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Prognostic and Predictive Protein Biomarkers in Laryngeal Squamous Cell Carcinoma—A Systematic Review

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Abstract

Background: Despite recent advances in clinical management of laryngeal squamous cell carcinoma (LSCC), the overall 5-year survival continues to be poor. Consequently, biomarkers of treatment response will need to be identified. Proteomic strategies are one way to attempt to identify such biomarkers. Methods: The Medline, Embase and Cochrane Library databases were systematically searched until 1st March 2014 using the terms "larynx", "squamous cell carcinoma", "proteomic", and "biomarker". Articles which met inclusion criteria were assessed for the type of biomarker investigated, the proteomic technique used, and whether any validation had been performed. Results: Six studies identified biomarkers, including UCRP, ceramides, uPA, MT1-MMP, stratifin, transferrin, albumin, S100 calcium-binding protein A9, stathmin, enolase, PLAU, IGFBP7, MMP14, THBS1, and transthyretin. Transferrin was the only biomarker to appear in more than one study. Conclusions: Our review identified several potential biomarkers of outcome in LSCC. Well designed studies will need to further validate their use in the future.

Keywords

Laryngeal, Squamous Cell Carcinoma, Cancer, Proteomics, Biomarker

1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is diagnosed in over 650,000 individuals annually worldwide and remains to be an important global health issue [1]. Laryngeal squamous cell carcinoma (LSCC) accounts for more than 150,000 of these cases, with an annual global mortality of approximately 83,000 individu-

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als [1]. Although extensive research had been undertaken on LSCC in the past decade in an attempt to identify biomarkers of treatment response or outcome, there continued to be significant morbidity and mortality associated with this condition, to the extent that the overall 5-year survival had reduced between 1975 and 2010 in the USA [2]. The prognosis is especially poor for patients with metastatic disease, with a 5-year survival of less than 50% [3]. Consequently, a better understanding of biomarkers of outcome associated with LSCC may have clinical use in improved treatment stratification and prognostication and may even inform the development of future novel targeted therapies.

In recent years, biomarker development in other tumor types has resulted in treatment targeting and improved outcomes for biomarker derived subsets of patients. A notable example is the use of detection of the HER-2 receptor in breast cancer biopsy tissue [4].

There are three main clinical applications for biomarkers in cancer—in the diagnosis and characterization of tumors, as well as the potential development of novel targeted therapy against certain cancers.

As a diagnostic tool, molecular biomarkers may aid in the diagnosis of occult metastasis as well as the early detection of local and regional spread [5] [6]. Biomarkers, such as desmoglein 3 and Tissue-Specific Mir-205, have been shown to be associated with metastatic head and neck cancer and hence have the potential to be diagnostic biomarkers for this condition [7] [8].

In the characterization of tumors, they are used to better determine prognosis and treatment selection [5]. Epidermal growth factor receptor (EGFR) and p16 are well characterized biomarkers which are differentially expressed in a subset of LSCC [9] [10], and may play a role in the carcinogenesis of LSCC [11] [12]. Various targeted therapies against EGFR positive HNSCC have been developed, including anti-EGFR antibodies such as cetuximab, which improve overall survival without adverse effects on quality of life [13] [14].

Recent advances in proteomic technology and techniques have resulted in the discovery of new biomarkers in HNSCC [15], using various proteomic techniques. Protein separation techniques, such as 2-dimensional (2D) differential in-gel electrophoresis, have allowed large numbers of proteins to be sampled in a reproducible manner [16] [17]. Moreover, the differential expression of protein biomarkers identified using proteomic techniques such as matrix-assisted laser desorption/ionization (MALDI) time of flight (ToF) mass spectrometry (MS) [18], surface enhanced laser desorption/ionization (SELDI) ToF MS [19], laser capture [20], liquid chromatography mass spectrometry (LC-MS) [21], as well as newer techniques such as isobaric tag for relative and absolute quantitation (iTRAQ) [22] has led to a better understanding of the carcinogenesis of certain types of cancers [23] [24]. Various types of tissue samples may be used for proteomic analysis, which include fresh frozen, formalin-fixed paraffin embedded, and cell lines [25] [26]. Slight differences may exist among results obtained from different types of tissue samples [26]. Although previous reviews have evaluated studies utilizing proteomic techniques to characterize salivary cancer biomarkers [27] [28] and colorectal cancer biomarkers [29], no study has assessed the use of this technique in the identification of LSCC biomarkers.

This study aims to review the current literature to evaluate biomarkers in LSCC identified using proteomic techniques. In particular, this review will assess the type of biomarker and its potential use in LSCC, the proteomic technique used, and whether or not any validation of the biomarker has been performed.

2. Methods

A literature search for relevant articles was performed electronically by two independent reviewers—MK, a surgical HMO and PG, an otolaryngologist/head and neck surgeon. The Medline, Embase and Cochrane Library databases were searched using the following terms in combination: [laryngeal or larynx or glottis or glottis or supraglottic or supraglottis or subglottic or subglottis (all fields)] and [“squamous cell carcinoma” or SCC or cancer (all fields)] and [biomarker (all fields)] and [proteomic or proteome or electrophoresis or Maldi or “laser desorption ionization” or spectrometry (all fields)] in Medline; and ((((“laryngeal or larynx or glottis or glottis or supraglottis or supraglottic or subglottis or subglottic”) and “squamous cell carcinoma” or SCC or cancer) and biomarker) and (proteomic or proteome or electrophoresis or Maldi or “laser desorption ionization” or spectrometry)). mp. [mp = title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] in Embase.

The search strategy is outlined in **Figure 1** and follows the PRISMA statement. All articles published from 1950 to 1st March 2014 in the above databases were searched for relevant articles. Full text articles retrieved

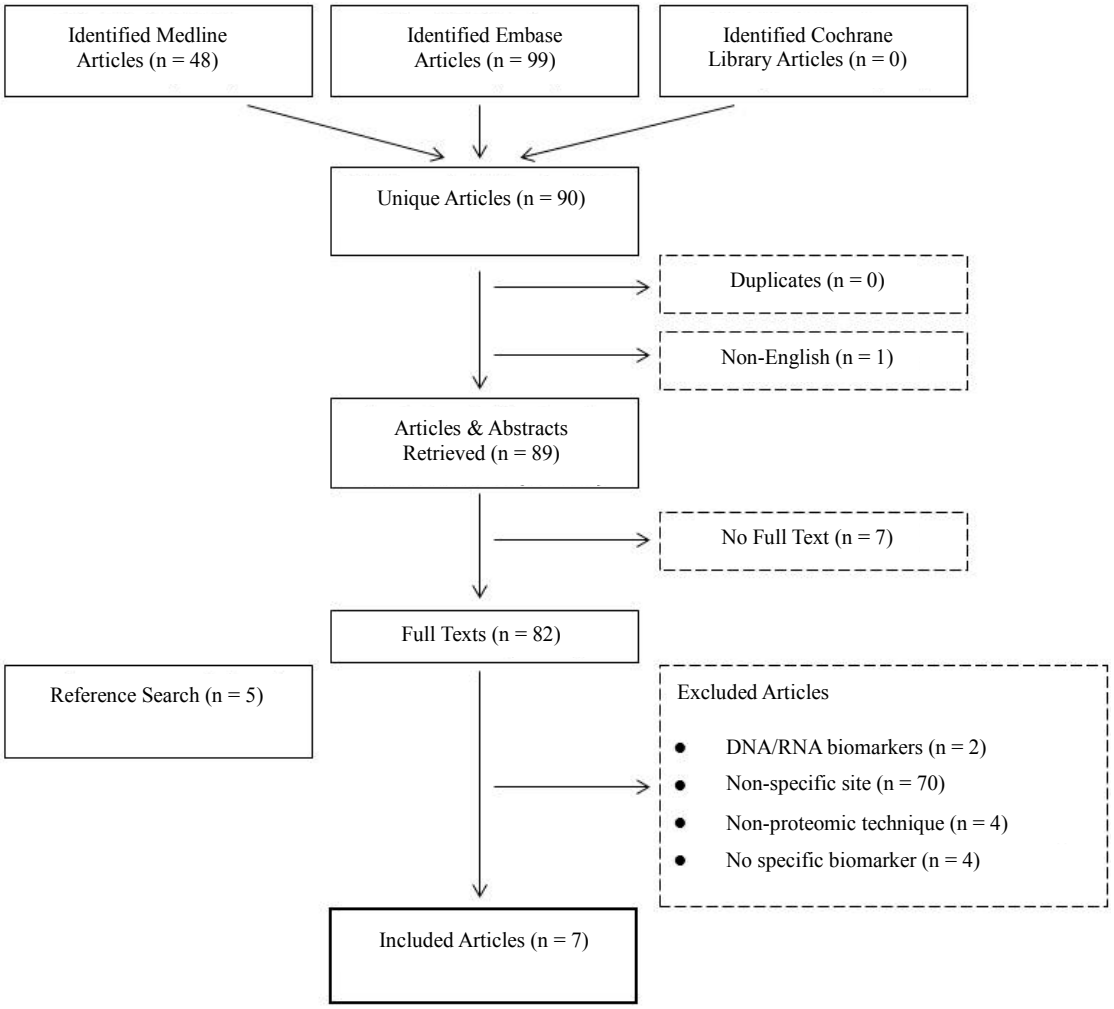

Figure 1. Systematic search strategy for studies included in this review.

were reviewed individually for eligibility into this study. Additionally, a manual search was performed on all reference lists of included articles.

Inclusion criteria were defined as articles which identify using a proteomic approach and/or validate biomarkers in laryngeal squamous cell carcinoma using immunohistochemical techniques on larger samples. Non-English articles, case reports, opinions, reviews and news articles were excluded from this study. Articles assessing DNA/RNA and those which did not identify specific biomarkers were also excluded.

Data retrieved from all unique articles identified include the date of publication, type of biomarker identified and its potential use in laryngeal SCC, proteomic technique used, source and tissue preparation, number of diseased and healthy subjects, and whether or not any validation of the biomarker had been performed. Due to the limited data available in the current literature, no statistical analysis could be performed.

3. Results

90 unique articles were identified in our search, with 7 meeting inclusion criteria for review in this study. The majority of excluded articles (n = 70) assessed head and neck SCC biomarkers on a non-specific anatomical site. **Table 1** outlines a summary of information retrieved from included studies.

Studies were published between 1996 and 2012, with 5 studies published in the last 10 years [23] [30]-[33]. A variety of proteomic techniques were identified, including liquid chromatography, mass spectrometry, electrospray ionization, matrix-assisted laser desorption/ionization, sodium dodecyl sulfate polyacrylamide gel electrophoresis, enzyme-linked immunosorbent assay and 2-dimensional electrophoresis.

Table 1. Summary of information obtained from included studies.

Authors	Year	Proteomic technique	Tissue source	n	Biomarkers identified	Validation
Chi *et al*.	2009	LC-MS/MS, LC-ESI-MALDI Tandem MS, MALDI-TOF	Oral cavity SCC cell lines—validated in laryngeal SCC	18	↑UCRP	Not in a separate population
Dowling *et al*.	2008	LC-MS, 2DE	Laryngeal SCC, salivary samples	4	↑S100 calcium binding protein A9, ↑beta fibrin, ↑transferrin, ↑immunoglobulin heavy chain constant region gamma, ↑cofilin, ↓transthyretin	S100 calcium binding protein validated by immunoblot analysis
Karahatay *et al*.	2007	LC/MS	Laryngeal SCC tissue	10	↑C16-, ↓C18-, ↑C24-, ↑C24:1-ceramides	Nil
Parolini *et al*.	1996	SDS-PAGE, ELISA	Laryngeal SCC tissue frozen or fixed in paraformaldehyde	70	↑uPA	uPA and mRNA expression using ISH
Sepiashvili *et al*.	2012	MS, 2DE	Laryngeal SCC cell lines	2	↑PLAU, ↑IGFBP7, ↑MMP14 and ↑THBS1	Validated using IHC and ELISA in 56 laryngeal SCC
Sewell *et al*.	2007	MALDI-TOF, MS, 2D-DIGE	Laryngeal SCC frozen tissue	2	↓stratifin, ↓S100 calcium-binding protein A9, ↓p21-ARC, ↓stathmin, and ↑enolase, ↑MAGE D3, ↑transferrin, ↑albumin, ↑tumor-associated calcium signal transducer	Nil
Yoshizaki *et al*.	1997	SDS-PAGE	Laryngeal SCC tissue fixed in formalin	9	↑MT1-MMP	Nil

LC: Liquid chromatography; MS: Mass spectrometry; ESI: Electrospray ionization; MALDI: Matrix-assisted laser desorption/ionization; TOF: Time of flight; SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis; ELISA: Enzyme-linked immunosorbent assay; 2D-DIGE: 2-dimensional difference gel electrophoresis; 2DE: 2-dimensional electrophoresis.

The number of samples in each study ranged between two to 70, 5 of the 6 studies having less than 50 samples. Laryngeal SCC samples were obtained from fresh tissue in four studies, saliva in one study and from cell lines in two studies. One study [30] validated biomarkers identified in oral cavity SCC using laryngeal SCC samples.

A number of laryngeal SCC biomarkers were identified in this review. These include ubiquitin cross-reactive protein (UCRP), C16-, C24-, C24:1-ceramides, urokinase plasminogen activator (uPA), membrane associated type 1 matrix metalloproteinase (MT1-MMP), stratifin, transferrin, albumin, tumor-associated calcium signal transducer, melanoma-associated antigen D3 (MAGE D3), S100 calcium-binding protein A9, p21-ARC, stathmin, enolase, plasminogen activator, urokinase (PLAU), insulin-like growth factor-binding protein 7 (IGFBP7), matrix metalloproteinase 14 (MMP14), Thrombospondin 1 (THBS1), beta fibrin, immunoglobulin heavy chain constant region gamma, cofilin, and transthyretin. A summary of the differential expression of laryngeal SCC biomarkers identified in this review are presented in **Table 2**. Transferrin was the only biomarker identified using proteomic techniques in more than one study. Only one study validated their identified biomarker using in a separate patient population [23] [31].

4. Discussion

LSCC remains to be associated with significant morbidity and mortality despite extensive research recently, being one of only two cancer types to have a reduction in 5 year survival over the last decades [1]-[3]. The lack of specific molecular biomarkers in the management of LSCC may contribute to these statistics [23]. A better understanding of biomarkers may therefore provide new management options in the diagnosis, characterization, and targeted therapy for LSCC [5]. Recent developments in biomarker research include p16 and EGFR, which are implicated in the carcinogenesis and targeted therapy for LSCC, respectively [9]-[14]. A review of LSCC biomarkers in 2004 by Almadori *et al*. [5] suggested the possible association of protein biomarkers S100A2 calcium binding protein and galectin-3 with the molecular characterization of LSCC.

Table 2. Differential expression of biomarkers identified.

Author	Up-regulated biomarkers	Down-regulated biomarkers
Chi *et al*. [30]	UCRP	
Dowling *et al*. [31]	S100 calcium binding protein Beta fibrin Transferrin Immunoglobulin heavy chain constant region gamma Cofilin	Transthyretin
Karahatay *et al*. [32]	C16- ceramide C24- ceramide C24:1- ceramide	C18- ceramide
Parolini *et al*. [35]	uPA	NA
Sepiashvili *et al*. [33]	PLAU IGFBP7 MMP14 THBS1	NA
Sewell *et al*. [23]	Enolase MAGE D3 Transferrin Albumin Tumor-associated calcium signal transducer	Stratifin S100 calcium-binding protein A9 p21-ARC Stathmin
Yoshizaki *et al*. [36]	MT1-MMP	NA

With the use of proteomic techniques such as 2DIGE and MALDI-TOF MS, it is now possible to sample large amount of proteins in the identification of potential cancer biomarkers [16]-[19]. This may aid in the process of identifying and validating biomarkers for LSCC. Although previous reviews had been conducted regarding the proteomic analysis of biomarkers associated with colorectal and endometrial cancer [29] [34], this is the first systematic review evaluating the use of proteomic techniques in the analysis of LSCC biomarkers.

This review has identified 25 proteins reported as differentially expressed by 7 studies [23] [30]-[33] [35] [36]. 2 of these proteins—S100 calcium binding protein A9 and transferrin were reported as differentially expressed by more than one study, with transferrin [23] [31] reported to be up-regulated in 2 studies, while the differential expression of S100 calcium binding protein A9 was inconsistently reported in 2 studies [23] [31]. A selection of identified proteins will be discussed below.

The S100 family is a subtype of calcium binding proteins consisting of at least 25 different proteins [37]. There is current evidence to suggest the role of S100 proteins in cancer cell differentiation [38], cell proliferation [39] [40], cell apoptosis [41] [42] and tumor metastasis [43] [44]. Members of the S100 family have been implicated in various types of cancers. For example, non-small cell lung cancer (NSCLC) had been associated with over-expression of S100A2, S100A8 and S100A9 [45]-[47], while S100A2 and S100A11 may be a prognostic marker for post-operative patients with pancreatic cancer [48] [49]. In HNSCC, patients with S100A2 positive LSCC had been shown to have better survival compared to S100A2 negative tumors [50]. S100A7 expression may also be implicated in the prognosis of HNSCC tumors [51]. Although serum S100 proteins may have potential implications in various types of cancers, there are still limited clinical applications for S100 proteins, with the only independent prognostic marker being serum S100B in melanoma [52] [53]. The differential reporting of S100 calcium binding protein by Dowling *et al*. and Sewell *et al*. as identified in this review may be secondary to the different sample sources (saliva and tumor tissue, respectively) and the use of control samples from the same patient by Dowling *et al.* [23] [31].

A number of biomarkers identified in this review may not have significance in the carcinogenesis of LSCC due to their non-specific nature in multiple disease processes, notably albumin and transferrin, which may be differentially expressed due to various physiological or pathological processes [54] [55]. Transthyretin has been shown to be a potential biomarker in pancreatic cancer [56] [57], but its association with multiple inflammatory disease processes may reduce its usefulness as a cancer biomarker [58]. Similarly, the use of fibrin and cofilin as LSCC biomarkers is limited by its non-specificity even though it is involved with various carcinogenic processes [59] [60].

Although Chi *et al*. identified the association of UCRP with LSCC, a protein previously shown to change tumor sensitivity to chemotherapy [61] [62], the lack of validation using proteomic techniques in LSCC samples highlights the need for further classification of this biomarker [30].

Stathmin over-expression is found in many types of cancers [63], and is involved in processes such as the regulation of cell migration [64] [65], which may have implications in the pathogenesis of LSCC. Likewise, IGFBP7 is differentially expressed in various cancers as a tumour suppressor protein [66] [67]. However, there is still insufficient evidence currently to demonstrate any significant associations between these proteins and LSCC.

Two biomarkers, uPA and MT1-MMP, were identified more than 10 years ago [32] [36]. uPA is a type of protease responsible for both proteolysis and fibrinolysis, processes which are required for tumor growth and metastasis [68]. Its expression is associated with various cancer types such as breast and colorectal tumors [69] [70]. It is the only biomarker identified in this review which had used a sample size of greater than 20. Moreover, recent studies have validated the over-expression of uPA in separate LSCC populations which suggests its potential as a biomarker in LSCC [71] [72]. MT1-MMP was also a novel biomarker and potential therapeutic target identified in the 1990s, but there had been limited studies regarding its use in LSCC since, with research focusing on other MMP subtypes such as MMP-2 and MMP-9 [73] [74].

Ceramides, a well-studied group of molecules, are involved in apoptotic cellular pathways [75]. They have been shown to assist in the induction of cell death by chemotherapeutic agents both in vivo and in situ [76] [77]. Karahatay *et al*. reported a lower level of C18-ceramide in patients with LSCC as well as an inverse relationship between ceramide levels and the risk of nodal metastasis [32]. Ceramides may therefore be an important prognostic marker in LSCC.

The small number of studies identified by this review limits the use of quantitative methods to analyse current LSCC biomarkers discovered by proteomic techniques, and may be a source of bias in this review. Although 25 different proteins were identified, only two were reported by more than one study. This highlights some of the pitfalls of using proteomic techniques in cancer biomarker research. Firstly, identified biomarkers require validation in a larger population with reproducible results [78]. Only one study validated the identified biomarker in a separate population and the vast majority of studies had sample sizes of less than 20. Secondly, using different proteomic techniques and technologies as well as different tissue preparations may produce varying results. There was great variability in the type of tissue analyzed and the proteomic technique used between studies. There was no standardized method of identifying LSCC biomarkers using proteomic techniques across the seven studies identified in this review. This highlights the need for further research in evaluating the molecular mechanisms for LSCC biomarkers by proteomic techniques, with the aim of developing novel diagnostic and therapeutic options for this condition.

5. Conclusion

Despite advances in biomarker research, LSCC remains to be associated with significant morbidity and mortality. This systematic review assessing LSCC biomarkers identified using proteomic techniques has found various differentially expressed protein biomarkers associated with LSCC, with no specific marker of clinical significance. Future studies may aim to further characterize these biomarkers to better understand their mechanism of action in LSCC, and validate their use in a clinical setting.

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