Biomarkers in Laryngeal Squamous Cell Carcinoma

Subjects: Otorhinolaryngology | Oncology | Biochemistry & Molecular Biology

Contributor: Barbara Verro, Carmelo Saraniti, Daniela Carlisi, Carlos Chiesa-Estomba, Antonino Maniaci, Jerome R. Lechien, Miguel Mayo, Nicolas Fakhry, Marianna Lauricella

Laryngeal squamous cell carcinoma is a prevalent cancer associated with poor prognosis in advanced stages. Despite advancements in diagnostic tools, there have been minimal improvements in therapeutic approaches. The potential new frontier lies in the realm of biomarkers.

Keywords: larynx ; laryngeal cancer ; biomarkers ; heat shock proteins ; metallothioneins ; heme oxygenase ; cyclooxygenase-2 ; nuclearfactor erythroid 2-related factor 2 ; micro ribonucleic acid

1. Introduction

Carcinoma of the larynx is the second most common cancer among head and neck cancers, with 177,422 new cases per year and 94,771 deaths per year worldwide [1]. Laryngeal cancer mainly affects the adult population aged between 55 and 65 years old, but cases in young adults (under the age of 40) have also been described [2]. To date, men are five times more affected than women, although there is a progressive increase in incidence in women due to the increased spread of the smoking habit; smoking habit and alcohol abuse represent the leading and main risk factors [3]. Other independent risk factors are occupation-related toxic agents such as polycyclic aromatic hydrocarbons, asbestos, wood dust, cement dust, etc. [4]. Laryngopharyngeal reflux represents another chronic stress factor that could increase the risk of laryngeal cancer, especially bile acids [5]. Regarding histology, squamous cell carcinoma (SCC) accounts for about 95% of laryngeal tumors, with the prevalence of low and moderate grades of differentiation [6]. The glottis is the most commonly affected laryngeal region (about 65–70% of cases), followed by the supraglottis and subglottis [7]. Biopsy with histological examination of the sample continues to be the gold standard for diagnosis. There are different surgical and non-surgical strategies to manage laryngeal squamous cell carcinoma (LSCC) [8]. Depending on the tumor stage, surgical therapy may involve organ preservation approaches (transoral laser microsurgery or transoral robotic surgery), open partial laryngectomies, or total laryngectomy. Non-surgical treatment may involve exclusive radiation therapy (RT), concurrent chemoradiotherapy (CRT), and, in some cases, immunotherapy. The choice of therapy, surgical or non-surgical, depends not only on the features of carcinoma but also on the patient in terms of age, comorbidities, preference, and socio-family background. Despite the fact that policies against smoking and alcohol have led to a lower incidence of laryngeal carcinoma (which today is around 60%) and despite the introduction of more sensitive diagnostic techniques [9], as well as more targeted therapies, 5-year overall survival (OS) has not changed significantly in recent decades leading to a negative prognosis due to late diagnosis in advanced stages in about 60% of cases with a survival rate below 50% [8][10].

So, based on these assumptions, in recent years, new studies have investigated the detection of biomarkers in laryngeal tumor tissue that could help to better characterize the LSCC and could better define a target population to whom a specific therapy may be proposed (RT, CRT, immunotherapy) with greater success rate. For instance, to date, there is an ongoing clinical trial about the possible relationship between the expression level of Excision Repair Crossing Complementation group 1 (ERCC1) biomarker and CRT or chemotherapy alone in locally advanced head and neck squamous cell carcinoma [ClinicalTrials.gov ID NCT02128906].

2. Biomarkers

2.1. Heat Shock Proteins (HSPs)

HSPs are stress-induced proteins involved in intracellular protection mechanisms. The main stress factors responsible for HSP induction are elevated temperature, oxidative stress, hypoxia, heavy metals, ethanol, infections, and radiation. Chaperone and HSP are usually used as synonyms. They are classified according to their molecular weight (expressed in kiloDaltons). They have canonical functions pertinent to maintaining protein homeostasis; indeed, they help the correct folding of many proteins and protect cells from protein misfolding, premature degradation, or aggregation [11]. They also have non-canonical functions, such as participation in immune system regulation, cell differentiation, and carcinogenesis

[12][13][14]. Typically, they are cytoprotective, but if qualitatively and/or quantitatively abnormal, they can become pathogenetic and cause a disease called chaperonopathy.

2.1.1. HSP27

HSP27 represents a small heat shock protein (sHSP) whose effects depend on its phosphorylation, oligomerization, and possible chimera formation with other sHSP. HSP27 usually inhibits apoptosis and necrosis by blocking the caspase system, and it is involved in nuclear protein folding regulation and cell differentiation, too [11]. This chaperone is overexpressed in different types of tumors (breast, lung, prostate, head, and neck) and plays a role in carcinogenesis by promoting anti-apoptotic activity [11][14][15]. In cancer, phosphorylation changes the affinity of HSP27 for its oncoprotein, leading to the activation of anti-apoptotic and pro-survival signaling pathways. This promotes tumor growth, metastatization, and chemoresistance.

In the case of LSCC, HSP27 is found both in the cytoplasm and in the nucleus. In particular, a study carried out on 50 samples of LSCC reported a correlation between the cytoplasmatic expression of phosphorylated HSP27 and nuclear expression of both phosphorylated and non-phosphorylated HSP27 and neck node metastases [16]. Moreover, the expression level of HSP27 is found to be related to tumor stage (p-value 0.0039) and grade of differentiation (p-value < 0.001) [17].

2.1.2. HSP70

HPS70 is involved in the control of cellular homeostasis. It plays a role in inhibiting apoptosis induced by reactive oxygen species (ROS). HSP70 overexpression is related to poor prognosis in endometrial and breast cancers [18]. Xu et al. suggested that the expression level of HSP70 was higher in the advanced stage than in the early stage of LSCC (p-value 0.015) [19]. In particular, overexpression of HSP70 induces radiotherapy (RT) resistance by inhibiting ROS-induced apoptosis promoted by X-ray.

2.1.3. HSP47

HSP47, also called colligin-2, is a collagen protein of the endoplasmic reticulum involved in the production of procollagens. It is overexpressed and related to carcinogenesis and poor prognosis in several tumors (oral cavity, breast) but not in all types of cancers [20].

However, in the larynx, HSP47 expression is higher in normal tissue than in LSCC, and it decreases with a decrease in the degree of differentiation of cancer (p-value < 0.001). A study showed that this chaperone inhibits cancer cell proliferation, induces apoptosis (via intrinsic and extrinsic pathways), and increases sensitivity to cisplatin. So, in this case, its high expression is related to better prognosis and longer OS (p-value 0.001).

2.2. Metallothioneins (MTs)

Metallothioneins are cysteine-rich proteins that bind metals (copper and zinc), protecting cells against ROS, radiation, and heavy metal toxicity. In fact, thanks to the thiol group of their cysteine, MT can bind heavy metals (cadmium, platinum, mercury), protecting cells from their toxicity, whereas, in physiological conditions, MTs bind zinc and copper, promoting cell proliferation. MT1 and MT2 are the most expressed among the 11 human MT isoforms. By the way, MT1 and MT2 expression is induced by different stimuli such as metals, oxidants, and stress. Studies have hypothesized the mechanisms of action of MTs in cancer progression. In particular, the binding of zinc by the MTs can lead to the following possible effects: (1) zinc promotes G1/S phase transition; therefore, low MT levels may arrest cancer cells in the G1 phase, inhibiting their proliferation, and (2) zinc is fundamental for the transcription of tumor suppressor protein as p53; therefore, high MT levels may remove zinc from p53, causing its inhibition and promoting uncontrolled cancer cell growth [21].

Also, in this case, MT expression depends on the type of cancer: upregulated in breast and nasopharyngeal tumors and downregulated in prostate and thyroid carcinoma. Moreover, their overexpression is related to poor prognosis in breast cancer and good prognosis in rectal carcinoma. In the case of lung cancer, MTs are expressed in squamous cell carcinoma, but they are not encountered in small cell cancer [21].

2.3. Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2)

Nrf2 is a transcription factor that plays a role in cellular protection against oxidative stress, one of the main causes of DNA damage and carcinogenesis [22]. Under physiological conditions, Kelch-like ECH-associated protein 1 (KEAP1) keeps low Nrf2 expression, whereas, in case of oxidative stress conditions, KEAP1 is inhibited by ROS, and Nrf2 is overexpressed

and reaches the nucleus where it activates its corresponding genes encoding cytoprotective enzymes such as heme oxygenase-1 (HO1) and NAD(P)H quinone oxidoreductase 1 (NQO1). Therefore, genetic changes, e.g., gain of function or amplification of Nrf2 or loss of functions or deletion of KEAP1, could lead to carcinogenesis and resistance to RT [23][24]. Its overexpression is related to poor prognosis in lung and esophageal squamous cell carcinoma [25][26].

2.4. Heme Oxygenase (HO)

Heme oxygenase-1 (HO-1) is an enzyme that protects cells (including cancer cells) against oxidative stress, inflammation, and apoptotic effect [27]. HO-1 is a target of Nrf2. Under cellular oxidative stress conditions, Nrf is activated and migrates into the cell nucleus, where it binds to the antioxidant response element (ARE) area of the HO-1 gene. Studies found that HO-1 is usually more expressed in cancer tissue (e.g., squamous cell carcinoma, lymphosarcoma, melanoma) than in close healthy tissues [28].

Lv et al. carried out a laboratory study on human LSCC Hep-2 cell lines to assess the role of HO-1 in reducing the pro-apoptotic action of cisplatin, one of the most used chemotherapeutics [29]. Cisplatin promotes cancer cell apoptosis by breaking DNA, inhibiting DNA synthesis, or inducing oxidative stress, leading to Nrf2 activation. In their study, the authors found that a high dose of cisplatin is needed to overcome the anti-apoptotic effect of HO-1; however, a high dose of chemotherapeutic means high toxicity and several side effects, too. So, being aware of the HO-1 mechanism of action in cancer, the authors suggest suppressing the enzyme expression to enhance LSCC chemosensitivity to cisplatin. This may lead to a lower cisplatin therapeutic dose and a lower risk of side effects.

2.5. Cyclooxygenase-2 (COX-2)

There are two isoforms of cyclooxygenase, COX-1, a constitutive enzyme, and COX-2, which is induced by stress factors (e.g., smoking, RT). COX-2 is an enzyme that catalyzes the production of prostaglandin E2 (PGE2) from arachidonic acid [30]. This enzyme is usually expressed at low levels in healthy tissue; its overexpression is due to inflammatory cytokines and oncogenes. High levels of COX-2 lead to increased production of PGEs that promote carcinogenesis, mainly by angiogenesis, but also by suppressing apoptosis and immune response [31]. This explains why it was found in some tumors such as breast, liver, endometrial, and laryngeal cancers and related to poor prognosis and high risk of recurrence [32][33][34][35].

Ranelletti et al. studied COX-2 expression in LSCC by immunohistochemistry. They found COX-2 immunostaining in well-differentiated laryngeal cancer cells (G1), whereas the poorly differentiated laryngeal cancer cells and the health cells close to the tumor did not express COX-2. By the way, they did not observe a statistically significant correlation between COX-2 expression and TNM stage (p-value 0.96), tumor site (p-value 0.17), and age (p-value 0.78) [36]. Similarly, other studies reported that epithelial immunohistochemical expression of COX-2 is mostly comparable between well-differentiated (G1) and moderate-differentiated (G2) cancer cells, whereas the enzyme is not found in poorly differentiated (G3) cancer cells (p-value 0.04) [37][38]. On the contrary, a study conducted on 80 patients affected by LSCC reported a relationship between COX-2 upregulation and advanced stages and poorly differentiated LSCC, correlating high expression levels of this marker with unfavorable prognosis [39].

A 2022 meta-analysis found that COX-2 overexpression is related to a higher risk of developing LSCC. The authors also reported a correlation between COX-2 expression and T-stage and lymph node metastases; however, this result was found only in the Asian but not the Caucasian population. In conclusion, this meta-analysis demonstrates a statistically significant association between COX-2 overexpression and worse prognosis in LSCC (p-value < 0.05) [40].

COX-2 role in LSCC sensitivity to radiation therapy (RT) was studied by Nix et al. [41]. They demonstrated that laryngeal cancer cells with higher levels of COX-2 are resistant to RT (p-value 0.004). So, they hypothesized that assessing the expression levels of this enzyme in pre-treatment LSCC could be a useful prognostic tool: its upregulation may predict the risk of RT failure, recommending surgery as first-line treatment in this case. Moreover, they assumed that selective COX-2 inhibitors, such as NS-398 and SC-236, may enhance sensitivity to RT in cancer cells with COX-2 overexpression [42][43]. By the way, studies demonstrated that inhibition of COX-2 is related to the proliferation of immune suppressor cells, such as the natural killer T (NKT) cells. In fact, in their study, Klatka et al. found that natural killer T (NKT) cell proliferation was lower in the laryngeal cancer group than in the control group (p-value < 0.0001). The authors also reported higher expression of NKT cells in the laryngeal cancer group treated with COX-2 inhibitor than in the laryngeal cancer group without this inhibition (p-value < 0.0001).

2.6. Micro Ribonucleic Acids (miRNAs)

MiRNAs are endogenous and non-coding RNAs, usually containing from 19 to 25 nucleotides, that silence target transcripts and impact gene expression. They can have tumor-suppressive or protooncogenic effects, depending on the type of cancer [44]. Epigenetic alterations and defects in enzymes involved in miRNA maturation cause miRNA dysregulation, leading to carcinogenesis. The identification of different expression profiles of miRNAs in the neoplastic tissue compared with normal tissue supports the hypothesis of a probable involvement of miRNAs in tumor development and progression. So, considering the important role of miRNAs in the control of protein expression, the detection of the protein pattern under the control of miRNAs could give important information about specific biomarkers of laryngeal carcinomas.

A recent study compared the expression of nine miRNAs in benign, premalignant, and malignant laryngeal lesions and found a statistically significant expression of *Hs_miR-21_5p, Hs_miR-218_3p,* and *Hs_miR-210_3p* only in the malignant laryngeal lesions. This result could suggest miRNA's role as a biomarker for laryngeal cancers [45][46][47][48]. In particular, Kinoshita et al. explained the role of *Hs_miR-218_3p* in the regulation of migration and invasion of tumor cells via local adhesion pathways: these data could clarify the mechanism of local recurrence and distant metastasis [49]. Also, Hs_*miR-744-3* upregulation seems to be related to neck node metastasis [50], while *Hs_miR-138* seems to be negatively correlated to distal metastases in LSCC [51].

As written above, cigarette smoking is one of the main risk factors for laryngeal cancer. So, many studies focused on its effect on miRNA changes and deregulation, leading to impairment of the p53 pathway, too [52][53]. Based on these assumptions, a study correlated smoke habits with miRNA changes in laryngeal cancer and found that *Hs_miR-202* is overexpressed in smokers for more than 20 years (*p*-value 0.005); however, its expression level is not found to be related to LSCC stage (*p*-value 0.087). *Hs_miR-548* is downregulated in smokers for more than 20 years (*p*-value 0.004), but its expression level statistically increases in pT4N+ LSCC (*p*-value 0.030). This study also demonstrated that the *Hs_miR-29a* expression level is statistically significantly lower in T1 than in T2 or T3 LSCC (*p*-value 0.037), without differences based on smoking habit duration (*p*-value 0.096). This result confirms that *Hs_miR-29a* expression is positively correlated with the TNM stage, suggesting its role as an oncogene in laryngeal cancer.

Recent in vitro studies on human LSCC Hep-2 cell lines found that *miR-33a*, *miR-199a-5p*, *miR-145, miR-34a,* and *miR-150-5p* overexpression promoted cancer cell apoptosis, suggesting a role as tumor suppressor [54][55][56][57][58]. A study carried out on 97 patients with LSCC demonstrated that low expression levels of *miR-196b* are related to worse overall survival (median survival: 48 months), while its overexpression is related to better survival (median survival: 81 months) (*p*-value 0.04) [59].

3. Conclusions

Despite the increased awareness of the carcinogenic risks related to smoking and alcohol habits and the now customary use of more sensitive diagnostic techniques, laryngeal cancer represents the second most common carcinoma among head and neck cancers, with a survival rate below 50% in advanced stages. Because of these data and poor prognosis, recent studies focus on the identification of biomarkers that may play a critical role in the pathogenesis of laryngeal squamous cell carcinoma or that may correlate with any factors (e.g., age, tumor stage, risk factors). However, the study of biomarkers in LSCC is still at the beginning, and further research is needed to achieve the main goal: the detection of a therapeutic strategy tailored for each patient in order to ensure the greatest success rate.

References

1. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 2018, 68, 394–424.
2. Bradley, P.J. Laryngeal cancer in nondrinker nonsmoker young patients: A distinct pathological entity? Curr. Opin. Otolaryngol. Head Neck Surg. 2016, 24, 140–147.
3. Johnson, D.E.; Burtness, B.; Leemans, C.R.; Lui, V.W.Y.; Bauman, J.E.; Grandis, J.R. Head and neck squamous cell carcinoma. Nat. Rev. Dis. Primers 2020, 6, 92.
4. Bayer, O.; Cámara, R.; Zeissig, S.R.; Ressing, M.; Dietz, A.; Locati, L.D.; Ramroth, H.; Singer, S. Occupation and cancer of the larynx: A systematic review and meta-analysis. Eur. Arch. Otorhinolaryngol. 2016, 273, 9–20.

5. Kim, S.Y.; Park, B.; Lim, H.; Kim, M.; Kong, I.G.; Choi, H.G. Increased risk of larynx cancer in patients with gastroesophageal reflux disease from a national sample cohort. Clin. Otolaryngol. 2019, 44, 534–540.
6. Ciolofan, M.S.; Vlăescu, A.N.; Mogoantă, C.A.; Ioniță, E.; Ioniță, I.; Căpitănescu, A.N.; Mitroi, M.R.; Anghelina, F. Clinical, Histological and Immunohistochemical Evaluation of Larynx Cancer. Curr. Health Sci. J. 2017, 43, 367–375.
7. Markou, K.; Christoforidou, A.; Karasmanis, I.; Tsiropoulos, G.; Triaridis, S.; Constantinidis, I.; Vital, V.; Nikolaou, A. Laryngeal cancer: Epidemiological data from Northern Greece and review of the literature. Hippokratia 2013, 17, 313–318.
8. Forastiere, A.A.; Ismaila, N.; Lewin, J.S.; Nathan, C.A.; Adelstein, D.J.; Eisbruch, A.; Fass, G.; Fisher, S.G.; Laurie, S.A.; Le, Q.T.; et al. Use of Larynx-Preservation Strategies in the Treatment of Laryngeal Cancer: American Society of Clinical Oncology Clinical Practice Guideline Update. J. Clin. Oncol. 2018, 36, 1143–1169.
9. 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. Otorhinolaryngol. 2021, 25, e471–e478.
10. Steuer, C.E.; El-Deiry, M.; Parks, J.R.; Higgins, K.A.; Saba, N.F. An update on larynx cancer. CA Cancer J. Clin. 2017, 67, 31–50.
11. Caruso Bavisotto, C.; Marino Gammazza, A.; Campanella, C.; Bucchieri, F.; Cappello, F. Extracellular heat shock proteins in cancer: From early diagnosis to new therapeutic approach. Semin. Cancer Biol. 2022, 86 Pt 1, 36–45.
12. D'anneo, A.; Bavisotto, C.C.; Gammazza, A.M.; Paladino, L.; Carlisi, D.; Cappello, F.; de Macario, E.C.; Macario, A.J.L.; Lauricella, M. Lipid chaperones and associated diseases: A group of chaperonopathies defining a new nosological entity with implications for medical research and practice. Cell Stress Chaperones 2020, 25, 805–820.
13. Gammazza, A.M.; Campanella, C.; Barone, R.; Bavisotto, C.C.; Gorska, M.; Wozniak, M.; Carini, F.; Cappello, F.; D'Anneo, A.; Lauricella, M.; et al. Doxorubicin anti-tumor mechanisms include Hsp60 post-translational modifications leading to the Hsp60/p53 complex dissociation and instauration of replicative senescence. Cancer Lett. 2017, 385, 75–86.
14. Campanella, C.; D'Anneo, A.; Gammazza, A.M.; Bavisotto, C.C.; Barone, R.; Emanuele, S.; Cascio, F.L.; Mocciaro, E.; Fais, S.; De Macario, E.C.; et al. The histone deacetylase inhibitor SAHA induces HSP60 nitration and its extracellular release by exosomal vesicles in human lung-derived carcinoma cells. Oncotarget 2016, 7, 28849–28867.
15. Voll, E.A.; Ogden, I.M.; Pavese, J.M.; Huang, X.; Xu, L.; Jovanovic, B.D.; Bergan, R.C. Heat shock protein 27 regulates human prostate cancer cell motility and metastatic progression. Oncotarget 2014, 5, 2648–2663.
16. Kaigorodova, E.V.; Zavyalova, M.V.; Bychkov, V.A.; Perelmuter, V.M.; Choynzonov, E.L. Functional state of the Hsp27 chaperone as a molecular marker of an unfavorable course of larynx cancer. Cancer Biomark. 2016, 17, 145–153.
17. Karam, J.; Fadous-Khalifé, M.C.; Tannous, R.; Fakhreddine, S.; Massoud, M.; Hadchity, J.; Aftimos, G.; Hadchity, E. Role of Krüppel-like factor 4 and heat shock protein 27 in cancer of the larynx. Mol. Clin. Oncol. 2017, 7, 808–814.
18. Rappa, F.; Farina, F.; Zummo, G.; David, S.; Campanella, C.; Carini, F.; Tomasello, G.; Damiani, P.; Cappello, F.; DEMacario, E.C.; et al. HSP-molecular chaperones in cancer biogenesis and tumor therapy: An overview. Anticancer Res. 2012, 32, 5139–5150.
19. Xu, J.; Wang, K.; Zhang, X.; Qiu, Y.; Huang, D.; Li, W.; Xiao, X.; Tian, Y. HSP70: A promising target for laryngeal carcinoma radiaotherapy by inhibiting cleavage and degradation of nucleolin. J. Exp. Clin. Cancer Res. 2010, 29, 106.
20. Zhu, J.; Xiong, G.; Fu, H.; Evers, B.M.; Zhou, B.P.; Xu, R. Chaperone Hsp47 Drives Malignant Growth and Invasion by Modulating an ECM Gene Network. Cancer Res. 2015, 75, 1580–1591.
21. Si, M.; Lang, J. The roles of metallothioneins in carcinogenesis. J. Hematol. Oncol. 2018, 11, 107.
22. Emanuele, S.; Celesia, A.; D'anneo, A.; Lauricella, M.; Carlisi, D.; De Blasio, A.; Giuliano, M. The Good and Bad of Nrf2: An Update in Cancer and New Perspectives in COVID-19. Int. J. Mol. Sci. 2021, 22, 7963.
23. Namani, A.; Matiur Rahaman, M.; Chen, M.; Tang, X. Gene-expression signature regulated by the KEAP1-NRF2-CUL3 axis is associated with a poor prognosis in head and neck squamous cell cancer. BMC Cancer 2018, 18, 46.
24. Jaramillo, M.C.; Zhang, D.D. The emerging role of the Nrf2-Keap1 signaling pathway in cancer. Genes Dev. 2013, 27, 2179–2191.
25. Sasaki, H.; Shitara, M.; Yokota, K.; Hikosaka, Y.; Moriyama, S.; Yano, M.; Fujii, Y. Increased NRF2 gene (NFE2L2) copy number correlates with mutations in lung squamous cell carcinomas. Mol. Med. Rep. 2012, 6, 391–394.
26. Xia, D.; Zhang, X.R.; Ma, Y.L.; Zhao, Z.J.; Zhao, R.; Wang, Y.Y. Nrf2 promotes esophageal squamous cell carcinoma (ESCC) resistance to radiotherapy through the CaMKIIα-associated activation of autophagy. Cell Biosci. 2020, 10, 90.

27. Chen, J.S.; Huang, P.H.; Wang, C.H.; Lin, F.Y.; Tsai, H.Y.; Wu, T.C.; Lin, S.J.; Chen, J.W. Nrf-2 mediated heme oxygenase-1 expression, an antioxidant-independent mechanism, contributes to anti-atherogenesis and vascular protective effects of Ginkgo biloba extract. Atherosclerosis 2011, 214, 301–309.
28. Dunn, L.L.; Midwinter, R.G.; Ni, J.; Hamid, H.A.; Parish, C.R.; Stocker, R. New insights into intracellular locations and functions of heme oxygenase-1. Antioxid. Redox Signal. 2014, 20, 1723–1742.
29. Lv, X.; Song, D.M.; Niu, Y.H.; Wang, B.S. Inhibition of heme oxygenase-1 enhances the chemosensitivity of laryngeal squamous cell cancer Hep-2 cells to cisplatin. Apoptosis 2016, 21, 489–501.
30. Cui, J.; Jia, J. Natural COX-2 Inhibitors as Promising Anti-inflammatory Agents: An Update. Curr. Med. Chem. 2021, 28, 3622–3646.
31. Gallo, O.; Masini, E.; Bianchi, B.; Bruschini, L.; Paglierani, M.; Franchi, A. Prognostic significance of cyclooxygenase-2 pathway and angiogenesis in head and neck squamous cell carcinoma. Hum. Pathol. 2002, 33, 708–714.
32. Hugo, H.J.; Saunders, C.; Ramsay, R.G.; Thompson, E.W. New Insights on COX-2 in Chronic Inflammation Driving Breast Cancer Growth and Metastasis. J. Mammary Gland. Biol. Neoplasia 2015, 20, 109–119.
33. Alves, A.F.; Baldissera, V.D.; Chiela, E.C.F.; Cerski, C.T.S.; Fontes, P.R.O.; Fernandes, M.D.C.; Porawski, M.; Giovenardi, M. Altered expression of COX-2 and TNF-α in patients with hepatocellular carcinoma. Rev. Esp. Enferm. Dig. 2019, 111, 364–370.
34. Li, M.; Li, M.; Wei, Y.; Xu, H. Prognostic and Clinical Significance of Cyclooxygenase-2 Overexpression in Endometrial Cancer: A Meta-Analysis. Front. Oncol. 2020, 10, 1202.
35. Sayar, C.; Sayar, H.; Özdemir, S.; Selçuk, T.; Görgülü, O.; Akbaş, Y.; Kemal Olgun, M. Cyclooxygenase-2 expression and clinical parameters in laryngeal squamous cell carcinoma, vocal fold nodule, and laryngeal atypical hyperplasia. Head Neck 2013, 35, 52–56.
36. Ranelletti, F.O.; Almadori, G.; Rocca, B.; Ferrandina, G.; Ciabattoni, G.; Habib, A.; Galli, J.; Maggiano, N.; Gessi, M.; Lauriola, L. Prognostic significance of cyclooxygenase-2 in laryngeal squamous cell carcinoma. Int. J. Cancer 2001, 95, 343–349.
37. Kourelis, K.; Vandoros, G.; Kourelis, T.; Papadas, T.; Goumas, P.; Sotiropoulou-Bonikou, G. Low COX2 in tumor and upregulation in stroma mark laryngeal squamous cell carcinoma progression. Laryngoscope 2009, 119, 1723–1729.
38. Kawata, R.; Hyo, S.; Maeda, T.; Urade, Y.; Takenaka, H. Simultaneous expression of cyclooxygenase-2 and microsomal prostaglandin E synthase in squamous cell carcinoma of the larynx. Acta Otolaryngol. 2006, 126, 627–632.
39. Chen, Y.F.; Luo, R.Z.; Li, Y.; Cui, B.K.; Song, M.; Yang, A.K.; Chen, W.K. High expression levels of COX-2 and P300 are associated with unfavorable survival in laryngeal squamous cell carcinoma. Eur. Arch. Otorhinolaryngol. 2013, 270, 1009–1017.
40. Du, J.; Feng, J.; Luo, D.; Peng, L. Prognostic and Clinical Significance of COX-2 Overexpression in Laryngeal Cancer: A Meta-Analysis. Front. Oncol. 2022, 12, 854946.
41. Nix, P.; Lind, M.; Greenman, J.; Stafford, N.; Cawkwell, L. Expression of Cox-2 protein in radioresistant laryngeal cancer. Ann. Oncol. 2004, 15, 797–801.
42. Tsujii, M.; DuBois, R.N. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell 1995, 83, 493–501.
43. Pyo, H.; Choy, H.; Amorino, G.P.; Kim, J.S.; Cao, Q.; Hercules, S.K.; DuBois, R.N. A selective cyclooxygenase-2 inhibitor, NS-398, enhances the effect of radiation in vitro and in vivo preferentially on the cells that express cyclooxygenase-2. Clin. Cancer Res. 2001, 7, 2998–3005.
44. Fabbri, M. miRNAs as molecular biomarkers of cancer. Expert Rev. Mol. Diagn. 2010, 10, 435–444.
45. Tuncturk, F.R.; Akalin, I.; Uzun, L.; Zenginkinet, T. Comparison of miRNA expressions among benign, premalignant and malignant lesions of the larynx: Could they be transformation biomarkers? J. Otolaryngol. Head Neck Surg. 2021, 50, 14.
46. Hu, A.; Huang, J.J.; Xu, W.H.; Jin, X.J.; Li, J.P.; Tang, Y.J.; Huang, X.F.; Cui, H.J.; Sun, G.B. miR-21 and miR-375 microRNAs as candidate diagnostic biomarkers in squamous cell carcinoma of the larynx: Association with patient survival. Am. J. Transl. Res. 2014, 6, 604–613.
47. Erkul, E.; Yilmaz, I.; Gungor, A.; Kurt, O.; Babayigit, M.A. MicroRNA-21 in laryngeal squamous cell carcinoma: Diagnostic and prognostic features. Laryngoscope 2017, 127, E62–E66.
48. Liu, M.; Wu, H.; Liu, T.; Li, Y.; Wang, F.; Wan, H.; Li, X.; Tang, H. Regulation of the cell cycle gene, BTG2, by miR-21 in human laryngeal carcinoma. Cell Res. 2009, 19, 828–837.

49. Kinoshita, T.; Hanazawa, T.; Nohata, N.; Kikkawa, N.; Enokida, H.; Yoshino, H.; Yamasaki, T.; Hidaka, H.; Nakagawa, M.; Okamoto, Y.; et al. Tumor suppressive microRNA-218 inhibits cancer cell migration and invasion through targeting laminin-332 in head and neck squamous cell carcinoma. Oncotarget 2012, 3, 1386–1400.
50. Li, J.Z.; Gao, W.; Lei, W.B.; Zhao, J.; Chan, J.Y.; Wei, W.I.; Ho, W.K.; Wong, T.S. MicroRNA 744-3p promotes MMP-9-mediated metastasis by simultaneously suppressing PDCD4 and PTEN in laryngeal squamous cell carcinoma. Oncotarget 2016, 7, 58218–58233.
51. Gao, S.; Wang, J.; Xie, J.; Zhang, T.; Dong, P. Role of miR-138 in the regulation of larynx carcinoma cell metastases. Tumour Biol. 2016, 37, 15601–15606.
52. Russ, R.; Slack, F.J. Cigarette-Smoke-Induced Dysregulation of MicroRNA Expression and Its Role in Lung Carcinogenesis. Pulm. Med. 2012, 2012, 791234.
53. Ahrendt, S.A.; Chow, J.T.; Yang, S.C.; Wu, L.; Zhang, M.J.; Jen, J.; Sidransky, D. Alcohol consumption and cigarette smoking increase the frequency of p53 mutations in non-small cell lung cancer. Cancer Res. 2000, 60, 3155–3159.
54. Karatas, O.F. Antiproliferative potential of miR-33a in laryngeal cancer Hep-2 cells via targeting PIM1. Head Neck 2018, 40, 2455–2461.
55. Li, D.J.; Wang, X.; Yin, W.H.; Niu, K.; Zhu, W.; Fang, N. MiR-199a-5p suppresses proliferation and invasion of human laryngeal cancer cells. Eur. Rev. Med. Pharmacol. Sci. 2020, 24, 12200–12207.
56. Karatas, O.F.; Yuceturk, B.; Suer, I.; Yilmaz, M.; Cansiz, H.; Solak, M.; Ittmann, M.; Ozen, M. Role of miR-145 in human laryngeal squamous cell carcinoma. Head Neck 2016, 38, 260–266.
57. Shen, Z.; Zhan, G.; Ye, D.; Ren, Y.; Cheng, L.; Wu, Z.; Guo, J. MicroRNA-34a affects the occurrence of laryngeal squamous cell carcinoma by targeting the antiapoptotic gene survivin. Med. Oncol. 2012, 29, 2473–2480.
58. Chen, H.; Cai, X.; Du, B.; Cai, J.; Luo, Z. MicroRNA-150-5p inhibits the proliferation and invasion of human larynx epidermiod cancer cells though regulating peptidyl-prolyl cis/trans isomerase. Braz. J. Otorhinolaryngol. 2023, 89, 383–392.
59. Luo, M.; Sun, G.; Sun, J.W. MiR-196b affects the progression and prognosis of human LSCC through targeting PCDH-17. Auris Nasus Larynx 2019, 46, 583–592.