Abstract

Introduction

Laryngeal squamous cell carcinoma represents an important cause of cancer-related death. Laryngeal squamous cell carcinoma is the second most common type of head and neck malignancies, with a total of 12,000 new cases diagnosed yearly in the United States. The clinical value of several molecules as molecular biomarkers for prognosis of laryngeal squamous cell carcinoma as well as for monitoring the response of laryngeal squamous cell carcinoma patients to treatment is high. This review summarises the clinical importance of tumour protein p53, proliferating cell nuclear antigen, marker of proliferation Ki-67, cyclins, cyclin-dependent kinase 4, inhibitors of cyclin-dependent kinases, epidermal growth factor receptor, vascular endothelial growth factor, A and its receptor, B-cell CLL/lymphoma 2 (BCL2) protein family members, kalikrein-related peptidase 11, 3,4-dihydroxy-L-phenylalanine decarboxylase and microRNAs.

Conclusion

Deregulation of protein or mRNA expression of these genes in laryngeal squamous cell carcinoma compared to benign laryngeal tumours, dysplasias, or normal tissues of the larynx suggests that these potential molecular biomarkers merit further validation owing to their important prognostic value in laryngeal squamous cell carcinoma treatment.

Molecular biomarkers of prognosis in laryngeal squamous cell carcinoma

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Introduction

Head and neck cancer is the sixth most commonly diagnosed cancer all over the world. The overwhelming majority of head and neck cases are characterised as squamous cell carcinomas (SCCs). Head and neck squamous cell carcinoma (HNSCC), which represents an important cause of cancer-related death, starts from squamous cells lining the moist, mucosal surfaces inside the mouth, the nose and the throat. Thus, such tumours can develop in nasal cavity, paranasal sinuses, oral cavity, larynx, trachea, hypolarynx, nasopharynx, oropharynx, ears and salivary glands. Laryngeal squamous cell carcinoma (LSCC) is the second most common type of HNSCC, with a total of 12,000 new cases diagnosed yearly in the US. The incidence of LSCC is greater in men than in women, especially for people between the sixth or seventh decade of their life. The causative risk factors of HNSCC include tobacco use and alcohol consumption as well as other habits that are likely to be implicated in laryngeal tumourigenesis.

Deregulation of the expression of many genes is related to many hallmarks of cancer, such as uncontrolled cell proliferation, impaired apoptosis, progressive loss of cell differentiation (anaplasia), epithelial–mesenchymal transition, metastasis and angiogenesis. Besides that, the clinical value of such molecules as molecular biomarkers for diagnosis, prognosis or monitoring of patient response to treatment is high. Molecular biomarkers are needed in clinical practice, to achieve individualisation of anticancer treatment. In this context, immunohistochemical assessment and quantification of messenger RNA (mRNA) expression of emerging molecular biomarkers will assist utmost the generation of novel screening tests possessing high sensitivity and specificity, as well as tailor-made therapies counteracting LSCC. Moreover, selective impairment of interactions between key players of biochemical processes could significantly decelerate tumour progression and, hence, elongate survival of LSCC patients. The aim of this review is to discuss the molecular biomarkers of prognosis in laryngeal squamous cell carcinoma.

Discussion

The author has referenced some of his own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

Tumour protein p53

Tumour protein p53, expressed by the TP53 gene, is a tumour-suppressor protein that keeps many genes under transcriptional control in response to many different cellular stresses. p53 can induce cell cycle arrest, senescence, DNA repair and apoptosis. Although the potential associations of p53 with clinicopathological features of LSCC as well as the prognostic significance of p53 expression in LSCC have been intensively studied during the last 20 years; the resulting data are contradictory. Variations in the selection of LSCC patients

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p53 overexpression does not seem to be associated with biological features of LSCC. In more detail, no significant associations have been found between immunohistochemically detected p53 expression and depth of tumour invasion, histological grade, status of regional lymph nodes, or TNM stage of LSCC patients as well as with progression of the disease. Although Hirvikoski et al. has proposed a favourable prognostic role for strong p53 immunostaining in LSCC, numerous studies showed that p53 is a rather unfavourable predictor of disease-free survival (DFS) and overall survival (OS). However, many other studies failed to demonstrate any prognostic significance for this tumour-suppressor protein. Hence, p53 immunopositivity could not predict tumour recurrence of radiotherapy-treated patients suffering from glottic LSCC. Consequently, larger studies are needed to examine p53 expression in laryngeal tumours of patients subjected to different treatment modalities and to assess its prognostic importance.

Proliferating cell nuclear antigen

Proliferating cell nuclear antigen (PCNA) is a cofactor of DNA polymerase delta. PCNA expression in paraffin-embedded tissue sections is indicative of the cancer cell proliferation and is associated with histological tumour differentiation, vascular and lymphatic invasion, depth of tumour invasion, nodal status, presence of neck metastases and locoregional recurrence. PCNA expression in preoperative biopsies has been proposed as a reliable predictor of presence of occult neck metastases. PCNA overexpression is also associated with low DFS and OS rates. Thus, immunohistochemical assessment of PCNA expression in LSCC could prove a valuable tool for determination of prognosis and selection of the appropriate therapeutic treatment.

Marker of proliferation Ki-67

Marker of proliferation Ki-67 (MKI67) is a nuclear protein, involved in ribosomal RNA (rRNA) synthesis and cell proliferation. MKI67 overexpression was associated with high histologic grade. MKI67 expression was also elevated in node-positive LSCC patients, compared to patients without local metastases. Furthermore, LSCC patients with high MKI67 expression in their tumour recurred locoregionally more frequently if treated with split-course radiotherapy than if treated with a continuous course of therapy. MKI67 overexpression can also predict the presence of occult neck metastases in LSCC. As a result, immunohistochemical assessment of MKI67 expression could select those patients who are more likely to benefit from elective neck dissection.

Cyclins, cyclin-dependent kinases and their inhibitors

Cyclins are members of a highly conserved protein family, which control the cell cycle. They form complexes with cyclin-dependent kinases and hence activate them. These cell-cycle regulators exhibit an important periodicity through the different phases of the cell cycle. The most studied cyclins in LSCC are cyclin A2 (CCNA2; in the past, known as cyclin A), cyclin B1 (CCNB1), cyclin D1 (CCND1), cyclin D3 (CCND3) and cyclin E1 (CCNE1; in the past, known as cyclin E). Protein expression levels of CCNB1, CCND1 and CCNE1 are related to the site of the tumour, depth of tumour invasion and stage of the disease. In more detail, CCNB1, CCND1 and CCNE1 immunopositivities are associated with the existence of LSCC located in the supraglottic larynx, highly invasive (T3 and T4) malignant neoplasms of the larynx and tumours being at an advanced clinical stage (III and IV). Moreover, overexpression of CCND1 and CCNE1 was linked to positive nodal status. Moreover, the CCNE1 gene was overexpressed in grade III LSCC, compared to grades I and II LSCC. Besides CCND1 protein overexpression, high CCND1 mRNA expression was observed in several LSCC specimens and was associated with local invasion and stage IV LSCC. It has been suggested that CCND1 gene amplification could account for high CCND1 mRNA levels that were measured in several LSCC specimens. It should also be noted that CCND1 gene amplification was associated with the presence of metastases in regional lymph nodes.

Cyclins also possess a significant prognostic potential in LSCC. CCNA2 immunopositivity was indicative of locoregional recurrence in LSCC, as the risk of locoregional relapse within a 5-year interval from the time of LSCC diagnosis was double for patients with CCNA2-positive LSCC than for those with CCNA2-negative laryngeal tumours. Overexpression of CCNA2 also predicted poor DFS and OS in LSCC patients who had been treated with surgery and postoperative radiotherapy. In addition, CCNB1 immunopositivity was shown to constitute an unfavourable—though not independent—prognosticator in this malignancy. The most significant unfavourable predictors of DFS and OS were CCND1 and CCNE1. In addition to these cyclins, high CCND3 mRNA expression was an independent prognosticator of poor outcome in LSCC.

The co-expression of CCND1 and CCND3 in laryngeal cancerous tissue specimens was a very strong and reliable prognosticator, as patients with CCND1+/CCND3+ tumours had the smallest OS rates than all the other LSCC patients, and patients with CCND1-/CCND3− tumours had the...
highest OS probabilities, whereas patients with CCND1−/CCND3+ or CCND1+/CCND3− LSCC had an intermediate prognosis13. Furthermore, co-expression of CCND1 and CDK4 predicted poor patient outcome in the total of LSCC patients, but also in the subgroup of early-stage (I and II) LSCC cases9, similar to CCNE1 over-expression7. Consequently, it appears that assessment of expression of cyclins could contribute to the appropriate management of LSCC patients by indicating those with poor prognosis and those who should be treated more aggressively.

The enzymatic activity of activated cyclin-dependent kinases can be inhibited by specific proteins, usually during the G1 phase of the cell cycle or in response to environmental stress or damaged DNA. The most representative examples of such proteins are cyclin-dependent kinase inhibitors, such as cyclin-dependent kinase inhibitor 1A (CDKN1A; also known as p21, CIP1 and WAF1), cyclin-dependent kinase inhibitor 1B (CDKN1B; also known as p27 and KIP1), cyclin-dependent kinase inhibitor 2A (CDKN2A; also known as p21, p16, p14, INK4A and ARF), and cyclin-dependent kinase inhibitor 2B (CDKN2B; also known as p15 and INK4B).

CDKN1A and CDKN1B immunoreactivities were related to clinicopathological characteristics of malignant laryngeal tumours. In particular, low CDKN1A expression was associated with grade III tumours and presence of metastases in regional lymph nodes in LSCC patients. Moreover, weak CDKN1B staining was associated with high depth of tumour invasion (T3 and T4) and advanced clinical stage (stages III and IV)12. As expected, LSCC patients with DKN1A-positive tumours had longer OS intervals than those with DKN1A-negative neoplasms14. CDKN1B expression is another independent favourable prognosis indicator in LSCC, as it predicts better DFS and OS rates.

On the other hand, patients with CDKN1B+/CCND3+ LSCC had a very poor prognosis, in terms of DFS and OS15. In basaloid LSCC—a rather uncommon but aggressive variant of LSCC—loss of CDKN1B expression was associated with high tumour aggressiveness and poor clinical outcome15. Moreover, deletion of CDKN2A and/or CDKN2B genes was associated with disease progression and poor OS in LSCC patients16. In general, loss of expression of cyclin-dependent kinase inhibitors predicts unfavourable prognosis in LSCC.

Epidermal growth factor receptor

The epidermal growth factor receptor (EGFR) is a transmembrane receptor exhibiting tyrosine kinase activity. EGFR is overexpressed in LSCC, in comparison with normal laryngeal tissue specimens. It has recently been suggested that EGFR expression could identify LSCC patients at high risk for locoregional recurrence. Overexpression of the EGFR gene at the protein level was also observed in poorly-differentiated LSCC and predicted an increased risk of regional metastatic recurrence9. EGFR immunopositivity was demonstrated to predict short-term relapse and poor outcome in LSCC patients, independently of other significant prognostic factors including the tumour extent, nodal status and histological grade18. Besides its high significance as a molecular prognostic biomarker, EGFR expression probably constitutes a predictive biomarker for the identification of LSCC patients who are most likely to benefit from accelerated radiotherapy with carbogen (98% O2; 2% CO2) and nicotinamide (ARCON), a therapeutic approach aiming at high locoregional control, in particular for LSCC with low EGFR levels19. Thus, positive EGFR expression detected at the time of diagnosis could assist decision-making concerning LSCC treatment.

Vascular endothelial growth factor A and its receptor

The vascular endothelial growth factor A (VEGFA) is produced by cancer and stroma cells and exerts its angiogenic action by binding to its receptors, one of which is fms-related tyrosine kinase 1 (FLT1)20. VEGFA levels in serum were significantly higher in patients with advanced-stage LSCC than in healthy controls, and this overexpression predicted a more aggressive LSCC phenotype and a worse clinical outcome21. VEGFA expression was significantly associated with tumour size, histological grade and presence of metastases in regional lymph nodes of LSCC patients. Furthermore, VEGFA and FLT1 mRNA expression was associated with high depth of tumour invasion and predicted independently short-term relapse of LSCC patients20. In addition, strong VEGFA immunostaining was an independent unfavourable prognosticator of OS22.

BCL2 family members

BCL2 family proteins promote or inhibit apoptosis and are, hence, either antiapoptotic (e.g. BCL2) or proapoptotic members (e.g. BAX). Nonetheless, some BCL2 family genes encode both antiapoptotic and proapoptotic protein isoforms. Expression of the antiapoptotic BCL2 protein is associated with positive nodal status and advanced TNM stage23. Moreover, mRNA expression of another member of the BCL2 family, BCL2L12, was shown to be downregulated in advanced-stage LSCC24. BCL2 immunopositivity, either alone or in combination with BCLXL expression and reduced BAX levels predicts radiotherapy failure, thus implying that inhibition of apoptosis accounts for radiotherapy resistance25.

Kallikrein-related peptidase 11

Kallikrein-related peptidase 11 (KLK11) is a trypsin-like serine peptidase. It is a member of the family of...
the tissue kallikrein and kallikrein-related peptidases. KLK11 mRNA expression was found to be dramatically lower in LSCC of primary or recurrent origin, in comparison with adjacent non-cancerous laryngeal tissue specimens. In addition, KLK11 mRNA positivity was more common among early-stage (I and II) carcinomas of the larynx than among laryngeal tumours of advanced TNM stage (III and IV). Patients with KLK11 mRNA-positive LSCC exhibited prolonged OS interval compared to those with KLK11 mRNA-negative LSCC.

Thus, KLK11 is likely to constitute a promising diagnostic and/or prognostic biomarker in this malignancy.

L-DOPA decarboxylase

L-DOPA decarboxylase (DDC) is a pyridoxal-phosphate-dependent enzyme catalysing the decarboxylation of 3,4-dihydroxy-L-phenylalanine (L-DOPA) to dopamine as well as the decarboxylation of 5-hydroxy-L-tryptophan (5-HTP) to serotonin. Quantitative mRNA expression analysis of the DDC gene in primary tumours of the larynx and their non-cancerous counterparts revealed its upregulation in LSCC. DDC mRNA expression was shown to constitute a potential diagnostic biomarker, which merits further evaluation in large cohorts of laryngeal tissue samples. Moreover, DDC mRNA expression status is inversely associated with progression of LSCC, as early-stage (I and II) tumours exhibit higher DDC mRNA levels than advanced-stage (III and IV) tumours, suggesting that DDC mRNA expression could represent a novel molecular prognostic biomarker in LSCC.

microRNAs

microRNAs (miRNAs) are small non-coding RNAs of approximately 19–25 nucleotides that post-transcriptionally regulate finely protein-coding gene expression. miRNAs bind to the 3' untranslated region of targeted mRNAs and trigger translational repression and, sometimes, mRNA degradation. miRNAs are heavily involved in laryngeal tumourigenesis and represent very promising biomarkers in LSCC as well as in other malignancies, as they can be accurately quantified in FFPE samples and blood plasma or serum of patients, owing to their stability.

Recently, a panel of 26 miRNAs being differentially expressed in the blood plasma of LSCC patients compared to the control cohort has been discovered. Of these, 16 miRNAs were significantly upregulated and 10 miRNAs were significantly downregulated in the LSCC patients' blood plasma. Most importantly, 17 miRNAs were shown to exist only in the blood plasma of LSCC patients. Thus, this LSCC-specific miRNA signature could serve as a non-invasive molecular biomarker for LSCC. Besides them, three other miRNAs (miR-155, miR-455-5p and miR-196a) were demonstrated to be overexpressed in laryngeal tumours, compared to their non-cancerous counterparts. High levels of miR-155, miR-455-5p and miR-196a were correlated with the progression of the disease. In particular, there was a progressive increase of miR-455-5p and miR-196a levels during laryngeal tumourigenesis, being very low in normal laryngeal tissues, intermediate in benign tumours, higher in dysplasias and very high in malignant tumours of the larynx.

miR-196a and miR-155 expression was more elevated in highly invasive (T3 and T4) LSCC than in low invasive (T1 and T2) tumours. miR-155 overexpression was also associated with poorly differentiated (grade III) LSCC. Interestingly, several miRNAs among those which are differentially expressed in LSCC patients could be combined in a multifactorial panel of biomarkers with diagnostic and/or prognostic purposes.

Conclusion

The clinical significance of all these potential diagnostic, prognostic and treatment-response biomarkers in LSCC has been intensively investigated so far. Deciphering of the signalling pathways that are implicated in LSCC progression and metastasis is likely to contribute utmost to the discovery of novel molecular biomarkers that could be applied in clinical practice. Deregulation of protein and/or mRNA expression in LSCC compared to benign laryngeal tumours, dysplasias, or normal laryngeal tissues suggests further validation for these potential biomarkers in larger cohorts of LSCC patients.

Abbreviations list

- CCN2, cyclin A2; CCNB1, cyclin B1; CCND1, cyclin D1; CCND3, cyclin D3; CCNE1, cyclin E1; CDKN1A, cyclin-dependent kinase inhibitor 1A; CDKN1B, cyclin-dependent kinase inhibitor 1B; CDKN2A, cyclin-dependent kinase inhibitor 2A; CDKN2B, cyclin-dependent kinase inhibitor 2B; DDC, L-DOPA decarboxylase; DFS, disease-free survival; EGFR, epidermal growth factor receptor; FLT1, fms-related tyrosine kinase 1; HNCC, head and neck squamous cell carcinoma; 5-HTP, 5-hydroxy-L-tryptophan; L-DOPA, 3,4-dihydroxy-L-phenylalanine; LSCC, laryngeal squamous cell carcinoma; mRNA, messenger RNA; miRNA, microRNA; MKI67, marker of proliferation Ki-67; mRNA, messenger RNA; OS, overall survival; PCNA, proliferating cell nuclear antigen; rRNA, ribosomal RNA; SSC, squamous cell carcinoma; TP53, tumour protein p53; VEGFA, vascular endothelial growth factor A.

References


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