REVIEW

Immunohistochemical Markers of Epithelial - Mesenchymal Transition in Oral Squamous Cell Carcinoma: A Systematic Review.

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Abstract:

Objective: The aim of this systematic review was to explore the prognostic significance of immunohistochemical markers of epithelial-mesenchymal transition (EMT) in oral squamous cell carcinoma (OSCC).
Methods: PubMed, EMBASE, and Scopus databases were thoroughly searched using various combinations of keywords, and Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines were followed for analysis.

Results: Database search yielded a total of 45 articles, out of which 13 articles were selected based on the inclusion and exclusion criteria. A total of 946 oral squamous cell carcinoma samples were evaluated for various immunohistochemical markers. The most commonly studied immunohistochemical marker of EMT was E-Cadherin followed by Beta-catenin, CD44, N-Cadherin, EMA, vimentin, SMA, ALDH1, cyclin D1, APC, Collagen IV and MMP 9. A panel of transcription factors such as TWIST1, SNAI1/2, ZEB1, and ZEB2 and other genes intimately related to EMT (CDH1 and LAMC2) at the invasive tumor front of OSCC tissues were also studied.

Conclusion: Biomarkers such as E-cadherin, EMA, and SMA are emerging as valuable tools for predicting prognostic and therapeutic outcomes in OSCC patients. The higher levels of EMT phenotype in OSCC tissues were characterized by reduced E-cadherin and β-catenin expression and overexpression of N-cadherin. Angiolymphatic invasion and lymph node metastasis are poor prognostic factors and can be predicted with higher ALDH1 and CD44 immunoexpression respectively. Co-expression of TWIST1 and ZEB2 was significantly prevalent in OSCC patients with poorer overall survival, especially in patients with no lymph node metastasis. The Snail family of zinc-finger transcription factors especially SNAI1/2 was also significantly overexpressed in OSCC. The prognostic value of these biomarkers is proven and can be employed for evolving new therapeutic modalities.

Keywords: Immunohistochemistry, oral squamous cell carcinoma, epithelial-mesenchymal transition.

Introduction

Head and neck cancer is the sixth most prevalent malignancy globally, and oral squamous cell carcinoma (OSCC) is the most common type among them. Life-threatening and untreatable recurrent diseases will significantly reduce the favorable therapeutic outcomes in OSCC patients. The factors mostly leading to a poor 5-year survival rate are tumor metastasis, recurrence, and drug resistance. The life expectancy in advanced cancer patients can be extended if we can overcome these factors[1].

Epithelial-mesenchymal transition (EMT) is a phenomenon characterised by reduced expression of epithelial genes (E-cadherin) and an increased expression of mesenchymal genes (N-cadherin) and EMT transcription factors. Together with an altered localization of the beta-catenin, the epithelial cells lose their phenotype and intercellular adhesions. Besides, there is an increased expression of vimentin signifying a mesenchymal change in the cytoskeleton. At the same time an increase in tenascin pointing towards the fact that matrix deposition enables the migration of cells. Significantly, the matrix metalloproteinase 9 (MMP9) over expression demonstrates the disruption of the basement membrane and the proneness of cells to infiltrate the underlying stroma[1,2].

It is an established fact that epithelial-mesenchymal transition (EMT) is a reversible dynamic process allowing a polarized epithelial cell to undergo multiple biochemical changes leading to a mesenchymal cell phenotype, which includes enhanced migratory capacity and invasiveness, increased resistance to apoptosis and a remarkable increased production of extracellular matrix (ECM) components[3-5]. In the invasive stage, tumor epithelial cells lose their intercellular junctions and apical-basal polarity, thus detaching from the basement membrane[6]. EMT – inducing transcription factors are responsible for the further alterations in the cell-ECM interactions and overexpression of mesenchymal phenotypes. The next stage involves the angiogenic switch enabling cancer cells to enter the blood circulation and their exit from the blood stream at a remote site which may lead to the formation of micro- and macro metastases. Cancer cells grow into metastatic tumors, once a proper distant site is reached[7].

The expression of specific genes involved in the epithelial phenotype repression and mesenchymal phenotype activation is the hallmark of epithelial-mesenchymal transition. The changes in the various factors during the epithelial-mesenchymal transition can be utilized as biomarkers and studies have suggested that these biomarkers can be used for predicting the prognosis of cancer. E-cadherin, CD44, Beta-catenin, N-cadherin, smooth muscle actin, ALDH1 and transcription factors such as TWIST1 and ZEB2 are promising biomarkers[7]. The aim of this systematic review was to explore the prognostic significance of immunohistochemical markers of EMT in oral squamous cell carcinoma.

Methods:

Protocol:

PubMed, EMBASE, and SCOPUS databases were thoroughly searched using combination of keywords: Oral Squamous Cell Carcinoma, immunohistochemical markers, epithelial-mesenchymal transition, and prognosis. The search was merged into reference manager software and the retrieved records were reviewed systematically. Articles in English language only were included for this study. This review was done according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Search strategy:

The primary articles were identified using the advanced search field with keywords. The search strategy was based on the combinations of the following main keywords: oral squamous cell carcinoma (AND) epithelial-mesenchymal transition (AND) immunohistochemical markers (AND) prognosis. In addition, manual search was also conducted to include certain references of articles. The main factors in the literature search were the relationship between immunohistochemical markers and prognostic outcomes in oral squamous cell carcinoma.

Eligibility criteria:

Following criteria were used for the study selection:

1) Cross-sectional, case-control or cohort studies published in English language.
2) Studies done in oral squamous cell carcinoma specimen.
3) Studies evaluating the association between immunohistochemical markers expression and overall survival (OS).

Exclusion criteria includes the following:

1) Book chapters, letters, conference abstracts, and abstracts without full text.
2) Studies out of areas of interest (eg; when the studies investigated other diseases or solid tumors).
3) Studies with insufficient useful data.
4) Studies with increased risk of bias.

Study selection and data extraction:

All search records obtained from different databases were analyzed thoroughly to retrieve 45 primary articles. Duplicated files were removed using the Endnote software. After an independent screening of the titles and abstracts based on the inclusion criteria, the eligible studies were selected. For all the included articles, the following descriptive data were collected: the name of the first author, country and year of conduction of the study, detection method, age, sex, sample size, immunohistochemical marker assessed, median or mean follow-up times, clinicopathological parameters, cut-off value, and related survival data. The primary outcome was the relationship between the immunohistochemical markers expression in OSCC patients.

Measures to assess the quality and risk of bias :

The publications were critically appraised separately by three authors in accordance with the Joanna Briggs Institute Reviewer's Manual of 2017 (https://joannabriggs.org/).

Results:

Database search yielded a total of 45 articles, out of which 13 articles were selected according to the inclusion and exclusion criteria. Table 1 shows the details of the collected articles. A total of 946 oral squamous cell carcinoma samples were analyzed in the selected studies. Immunohistochemical markers used in these studies were E-cadherin, Beta-catenin, N-cadherin, CD44, ALDH1, TGF beta, Ki 67, Cyclin D1, Collagen IV, APC, SMA, EMA, EMMPRIN, MMP9, SNAIL, TWIST1, ZEB1, ZEB2, CDH1, AND LAMC2.

TABLE 1: SUMMARY OF ARTICLES INCLUDED IN THE SYSTEMATIC REVIEW

SL NO.	AUTHOR & YEAR	TOTAL NUMBER OF SAMPLES	TECHNIQUE USED	IMMUNHISTOCHEMICAL MARKERS
1	Ortiz RC et al[8], 2018	75	IHC	CD44, ALDH1
2	Essa AAM et al[9], 2022	114	IHC	CD44, MMP9, Ki67, CD31, TGF Beta
3	Irani S et al[10], 2014	63	IHC	CD44, E-Cadherin
4	Patil R et al[11], 2014	30	IHC	MMP 9
5	Khan SM et al[12], 2021	30	IHC	EMMPRIN
6	Nambiyar K et al[13], 2021	45	IHC	E-cadherin, EMA, vimentin, and SMA

7	Angadi PV et al[14], 2016	60	IHC	E-cadherin, β-catenin, and N-cadherin
8	da Silva SD et al[15], 2015	102	IHC	E-cadherin, catenin (a, b, and c), APC, collagen IV, Ki-67, cyclin D1, and CD44
9	Hanemann JA et al[16], 2014	71	IHC	E-cadherin and β-catenin
10	Rosado P et al[17], 2013	69	IHC	E-cadherin and β -catenin
11	Hong KO et al[18], 2018	56	IHC	Snail and Twist
12	da Silva SD et al[19], 2014	108	IHC, complimentary DNA microarray platform coupled to quantitative reverse transcriptase-polymerase chain reaction	TWIST1
13	Kong YH et al[20], 2015	148	IHC	TWIST1, SNAI1/2, ZEB1 and ZEB2) and other genes intimately related to EMT (CDH1 and LAMC2)

Discussion:

The enhanced migratory capacity, invasiveness, increased resistance to apoptosis and remarkably increased production of extracellular matrix (ECM) components are some of the notorious pathologic characteristics of tumor cells. These biochemical changes are acquired by the tumor cells during the epithelial-mesenchymal transition process. Loss of intercellular junctions and apical-basal polarity and subsequent detachment of tumor epithelial cells from the basement membrane are evident in the invasive stage. Furthermore, altered cell-ECM interactions and overexpression of mesenchymal phenotypes are developed via EMT-inducing transcription factors[7]. Thus, with in-depth study, various immuno histochemical markers may emerge as promising prognostic and diagnostic tools in the future.

Cancer stem cells are the most migratory and highly

metastatic cellular subpopulation within the tumor. They have features of both cancer cells and stem cells, including self-renewal and resistance to apoptosis, and are responsible for tumor progression. Studies have established a link between the CSC phenotype and the process of epithelial-mesenchymal transition (EMT), characterized by the loss of cell polarity and cell-cell adhesion, as well as the gain of mesenchymal morphology, which allows them to migrate beyond the primary tumor. Moreover, CSCs that undergo EMT have the ability to invade adjacent stromal tissues and acquire the migratory capacity by entering the blood or lymph vessels. Subsequently by the reverse process of mesenchymal- epithelial transition (MET) they colonize at distant sites and generate metastasis[8].

The most known marker of CSC is CD44, a transmembrane glycoprotein, and receptor for hyaluronan (HA). The wide association with cell adhesion, angiogenesis, cell migration, and cell proliferation in tumorigenesis makes this marker a center of study attraction. Studies have demonstrated that the association of CD44 with ALDH1 activity could increase tumorigenesis[8].

Aldehyde dehydrogenase 1 (ALDH1), is a detoxifying enzyme involved in cell differentiation, detoxification, and drug resistance. It is highly expressed in normal and cancer stem cells, including head and neck cancers. In the extracellular domain CD44 interaction with ligands is crucial for cell signaling regulation. Hence it is clear that CD44 acted as a protein responsible for cellular attachment to the extracellular matrix, angiogenesis, migration, and invasion depending on its splicing variant. CD44 is also a known marker of cancer stem cells in various cancer types and carcinoma cells produce many types of CD44. A study conducted by Ortiz RC et al in 75 OSCC samples revealed that CD44 high cells were associated with cervical lymph node metastasis while ALDH1high immunostaining was associated with angiolymphatic invasion, both considered important parameters for OSCC poor prognosis8. Another study by Essa AAM et al proved that the association of CD44 with MMP-9 and TGF-beta may increase CD44 expression on OSCC-associated blood vessels, and they suggested that CD44 might be a useful therapeutic target for anti-angiogenic therapies[9].

Matrix metalloproteinase (MMP) is a zinc-dependent endopeptidase which can degrade several types of collagens in the ECM. They have important roles in tissue repair, ECM remodeling, and cancer metastasis. MMP-9 belongs to gelatinase subgroup of MMP and known as multifunctional modulator. They have critical role in very complex cell signaling cascades. MMP-9 facilitates tumor cell invasion through proteolytic degradation of ECM components (including types III, IV, and V collagens, as well as gelatin) and results in the discharge of growth factors such as vascular endothelial growth factor that enhance angiogenesis and tumor progression. Antiangiogenic endostatin, angiostatin, and tumstatin are released during the same time. Patil R et al found that overexpression of the MMP-9 indicated nodal metastasis in many cases of OSCC. Since higher expression of MMP-9 is associated with advanced stages of OSCC it can be utilized to predict the carcinoma invasion and progression[11]. Thus, MMP-9 may be a useful tool to determine the prognosis of patients with OSCC.

Extracellular matrix metalloproteinase inducer (EMMPRIN), also known as CD147, is a membrane-bound glycoprotein and is found on the surface of tumor cells. It is involved in a variety of physiological and pathological activities. It has the ability to function as inducer of matrix metalloproteinases (MMPs). It is a transmembrane protein that regulates the turnover, and remodeling of the extracellular matrix and at the same time, it is an important mediator of cell and stromal interactions also. Dysregulation of EMMPRIN has been noted in the progression of cancer. Many studies have shown that EMMPRIN is central in the promotion of tumor invasion, growth/progression, and metastasis as it upregulates MMPs secreted from adjacent fibroblasts. Furthermore, EMMPRIN influences the production of several proinflammatory cytokines that have been directly associated with cancer. EMMPRIN also promotes neovascularization through the expression of vascular endothelial growth factor. Khan SM et al proved that EMMPRIN expression increased in advanced clinical stages and higher histological grades of OSCC, suggesting that it might be involved in the progression and spread of these tumors. Thus, EMMPRIN can be used as an effective biological prognostic marker to identify high-risk OSCC patients[12].

E-cadherin, a transmembrane glycoprotein, is responsible for cell-cell adhesion in epithelial tissues and the maintenance of structural integrity. The loss of E-cadherin expression increases the mobility of epithelial cells and their ability for local invasion. The change of expression of cadherin from E-cadherin to N-cadherin in the neoplastic cells, known as cadherin switch, promotes cancer progression. A study conducted by Nambiyar K et

al showed that the expression of E-cadherin was significantly reduced in cases with lymph node metastasis[13].

Twist1, a highly conserved, basic helix-loop-helix transcription factor mapped at 7q21.2. It has a bifunctional role, acting as an activator or a repressor, depending on post-translational modifications and physiologic contexts. Twist1 induces gene transactivation through cis binding to E-box regulatory regions, which are present in several target genes, and this involves complex homodimerization and heterodimerization mechanisms regulated by protein phosphorylation. In the case of gene repression, Twist1 can repress genes by regulating chromatin remodeling through histone acetyltransferase-dependent=histone deacetylase-dependent mechanisms and through the inhibition of DNA binding activity of transcription factors. The implication of Twist1 in cell migration is attributed primarily to its ability to contribute to EMT, eg, through the down-regulation of E-cadherin and the upregulation of mesenchymal markers like vimentin, fibronectin, and N-cadherin. Kong YH et al proved that co-expression of TWIST1 and ZEB2 was significantly prevalent in OSCC patients with poorer overall survival, particularly in patients with no lymph node metastasis[20].

The Snail family of zinc-finger transcription factors is composed of a highly conserved carboxy-terminal region containing four to six C2H2-type zinc fingers. These are associated with the mediation of sequence-specific interactions with DNA promoters containing an E-box sequence (CAGGTG). Snail1 (Snail), Snail2 (Slug), and Snail3 (Smuc) are the three members of the Snail family seen in vertebrates. Among these Snail and Slug are critical in regulating the multiple signaling pathways involved in EMT and their activation. Snail is the most important transcriptional repressor of E-cadherin and is closely associated with tumor metastasis. Furthermore, Snail and Slug down regulate the expression of other epithelial markers, including claudins and occludins. The major concern lies in the therapeutic resistance caused by the increased expression and accumulation of Snail in the nucleus[21]. A study by Kong YH et al revealed that SNAI1/2 was significantly overexpressed in greater than 70% of OSCC specimens. In addition, they pointed out that Snail could be considered a master regulator of EMT since it controls the expression of matrix metalloproteinases (MMPs) and other transcription factors (i.e., TWIST1, ZEB1, ZEB2)[20].

The prognostic value of these IHC markers in epithelial-mesenchymal transition play an important role in the prognosis and can be utilized to lighten up new strategies in cancer therapeutics. Ever-increasing incidence of cancer is a threat to humankind and advanced research to implement better modalities is mandatory for increasing the survival rate and decreasing the morbidity and mortality of OSCC patients.

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