, 80 kDa; Cell Signaling Technology, Danvers, MA, USA), asparagine synthetase (ASNS, 64 kDa; Cell Signaling Technology, Danvers, MA, USA).

After washing, membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Promega, Milan, Italy) for 1 hour at room temperature. Protein bands were detected by enhanced chemiluminescence (ECL) and quantified with a ChemiDoc imaging system (Bio-Rad Laboratories, Hercules, CA, USA), followed by densitometric analysis (Quantity One software, version 4.6.6). Protein expression levels were normalized

to the housekeeping protein mucin-1 (MUC-1, 122 kDa; Santa Cruz Biotechnology, Dallas, TX, USA). All experiments were conducted in triplicate.

c. Statistical Analysis

Descriptive statistics were expressed as numbers and percentages or mean ± standard deviation (SD).

Data are presented as median values. Paired comparisons of expression levels between LSCC samples and adjacent healthy ones were performed using the Wilcoxon signed-rank test. Associations between protein expression levels and clinical parameters were analysed using Spearman's rank correlation coefficient for continuous variables (e.g., age and CRP) and the Kruskal–Wallis test for categorical variables. Statistical significance was indicated as follows: $*p<0.05$, $**p<0.01$, $***p<0.001$.

RESULTS

a. Clinical Data

A total of 35 patients were included in this project work. Recruitment was conducted according to the predefined inclusion criteria, as described in the Materials and Methods section. The study population was composed by 28 (80%) males and 7 (20%) females with an average age of 62.11 ± 8.71 years old (range 43 – 79).

As regards exposure to the main risk factors: 8 (22.86%) patients are former smokers and 25 (71.43%) patients are still smokers with consumption of 30.78 ± 20.63 cigarettes per day on average (range 6 – 100); 13 (37.14%) patients drink alcohol daily, of which only 6 are heavy drinkers, 1 patient is former alcohol abuser. Most patients (74.29%) had no history of LPR. In 16 (45.71%) patients, the serum CRP was found to be higher than 5, which is an indicator of inflammation (Table 1).

Table 1. Characteristics of recruited patients

Characteristics	N (%)
Gender	
Female	7 (20)
Male	28 (80)
Age (years)	
Mean ± SD	62.11 ± 8.71
< 59 years old	9 (25.71)
≥ 59 years old	26 (74.29)
By gender	
Female (7)	57.86 ± 9.70
Male (28)	63.18 ± 8.31
Range	43 – 79
Risk factors	
Tobacco use	
Non-smoker	2 (5.71)
Smoker < 20 cigarettes/day	4 (11.43)
Smoker ≥ 20 cigarettes/day	21 (60)
Former smoker	8 (22.86)
Mean cigarettes/day ± SD	27.24 ± 18.18
Alcohol abuse	
Non-drinker	21 (60)

Drinker < 4 drinks/day	7 (20)
Drinker ≥ 4 drinks/day	6 (17.14)
Former drinker	1 (2.86)
Mean drinks/day ± SD	4.54 ± 3.12
LPR	
Yes	9 (25.71)
No	26 (74.29)
Family history of cancer (mother, father, sisters, brothers)	
Yes (lung, breast, prostate, melanoma, lymphoma, gallbladder)	13 (37.14)
No	22 (62.86)
CRP (mg/L)	
Cut-off: < 5 mg/L	
< 5	19 (54.29)
≥ 5	16 (45.71)
Total	35 (100)
SD: standard deviation; LPR: laryngo-pharyngeal reflux; CRP: C-reactive protein	

Thus, 80 samples (35 patients) have been collected for this project work: 41 LSCC specimen (cases) and 39 samples of the adjacent laryngeal health tissue (controls). Table 2 summarizes the characteristics of LSCC samples, including laryngeal subsite involvement, pTNM stage, PD-L1 and p16 expression levels, and degree of differentiation. The majority of patients presented with glottic tumours (21), followed by supraglottic (n = 5), transglottic (n = 5), and pharyngolaryngeal (n = 4) SCC. Combined CPS was ≥ 1 in 19 cases and only 2 biopsies expressed p16 marker. Most patients underwent surgery as follows: 8 patients underwent transoral laser microsurgery (TLM), 12 patients underwent OPHL, and 13 patients underwent TL. Two patients did not undergo surgical treatment and were managed with definitive chemoradiotherapy (CRT) with curative intent. Tumour specimens and paired healthy adjacent mucosal tissue were obtained from pre-treatment diagnostic biopsies and included in the analysis. Of these 33 patients, 9 underwent adjuvant therapy: 2 RT and 7 CRT (Table 2). The follow-up (up to 01/10/2025) lasted on average 23.49 ± 8.62 months (range 2.59 – 34.53); three patients died during follow-up due to metastases.

Table 2. Features of laryngeal squamous cell carcinoma	
Parameters	**N (%)**
Laryngeal involvement	
Supraglottic SCC	5 (14.29)
Glottic SCC	21 (60)
Trans-glottic SCC	5 (14.29)
Pharyngo-laryngeal SCC	4 (11.42)
pT	
Cis	1 (2.85)
T1	4 (11.43)
T2	14 (40)
T3	11 (31.43)
T4a	5 (14.29)
pN	
Nx	8 (22.86)
N0	18 (51.43)
N1	3 (8.57)
N2b	1 (2.85)
N2c	2 (5.72)
N3b	3 (8.57)
PD-L1	
CPS < 1	4 (11.43)
CPS ≥ 1	19 (54.28)
CPS ≥ 20	5 (14.29)
Miss data	7 (20)
+p16	
Negative	29 (82.85)
Equivocal	0 (0)
Positive	2 (5.72)
Miss data	4 (11.43)
Degree of differentiation	
G1	2 (5.72)
G2	24 (68.57)
G3	4 (11.43)
Miss data	5 (14.29)
Therapy	
Surgery	
TLM (cordectomies, ESL)	8 (22.86)

OPHL	12 (34.28)
TL	13 (37.14)
Definitive CRT	2 (5.72)
Adjuvant therapy	
RT	2 (5.72)
CT	0 (0)
CRT	9 (25.71)
Specimens (35)	
Supraglottis	19 (54.28)
Glottis	10 (28.58)
Subglottis	3 (8.57)
Trans-glottic	3 (8.57)
Total	35 (100)

SCC: squamous cell carcinoma; TNM: Tumour-Nodes-Metastasis; PD-L1: Programmed Death-1 Ligand 1; CPS: Combined Positive Score; TLM: transoral laser microsurgery; ESL: endoscopic supraglottic laryngectomy; OPHL: open partial horizontal laryngectomy; TL: total laryngectomy; RT: radiotherapy; CT: chemotherapy; CRT: chemoradiotherapy

b. Biochemical Data

Analysis of protein expression between LSCC tissue and adjacent healthy tissue showed a significant increase for most of the proteins analyzed (Table 3, Figures 4, 5 and 6). The differential expression of all investigated proteins between LSCC and paired adjacent non-tumour tissues remained statistically significant after Benjamini–Hochberg correction for multiple comparisons, supporting the reliability of the observed alterations in redox- and metabolism-related pathways.

In particular, SOD, CAT, HO-1, MT, VIM and ASNS showed a highly significant difference ($p < 0.001$), with median values significantly higher in LSCC tissue than in in adjacent normal tissue.

NRF2 and G6PD were also significantly increased ($p < 0.01$), suggesting an involvement in oxidative stress response mechanisms and tumour cellular metabolism.

A more moderate but still significant increase was observed for ASCT2 and GLS ($p < 0.05$), indicating a possible activation of amino acid metabolism in neoplastic cells.

Interestingly, GFAT showed a highly significant difference ($p < 0.001$), although with a less marked increase than other proteins, suggesting a possible role in tumour metabolic regulation.

Overall, these findings support the hypothesis of a marked alteration of redox and metabolic pathways in tumour tissue compared to adjacent normal tissue.

Table 3. Comparison of protein expression levels between LSCC and adjacent healthy tissues (Wilcoxon Paired Analysis with Benjamini–Hochberg FDR correction)

Protein	N° samples analyzed	LSCC (median)	N-LSCC (median)	Raw p-value	FDR-adjusted p-value	Significance
SOD	24	1.67	0.625	8.34×10^{-7}	9.17×10^{-6}	***
CAT	25	1.3	0.74	4.06×10^{-5}	1.12×10^{-4}	***
HO-1	18	1.435	0.595	2.29×10^{-5}	8.40×10^{-5}	***
MT	16	1.44	0.6	4.22×10^{-4}	6.63×10^{-4}	***
VIM	15	1.67	0.36	6.10×10^{-5}	1.34×10^{-4}	***
NRF2	14	1.41	0.665	0.00188	0.00259	**
G6PD	25	1.42	0.72	0.00807	0.00986	**
GFAT	20	0.64	0.53	0.00014	0.000257	***
ASCT2	23	1.15	0.47	0.0285	0.0285	*
GLS	23	0.56	0.47	0.0149	0.0164	*
ASNS	26	1.095	0.78	2.10×10^{-5}	8.40×10^{-5}	***

* $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns = not significant

FDR: false discovery rate; LSCC: laryngeal squamous cell carcinoma; SOD: superoxide dismutase; CAT: catalase, HO-1: heme-oxygenase 1; MT: metallothionein; VIM: vimentin; G6PD: glucose-6-phosphate dehydrogenase; ASCT2: alanine-serine-cysteine transporter 2; GLS: glutaminase; GFAT: glutamine:fructose-6-phosphate amidotransferase; ASNS: asparagine synthetase

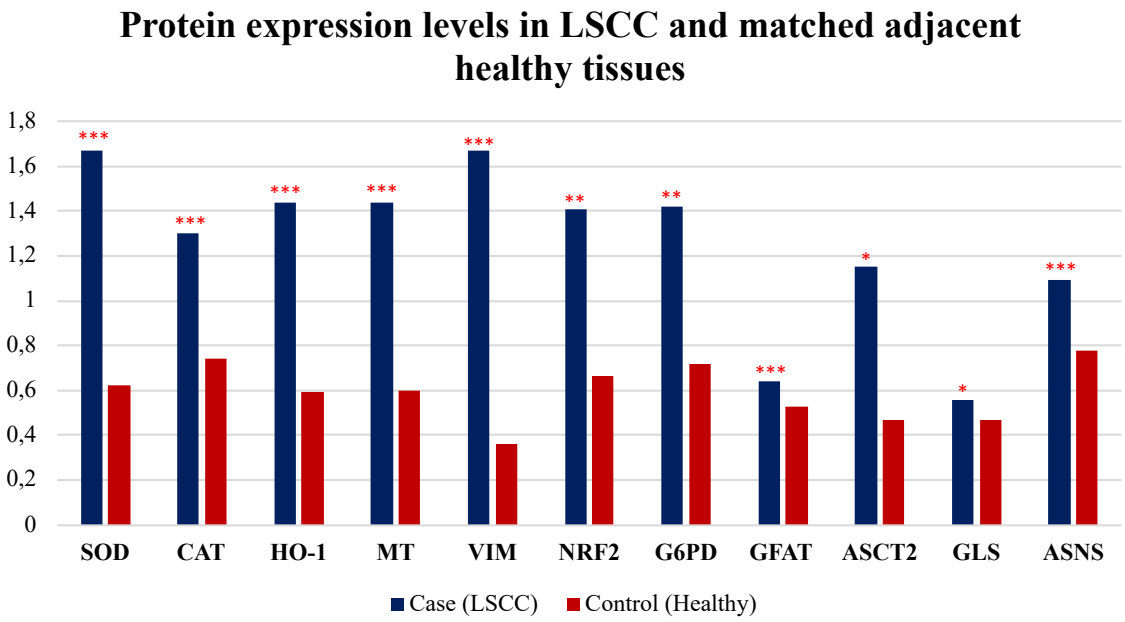
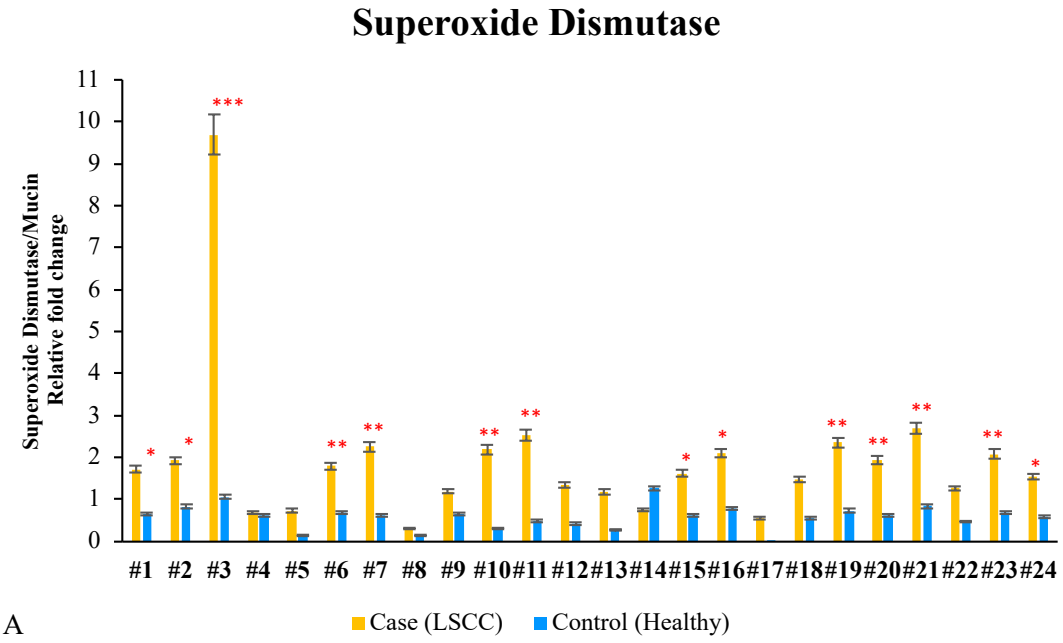
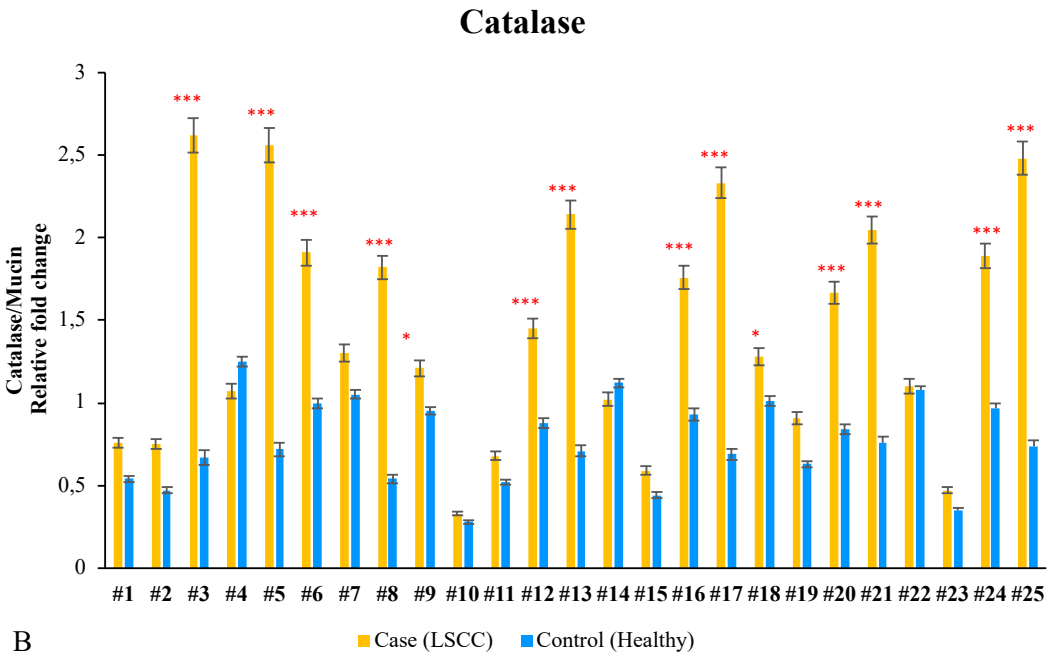


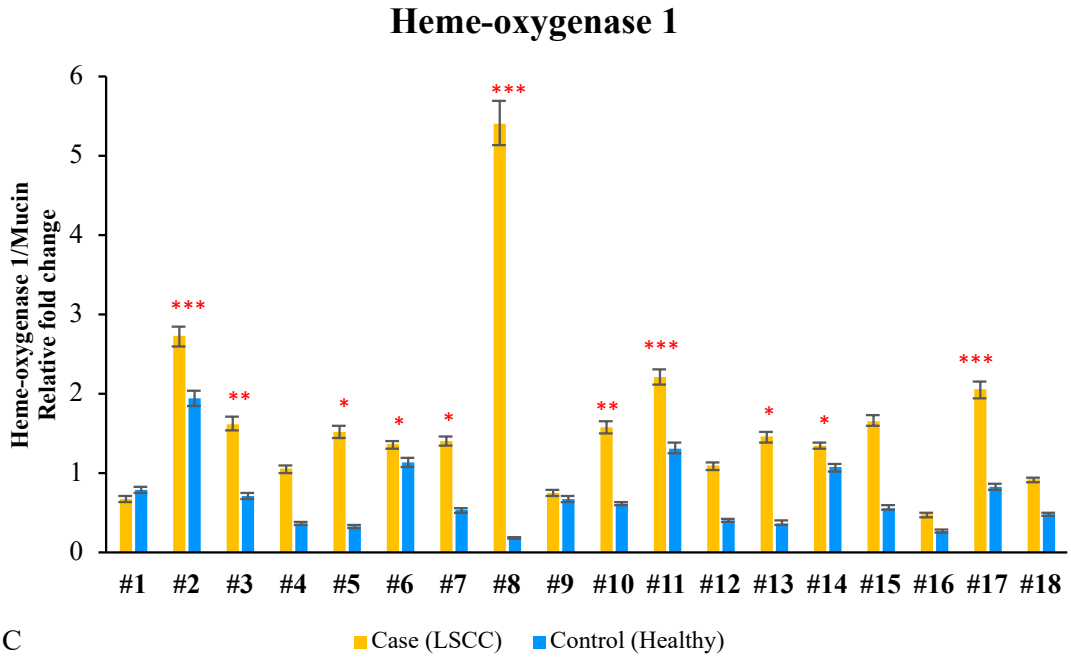
Figure 4 Comparison of protein expression levels between LSCC (blue) and adjacent healthy (red) tissues.
*Data are presented as median values. Differences between paired samples were evaluated using the Wilcoxon signed-rank test. Statistical significance is indicated as follows: *p < 0.05, **p < 0.01, ***p < 0.001. Superoxide Dismutase (SOD), Catalase (CAT), Heme-oxygenase 1 (HO-1), Vimentin (VIM), Nuclear factor erythroid 2-related factor 2 (NRF2), Metallothionein (MT), glucose-6-phosphate dehydrogenase (G6PD), glutamine:fructose-6-phosphate amidotransferase (GFAT), alanine-serine-cysteine transporter 2 (ASCT2), glutaminase (GLC), asparagine synthetase (ASNS).*



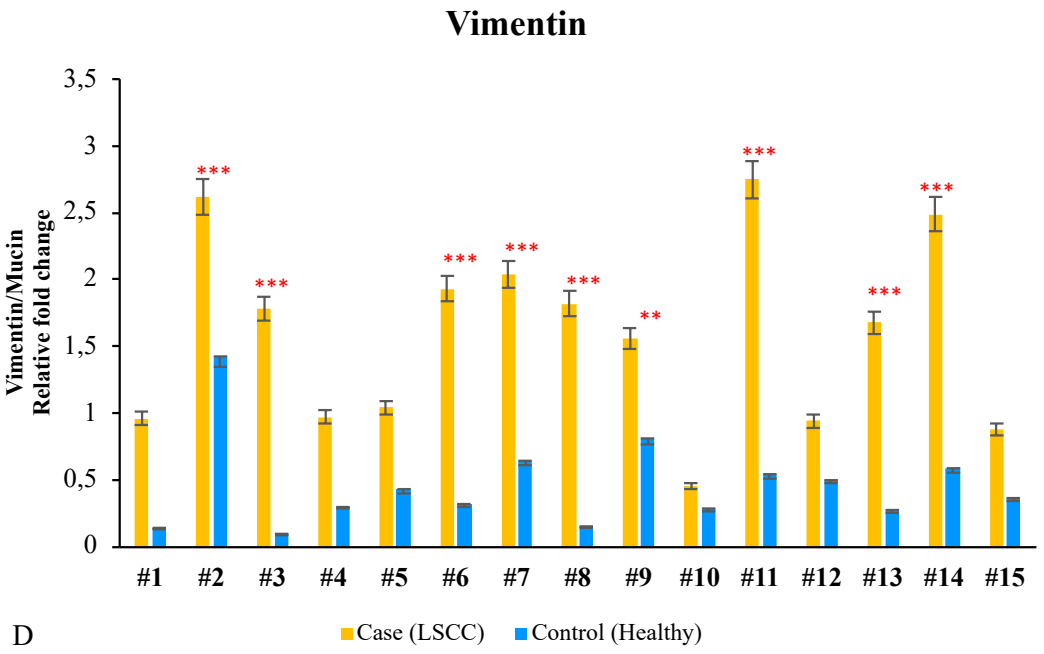
A


Catalase
Catalase/Mucin
Relative fold change
3
2,5
2
1,5
1
0,5
0
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12 #13 #14 #15 #16 #17 #18 #19 #20 #21 #22 #23 #24 #25
Case (LSCC)
Control (Healthy)

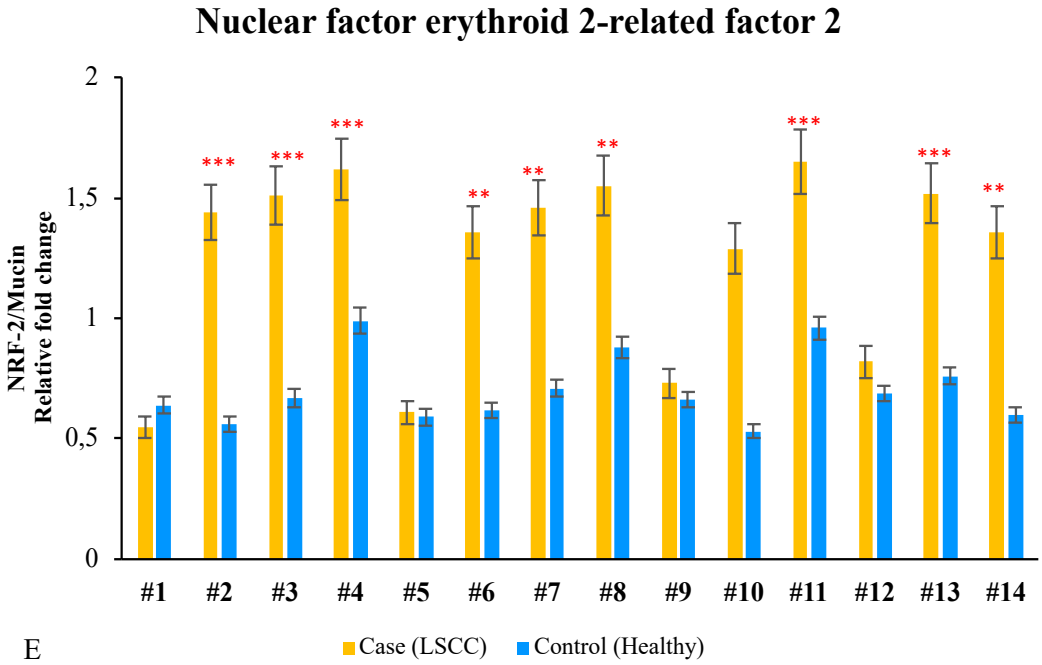
B


Heme-oxygenase 1
Heme-oxygenase 1/Mucin
Relative fold change
6
5
4
3
2
1
0
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12 #13 #14 #15 #16 #17 #18
Case (LSCC)
Control (Healthy)

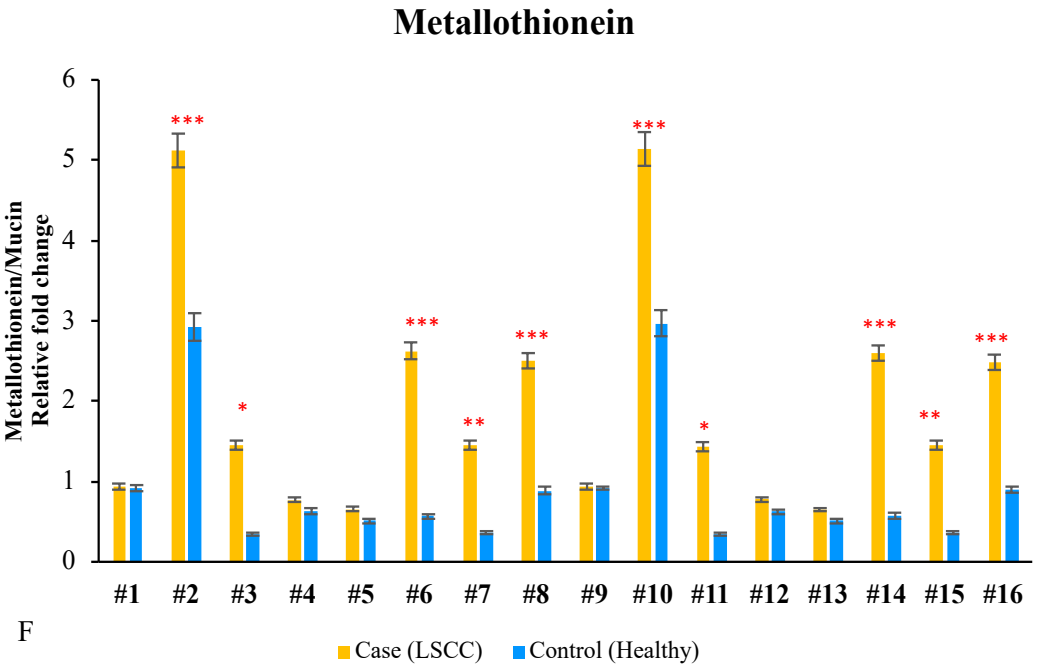
C


Vimentin
Vimentin/Mucin
Relative fold change
3,5
3
2,5
2
1,5
1
0,5
0
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12 #13 #14 #15
Case (LSCC)
Control (Healthy)

D

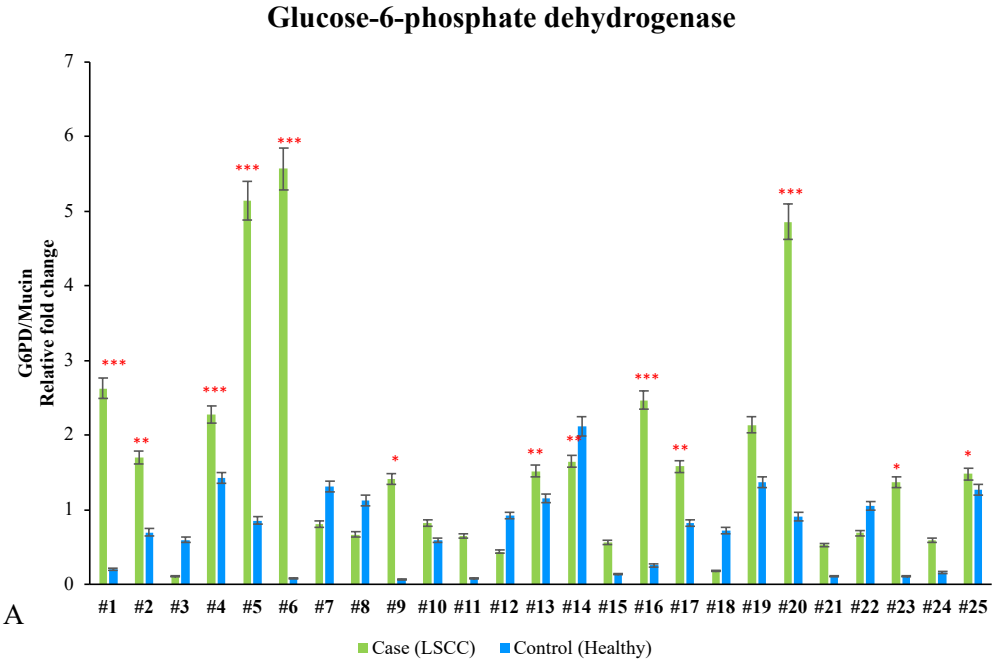

Nuclear factor erythroid 2-related factor 2
NRF-2/Mucin
Relative fold change
2
1,5
1
0,5
0
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12 #13 #14
Case (LSCC)
Control (Healthy)

E



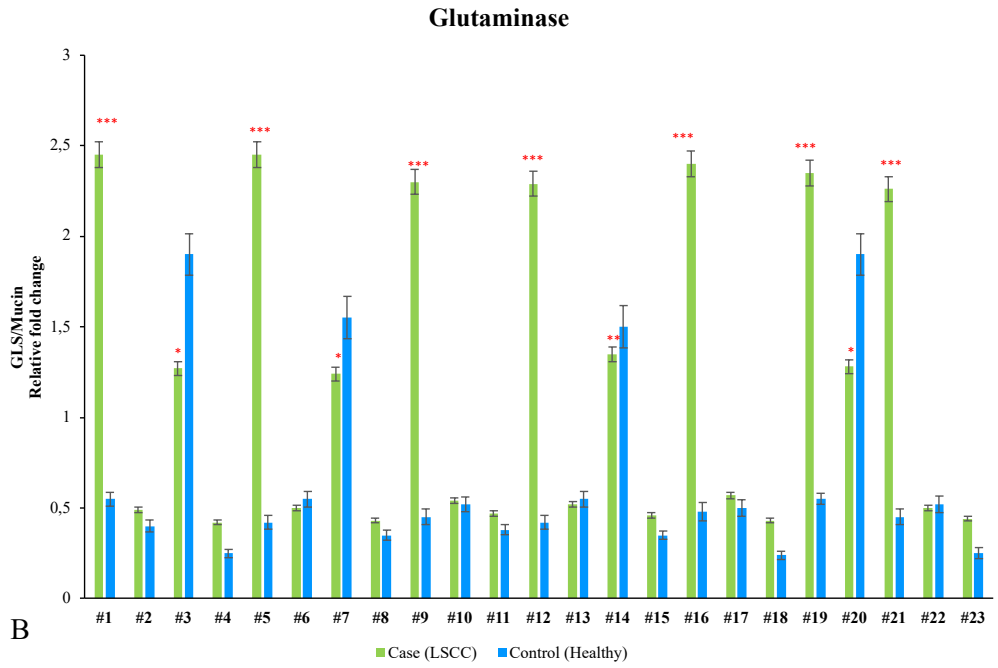
F

Figure 5 Biochemical markers of oxidative stress and tumour ptogression in LSCC.
*Western blotting analysis of **(A)** Superoxide Dismutase, **(B)** Catalase, **(C)** Heme-oxygenase 1, **(D)** Vimentin, **(E)** Nuclear factor erythroid 2-related factor 2 (NRF2) and **(F)** Metallothionein in LSCC samples (yellow) and adjacent healthy tissues (blue) (X-axis). Data represent densitometric analysis performed using Quantity One software. Protein levels were normalized to Mucin and expressed as relative fold change compared to healthy tissue. Values represent the mean of three independent experiments. Error bars indicate the standard error of the mean. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ respect to healthy tissues.*

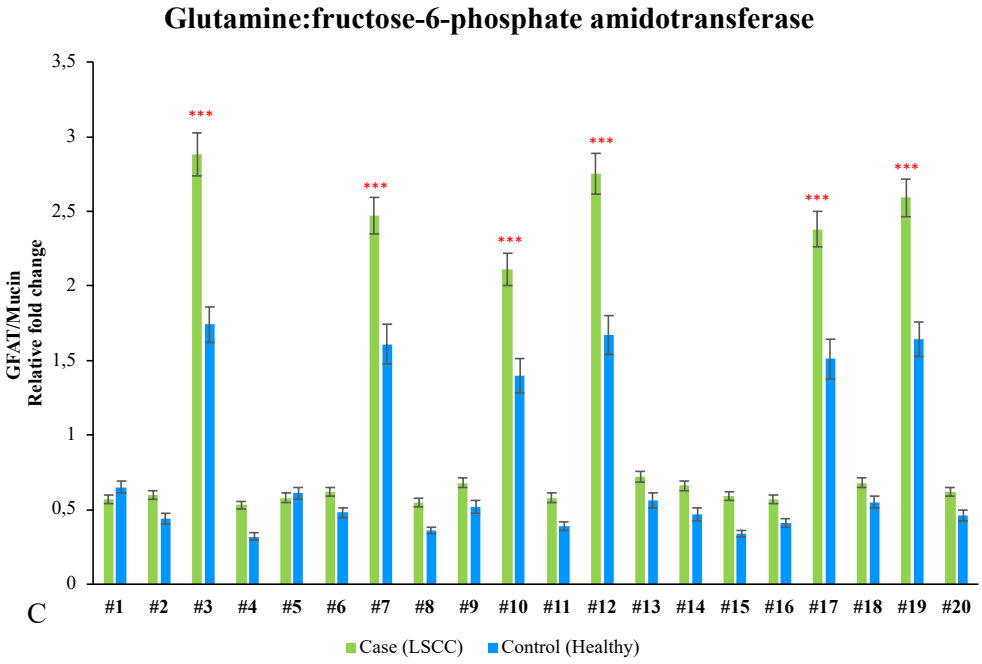


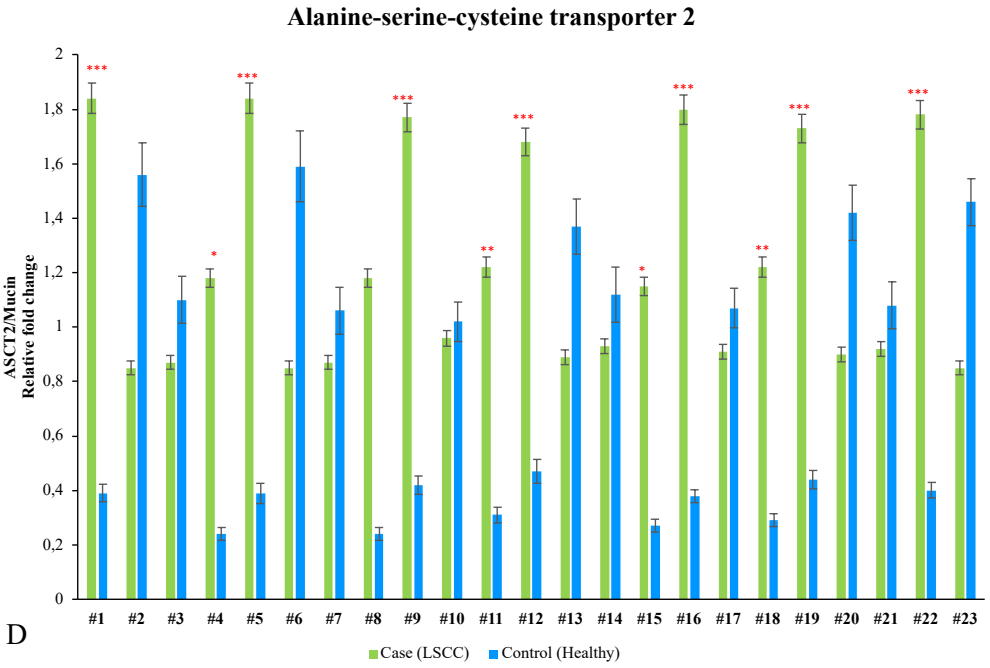
A

B

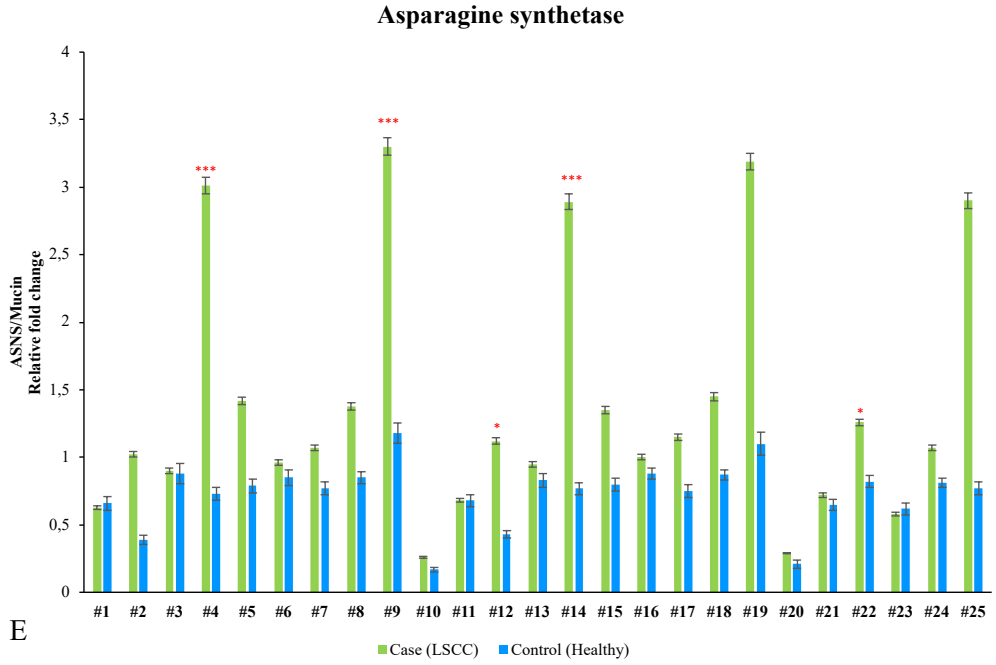

Glutaminase
GLS/Mucin
Relative fold change
3
2,5
2
1,5
1
0,5
0
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12 #13 #14 #15 #16 #17 #18 #19 #20 #21 #22 #23
Case (LSCC)
Control (Healthy)

C


Glutamine:fructose-6-phosphate amidotransferase
GFAT/Mucin
Relative fold change
3,5
3
2,5
2
1,5
1
0,5
0
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12 #13 #14 #15 #16 #17 #18 #19 #20
Case (LSCC)
Control (Healthy)



D



E

Figure 6 Biochemical markers of carbohydrate and amino acid reprograming metabolism in LSCC.
Western blotting analysis of ***(A)*** *glucose-6-phosphate dehydrogenase (G6PD),* ***(B)*** *glutaminase (GLC),* ***(C)*** *glutamine:fructose-6-phosphate amidotransferase (GFAT),* ***(D)*** *alanine-serine-cysteine transporter 2 (ASCT2),* ***(E)*** *asparagine synthetase (ASNS) in LSCC samples (green) and adjacent healthy tissues (blue) (X-axis). Data represent densitometric analysis performed using Quantity One software. Protein levels were normalized to Mucin and expressed as relative fold change compared to healthy tissue. Values represent the mean of three independent experiments. Error bars indicate the standard error of the mean. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ respect to healthy tissues.*

c. Clinical – Biochemical Correlation

Analysis of correlations between increased protein expression (LSCC vs. adjacent normal tissue) and clinicopathological parameters revealed significant associations limited to specific proteins (Figure 7).

Correlation Between Protein Expression and Clinical Parameters (p-value)

Protein	Age	Sex	Smoking	Alcohol	GERD	CRP	Laryngeal site	Grade of differentiation	PD-L1	pTNM	p16	Lymph nodes
SOD	0.597	0.474	0.864	0.482	0.127	0.322	0.994	0.359	0.607	0.223	0.175	0.894
CAT	0.806	0.242	0.385	0.529	0.308	0.677	NA	0.347	0.913	0.641	NA	0.497
HO-1	0.798	0.153	0.914	0.566	0.524	NA	0.210	0.597	0.155	0.295	NA	0.691
MT	0.143	0.143	0.036 *	0.398	0.329	NA	0.006 **	0.003**	0.072	0.022*	NA	0.226
VIM	0.192	0.602	0.493	0.965	0.734	NA	0.237	0.416	0.896	0.692	0.247	0.361
NRF2	0.854	0.938	0.844	0.110	0.180	0.265	0.110	0.158	0.749	0.158	NA	NA
G6PD	0.648	0.468	0.203	0.827	0.415	0.038*	0.590	0.071	0.946	0.824	0.483	0.589
GFAT	0.529	NA	NA	NA	0.037*	0.029*	NA	0.037*	0.037*	0.108	0.620	0.620
ASCT2	0.726	NA	NA	NA	0.371	0.913	0.285	0.371	0.371	0.495	0.285	0.285
GLS	0.223	NA	NA	NA	0.117	0.146	0.547	0.117	0.117	0.107	0.547	0.547
ASNS	0.465	0.977	0.132	0.523	0.656	0.907	0.304	0.483	0.763	0.611	0.336	0.692

* p<0.05, ** p<0.01, *** p<0.001, ns = not significant

LSCC: laryngeal suqmous cell carcinoma; SOD: superoxide dismutase; CAT: catalase, HO-1: heme-oxygenase 1; MT: metallothionein; VIM: vimentin; G6PD: glucose-6-phosphate dehydrogenase; ASCT2: alanine-serine-cysteine transporter 2; GLS: glutaminase; GFAT: glutamine:fructose-6-phosphate amidotransferase; ASNS: asparagine synthetase; LPR: laryngopharyngeal reflux; CRP: C-reactive protein

Figure 7 Correlation between protein expression and clinical parameters
Statistical analysis was performed to evaluate the association between protein expression levels and clinical characteristics. Spearman's rank correlation coefficient was used for continuous variables (e.g., age, CRP), whereas the Kruskal–Wallis test was applied for categorical variables. Results are reported as p-values, with statistical significance set at $p < 0.05$

In particular, MT showed a significant correlation with several clinical parameters, including degree of differentiation ($p = 0.0033$), laryngeal site ($p = 0.0060$), and pTNM stage ($p = 0.0217$), suggesting a possible role in tumour progression and disease aggressiveness. Furthermore, a significant association with cigarette smoking was observed ($p = 0.0357$), indicating a possible link with environmental risk factors (Figure 8).

Regarding GFAT, significant correlations with CRP emerged ($p = 0.0288$), suggesting a possible link with systemic inflammatory status. Further associations were observed with GERD, degree of differentiation and PD-L1 ($p < 0.05$), indicating a potential involvement in the mechanisms of interaction between tumour metabolism, inflammation and immune response.

Finally, G6PD showed a significant correlation with CRP levels ($p = 0.0375$), strengthening the hypothesis of an interaction between oxidative metabolism and systemic inflammation (Figure 9).

Overall, these results suggest that, although most of the proteins analysed do not show significant associations with clinical parameters, some specific molecules, particularly MT

and GFAT, could represent biomarkers potentially relevant for biological and clinical tumour characterization.

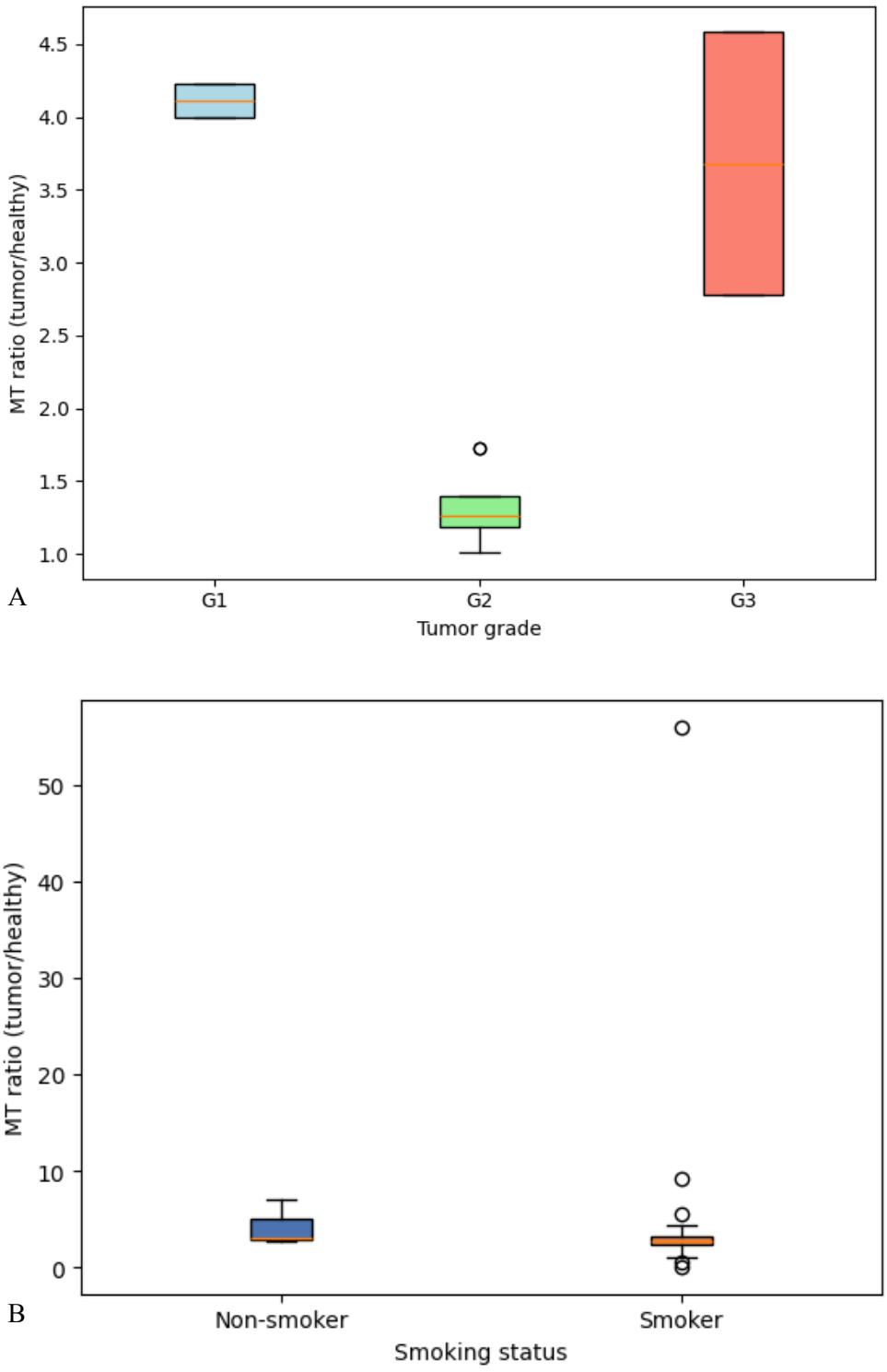


Figure 8 Metallothionein (MT) expression according to tumour differentiation grade and smoking status.
Box plots show the distribution of MT expression, expressed as tumour-to-normal ratio, across different tumour differentiation grades (G1–G3) and smoking status. The interquartile range (IQR) is represented by the box, with the median indicated by a horizontal line. The whiskers cover the entire data range and outliers are shown as discrete points. ***(A)*** *MT expression differed significantly across tumour grades ($p = 0.0033$), with higher-grade tumours tending to show increased and more variable expression levels.* ***(B)*** *A significant difference was also observed with smoking status ($p = 0.036$).*

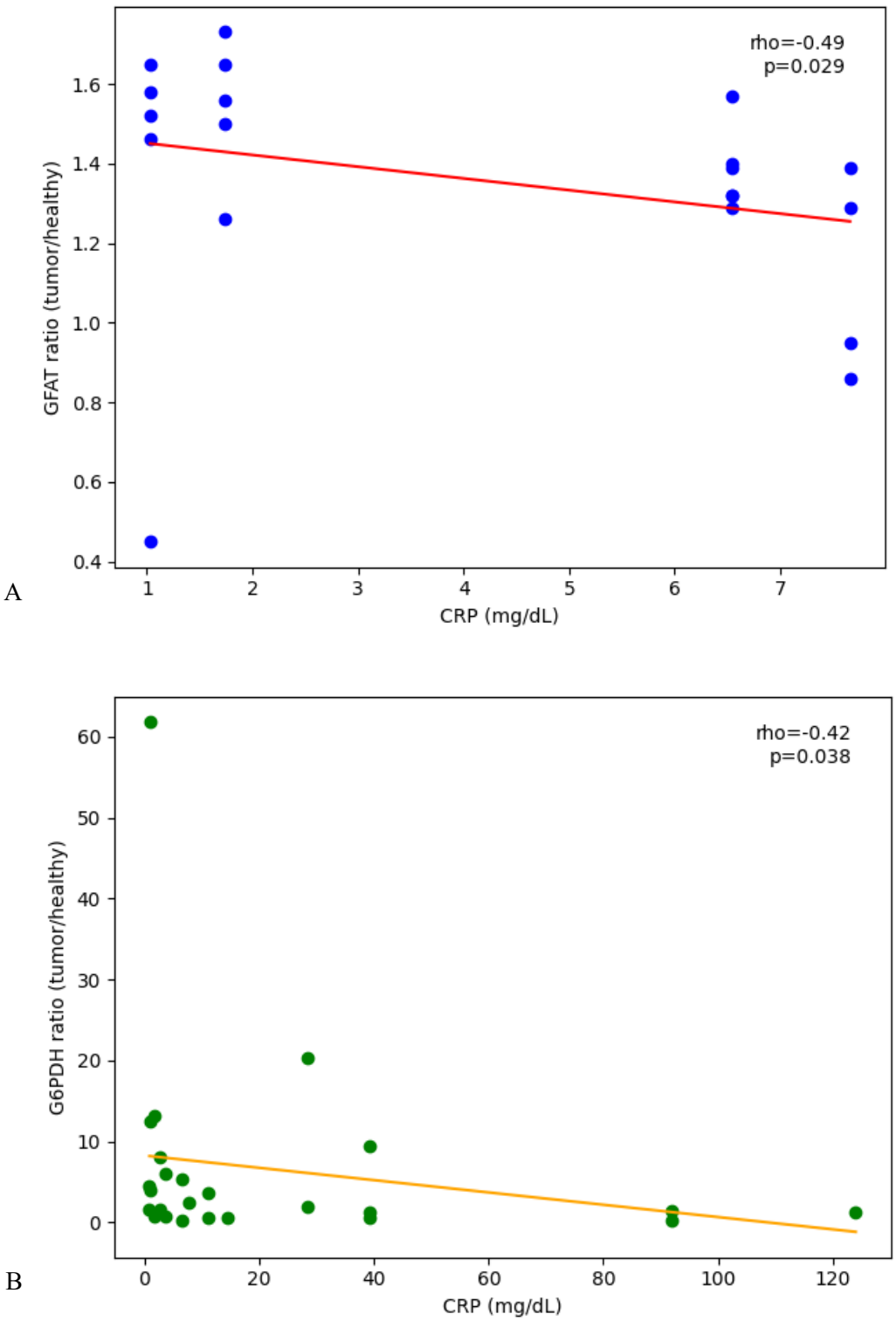


Figure 9 Relationship between metabolic protein expression and C-reactive protein (CRP) levels.
Scatter plots showing the relationship between protein expression (tumour-to-normal ratio) and CRP levels. Each point represents a single patient, and the line indicates the overall trend. ***(A)*** *A significant positive correlation was observed between GFAT expression and CRP levels (Spearman ρ shown in the figure, p = 0.0288), indicating that increased GFAT expression is associated with increased systemic inflammation.* ***(B)*** *Similarly, G6PD expression was positively correlated with CRP levels, suggesting a potential link between metabolic activity and inflammatory status.*

Correlation analyses were limited to biologically relevant protein groups involved in oxidative stress response and metabolic pathways (Table 4 and Figure 10).

Within the glutamine metabolism pathway, a significant positive correlation was observed between ASCT2 and GLS expression (Spearman $\rho = 0.60$, $p = 0.0024$), indicating a coordinated upregulation of glutamine transport and utilization in tumour tissue.

Within the redox pathway, NRF2 expression was positively associated with MT ($\rho \approx 0.64$, $p < 0.01$), supporting a linked activation of antioxidant responses.

Interestingly, a significant inverse correlation between GLS and MT expression was identified ($\rho \approx -0.71$, $p \approx 0.001$), suggesting a potential balance between metabolic activity and redox regulation.

Overall, these findings indicate the presence of coordinated and potentially competing adaptive mechanisms involving glutamine metabolism and oxidative stress response in tumour tissue (Figure 11).

Table 4. Correlation analysis among biologically related proteins

Protein 1	Protein 2	Spearman rho	p-value	Significance
NRF2	HO-1	-0.20	0.4780	ns
NRF2	MT	0.64	0.0097	**
SOD	CAT	0.12	0.5628	ns
HO-1	SOD	-0.01	0.9805	ns
HO-1	CAT	0.35	0.1423	ns
ASCT2	GLS	0.62	0.0011	**
ASCT2	ASNS	0.29	0.1641	ns
GLS	ASNS	0.18	0.4005	ns
GFAT	G6PD	-0.01	0.9548	ns
GFAT	ASCT2	0.00	1.0000	ns
GFAT	GLS	-0.43	0.0515	ns
NRF2	GFAT	0.55	0.0336	*
MT	GLS	-0.71	0.0013	**

* p<0.05, ** p<0.01, *** p<0.001, ns = not significant

SOD: superoxide dismutase; CAT: catalase, HO-1: heme-oxygenase 1; MT: metallothionein; VIM: vimentin; G6PD: glucose-6-phosphate dehydrogenase; ASCT2: alanine-serine-cysteine transporter 2; GLS: glutaminase; GFAT: glutamine:fructose-6-phosphate amidotransferase; ASNS: asparagine synthetase

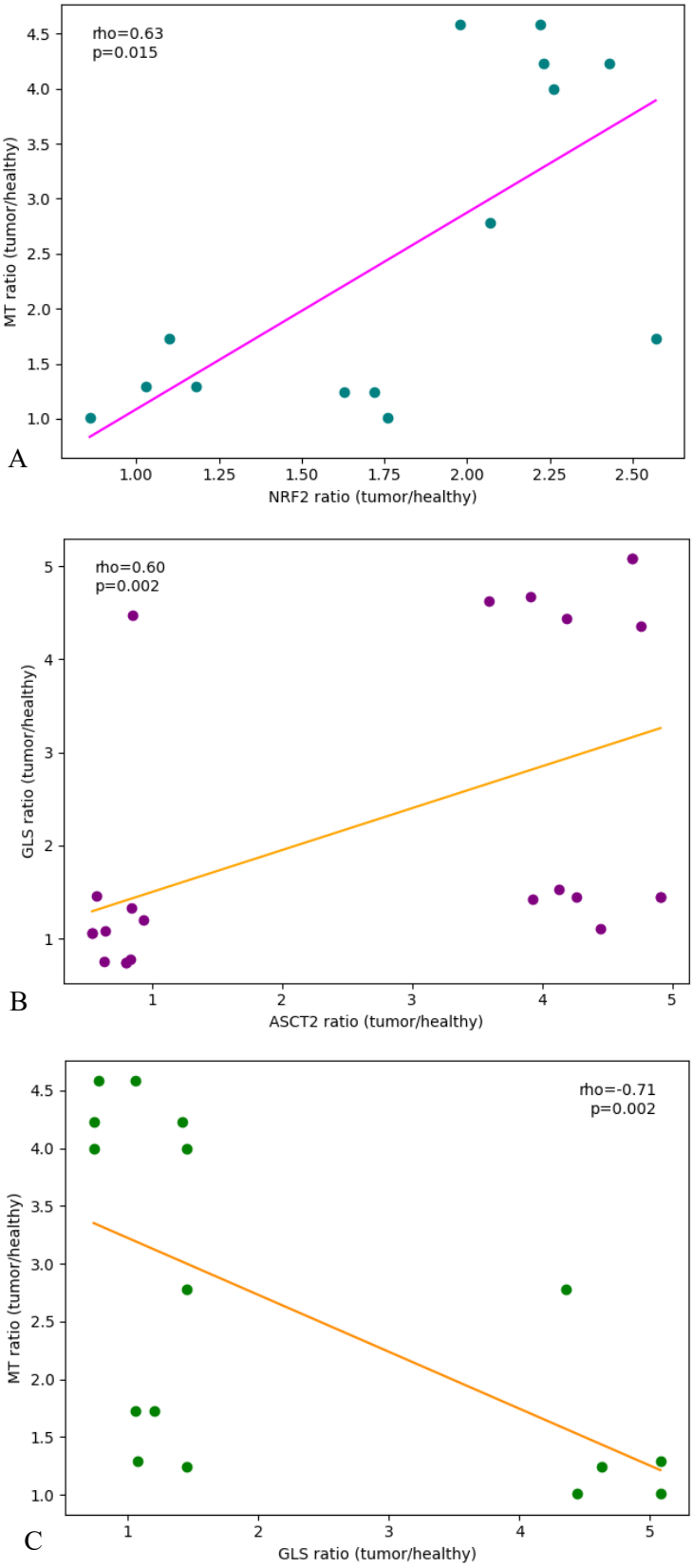


Figure 10 Correlation between metabolic and redox-related protein expression.
Scatter plots showing the relationships between selected proteins, all expressed as tumour-to-normal ratio. Each point represents an individual patient. (A) A significant positive correlation was observed between ASCT2 and GLS expression (Spearman ρ = 0.60, p = 0.0024), indicating that increased ASCT2 expression is associated with higher GLS expression. (B) A further positive correlation was identified between NRF2 and MT expression, suggesting a coordinated activation of antioxidant response mechanisms. (C) In contrast, a significant inverse correlation was observed between GLS and MT expression, indicating a potential balance between metabolic activity and redox regulation.

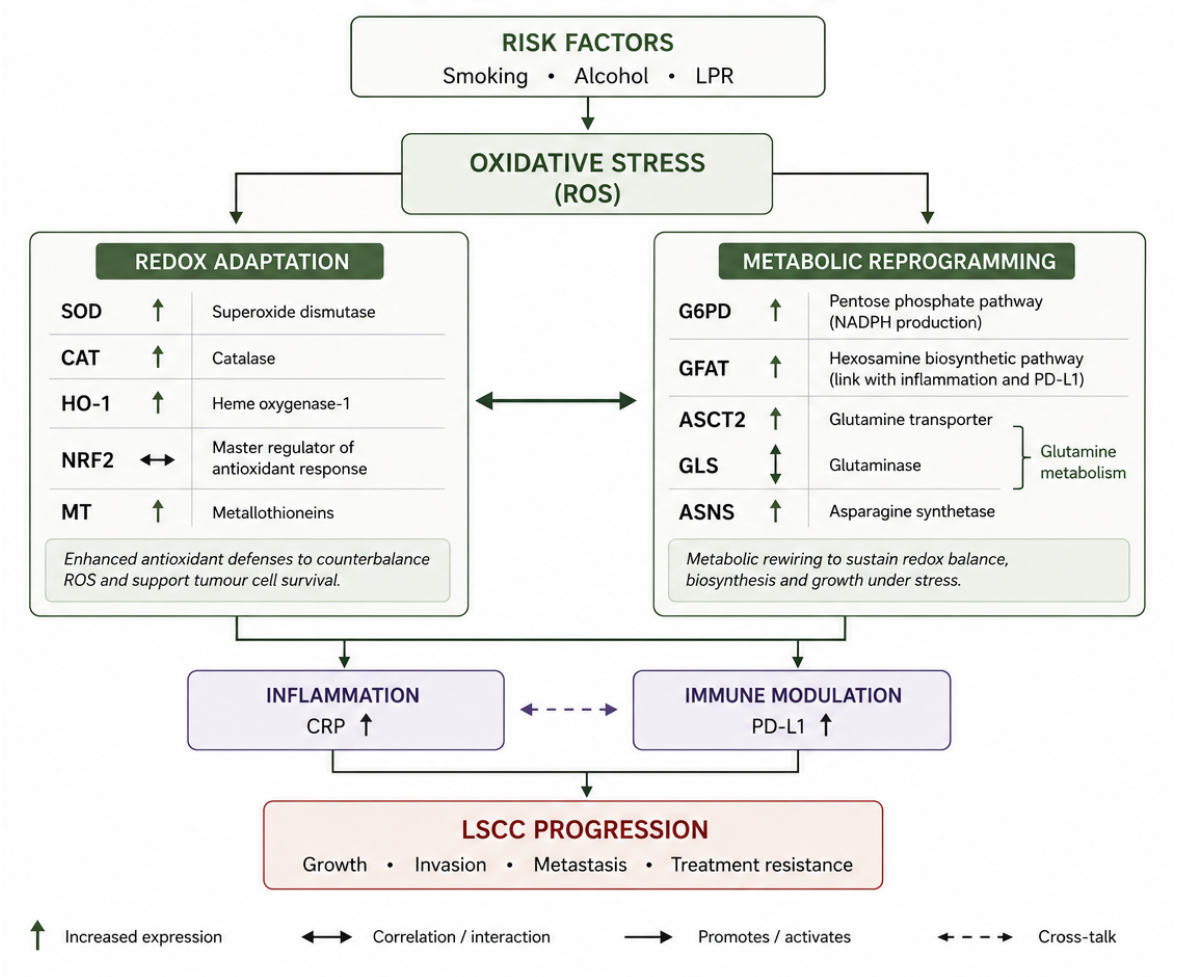


Figure 11 Proposed interaction between oxidative stress, metabolic reprogramming, and tumour progression in laryngeal squamous cell carcinoma (LSCC).

Chronic exposure to risk factors such as tobacco smoking, alcohol consumption, and laryngopharyngeal reflux (LPR) promotes oxidative stress and the production of reactive oxygen species (ROS). In response, tumour cells activate adaptive antioxidant mechanisms (SOD, CAT, HO-1, NRF2, and MT) along with metabolic reprogramming pathways involving G6PD, GFAT, ASCT2, GLS, and ASNS. The interplay between redox adaptation and metabolic rewiring contributes to inflammation, immune modulation, and tumour progression, supporting growth, survival, invasion, and resistance to LSCC treatment. The model summarizes the main biological pathways that emerged from the results of the present study and from the available literature.

DISCUSSION

Overall, LSCC is a complex, multifactorial malignancy resulting from interactions among environmental factors, lifestyle habits, and molecular alterations. Among the main etiological determinants, cigarette smoking and alcohol consumption play a central role, playing a role in both the onset and progression of the disease by mechanisms closely linked to oxidative stress.

Oxidative stress is among the main biological factors involved in laryngeal carcinogenesis. Excessive production of ROS, combined with reduced antioxidant capacity, alters the cellular redox balance, leading to damage to DNA, proteins, and lipids. Such imbalance promotes genomic instability, oncogene activation, and inactivation of tumour suppressor genes, as well as modulating key pathways engaged in cell proliferation, survival, and apoptosis. At the same time, oxidative stress integrates with chronic inflammatory processes, establishing a vicious cycle that contributes to neoplastic transformation and the maintenance of a TME favourable to disease progression.

In this context, an increasingly important role is attributed to tumour metabolism and the concept of metabolic reprogramming. Neoplastic cells not only undergo passive metabolic alterations but also reorganize metabolic flows to support growth, survival, and adaptation to adverse microenvironmental conditions. In LSCC, this reprogramming involves multiple pathways, including carbohydrate metabolism (with PPP activation and increased NADPH production), amino acid metabolism (particularly glutamine), and lipid metabolism [186]. These responses allow not only to meet the biosynthetic needs of tumour cells but also to maintain redox homeostasis, protecting cells from oxidative stress while promoting resistance against treatments.

In this context, the present study focused on a selected panel of biomarkers involved in these processes, aiming to explore their contribution to the pathophysiology of LSCC. In particular, key enzymes of the antioxidant system, including SOD, CAT, and HO-1, which are key components in regulating cellular redox balance, were analysed. These enzymes have a critical role in ROS detoxification, helping to limit oxidative damage; however, in tumour cells, their expression can be altered and sometimes adaptive, promoting survival under conditions of high oxidative stress. The results obtained highlight a marked alteration of redox and metabolic pathways in tumour tissue compared to adjacent healthy tissue. In particular, increased expression of SOD, CAT, and HO-1 in LSCC tissues suggests coordinated activation of antioxidant systems. This finding is consistent with the known state of chronic oxidative stress that characterizes the tumour microenvironment, in which

neoplastic cells develop adaptive mechanisms to survive elevated levels of ROS. Previous studies investigating SOD activity in LSCC have reported higher levels in tumour tissue compared to adjacent non-cancerous tissue, consistent with our findings [117, 118, 187]. In contrast, Kalayci et al. [119] didn't observe a statistically significant increase in SOD activity when comparing LSCC tissue with adjacent non-cancerous tissue. Their results revealed only a slight, non-significant decrease in SOD activity in tumour samples ($p > 0.05$). Based on the interpretation proposed by Biri et al. [188] in the context of prostate cancer, the authors suggested that this lack of upregulation of SOD may reflect an insufficient antioxidant response in malignant tissues, which may fail to effectively neutralize the excess ROS generated during carcinogenesis. This impaired enzymatic defense could contribute to persistent oxidative stress, potentially promoting DNA damage and tumour progression. Evidence on the expression and role of HO-1 in LSCC remains limited. However, one study demonstrated that its high expression counteracts the pro-apoptotic effects of cisplatin in LSCC Hep-2 cells, thereby reducing treatment sensitivity by modulating oxidative stress and apoptotic signaling pathways [141]. The study by Tang et al. reports that, in LSCC patients, serum HO-1 levels were lower than in healthy controls, and that these levels were inversely correlated with tumour stage and the presence of neck node metastases: more advanced tumours with neck node involvement showed even lower serum HO-1 values [142].

Other redox-sensitive mediators were considered, such as the NRF2, the main regulator of antioxidant response, and MTs, proteins involved in metal detoxification and protection from oxidative stress. Both are frequently deregulated in tumours, causing the ability of neoplastic cells to adapt to stressful conditions and develop resistance against treatments. In this study, MT expression was found to be significantly associated with key clinical and molecular features, pointing to a potential role in tumour biology. In particular, the association with laryngeal subsite may reflect underlying biological differences between anatomical regions, possibly related to variations in exposure to carcinogens or local microenvironmental factors. Furthermore, the significant association between MT expression and pTNM stage further supports its link with tumour progression, as higher expression levels appear to be associated with more advanced disease. Taken together, these results indicate that MT may not represent a passive marker but may be actively involved in the mechanisms underlying tumour growth and aggressiveness. Particularly, noteworthy is the association with smoking. Indeed, tobacco exposure is an established source of oxidative stress, and MTs are known to play a role in cellular defence against oxidative damage. It is therefore plausible that increased MT expression reflects an adaptive response to smoking-induced chronic oxidative stress. However, in the context of cancer, this defensive mechanism may

paradoxically contribute to the survival and progression of tumour cells. Interestingly, MT expression showed a significant association with tumour differentiation; however, the observed pattern was not linear, with higher expression levels detected in both G1 and G3 tumours compared to G2 lesions. This finding indicates that MT expression may not simply reflect increased tumour aggressiveness but rather different biological states during tumour progression. Pastuszewski et al. reported the opposite trend, with higher MT expression in G2 tumours compared to G1 and G3, although the difference was not statistically significant [147]. Therefore, the relationship between MT expression and tumour differentiation remains controversial. Taken together, these data suggest that MT expression may not increase simply with tumour aggressiveness, but may instead reflect complex and context-specific adaptive reactions to oxidative stress, cell proliferation, and differentiation. Further studies on larger cohorts are needed to clarify the biological significance of this association. Furthermore, Ioachim et al. analysed the immunohistochemical expression of MT in benign, premalignant and malignant laryngeal tissues. The authors reported a progressive, statistically significant increase in MT expression from benign to malignant tissue ($p < 0.0001$), suggesting a possible role for MT in the neoplastic transformation of the larynx. However, no significant difference in MT expression was observed between carcinoma in situ and infiltrating carcinoma, suggesting that MT may play a relevant role in the progression of LSCC and may represent a potential biomarker useful for assessing tumour status and aggressiveness [148].

In addition to its clinical associations, MT also showed significant correlations with other proteins involved in metabolic and redox pathways: a positive correlation was observed between MT and NRF2 expression, consistent with the well-established role of NRF2 as a central regulator of antioxidant responses. This finding suggests that MT may be part of a coordinated cellular program aimed at counteracting oxidative stress in tumour cells. Interestingly, a strong inverse correlation was identified between MT and GLS expression. While GLS is a key enzyme in glutamine metabolism and supports tumour growth through metabolic reprogramming, MT is primarily involved in redox homeostasis and cellular protection. This inverse relationship may reflect the presence of alternative adaptive strategies adopted by tumour cells, in which metabolic activation and antioxidant defence are differentially regulated. In this context, tumour cells with high metabolic activity may rely more on glutamine utilization, whereas others may preferentially activate antioxidant mechanisms to cope with oxidative stress. Overall, these findings support the hypothesis that MT is not only a marker of tumour progression but also part of a larger network linking metabolic reprogramming and redox regulation. The observed correlations highlight the

complexity of tumour adaptation mechanisms and suggest the existence of a functional interaction between metabolic pathways and oxidative stress responses. Li et al. evaluated the expression and correlations between NRF2 and HO-1 in advanced LSCC samples and found that their increased expression was significantly associated with a more advanced tumour stage (III-IV) and greater perineural and vascular infiltration, suggesting that deregulation of NRF2 promotes aggressive biological characteristics of laryngeal carcinoma [7].

In parallel, the evaluation of VIM, a key marker of the EMT process, closely related to invasiveness and metastatic capacity, was included. VIM expression therefore reflects not only cytoskeletal structural alterations, but also the impact of oxidative stress on tumour progression processes. Although VIM has consistently been associated with EMT and poor prognosis in LSCC [156-157], the absence of significant associations in our cohort may suggest that EMT-related changes are not uniformly represented across all LSCC subtypes. Alternatively, VIM expression alone may be insufficient to capture the complexity of EMT processes, which involve multiple interacting molecular pathways.

A further focus was placed on tumour metabolism, particularly on enzymes and transporters involved in metabolic reprogramming. Among these, G6PD, a key enzyme in the PPP, has been analysed for its role in NADPH production, which is critical for both cellular biosynthesis and the maintenance of redox homeostasis. In the present study, a significant positive correlation was observed between G6PD expression and CRP levels, a well-established marker of systemic inflammation, suggesting that activation of the PPP may be linked not only to tumour metabolism but also to systemic inflammatory responses, supporting the concept of a close interaction between metabolic adaptation and tumour-host inflammatory signalling.

Tumours with higher inflammatory status may exhibit increased activation of metabolic pathways that support growth and adaptation to oxidative stress. In this context, elevated ROS levels may favor increased G6PD activity, which can provide a selective advantage to tumour cells by enhancing antioxidant capacity and promoting survival through NADPH production. Overall, these findings support a connection between metabolic adaptation and the tumour microenvironment.

Furthermore, amino acid metabolism, with particular attention to glutamine, was investigated through the study of the transporter ASCT2 and the enzyme GLS, both implicated in the phenomenon of “glutamine addiction” typical of tumour cells [189]. The evidence available in the LSCC remains limited, highlighting a significant gap in current knowledge. In particular, the only study conducted on LSCC samples demonstrated that

ASCT2 overexpression was significantly associated with more aggressive tumour behavior and worse clinical outcomes in patients with advanced LSCC ($p = 0.039$) [190]. In our study, although no direct associations with clinical parameters were observed, a significant positive correlation between ASCT2 and GLS expression was identified. Given the role of ASCT2 as a glutamine transporter and GLS as a key enzyme in glutamine metabolism, this finding implies a coordinated activation of glutamine uptake and utilization in tumour cells. This coordinated upregulation may reflect an increased dependence on glutamine metabolism to support tumour growth and metabolic demands. Thus, even in the absence of direct clinical correlations, our data provide further insight into the metabolic role of ASCT2 in LSCC, supporting its involvement in tumour metabolic reprogramming.

An interesting result concerns the GFAT, which showed a highly significant difference despite a relatively modest quantitative increase. This suggests that GFAT may play an important regulatory role in glucose metabolism via the HBP, rather than merely reflecting a quantitative change. This model may reflect GFAT's involvement in metabolic reprogramming processes that don't necessarily require large expression shifts but instead depend on exact regulatory control. Furthermore, the significant correlation observed with CRP suggests that its expression could be linked to the patient's systemic inflammatory status. This finding supports the idea that metabolic alterations in tumour cells are not isolated events but rather occur within a broader inflammatory context that may influence tumour behaviour. Associations with LPR, degree of differentiation, and PD-L1 expression further strengthen the potential involvement of GFAT across multiple aspects of tumour biology. The association between GFAT expression and LPR can be interpreted by considering the chronic inflammatory and oxidative microenvironment induced by reflux. Persistent laryngeal exposure to gastric contents may promote local inflammation and increased ROS production, thereby triggering adaptive metabolic responses in tumour cells. In this context, upregulation of GFAT may reflect activation of HBP, which facilitates cellular adaptation by modulation of protein glycosylation and signalling processes. The association between GFAT and PD-L1 expression may further support a link between metabolic reprogramming and immune modulation. In an inflammatory microenvironment, such as that induced by LPR, cytokine signalling is known to promote PD-L1 expression [191]. In parallel, GFAT upregulation may enhance HBP, potentially influencing protein glycosylation processes that contribute to PD-L1 stability and function. This combined effect may facilitate immune evasion of the tumour.

Furthermore, the relationship with differentiation may indicate a role in tumour progression. Taken together, these results indicate a contribution of GFAT to the interaction between metabolic reprogramming, inflammatory processes, and the immune response.

In our study, an increase in ASNS expression was found in tumour tissue compared to normal samples, suggesting an adaptive metabolic response in LSCC. Tumour cells are often exposed to conditions of metabolic stress, including nutrient limitation and increased biosynthetic demand, which can drive upregulation of enzymes involved in amino acid metabolism. In this context, ASNS overexpression may help maintain the availability of intracellular amino acids, supporting protein synthesis and cell survival. This interpretation is consistent with previous evidence highlighting alterations in amino acid metabolism in HNCs, as well as the involvement of ASNS in tumour cell adaptation and response to stress conditions [192].

Although no significant associations with clinical or molecular parameters were observed in our cohort, increased ASNS expression supports its potential role as part of the metabolic reprogramming processes underlying tumour progression.

Overall, the data support the hypothesis that tumour tissue exhibits a dual adaptive strategy: on the one hand, the enhancement of antioxidant systems to counteract oxidative stress, and on the other, profound metabolic reprogramming aimed at supporting cell growth and survival. The integrated analysis performed in this study suggests that these pathways should be studied as components of a common biological network rather than as isolated molecular events. These mechanisms could represent potential therapeutic targets, particularly in HNCs, where oxidative stress and glutamine metabolism play a central role.

a. Limitations

However, some limitations should be taken into account when interpreting the results of this study. First, the relatively small sample size may have reduced the statistical power of the analyses, limited the ability to detect weaker associations, and limited the robustness of the subgroup analyses. Furthermore, the cohort was characterized by some degree of clinical and pathological heterogeneity, which may have contributed to variability in protein expression patterns. Consequently, the generalizability of the results may be limited, and the results should be interpreted as preliminary until confirmed in larger multicentre cohorts. Second, because more biomarkers were studied and tissue availability was limited, not all proteins could be assessed in every patient. Consequently, the number of cases included in each analysis varied among biomarkers. Furthermore, exploratory analyses investigating associations between protein expression and clinicopathological parameters involved

multiple statistical comparisons, which may have increased the risk of false-positive results. Therefore, these associations should be interpreted with caution and considered hypothesis-generating until validated in independent cohorts. Third, the study was primarily descriptive and correlative in nature; therefore, no causal relationship could be established between changes in protein expression and tumour behaviour. The biological interpretation of some results remains speculative, as no functional experiments were performed in vitro or in vivo to establish causal relationships or confirm the mechanistic role of the analysed proteins. Consequently, the observed associations should be considered hypothesis-generating rather than mechanistic evidence. Further studies combining larger patient cohorts with functional investigations will be needed to clarify the biological and clinical significance of these biomarkers. Furthermore, protein expression was assessed exclusively by Western blot analysis. Although this technique provides quantitative information on protein abundance, complementary approaches such as immunohistochemistry, RT-qPCR, and integrative multi-omics analyses may offer further insight into tissue localization, transcriptional regulation, along with the broader molecular context of the biomarkers studied. Finally, another limitation of this study is the use of adjacent non-tumour mucosa as reference tissue. Although histologically free of malignant tumours, these samples were obtained from patients with LSCC and may not represent a truly normal laryngeal mucosa. According to the concept of field cancerization [193], originally proposed by Slaughter and subsequently supported by molecular evidence from Braakhuis et al., genetically modified but histologically normal-appearing epithelial fields may exist throughout the upper aerodigestive tract due to chronic exposure to carcinogens. Therefore, molecular alterations may already be present in the adjacent mucosa, may lead to an underestimation of biological differences between tumour and control tissues.

CONCLUSION AND FUTURE PERSPECTIVE

In conclusion, the present study provides an integrated characterization of metabolic and oxidative stress–related alterations in LSCC, highlighting the coexistence of redox imbalance and metabolic reprogramming within tumour tissue. Overall, the data support the hypothesis that LSCC is characterized by marked alterations in antioxidant defence systems and metabolic pathways, which jointly contribute to tumour adaptation, progression, and survival. Rather instead of representing independent phenomena, oxidative stress responses and metabolic changes appear to be closely interconnected processes which shape tumour behaviour and the interaction between tumour cells and their microenvironment. Notably, specific associations among MT, GFAT, and G6PD are identified, suggesting potential links among oxidative stress, inflammation, immune modulation, and metabolic adaptation. Although most of the proteins analysed showed no significant associations with clinicopathological parameters, the overall observed pattern supports the existence of a complex biological network linking redox regulation and tumour metabolism in LSCC.

Despite the data provided, this study remains primarily descriptive and hypothesis-generating, and several aspects remain to be clarified. First, larger multicentre studies are needed to validate the clinical relevance of the identified biomarkers and confirm their prognostic and predictive value in more representative patient populations. Second, future inquiries should focus on the functional characterization of these molecules. Understanding whether their altered expression directly contributes to tumour progression or instead represents an adaptive response to oxidative stress may provide important information for therapeutic targeting. Experimental models, including both in vitro and in vivo approaches, will be essential to expound these mechanisms. Furthermore, integrating redox biology with metabolic profiling represents an especially promising area for future research. Multi-omics methods integrating proteomics, transcriptomics, and metabolomics can help detect distinct molecular subtypes of LSCC and deliver a more comprehensive understanding of tumour heterogeneity. From a translational perspective, a more in-depth characterization of these pathways may facilitate the identification of clinically relevant biomarkers for risk stratification and treatment selection. Furthermore, targeting the interaction between oxidative stress and metabolic adaptation may represent a possible therapeutic strategy worthy of further investigation.

Overall, this work contributes to the growing understanding of LSCC biology and supports the concept that oxidative stress and metabolic reprogramming should be considered interconnected hallmarks of disease progression. By highlighting to the interplay

among redox regulation, inflammation, immune modulation, and metabolic adaptation, the present study provides a platform for future investigations to enhance biomarker development and identify novel therapeutic opportunities in LSCC.

REFERENCES

1. Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. Nat Rev Dis Primers. 2020 Nov 26;6(1):92. doi: 10.1038/s41572-020-00224-3. Erratum in: Nat Rev Dis Primers. 2023 Jan 19;9(1):4. doi: 10.1038/s41572-023-00418-5. PMID: 33243986; PMCID: PMC7944998.
2. Barsouk A, Aluru JS, Rawla P, Saginala K, Barsouk A. Epidemiology, Risk Factors, and Prevention of Head and Neck Squamous Cell Carcinoma. Med Sci (Basel). 2023 Jun 13;11(2):42. doi: 10.3390/medsci11020042. PMID: 37367741; PMCID: PMC10304137.
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May;71(3):209-249. doi: 10.3322/caac.21660. Epub 2021 Feb 4. PMID: 33538338.
4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018 Nov;68(6):394-424. doi: 10.3322/caac.21492. Epub 2018 Sep 12. Erratum in: CA Cancer J Clin. 2020 Jul;70(4):313. PMID: 30207593
5. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin. 2023 Jan;73(1):17-48. doi: 10.3322/caac.21763. PMID: 36633525.
6. Mousavi SE, Ilaghi M, Aslani A, Najafi M, Yekta Z, Nejadghaderi SA. Laryngeal cancer incidence trends in the United States over 2000-2020: a population-based analysis. Arch Public Health. 2024 Jul 10;82(1):106. doi: 10.1186/s13690-024-01333-1. PMID: 38987838; PMCID: PMC11234729.
7. Li C, Wu H, Wang S, Zhu J. Expression and correlation of NRF2, KEAP1, NQO-1 and HO-1 in advanced squamous cell car-cinoma of the larynx and their association with clinicopathologic features. Mol Med Rep. 2016 Dec;14(6):5171-5179. doi: 10.3892/mmr.2016.5913. Epub 2016 Nov 1. PMID: 27840932; PMCID: PMC5355699
8. Bradley PJ. Laryngeal cancer in nondrinker nonsmoker young patients: a distinct pathological entity? Curr Opin Oto-laryngol Head Neck Surg. 2016 Apr;24(2):140-7. doi: 10.1097/MOO.0000000000000230. PMID: 26679783

9. Li H, Li EY, Kejner AE. Treatment modality and outcomes in larynx cancer patients: A sex-based evaluation. Head Neck. 2019 Nov;41(11):3764-3774. doi: 10.1002/hed.25897. Epub 2019 Aug 7. PMID: 31392796
10. Tosakoon S, Lawrence WR, Shiels MS, Jackson SS. Sex Differences in Cancer Incidence Rates by Race and Ethnicity: Results from the Surveillance, Epidemiology, and End Results (SEER) Registry (2000-2019). Cancers (Basel). 2024 Feb 29;16(5):989. doi: 10.3390/cancers16050989. PMID: 38473350; PMCID: PMC10930733
11. Ramroth H, Dietz A, Becher H. Intensity and inhalation of smoking in the aetiology of laryngeal cancer. Int J Environ Res Public Health. 2011 Apr;8(4):976-84. doi: 10.3390/ijerph8040976. Epub 2011 Apr 1. PMID: 21695025; PMCID: PMC3118874
12. Verro B, Saraniti G, Fiumara S, Ottoveggio G, Saraniti C. Smoking and alcohol habits in head and neck cancers: How many patients stop after diagnosis? J Cancer Policy. 2024 Sep;41:100498. doi: 10.1016/j.jcpo.2024.100498. Epub 2024 Jul 25. PMID: 39059763.
13. Liberale C, Soloperto D, Marchioni A, Monzani D, Sacchetto L. Updates on Larynx Cancer: Risk Factors and Oncogenesis. Int J Mol Sci. 2023 Aug 18;24(16):12913. doi: 10.3390/ijms241612913. PMID: 37629093; PMCID: PMC10454133
14. Rumgay H, Murphy N, Ferrari P, Soerjomataram I. Alcohol and Cancer: Epidemiology and Biological Mechanisms. Nutri-ents. 2021 Sep 11;13(9):3173. doi: 10.3390/nu13093173. PMID: 34579050; PMCID: PMC8470184
15. Levesque C, Sanger N, Edalati H, Sohi I, Shield KD, Sherk A, et al. A systematic review of relative risks for the relationship between chronic alcohol use and the occurrence of disease. Alcohol Clin Exp Res (Hoboken). 2023 Jul;47(7):1238-1255. doi: 10.1111/acer.15121. Epub 2023 Jul 9. PMID: 37422765
16. Ferster APO, Schubart J, Kim Y, Goldenberg D. Association Between Laryngeal Cancer and Asbestos Exposure: A Systematic Review. JAMA Otolaryngol Head Neck Surg. 2017 Apr 1;143(4):409-416. doi: 10.1001/jamaoto.2016.3421. PMID: 27918783
17. Wagner M, Bolm-Audorff U, Hegewald J, Fishta A, Schlattmann P, Schmitt J, et al. Occupational polycyclic aromatic hydro-carbon exposure and risk of larynx cancer: a systematic review and meta-analysis. Occup Environ Med. 2015 Mar;72(3):226-33. doi: 10.1136/oemed-2014-102317. Epub 2014 Nov 14. PMID: 25398415
18. Bayer O, Cámara R, Zeissig SR, Ressing M, Dietz A, Locati LD, et al. Occupation and cancer of the lar-ynx: a systematic review and meta-analysis. Eur Arch

Otorhinolaryngol. 2016 Jan;273(1):9-20. doi: 10.1007/s00405-014-3321-y. Epub 2014 Oct 14. PMID: 25311307

19. McCormick CA, Samuels TL, Battle MA, Frolkis T, Blumin JH, Bock JM, et al. H+/K+ATPase Expression in the Larynx of Laryngopharyngeal Reflux and Laryngeal Cancer Patients. Laryngoscope. 2021 Jan;131(1):130-135. doi: 10.1002/lary.28643. Epub 2020 Apr 6. PMID: 32250454
20. Kim SY, Park B, Lim H, Kim M, Kong IG, Choi HG. Increased risk of larynx cancer in patients with gastroesophageal reflux disease from a national sample cohort. Clin Otolaryngol. 2019 Jul;44(4):534-540. doi: 10.1111/coa.13328. Epub 2019 Apr 26. PMID: 30884136
21. Garavello W, Lucenteforte E, Bosetti C, Talamini R, Levi F, Tavani A, et al. Diet diversity and the risk of laryngeal cancer: a case-control study from Italy and Switzerland. Oral Oncol. 2009 Jan;45(1):85-9. doi: 10.1016/j.oraloncology.2008.02.011. Epub 2008 May 19. PMID: 18487075
22. Pezzuto F, Buonaguro L, Caponigro F, Ionna F, Starita N, Annunziata C, et al. Update on Head and Neck Cancer: Current Knowledge on Epidemiology, Risk Factors, Molecular Features and Novel Therapies. Oncology. 2015;89(3):125-36. doi: 10.1159/000381717. Epub 2015 May 8. PMID: 25967534.
23. Chen X, Gao L, Sturgis EM, Liang Z, Zhu Y, Xia X, et al. HPV16 DNA and integration in normal and malignant epithelium: implications for the etiology of laryngeal squamous cell carcinoma. Ann Oncol. 2017 May 1;28(5):1105-1110. doi: 10.1093/annonc/mdx027. PMID: 28327951; PMCID: PMC5406756.
24. Markou K, Christoforidou A, Karasmanis I, Tsiropoulos G, Triaridis S, Constantinidis I, Vital V, Nikolaou A. Laryn-geal cancer: epidemiological data from Northern Greece and review of the literature. Hippokratia. 2013 Oct;17(4):313-8. PMID: 25031508; PMCID: PMC4097410
25. Rocha BMM, Dolan RD, Paiva CE, McGovern J, Paiva BSR, Preto DD, et al. Inflammation and Performance Status: The Cornerstones of Prognosis in Advanced Cancer. J Pain Symptom Manage. 2023 Apr;65(4):348-357. doi: 10.1016/j.jpainsymman.2022.11.021. Epub 2022 Dec 7. PMID: 36493981
26. Dantas TS, de Barros Silva PG, Sousa EF, da Cunha MDP, de Aguiar ASW, Costa FWG, et al. Influence of Educational Level, Stage, and Histological Type on Survival of Oral Cancer in a Brazilian Population: A Retrospective Study of 10 Years Observation. Medicine (Baltimore). 2016 Jan;95(3):e2314. doi: 10.1097/MD.0000000000002314. PMID: 26817864; PMCID: PMC4998238.

27. Ciolofan MS, Vlăescu AN, Mogoantă CA, Ioniță E, Ioniță I, Căpitănescu AN, et al. Clinical, Histological and Immunohistochemical Evaluation of Larynx Cancer. Curr Health Sci J. 2017 Oct-Dec;43(4):367-375. doi: 10.12865/CHSJ.43.04.14. Epub 2017 Dec 28. PMID: 30595905; PMCID: PMC6286454
28. Marioni G, Marchese-Ragona R, Cartei G, Marchese F, Staffieri A. Current opinion in diagnosis and treatment of laryngeal carcinoma. Cancer Treat Rev. 2006 Nov;32(7):504-15. doi: 10.1016/j.ctrv.2006.07.002. Epub 2006 Aug 22. PMID: 16920269.
29. Forastiere AA, Ismaila N, Lewin JS, Nathan CA, Adelstein DJ, Eisbruch A, et al. Use of Lar-ynx-Preservation Strategies in the Treatment of Laryngeal Cancer: American Society of Clinical Oncology Clinical Prac-tice Guideline Update. J Clin Oncol. 2018 Apr 10;36(11):1143-1169. doi: 10.1200/JCO.2017.75.7385. Epub 2017 Nov 27. PMID: 29172863
30. Baird BJ, Sung CK, Beadle BM, Divi V. Treatment of early-stage laryngeal cancer: A comparison of treatment options. Oral Oncol. 2018 Dec;87:8-16. doi: 10.1016/j.oraloncology.2018.09.012. Epub 2018 Oct 16. PMID: 30527248.
31. García Lorenzo J, Montoro Martínez V, Rigo Quera A, Codina Aroca A, López Vilas M, Quer Agustí M, et al. Modifications in the treatment of advanced laryngeal cancer throughout the last 30 years. Eur Arch Otorhinolaryngol. 2017 Sep;274(9):3449-3455. doi: 10.1007/s00405-017-4639-z. Epub 2017 Jun 17. PMID: 28625009.
32. Saraniti C, Chianetta E, Greco G, Mat Lazim N, Verro B. The Impact of Narrow-band Imaging on the Pre- and Intra- operative Assessments of Neoplastic and Preneoplastic Laryngeal Lesions. A Systematic Review. Int Arch Otorhino-laryngol. 2021 Jul;25(3):e471-e478. doi: 10.1055/s-0040-1719119. Epub 2020 Nov 30. PMID: 34377187; PMCID: PMC8321643
33. Steuer CE, El-Deiry M, Parks JR, Higgins KA, Saba NF. An update on larynx cancer. CA Cancer J Clin. 2017 Jan;67(1):31-50. doi: 10.3322/caac.21386. Epub 2016 Nov 29. PMID: 27898173
34. Bhat GR, Hyole RG, Li J. Head and neck cancer: Current challenges and future perspectives. Adv Cancer Res. 2021;152:67-102. doi: 10.1016/bs.acr.2021.05.002. Epub 2021 Jun 16. PMID: 34353444
35. Saxena P, Selvaraj K, Khare SK, Chaudhary N. Superoxide dismutase as multipotent therapeutic antioxidant enzyme: Role in human diseases. Biotechnol Lett. 2022 Jan;44(1):1-22. doi: 10.1007/s10529-021-03200-3. Epub 2021 Nov 3. PMID: 34734354.

36. Sharma A, Rajappa M, Saxena A, Sharma M. Antioxidant status in advanced cervical cancer patients undergoing neoadjuvant chemoradiation. Br J Biomed Sci. 2007;64(1):23-7. doi: 10.1080/09674845.2007.11732751. PMID: 17444415.
37. Ivanova D, Bakalova R, Lazarova D, Gadjeva V, Zhelev Z. The impact of reactive oxygen species on anticancer therapeutic strategies. Adv Clin Exp Med. 2013 Nov-Dec;22(6):899-908. PMID: 24431321.
38. Sullivan LB, Chandel NS. Mitochondrial reactive oxygen species and cancer. Cancer Metab. 2014 Nov 28;2:17. doi: 10.1186/2049-3002-2-17. PMID: 25671107; PMCID: PMC4323058.
39. Tafani M, Sansone L, Limana F, Arcangeli T, De Santis E, Polese M, et al. The Interplay of Reactive Oxygen Species, Hypoxia, Inflammation, and Sirtuins in Cancer Initiation and Progression. Oxid Med Cell Longev. 2016;2016:3907147. doi: 10.1155/2016/3907147. Epub 2015 Dec 20. PMID: 26798421; PMCID: PMC4699039.
40. Nowinska K, Chmielewska M, Piotrowska A, Pula B, Pastuszewski W, Krecicki T, et al. Correlation between levels of expression of minichromosome maintenance proteins, Ki-67 proliferation antigen and metallothionein I/II in laryngeal squamous cell cancer. Int J Oncol. 2016 Feb;48(2):635-45. doi: 10.3892/ijo.2015.3273. Epub 2015 Nov 27. PMID: 26648405.
41. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J. 2012 Jan;5(1):9-19. doi: 10.1097/WOX.0b013e3182439613. Epub 2012 Jan 13. PMID: 23268465; PMCID: PMC3488923.
42. Klaunig JE, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis. Toxicol Pathol. 2010 Jan;38(1):96-109. doi: 10.1177/0192623309356453. Epub 2009 Dec 17. PMID: 20019356.
43. Guina T, Biasi F, Calfapietra S, Nano M, Poli G. Inflammatory and redox reactions in colorectal carcinogenesis. Ann N Y Acad Sci. 2015 Mar;1340:95-103. doi: 10.1111/nyas.12734. Epub 2015 Feb 27. PMID: 25727454.
44. Murata M, Thanan R, Ma N, Kawanishi S. Role of nitrative and oxidative DNA damage in inflammation-related carcinogenesis. J Biomed Biotechnol. 2012;2012:623019. doi: 10.1155/2012/623019. Epub 2012 Jan 26. PMID: 22363173; PMCID: PMC3272848.

45. Huo L, Li CW, Huang TH, Lam YC, Xia W, Tu C, et al. Activation of Keap1/Nrf2 signaling pathway by nuclear epidermal growth factor receptor in cancer cells. Am J Transl Res. 2014 Nov 22;6(6):649-63. PMID: 25628777; PMCID: PMC4297334.
46. Korbecki J, Baranowska-Bosiacka I, Gutowska I, Chlubek D. The effect of reactive oxygen species on the synthesis of prostanoids from arachidonic acid. J Physiol Pharmacol. 2013 Aug;64(4):409-21. PMID: 24101387.
47. Matsuzawa A, Ichijo H. Redox control of cell fate by MAP kinase: physiological roles of ASK1-MAP kinase pathway in stress signaling. Biochim Biophys Acta. 2008 Nov;1780(11):1325-36. doi: 10.1016/j.bbagen.2007.12.011. Epub 2008 Jan 16. PMID: 18206122.
48. Barrera G. Oxidative stress and lipid peroxidation products in cancer progression and therapy. ISRN Oncol. 2012;2012:137289. doi: 10.5402/2012/137289. Epub 2012 Oct 17. PMID: 23119185; PMCID: PMC3483701.
49. Verro B, Saraniti C, Carlisi D, Chiesa-Estomba C, Maniaci A, Lechien JR, et al. Biomarkers in Laryngeal Squamous Cell Carcinoma: The Literature Review. Cancers (Basel). 2023 Oct 22;15(20):5096. doi: 10.3390/cancers15205096. PMID: 37894464; PMCID: PMC10604942.
50. Abreu IA, Cabelli DE. Superoxide dismutases-a review of the metal-associated mechanistic variations. Biochim Biophys Acta. 2010 Feb;1804(2):263-74. doi: 10.1016/j.bbapap.2009.11.005. Epub 2009 Nov 13. PMID: 19914406.
51. Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, Dhama K. Oxidative stress, prooxidants, and antioxidants: the interplay. Biomed Res Int. 2014;2014:761264. doi: 10.1155/2014/761264. Epub 2014 Jan 23. PMID: 24587990; PMCID: PMC3920909.
52. Song MA, Benowitz NL, Berman M, Brasky TM, Cummings KM, Hatsukami DK, et al. Cigarette Filter Ventilation and its Relationship to Increasing Rates of Lung Adenocarcinoma. J Natl Cancer Inst. 2017 Dec 1;109(12):djx075. doi: 10.1093/jnci/djx075. PMID: 28525914; PMCID: PMC6059254.
53. O'Connor RJ, Hurley PJ. Existing technologies to reduce specific toxicant emissions in cigarette smoke. Tob Control. 2008 Sep;17 Suppl 1:i39-48. doi: 10.1136/tc.2007.023689. PMID: 18768458.
54. National Center for Chronic Disease Prevention and Health Promotion (US) Office on Smoking and Health. The Health Consequences of Smoking—50 Years of Progress: A Report of the Surgeon General. Atlanta (GA): Centers for Disease

Control and Prevention (US); 2014. Available from: https://www.ncbi.nlm.nih.gov/books/NBK179276/

55. Kozlowski LT, O'Connor RJ. Cigarette filter ventilation is a defective design because of misleading taste, bigger puffs, and blocked vents. Tob Control. 2002 Mar;11 Suppl 1(Suppl 1):I40-50. doi: 10.1136/tc.11.suppl_1.i40. PMID: 11893814; PMCID: PMC1766061.
56. Horinouchi T, Higashi T, Mazaki Y, Miwa S. Carbonyl Compounds in the Gas Phase of Cigarette Mainstream Smoke and Their Pharmacological Properties. Biol Pharm Bull. 2016;39(6):909-14. doi: 10.1248/bpb.b16-00025. PMID: 27251492.
57. Caliri AW, Tommasi S, Besaratinia A. Relationships among smoking, oxidative stress, inflammation, macromolecular damage, and cancer. Mutat Res Rev Mutat Res. 2021 Jan-Jun;787:108365. doi: 10.1016/j.mrrev.2021.108365. Epub 2021 Jan 11. PMID: 34083039; PMCID: PMC8287787.
58. Hecht SS. Lung carcinogenesis by tobacco smoke. Int J Cancer. 2012 Dec 15;131(12):2724-32. doi: 10.1002/ijc.27816. Epub 2012 Oct 3. PMID: 22945513; PMCID: PMC3479369.
59. Pryor WA, Stone K. Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. Ann N Y Acad Sci. 1993 May 28;686:12-27; discussion 27-8. doi: 10.1111/j.1749-6632.1993.tb39148.x. PMID: 8512242.
60. Pryor WA. Cigarette smoke radicals and the role of free radicals in chemical carcinogenicity. Environ Health Perspect. 1997 Jun;105 Suppl 4(Suppl 4):875-82. doi: 10.1289/ehp.97105s4875. PMID: 9255574; PMCID: PMC1470037.
61. Goel R, Bitzer Z, Reilly SM, Trushin N, Foulds J, Muscat J, et al. Variation in Free Radical Yields from U.S. Marketed Cigarettes. Chem Res Toxicol. 2017 Apr 17;30(4):1038-1045. doi: 10.1021/acs.chemrestox.6b00359. Epub 2017 Mar 20. PMID: 28269983; PMCID: PMC5539526.
62. Goel R, Bitzer ZT, Reilly SM, Foulds J, Muscat J, Elias RJ, et al. Influence of Smoking Puff Parameters and Tobacco Varieties on Free Radicals Yields in Cigarette Mainstream Smoke. Chem Res Toxicol. 2018 May 21;31(5):325-331. doi: 10.1021/acs.chemrestox.8b00011. Epub 2018 May 7. PMID: 29701955; PMCID: PMC6471496.
63. Cadet J, Davies KJA, Medeiros MH, Di Mascio P, Wagner JR. Formation and repair of oxidatively generated damage in cellular DNA. Free Radic Biol Med. 2017 Jun;107:13-34. doi: 10.1016/j.freeradbiomed.2016.12.049. Epub 2017 Jan 2. PMID: 28057600; PMCID: PMC5457722.

64. Bandeira CM, de Almeida AÁ, Carta CFL, Almeida AA, de Figueiredo FAT, Sandrim VC, et al. Tobacco influence in heavy metals levels in head and neck cancer cases. Environ Sci Pollut Res Int. 2018 Sep;25(27):27650-27656. doi: 10.1007/s11356-018-2668-9. Epub 2018 Jul 28. PMID: 30056540.
65. International Agency for Research on Cancer (IARC), IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Tobacco Smoke and Involuntary Smoking, Lyon, France, 2004.
66. Caldecott KW. DNA single-strand break repair and spinocerebellar ataxia. Cell. 2003 Jan 10;112(1):7-10. doi: 10.1016/s0092-8674(02)01247-3. PMID: 12526788.
67. Ray Chaudhuri A, Nussenzweig A. The multifaceted roles of PARP1 in DNA repair and chromatin remodelling. Nat Rev Mol Cell Biol. 2017 Oct;18(10):610-621. doi: 10.1038/nrm.2017.53. Epub 2017 Jul 5. PMID: 28676700; PMCID: PMC6591728.
68. Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. Annu Rev Pharmacol Toxicol. 2005;45:51-88. doi: 10.1146/annurev.pharmtox.45.120403.095857. PMID: 15822171.
69. Benhar M. Roles of mammalian glutathione peroxidase and thioredoxin reductase enzymes in the cellular response to nitrosative stress. Free Radic Biol Med. 2018 Nov 1;127:160-164. doi: 10.1016/j.freeradbiomed.2018.01.028. Epub 2018 Feb 3. PMID: 29378334.
70. Orhan H, Evelo CT, Sahin G. Erythrocyte antioxidant defense response against cigarette smoking in humans--the glutathione S-transferase vulnerability. J Biochem Mol Toxicol. 2005;19(4):226-33. doi: 10.1002/jbt.20088. PMID: 16173057.
71. Vlahos R, Bozinovski S. Glutathione peroxidase-1 as a novel therapeutic target for COPD. Redox Rep. 2013;18(4):142-9. doi: 10.1179/1351000213Y.0000000053. PMID: 23849338; PMCID: PMC6837321.
72. Yan M, Lo JC, Edwards JT, Baran PS. Radicals: Reactive Intermediates with Translational Potential. J Am Chem Soc. 2016 Oct 5;138(39):12692-12714. doi: 10.1021/jacs.6b08856. Epub 2016 Sep 26. PMID: 27631602; PMCID: PMC5054485.
73. Helfinger V, Schröder K. Redox control in cancer development and progression. Mol Aspects Med. 2018 Oct;63:88-98. doi: 10.1016/j.mam.2018.02.003. Epub 2018 Mar 5. PMID: 29501614.
74. Leuti A, Maccarrone M, Chiurchiù V. Proresolving Lipid Mediators: Endogenous Modulators of Oxidative Stress. Oxid Med Cell Longev. 2019 Jun 18;2019:8107265. doi: 10.1155/2019/8107265. Erratum in: Oxid Med Cell Longev. 2019 Dec

12;2019:1759464. doi: 10.1155/2019/1759464. PMID: 31316721; PMCID: PMC6604337.

75. Tallima H, El Ridi R. Arachidonic acid: Physiological roles and potential health benefits - A review. J Adv Res. 2017 Nov 24;11:33-41. doi: 10.1016/j.jare.2017.11.004. PMID: 30034874; PMCID: PMC6052655.
76. Kay J, Thadhani E, Samson L, Engelward B. Inflammation-induced DNA damage, mutations and cancer. DNA Repair (Amst). 2019 Nov;83:102673. doi: 10.1016/j.dnarep.2019.102673. Epub 2019 Jul 25. PMID: 31387777; PMCID: PMC6801086.
77. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Alcohol consumption and ethyl carbamate. IARC Monogr Eval Carcinog Risks Hum. 2010;96:3-1383. PMID: 21735939; PMCID: PMC4781168.
78. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Personal habits and indoor combustions. IARC Monogr Eval Carcinog Risks Hum. 2012;100(Pt E):1-538. PMID: 23193840; PMCID: PMC4781577.
79. Clinton SK, Giovannucci EL, Hursting SD. The World Cancer Research Fund/American Institute for Cancer Research Third Expert Report on Diet, Nutrition, Physical Activity, and Cancer: Impact and Future Directions. J Nutr. 2020 Apr 1;150(4):663-671. doi: 10.1093/jn/nxz268. PMID: 31758189; PMCID: PMC7317613.
80. Rossi M, Jahanzaib Anwar M, Usman A, Keshavarzian A, Bishehsari F. Colorectal Cancer and Alcohol Consumption-Populations to Molecules. Cancers (Basel). 2018 Jan 30;10(2):38. doi: 10.3390/cancers10020038. Erratum in: Cancers (Basel). 2024 Nov 29;16(23):3999. doi: 10.3390/cancers16233999. PMID: 29385712; PMCID: PMC5836070.
81. Seitz HK, Stickel F. Molecular mechanisms of alcohol-mediated carcinogenesis. Nat Rev Cancer. 2007 Aug;7(8):599-612. doi: 10.1038/nrc2191. PMID: 17646865.
82. Barve S, Chen SY, Kirpich I, Watson WH, Mcclain C. Development, Prevention, and Treatment of Alcohol-Induced Organ Injury: The Role of Nutrition. Alcohol Res. 2017;38(2):289-302. PMID: 28988580; PMCID: PMC5513692.
83. Millonig G, Wang Y, Homann N, Bernhardt F, Qin H, Mueller S, et al. Ethanol-mediated carcinogenesis in the human esophagus implicates CYP2E1 induction and the generation of carcinogenic DNA-lesions. Int J Cancer. 2011 Feb 1;128(3):533-40. doi: 10.1002/ijc.25604. PMID: 20715111.

84. Albano E. Alcohol, oxidative stress and free radical damage. Proc Nutr Soc. 2006 Aug;65(3):278-90. doi: 10.1079/pns2006496. PMID: 16923312.
85. Ratna A, Mandrekar P. Alcohol and Cancer: Mechanisms and Therapies. Biomolecules. 2017 Aug 14;7(3):61. doi: 10.3390/biom7030061. PMID: 28805741; PMCID: PMC5618242.
86. Han Y, Chen JZ. Oxidative stress induces mitochondrial DNA damage and cytotoxicity through independent mechanisms in human cancer cells. Biomed Res Int. 2013;2013:825065. doi: 10.1155/2013/825065. Epub 2012 Dec 26. PMID: 23509785; PMCID: PMC3591153
87. Tuli HS, Kaur J, Vashishth K, Sak K, Sharma U, Choudhary R, et al. Molecular mechanisms behind ROS regulation in cancer: A balancing act between augmented tumorigenesis and cell apoptosis. Arch Toxicol. 2023 Jan;97(1):103-120. doi: 10.1007/s00204-022-03421-z. Epub 2022 Nov 28. PMID: 36443493
88. Zheng X, Tang P, Li H, Ye T, Zhu X, He W, et al. Cucurbitacin E elicits apoptosis in laryngeal squamous cell carcinoma by enhancing reactive oxygen species-regulated mitochondrial dysfunction and endoplasmic reticulum stress. Am J Cancer Res. 2024 Aug 25;14(8):3905-3921. doi: 10.62347/HPQQ9412. PMID: 39267666; PMCID: PMC11387858
89. Lubiński J, Jaworowska E, Derkacz R, Marciniak W, Białkowska K, Baszuk P, et al. Survival of Laryngeal Cancer Patients Depending on Zinc Serum Level and Oxidative Stress Genotypes. Biomolecules. 2021 Jun 10;11(6):865. doi: 10.3390/biom11060865. PMID: 34200699; PMCID: PMC8228711
90. Yuzhalin AE, Kutikhin AG. Inherited variations in the SOD and GPX gene families and cancer risk. Free Radic Res. 2012 May;46(5):581-99. doi: 10.3109/10715762.2012.658515. Epub 2012 Feb 8. PMID: 22257147
91. Bastaki M, Huen K, Manzanillo P, Chande N, Chen C, Balmes JR, et al. Genotype-activity relationship for Mn-superoxide dismutase, glutathione peroxidase 1 and catalase in humans. Pharmacogenet Genomics. 2006 Apr;16(4):279-86. doi: 10.1097/01.fpc.0000199498.08725.9c. PMID: 16538174
92. Emanuele S, D'Anneo A, Calvaruso G, Cernigliaro C, Giuliano M, Lauricella M. The Double-Edged Sword Profile of Redox Signaling: Oxidative Events As Molecular Switches in the Balance between Cell Physiology and Cancer. Chem Res Toxicol. 2018 Apr 16;31(4):201-210. doi: 10.1021/acs.chemrestox.7b00311. Epub 2018 Mar 14. PMID: 29513521

93. Bozan N, Demir H, Gürsoy T, Özkan H, Düzenli U, Sarıkaya E, et al. Alterations in oxidative stress markers in laryngeal carcinoma patients. J Chin Med Assoc. 2018 Sep;81(9):811-815. doi: 10.1016/j.jcma.2018.02.004. Epub 2018 May 31. PMID: 29778552
94. Zhou G, Liu Z, Myers JN. TP53 Mutations in Head and Neck Squamous Cell Carcinoma and Their Impact on Disease Progression and Treatment Response. J Cell Biochem. 2016 Dec;117(12):2682-2692. doi: 10.1002/jcb.25592. Epub 2016 Jun 3. PMID: 27166782; PMCID: PMC5493146.
95. Yu X, Li Z. The role of microRNAs expression in laryngeal cancer. Oncotarget. 2015 Sep 15;6(27):23297-305. doi: 10.18632/oncotarget.4195. PMID: 26079642; PMCID: PMC4695119.
96. Zhou C, Ye M, Ni S, Li Q, Ye D, Li J, Shen Z, Deng H. DNA methylation biomarkers for head and neck squamous cell carcinoma. Epigenetics. 2018;13(4):398-409. doi: 10.1080/15592294.2018.1465790. Epub 2018 Aug 10. PMID: 29927694; PMCID: PMC6140809.
97. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002 Dec 19-26;420(6917):860-7. doi: 10.1038/nature01322. PMID: 12490959; PMCID: PMC2803035.
98. Gonzalez H, Hagerling C, Werb Z. Roles of the immune system in cancer: from tumor initiation to metastatic progression. Genes Dev. 2018 Oct 1;32(19-20):1267-1284. doi: 10.1101/gad.314617.118. PMID: 30275043; PMCID: PMC6169832.
99. Forest KT, Langford PR, Kroll JS, Getzoff ED. Cu,Zn superoxide dismutase structure from a microbial pathogen establishes a class with a conserved dimer interface. J Mol Biol. 2000 Feb 11;296(1):145-53. doi: 10.1006/jmbi.1999.3448. PMID: 10656823.
100. Atzenhofer W, Regelsberger G, Jacob U, Peschek G, Furtmüller P, Huber R, et al. The 2.0A resolution structure of the catalytic portion of a cyanobacterial membrane-bound manganese superoxide dismutase. J Mol Biol. 2002 Aug 16;321(3):479-89. doi: 10.1016/s0022-2836(02)00624-1. PMID: 12162960.
101. Antonyuk SV, Strange RW, Marklund SL, Hasnain SS. The structure of human extracellular copper-zinc superoxide dismutase at 1.7 A resolution: insights into heparin and collagen binding. J Mol Biol. 2009 May 1;388(2):310-26. doi: 10.1016/j.jmb.2009.03.026. Epub 2009 Mar 14. PMID: 19289127.
102. Griess B, Tom E, Domann F, Teoh-Fitzgerald M. Extracellular superoxide dismutase and its role in cancer. Free Radic Biol Med. 2017 Nov;112:464-479. doi:

10.1016/j.freeradbiomed.2017.08.013. Epub 2017 Aug 24. PMID: 28842347; PMCID: PMC5685559.

103. Mohideen K, Chandrasekaran K, M K, T J, Dhungel S, Ghosh S. Assessment of Antioxidant Enzyme Superoxide Dismutase (SOD) in Oral Cancer: Systematic Review and Meta-Analysis. Dis Markers. 2024 Mar 16;2024:2264251. doi: 10.1155/2024/2264251. PMID: 38525070; PMCID: PMC10960654.

104. Babiuch K, Bednarczyk A, Gawlik K, Pawlica-Gosiewska D, Kęsek B, Darczuk D, et al. Evaluation of enzymatic and non-enzymatic antioxidant status and biomarkers of oxidative stress in saliva of patients with oral squamous cell carcinoma and oral leukoplakia: a pilot study. Acta Odontol Scand. 2019 Aug;77(6):408-418. doi: 10.1080/00016357.2019.1578409. Epub 2019 Mar 11. PMID: 30857448.

105. Huo W, Li ZM, Pan XY, Bao YM, An LJ. Antioxidant enzyme levels in pathogenesis of oral squamous cell carcinoma (OSCC). Drug Res (Stuttg). 2014 Nov;64(11):629-32. doi: 10.1055/s-0034-1370916. Epub 2014 Mar 12. PMID: 24623030.

106. Fu TY, Hou YY, Chu ST, Liu CF, Huang CH, Chen HC, et al. Manganese superoxide dismutase and glutathione peroxidase as prognostic markers in patients with buccal mucosal squamous cell carcinomas. Head Neck. 2011 Nov;33(11):1606-15. doi: 10.1002/hed.21653. Epub 2011 Jan 10. PMID: 21990225.

107. Strycharz-Dudziak M, Fołtyn S, Dworzański J, Kiełczykowska M, Malm M, Drop B, et al. Glutathione Peroxidase (GPx) and Superoxide Dismutase (SOD) in Oropharyngeal Cancer Associated with EBV and HPV Coinfection. Viruses. 2020 Sep 9;12(9):1008. doi: 10.3390/v12091008. PMID: 32917014; PMCID: PMC7551554.

108. Liu Z, Li S, Cai Y, Wang A, He Q, Zheng C, et al. Manganese superoxide dismutase induces migration and invasion of tongue squamous cell carcinoma via H2O2-dependent Snail signaling. Free Radic Biol Med. 2012 Jul 1;53(1):44-50. doi: 10.1016/j.freeradbiomed.2012.04.031. Epub 2012 May 9. PMID: 22580338; PMCID: PMC3377784.

109. Liu X, Wang A, Lo Muzio L, Kolokythas A, Sheng S, Rubini C, et al. Deregulation of manganese superoxide dismutase (SOD2) expression and lymph node metastasis in tongue squamous cell carcinoma. BMC Cancer. 2010 Jul 9;10:365. doi: 10.1186/1471-2407-10-365. PMID: 20618948; PMCID: PMC2911422.

110. Hardy RG, Vicente-Dueñas C, González-Herrero I, Anderson C, Flores T, Hughes S, et al. Snail family transcription factors are implicated in thyroid carcinogenesis. Am J Pathol. 2007 Sep;171(3):1037-46. doi: 10.2353/ajpath.2007.061211. PMID: 17724139; PMCID: PMC1959496.

111. Parascandolo A, Rappa F, Cappello F, Kim J, Cantu DA, Chen H, et al. Extracellular Superoxide Dismutase Expression in Papillary Thyroid Cancer Mesenchymal Stem/Stromal Cells Modulates Cancer Cell Growth and Migration. Sci Rep. 2017 Feb 20;7:41416. doi: 10.1038/srep41416. PMID: 28216675; PMCID: PMC5316948.

112. Cammarota F, de Vita G, Salvatore M, Laukkanen MO. Ras oncogene-mediated progressive silencing of extracellular superoxide dismutase in tumorigenesis. Biomed Res Int. 2015;2015:780409. doi: 10.1155/2015/780409. Epub 2015 Oct 15. PMID: 26550576; PMCID: PMC4624945.

113. Laukkanen MO. Extracellular Superoxide Dismutase: Growth Promoter or Tumor Suppressor? Oxid Med Cell Longev. 2016;2016:3612589. doi: 10.1155/2016/3612589. Epub 2016 May 12. PMID: 27293512; PMCID: PMC4880707.

114. Laatikainen LE, Castellone MD, Hebrant A, Hoste C, Cantisani MC, Laurila JP, et al. Extracellular superoxide dismutase is a thyroid differentiation marker down-regulated in cancer. Endocr Relat Cancer. 2010 Aug 16;17(3):785-96. doi: 10.1677/ERC-10-0021. PMID: 20576801.

115. Castellone MD, Langella A, Cantara S, Laurila JP, Laatikainen LE, Bellelli R, et al. Extracellular superoxide dismutase induces mouse embryonic fibroblast proliferative burst, growth arrest, immortalization, and consequent in vivo tumorigenesis. Antioxid Redox Signal. 2014 Oct 1;21(10):1460-74. doi: 10.1089/ars.2013.5475. Epub 2014 Feb 19. PMID: 24328532.

116. Li S, Mao Y, Zhou T, Luo C, Xie J, Qi W, et al. Manganese superoxide dismutase mediates anoikis resistance and tumor metastasis in nasopharyngeal carcinoma. Oncotarget. 2016 May 31;7(22):32408-20. doi: 10.18632/oncotarget.8717. PMID: 27083052; PMCID: PMC5078022.

117. Durak I, Işik AC, Canbolat O, Akyol O, Kavutçu M. Adenosine deaminase, 5' nucleotidase, xanthine oxidase, superoxide dismutase, and catalase activities in cancerous and noncancerous human laryngeal tissues. Free Radic Biol Med. 1993 Dec;15(6):681-4. doi: 10.1016/0891-5849(93)90174-s. PMID: 8138195

118. Inci E, Civelek S, Seven A, Inci F, Korkut N, Burçax G. Laryngeal cancer: in relation to oxidative stress. Tohoku J Exp Med. 2003 May;200(1):17-23. doi: 10.1620/tjem.200.17. PMID: 12862307

119. Kalayci A, Ozturk A, Ozturk K, Karagozoglu E, Dolanmaz D. Superoxide dismutase and glutathione peroxidase enzyme activities in larynx carcinoma. Acta Otolaryngol. 2005 Mar;125(3):312-5. doi: 10.1080/00016480410023074. PMID: 15966704

120. Glorieux C, Calderon PB. Catalase, a remarkable enzyme: targeting the oldest antioxidant enzyme to find a new cancer treatment approach. Biol Chem. 2017 Sep 26;398(10):1095-1108. doi: 10.1515/hsz-2017-0131. PMID: 28384098.

121. Rainis T, Maor I, Lanir A, Shnizer S, Lavy A. Enhanced oxidative stress and leucocyte activation in neoplastic tissues of the colon. Dig Dis Sci. 2007 Feb;52(2):526-30. doi: 10.1007/s10620-006-9177-2. Epub 2006 Dec 29. PMID: 17195121.

122. Sander CS, Hamm F, Elsner P, Thiele JJ. Oxidative stress in malignant melanoma and non-melanoma skin cancer. Br J Dermatol. 2003 May;148(5):913-22. doi: 10.1046/j.1365-2133.2003.05303.x. PMID: 12786821.

123. Kwei KA, Finch JS, Thompson EJ, Bowden GT. Transcriptional repression of catalase in mouse skin tumor progression. Neoplasia. 2004 Sep-Oct;6(5):440-8. doi: 10.1593/neo.04127. PMID: 15548352; PMCID: PMC1550329.

124. Beck R, Pedrosa RC, Dejeans N, Glorieux C, Levêque P, Gallez B, et al. Ascorbate/menadione-induced oxidative stress kills cancer cells that express normal or mutated forms of the oncogenic protein Bcr-Abl. An in vitro and in vivo mechanistic study. Invest New Drugs. 2011 Oct;29(5):891-900. doi: 10.1007/s10637-010-9441-3. Epub 2010 May 8. PMID: 20454833.

125. Glorieux C, Sandoval JM, Fattaccioli A, Dejeans N, Garbe JC, Dieu M, et al. Chromatin remodeling regulates catalase expression during cancer cells adaptation to chronic oxidative stress. Free Radic Biol Med. 2016 Oct;99:436-450. doi: 10.1016/j.freeradbiomed.2016.08.031. Epub 2016 Aug 31. PMID: 27591797.

126. Glorieux C, Buc Calderon P. Targeting catalase in cancer. Redox Biol. 2024 Nov;77:103404. doi: 10.1016/j.redox.2024.103404. Epub 2024 Oct 19. PMID: 39447253; PMCID: PMC11539659.

127. Wang S, Yang S, Vlantis AC, Liu SY, Ng EK, Chan AB, et al. Expression of Antioxidant Molecules and Heat Shock Protein 27 in Thyroid Tumors. J Cell

Biochem. 2016 Nov;117(11):2473-81. doi: 10.1002/jcb.25539. Epub 2016 Aug 16. PMID: 26970173.

128. Alakuş H, Kaya M, Özer H, Eğilmez HR, Karadayı K. Nuclear factor erythroid-2-related factor 2 (Nrf2) is a potential prognostic factor in patients with gastric adenocarcinoma. Arab J Gastroenterol. 2023;24(1):5-10. doi:10.1016/j.ajg.2022.10.010

129. Menegon S, Columbano A, Giordano S. The Dual Roles of NRF2 in Cancer. Trends Mol Med. 2016;22(7):578-593. doi:10.1016/j.molmed.2016.05.002

130. Cheraghi O, Dabirmanesh B, Ghazi F, et al. The effect of Nrf2 deletion on the proteomic signature in a human colorectal cancer cell line. BMC Cancer. 2022;22(1):979. Published 2022 Sep 13. doi:10.1186/s12885-022-10055-y

131. Jana S, Patra K, Jana J, Mandal DP, Bhattacharjee S. Nrf-2 transcriptionally activates P21Cip/WAF1 and promotes A549 cell survival against oxidative stress induced by H2O2. Chem Biol Interact. 2018;285:59-68. doi:10.1016/j.cbi.2018.02.030

132. Zhou Z, Xu J, Bao X, Shi J, Liu B, Chen Y, et al. Nuclear Nrf2 Activity in Laryngeal Carcinoma is Regulated by SENP3 After Cisplatin-Induced Reactive Oxygen Species Stress. J Cancer. 2019 Jun 9;10(15):3427-3434. doi: 10.7150/jca.30318. PMID: 31293646; PMCID: PMC6603410.

133. Sheth S, Farquhar DR, Schrank TP, Stepp W, Mazet al Lenze N, Little P, Jo H, Major MB, Chera BS, Zevallos JP, Hayes DN. Correlation of alterations in the KEAP1/CUL3/NFE2L2 pathway with radiation failure in larynx squamous cell carcinoma. Laryngoscope Investig Otolaryngol. 2021 Jun 3;6(4):699-707. doi: 10.1002/lio2.588. PMID: 34401494; PMCID: PMC8356873.

134. Motterlini R, Foresti R. Heme oxygenase-1 as a target for drug discovery. Antioxid Redox Signal. 2014 Apr 10;20(11):1810-26. doi: 10.1089/ars.2013.5658. Epub 2014 Feb 28. PMID: 24180608.

135. Loboda A, Jozkowicz A, Dulak J. HO-1/CO system in tumor growth, angiogenesis and metabolism - Targeting HO-1 as an anti-tumor therapy. Vascul Pharmacol. 2015 Nov;74:11-22. doi: 10.1016/j.vph.2015.09.004. Epub 2015 Sep 25. PMID: 26392237.

136. Yanagawa T, Omura K, Harada H, Nakaso K, Iwasa S, Koyama Y, et al. Heme oxygenase-1 expression predicts cervical lymph node metastasis of tongue squamous cell carcinomas. Oral Oncol. 2004 Jan;40(1):21-7. doi: 10.1016/s1368-8375(03)00128-3. PMID: 14662411.

137. Chang KW, Lee TC, Yeh WI, Chung MY, Liu CJ, Chi LY, et al. Polymorphism in heme oxygenase-1 (HO-1) promoter is related to the risk of oral squamous cell carcinoma occurring on male areca chewers. Br J Cancer. 2004 Oct 18;91(8):1551-5. doi: 10.1038/sj.bjc.6602186. PMID: 15365571; PMCID: PMC2409944.

138. Lee SS, Yang SF, Tsai CH, Chou MC, Chou MY, Chang YC. Upregulation of heme oxygenase-1 expression in areca-quid-chewing-associated oral squamous cell carcinoma. J Formos Med Assoc. 2008 May;107(5):355-63. doi: 10.1016/S0929-6646(08)60100-X. PMID: 18492619

139. Gandini NA, Fermento ME, Salomón DG, Blasco J, Patel V, Gutkind JS, et al. Nuclear localization of heme oxygenase-1 is associated with tumor progression of head and neck squamous cell carcinomas. Exp Mol Pathol. 2012 Oct;93(2):237-45. doi: 10.1016/j.yexmp.2012.05.001. Epub 2012 May 8. PMID: 22580187.

140. Yang PS, Hsu YC, Lee JJ, Chen MJ, Huang SY, Cheng SP. Heme Oxygenase-1 Inhibitors Induce Cell Cycle Arrest and Suppress Tumor Growth in Thyroid Cancer Cells. Int J Mol Sci. 2018 Aug 24;19(9):2502. doi: 10.3390/ijms19092502. PMID: 30149527; PMCID: PMC6163304.

141. Lv X, Song DM, Niu YH, Wang BS. Inhibition of heme oxygenase-1 enhances the chemosensitivity of laryngeal squamous cell cancer Hep-2 cells to cisplatin. Apoptosis. 2016;21(4):489-501. doi:10.1007/s10495-016-1216-7

142. Tang D, Tang WJ, Shi XL, Li WP, Zhou H, Lu LM, et al. Association of the microsatellite (GT)n repeat polymorphisms of the HO-1 gene promoter and corresponding serum levels with the risk of laryngeal squamous cell carcinoma. Acta Otolaryngol. 2016 Aug;136(8):806-11. doi: 10.3109/00016489.2016.1157265. Epub 2016 Mar 24. PMID: 27400252.

143. Brazão-Silva MT, Rodrigues MF, Eisenberg AL, Dias FL, de Castro LM, Nunes FD, et al. Metallothionein gene expression is altered in oral cancer and may predict metastasis and patient outcomes. Histopathology. 2015 Sep;67(3):358-67. doi: 10.1111/his.12660. Epub 2015 Mar 8. PMID: 25640883.

144. Thirumoorthy N, Manisenthil Kumar KT, Shyam Sundar A, Panayappan L, Chatterjee M. Metallothionein: an overview. World J Gastroenterol. 2007;13(7):993-996. doi:10.3748/wjg.v13.i7.993

145. Bezerra GMDR, Pontes FSC, Conte Neto N, Nascimento LSD, Souza LL, Pinto Junior DDS, et al. Nuclear metallothionein in oral squamous cell carcinoma: clinicopathological parameters and patient survival. Braz Oral Res. 2018 Oct 11;32:e105. doi: 10.1590/1807-3107bor-2018.vol32.0105. PMID: 30328895.

146. Li D, Peng W, Wu B, Liu H, Zhang R, Zhou R, et al. Metallothionein MT1M Suppresses Carcinogenesis of Esophageal Carcinoma Cells through Inhibition of the Epithelial-Mesenchymal Transition and the SOD1/PI3K Axis. Mol Cells. 2021 Apr 30;44(4):267-278. doi: 10.14348/molcells.2021.2179. PMID: 33820882; PMCID: PMC8112171.

147. Pastuszewski W, Dziegiel P, Krecicki T, Podhorska-Okolow M, Ciesielska U, Gorzynska E, et al. Prognostic significance of metallothionein, p53 protein and Ki-67 antigen expression in laryngeal cancer. Anticancer Res. 2007 Jan-Feb;27(1A):335-42. PMID: 17352251.

148. Ioachim E, Assimakopoulos D, Peschos D, Zissi A, Skevas A, Agnantis NJ. Immunohistochemical expression of metallothionein in benign premalignant and malignant epithelium of the larynx: correlation with p53 and proliferative cell nuclear antigen. Pathol Res Pract. 1999;195(12):809-814. doi:10.1016/S0344-0338(99)80102-2

149. Starska K, Krześlak A, Forma E, Olszewski J, Lewy-Trenda I, Osuch-Wójcikiewicz E, et al. Genetic polymorphism of metallothionein 2A and risk of laryngeal cancer in a Polish population. Med Oncol. 2014 Jul;31(7):75. doi: 10.1007/s12032-014-0075-8. Epub 2014 Jun 22. PMID: 24952512.

150. van Loon K, van Breest Smallenburg ME, Huijbers EJM, Griffioen AW, van Beijnum JR. Extracellular vimentin as a versatile immune suppressive protein in cancer. Biochim Biophys Acta Rev Cancer. 2023;1878(6):188985. doi:10.1016/j.bbcan.2023.188985

151. Meyer HJ, Höhn AK, Surov A. Associations Between ADC and Tumor Infiltrating Lymphocytes, Tumor-Stroma Ratio and Vimentin Expression in Head and Neck Squamous Cell Cancer. Acad Radiol. 2022;29 Suppl 3:S107-S113. doi:10.1016/j.acra.2021.05.007

152. Chen Z, Fang Z, Ma J. Regulatory mechanisms and clinical significance of vimentin in breast cancer. Biomed Pharmacother. 2021;133:111068. doi:10.1016/j.biopha.2020.111068

153. Kalaei Z, Shekarchi AA, Hojjat-Farsangi M, Jalali P, Jadidi-Niaragh F. The prognostic and therapeutic potential of vimentin in colorectal cancer. Mol Biol Rep. 2024;51(1):1027. Published 2024 Sep 30. doi:10.1007/s11033-024-09965-w

154. Yin S, Chen FF, Yang GF. Vimentin immunohistochemical expression as a prognostic factor in gastric cancer: A meta-analysis. Pathol Res Pract. 2018;214(9):1376-1380. doi:10.1016/j.prp.2018.07.014

155. Xuan B, Ghosh D, Jiang J, Shao R, Dawson MR. Vimentin filaments drive migratory persistence in polyploidal cancer cells. Proc Natl Acad Sci U S A. 2020;117(43):26756-26765. doi:10.1073/pnas.2011912117

156. Karamagkiolas S, Giotakis I, Kyrodimos E, Giotakis EI, Kataki A, Karagianni F, et al. Expression of vimentin (VIM) and metastasis-associated 1 (MTA1) protein in laryngeal squamous cell carcinoma are associated with prognostic outcome of patients. Am J Otolaryngol. 2019 Jul-Aug;40(4):487-493. doi: 10.1016/j.amjoto.2019.04.002. Epub 2019 Apr 3. PMID: 30979652.

157. Mizdrak I, Mizdrak M, Racetin A, Bošković B, Benzon B, Durdov MG, et al. Expression of Connexins 37, 40 and 45, Pannexin 1 and Vimentin in Laryngeal Squamous Cell Carcinomas. Genes (Basel). 2023 Feb 9;14(2):446. doi: 10.3390/genes14020446. PMID: 36833374; PMCID: PMC9956287.

158. Kroemer G, Pouyssegur J. Tumor cell metabolism: cancer's Achilles' heel. Cancer Cell. 2008 Jun;13(6):472-82. doi: 10.1016/j.ccr.2008.05.005. PMID: 18538731.

159. Xia L, Oyang L, Lin J, Tan S, Han Y, Wu N, et al. The cancer metabolic reprogramming and immune response. Mol Cancer. 2021 Feb 5;20(1):28. doi: 10.1186/s12943-021-01316-8. PMID: 33546704; PMCID: PMC7863491.

160. Ohashi T, Terazawa K, Shibata H, Inoue N, Ogawa T. Metabolic profiling analysis of head and neck squamous cell carcinoma. Oral Dis. 2024 Mar;30(2):342-352. doi: 10.1111/odi.14432. Epub 2022 Nov 28. PMID: 36349421.

161. Hou YJ, Yang XX, Meng HX. Mitochondrial metabolism in laryngeal cancer: therapeutic mechanisms and prospects. Biochim Biophys Acta Rev Cancer. 2025 Jul;1880(3):189335. doi: 10.1016/j.bbcan.2025.189335. Epub 2025 Apr 29. PMID: 40311711.

162. Zheng D, Pu X, Deng X, Liu C, Li S. Metabolic profiles in laryngeal cancer defined two distinct molecular subtypes with divergent prognoses. Front Immunol. 2025 May 22;16:1512502. doi: 10.3389/fimmu.2025.1512502. PMID: 40475768; PMCID: PMC12137314.

163. Ma K, Mao Q, Fei B, Ni T, Zhang Z, Ni H. Metabolic reprogramming and immune microenvironment characteristics in laryngeal carcinoma: advances in immunotherapy. Front Immunol. 2025 Apr 30;16:1589243. doi: 10.3389/fimmu.2025.1589243. PMID: 40370437; PMCID: PMC12075248.

164. Zhang C, Zhang Z, Zhu Y, Qin S. Glucose-6-phosphate dehydrogenase: a biomarker and potential therapeutic target for cancer. Anticancer Agents Med Chem. 2014 Feb;14(2):280-9. doi: 10.2174/18715206113136660337. PMID: 24066844.

165. Zhen X, Zhang M, Hao S, Sun J. Glucose-6-phosphate dehydrogenase and transketolase: Key factors in breast cancer progression and therapy. Biomed Pharmacother. 2024 Jul;176:116935. doi: 10.1016/j.biopha.2024.116935. Epub 2024 Jun 14. PMID: 38876050.

166. Pes GM, Errigo A, Soro S, Longo NP, Dore MP. Glucose-6-phosphate dehydrogenase deficiency reduces susceptibility to cancer of endodermal origin. Acta Oncol. 2019 Sep;58(9):1205-1211. doi: 10.1080/0284186X.2019.1616815. Epub 2019 May 21. PMID: 31109224.

167. Wang X, Li X, Zhang X, Fan R, Gu H, Shi Y, et al. Glucose-6-phosphate dehydrogenase expression is correlated with poor clinical prognosis in esophageal squamous cell carcinoma. Eur J Surg Oncol. 2015 Oct;41(10):1293-9. doi: 10.1016/j.ejso.2015.08.155. Epub 2015 Aug 24. PMID: 26329784.

168. Abdullah A, Kumbrink J, Liokatis P, Mock A, Abdullah A, Dewenter I, et al. The Role of Glucose-6-Phosphate Dehydrogenase in Skin Cancer Metabolism: A Paradigm Shift in Treatment Approaches. Cancers (Basel). 2024 Dec 27;17(1):48. doi: 10.3390/cancers17010048. PMID: 39796677; PMCID: PMC11718909.

169. Li R, Wang W, Yang Y, Gu C. Exploring the role of glucose-6-phosphate dehydrogenase in cancer (Review). Oncol Rep. 2020 Dec;44(6):2325-2336. doi: 10.3892/or.2020.7803. Epub 2020 Oct 12. PMID: 33125150.

170. Cheng AJ, Chiu DT, See LC, Liao CT, Chen IH, Chang JT. Poor prognosis in nasopharyngeal cancer patients with low glucose-6-phosphate-dehydrogenase activity. Jpn J Cancer Res. 2001 May;92(5):576-81. doi: 10.1111/j.1349-7006.2001.tb01132.x. PMID: 11376568; PMCID: PMC5926748.

171. Dessì S, Batetta B, Pani P, Barra S, Miranda F, Puxeddu P. Glucose-6-phosphate dehydrogenase (G6PD) activity in tumoral tissues of G6PD-deficient subjects affected by larynx carcinoma. Cancer Lett. 1990;53(2-3):159-162. doi:10.1016/0304-3835(90)90209-g

172. Nan D, Yao W, Huang L, Liu R, Chen X, Xia W, et al. Glutamine and cancer: metabolism, immune microenvironment, and therapeutic targets. Cell Commun Signal. 2025 Jan 24;23(1):45. doi: 10.1186/s12964-024-02018-6. PMID: 39856712; PMCID: PMC11760113.

173. Jin J, Byun JK, Choi YK, Park KG. Targeting glutamine metabolism as a therapeutic strategy for cancer. Exp Mol Med. 2023 Apr;55(4):706-715. doi: 10.1038/s12276-023-00971-9. Epub 2023 Apr 3. PMID: 37009798; PMCID: PMC10167356.

174. Guo S, Wang X, Wang Y, Bai J, Liu Y, Shao Z. The potential therapeutic targets of glutamine metabolism in head and neck squamous cell carcinoma. Biomed Pharmacother. 2024 Jul;176:116906. doi: 10.1016/j.biopha.2024.116906. Epub 2024 Jun 14. PMID: 38876051.

175. Basheer HA, Elsalem L, Salem A, Tailor A, Hunter K, Afarinkia K. The Expression of Glutaminases and their Association with Clinicopathological Parameters in the Head and Neck Cancers. Curr Cancer Drug Targets. 2022;22(2):169-179. doi: 10.2174/1568009622666211224111425. PMID: 34951574.

176. Yoo HC, Park SJ, Nam M, Kang J, Kim K, Yeo JH, et al. A Variant of SLC1A5 Is a Mitochondrial Glutamine Transporter for Metabolic Reprogramming in Cancer Cells. Cell Metab. 2020 Feb 4;31(2):267-283.e12. doi: 10.1016/j.cmet.2019.11.020. Epub 2019 Dec 19. PMID: 31866442.

177. Luo JT, Wang YF, Wang Y, Wang CL, Liu RY, Zhang Z. CircMAT2B facilitates the progression of head and neck squamous cell carcinoma via sponging miR-491-5p to trigger ASCT2-mediated glutaminolysis. Mol Cell Biochem. 2023 May;478(5):1067-1081. doi: 10.1007/s11010-022-04565-3. Epub 2022 Oct 11. PMID: 36219356.

178. Zhang Z, Liu R, Shuai Y, Huang Y, Jin R, Wang X, et al. ASCT2 (SLC1A5)-dependent glutamine uptake is involved in the progression of head and neck squamous cell carcinoma. Br J Cancer. 2020 Jan;122(1):82-93. doi: 10.1038/s41416-019-0637-9. Epub 2019 Dec 10. PMID: 31819178; PMCID: PMC6964701.

179. Paneque A, Fortus H, Zheng J, Werlen G, Jacinto E. The Hexosamine Biosynthesis Pathway: Regulation and Function. Genes (Basel). 2023 Apr 18;14(4):933. doi: 10.3390/genes14040933. PMID: 37107691; PMCID: PMC10138107.

180. Yang C, Chu F, Chen X, Meng F, Li Y, Chen J, et al. Glutamine: fructose-6-phosphate amidotransferase (GFAT) in the pathology of diseases: a review. Cell Death Discov. 2025 Dec 14;12(1):52. doi: 10.1038/s41420-025-02898-8. PMID: 41390473; PMCID: PMC12847842.

181. Jeong M, Kim BC, Choi HJ, Lee GT, Jang SM, Kim KB. Asparagine synthetase (ASNS) Drives Tumorigenicity in Small Cell Lung Cancer. Biomedicines. 2025 Dec 15;13(12):3087. doi: 10.3390/biomedicines13123087. PMID: 41463097; PMCID: PMC12731195.

182. O'Keefe EL, DiNicolantonio JJ, O'Keefe JH, Lavie CJ. Alcohol and CV Health: Jekyll and Hyde J-Curves. Prog Cardiovasc Dis. 2018 May-Jun;61(1):68-75. doi: 10.1016/j.pcad.2018.02.001. Epub 2018 Feb 16. PMID: 29458056.

183. Brierley J, Giuliani M, O'Sullivan B, Rous B, Van Eycken E, editors. TNM Classification of Malignant Tumours. 9th ed. New York: Wiley; 2025

184. College of American Pathologists. Head and Neck Biomarker Reporting Template (Version 2.0.0.1). 2021. Available online:https://documents.cap.org/protocols/HN.Bmk_2.0.0.1.REL_CAPCP.pdf (accessed on 20 March 2024).

185. Agilent Technologies. PD-L1 IHC 22C3 pharmDx Interpretation Manual: Head and Neck Squamous Cell Carcinoma (HN-SCC). Available online: https://www.agilent.com/cs/library/usermanuals/public/29314_22c3_pharmDx_hnscc_interpretation_manual_us.pdf (accessed on 20 March 2024).

186. Verro B, Oliveri R, Pratelli G, Lauricella M, Di Liberto D, Blasio AD, et al. Metabolism and Metabolic Reprogramming in Laryngeal Squamous Cell Carcinoma. Biomedicines. 2026; 14(5):959. doi: 10.3390/biomedicines14050959

187. Verro B, Saraniti C, Di Liberto D, Pratelli G, Lauricella M, Carlisi D. Oxidative Stress Biomarkers in Laryngeal Squamous Cell Carcinoma and Their Clinical Implications: Preliminary Results. Biomedicines. 2025 Mar 8;13(3):667. doi: 10.3390/biomedicines13030667. PMID: 40149643; PMCID: PMC11940273.

188. Biri H, Oztürk HS, Kaçmaz M, Karaca K, Tokuçoğlu H, Durak I. Activities of DNA turnover and free radical metabolizing enzymes in cancerous human prostate tissue. Cancer Invest. 1999;17(5):314-9. doi: 10.3109/07357909909032872. PMID: 10370358

189. Wise DR, Thompson CB. Glutamine addiction: a new therapeutic target in cancer. Trends Biochem Sci. 2010 Aug;35(8):427-33. doi: 10.1016/j.tibs.2010.05.003. PMID: 20570523; PMCID: PMC2917518.

190. Nikkuni O, Kaira K, Toyoda M, Shino M, Sakakura K, Takahashi K, et al. Expression of Amino Acid Transporters (LAT1 and ASCT2) in Patients with Stage III/IV Laryngeal Squamous Cell Carcinoma. Pathol Oncol Res. 2015

Sep;21(4):1175-81. doi: 10.1007/s12253-015-9954-3. Epub 2015 May 30. PMID: 26024742.

191. Munir S, Lundsager MT, Jørgensen MA, Hansen M, Petersen TH, Bonefeld CM, et al. Inflammation induced PD-L1-specific T cells. Cell Stress. 2019 Sep 13;3(10):319-327. doi: 10.15698/cst2019.10.201. PMID: 31656949; PMCID: PMC6789434.

192. Liu RY, Dong Z, Liu J, Zhou L, Huang W, Khoo SK, et al. Overexpression of asparagine synthetase and matrix metalloproteinase 19 confers cisplatin sensitivity in nasopharyngeal carcinoma cells. Mol Cancer Ther. 2013 Oct;12(10):2157-66. doi: 10.1158/1535-7163.MCT-12-1190. Epub 2013 Aug 16. PMID: 23956056; PMCID: PMC3795908.

193. Braakhuis BJ, Tabor MP, Kummer JA, Leemans CR, Brakenhoff RH. A genetic explanation of Slaughter's concept of field cancerization: evidence and clinical implications. Cancer Res. 2003 Apr 15;63(8):1727-30. PMID: 12702551.