Membrane-organizing extension spike protein and its role as an emerging biomarker in oral squamous cell carcinoma

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Abstract

Oral squamous cell carcinoma (OSCC) is the most malignant tumor worldwide with a relatively poor prognosis. This can be due to lack of using new specific biomarkers as a mode of pristine interventional therapy for detecting the lesions at an early stage, thereby not allowing it to proceed to a severe advanced stage. Biomarkers, being the products of malignant cells, can prove to be promising prognostic factors in understanding the molecular pathogenesis of oral cancer. One such biomarker is membrane-organizing extension spike protein (MOESIN). Belonging to the family of ezrin/radixin/MOESIN proteins, MOESIN acts as a structural linker between plasma membrane and actin filament of the cell moiety and is involved in regulating many fundamental cellular processes such as cell morphology, adhesion and motility. This narrative review is a systematic compilation on MOESIN and its role as an emerging biomarker in OSCC.

Keywords: Amino acid motifs, cytoskeleton protein, membrane-organizing extension spike protein, microfilament proteins, MSN protein, oral cancer

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common malignancy accounting for about 95% of cancer cases worldwide.[1-3] Choosing the best therapy for OSCC is dependent on both patient factors (nutritional status, associated diseases and oral behaviors) and tumor factors (size, site, histology and biologic behavior).[4] Despite the tremendous advancements in detection and treatment of OSCC, the prognosis still remains poor, with overall 5-year survival rate being as low as 50%–60%.[5-7] This can be partly imparted due to lack of use of new emerging biomarkers that can identify and bestow opportunities for effective pristine interventional treatment strategies and

harmonize with the severity of the disease progression. Biomarkers, being the products of malignant cells, can serve as targets for intervention of therapy and can, therefore, prove to be more promising prognostic factors in understanding the molecular pathogenesis of OSCC.[8] Biomarkers can also be used to assess the rate of malignant transformation, thereby aiding in early prophylactic conciliation of the disease progression.

A well-controlled balance between the cellular proliferation and differentiation is important for maintaining the normal development of epithelial integrity of the oral cavity. Any imbalance that can occur due to the confounding

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variables (tobacco, alcohol and genetics), divulges the expression of various proteins that regulate or modulate different signaling pathways that are basically involved in cell growth, differentiation, protein synthesis, cell adhesion and motility, thereby contributing to the process of carcinogenesis and metastatic cascade.[9,10] Tumor metastasis and systemic dissemination of malignant cells involves metastatic colonization and dynamic polarization of cytoskeleton, further orchestrating the tumor microenvironment.[11] Furthermore, the rate of malignant transformation fluctuates based on multiple variables, such as age, gender, population and histopathological grading, that further truncate the mortality rates.[12] The most common traditional method used to determine the rate of malignant transformation and prognosis is histopathological grading of OSCC (well-differentiated, moderately differentiated and poorly differentiated types). However, this is considered to be subjective and meager, leading to inaccurate results and poor prognosis.[13] To correlate further, the epigenetic and molecular changes that can occur within a cell during the carcinogenetic process can be detected by an in-detail study of cell moiety using new emerging biomarkers. Few specific and nonspecific markers were introduced in the past, but their day-to-day applications, on clinical basis, are still lacking. This can be one of the contributing factors for lack of early detection and poor prognosis. Hence, there is a need for introducing new emerging biomarkers as an interventional therapy for effectively addressing OSCC at an early stage, thus preventing it to further proceed to advance severe stage. One such biomarker can be membrane-organizing extension spike protein (MOESIN).

MEMBRANE-ORGANIZING EXTENSION SPIKE PROTEIN AND ITS PHYSICAL PROPERTIES

MOESIN, a 577-amino acid polypeptide, belongs to a group of ezrin/radixin/MOESIN (ERM) proteins.[14-16] It shares 71.7% sequence identity with the mouse ezrin.[14,17] The apparent molecular mass of MOESIN is 75 kDa.[15] These groups of proteins act as a structural linker between plasma membrane and actin filament of the cell moiety and are located beneath the cellular protrusions (microvilli, filopodia, uropods, microspikes ruffling membranes and retraction fibers) and cell adhesion sites.[15,17-19] MOESIN is primarily expressed in the cytoplasm and is concentrated at the actin-rich cell structures.[20] The tissue distribution of MOESIN is considered to be highest in the lung and spleen and lowest in the kidney. It is also expressed in some specialized epithelia such as ductal epithelium of exocrine glands and basal layers of the esophageal epithelium.[18] The main function of MOESIN is to link the F-actin to cell membrane proteins after phosphorylation and form conformational changes that are essential for cell configuration.[21]

STRUCTURE OF MEMBRANE-ORGANIZING EXTENSION SPIKE PROTEIN

Traced back to history, MOESIN was initially isolated as an extracellular heparin-binding protein in cultured bovine smooth muscle, and later identified as an intracellular protein without a signal or transmembrane sequence.[16,22,23]

Structurally, MOESIN is a part of "the band four point one and ERM domain" called the FERM domain (4.1 protein, ezrin, radixin and MOESIN). This domain consists of two parts: N-terminal FERM domain and the C-terminal tail domain. Both of these are connected to each other by a central helical domain. The amino-terminal domain has membranous proteins and the C terminal domain links with the F-actin. Three modules – F1, F2 and F3 – are present on N-terminal part which bind to integral membrane proteins, scaffold proteins and the Rho-related proteins (such as the Rho-guanosine 5'-diphosphate-dissociation inhibitor and Dbl)[24-31] and the PIP2. It exists in two states: a closed/inactive state and a phosphorylated active/opened state. In the closed state, the FERM domain is tightly bound to the tail domain, masking the binding sites for other molecules, and in the open state, it tethers between actin and receptors on the plasma membrane.[17] The FERM domain of MOESIN binds to the DH/PH domain of GEFs and interferes with them on the cell membrane, preventing them from activating Rho GTPase.[32] Therefore, removal (knockout) of MOESIN enhances the activities of Rho GTPase and induces cell protrusion in the wrong direction, thereby abolishing the ability of chemotaxis to catch bacteria.[33,34]

FUNCTIONS OF MEMBRANE-ORGANIZING EXTENSION SPIKE PROTEIN

1. Regulation of various physiological and pathological processes such as cell morphology, cell adhesion, cell motility and metastasis[35]
2. Formation of protrusions and immunological synapse[36-38]
3. Plays a role in mitotic divison. This hypothesis was confirmed by conducting an experimental trial on *Drosophila melanogaster*, where reduced levels of MOESIN using RNA interference resulted in multiple cell shape abnormalities and delay in anaphase onset[39-42]
4. Maintenance of oral epithelial and structural integrity by remaining consolidated in the epithelial layer.[43] Studies have also documented that loss of MOESIN

can lead to disruption of overall morphology and epithelial integrity[43-46]

5. Together with the binding partner, Bitesize which is the cytoplasmic protein, MOESIN remains recruited in the apical-basal domain, thus playing a role in maintaining the basal cell polarity by formation of adherens junctions

6. Specific role in immunology as it is present dominantly present in few of the immune cells such as neutrophils, lymphocytes, mast cells and platelets. It is the dominant ERM protein in lymphocytes, where it has been implicated in the egress of T- and B-cells from the secondary lymphoid organs.[47-49]

EVIDENCE-BASED STUDIES ON CORRELATION OF MOESIN WITH ORAL SQUAMOUS CELL CARCINOMA AND ITS HYPOTHESIS

MOESIN is upregulated in multiple human cancers, including breast cancers, prostate cancers, pancreatic cancers, lung cancers, melanoma and OSCC.[20] Few evidence-based studies of correlation of MOESIN with OSCC and its hypothetical phenomenon are documented below:

1. Hiroichi Kobayashi (2003) conducted a study to evaluate the expression of MOESIN on paraffin-embedded tissue of 59 cases of OSCC, 35 cases of oral epithelial dysplasia (OED), 17 cases of verrucous carcinoma (VC) and 5 cases of normal epithelium (as control). After approval from Shinshu University, School of Medicine, Ethical Committee, all the samples of OSCC, OED, VC and normal epithelium were fixed in 10% neutral buffered formalin. The WHO classification for tumor differentiation and clinical staging using international union against cancer (1997) was applied. Mouse monoclonal antibody provided by Dr. Tsukita of Kyoto University obtained using chicken gizzard as an antigen was used. Immunohistochemical detection was done using indirect peroxidase method. Sections stained were evaluated by two intraobserver examiners to avoid bias. The expression of staining patterns was divided into three groups: membranous, mixed and cytoplasmic expressions. The data obtained were subjected to Kruskal–Wallis rank test, Mann–Whitney *U*-test and Scheffe's test. This study results inferred high cytoplasmic expression of MOESIN in OSCC, high membranous expression in OED and mixed pattern in VC. The authors concluded that MOESIN was uniquely associated with squamous cell phenotype and can be used as a new emerging biomarker in diagnostic histopathology

2. Hiroichi Kobayashi (2004) conducted a study wherein the authors performed an immunohistochemical staining of MOESIN on 103 paraffin-embedded specimens of primary OSCC cases. After clearance from the Institutional Ethical Committee of Shinshu University School of Medicine, tissue samples of primary (*n* = 103) and metastatic (*n* = 30) lesions of OSCCs were collected. The study population consisted of 59 men and 44 women averaging 65.0 years of age (range, 27–88 years). The tumor grading was done as per the WHO classification and mode of invasion was classified according to Jakobson's classification. Mouse monoclonal antibody (CR-22) was used. Two cell lines derived from a single human tongue cancer (SQUU-A and SQUU-B) from nude mice transplanted sections. Formalin-fixed, paraffin-embedded sections were made and stained with the anti-MOESIN antibody. Sections were examined by two independent researchers. MOESIN expression of neoplastic cell in primary lesions was classified as follows: membranous pattern (membranous expression of MOESIN was dominant), mixed pattern (membranous expression and cytoplasmic expression were dominant) and cytoplasmic pattern (cytoplasmic expression was dominant). The cellular distribution pattern of MOESIN differed substantially in primary tumors and metastatic lymph nodes. Membranous or cytoplasmic patterns were seen in the primary tumor of the patient, and metastatic tumors in lymph nodes showed the cytoplasmic distribution pattern. Univariate regression and multivariate analysis using the Cox proportional hazards model inferred (*P* = 0.0470) that was statistically significant. Mixed or predominantly cytoplasmic expression patterns were seen in cell lines of SQUU-A cells with low metastatic potential whereas SQUU-B cell lines with high metastatic activity exhibited a downregulation of membranous expression and an increase in cytoplasmic expression. They concluded that that tumor cells with cytoplasmic expression of MOESIN showed a higher incidence of lymph node metastasis than tumor cells with membranous expression of MOESIN[35]

3. Karawan Khaleel Jubai (2016) conducted a comparative study on 46 formalin-fixed, paraffin-embedded tissue blocks of 30 OSCCs and 12 oral VCs (OVCs). The samples were stained with three markers, i.e., MOESIN, CK14 and MMP7. The expression patterns of all the markers were correlated with histopathological grading system. MOESIN expression was found to be 86.7% of SCC group. MOESIN showed cytoplasmic expression pattern in OSCC and membranous pattern in OVC

4. The shift in localization and expression pattern of MOESIN was hypothesized as follows: the conformational and functional changes of MOESIN result in redistribution of this molecule in tumor cells, and according to carcinogenesis, it is possible that increased membranous degradation in more aggressive neoplasms and mutation of MOESIN gene cannot cross-link between plasma membrane and actin filament[2]

5. Francisco Bárbara Abreu Barros *et al.* (2018) conducted a study to evaluate the participation of MOESIN and podoplanin in the invasive tumor front of OSCCs and their influence on patients' prognosis. The study was conducted at the Head and Neck Surgery and Otorhinolaryngology Department of A. C. Camargo Cancer Hospital, São Paulo, Brazil, during the period of 1963–2012. The total sample considered was 84 surgical specimens of OSCC involving the tongue, floor of the mouth, inferior gingiva and retromolar area. Clinical data and histopathological variables were considered. Immunoexpressions of MOESIN were evaluated applying the semiquantitative score method as given by Faustino *et al.* (2008). The target field of interest was the invasive front of tumor. Approximatex 10 microscopic fields of each sample were observed under ×400 magnification, and to avoid performance bias, the stained sections were examined by two pathologists. The scoring system was applied and classified into three groups from 0 to 2. The data were subjected to SPSS Statistical Software version 21.0 (SPSS Inc., Chicago, IL, USA).

Few previous study[24] have hypothesized that MOESIN gets translocated from plasma membrane to the cytoplasm of neoplastic cells and may reduce the ability to form cell cell contacts, as well as, influence the cytoskeleton remodeling and tumor invasion process. Over expression / strong expression of MOESIN in cytoplasm of malignant cells thereby can be considered as one of the prognostic markers in OSCC, thus concluding that strong MOESIN expression by malignant cells may help to determine patients with OSCC and poor prognosis.[21]

CONCLUSION

This narrative review is a systematic compilation on MOESIN and its role as an emerging biomarker in OSCC. Knowledge about this new biomarker is essential for its application as pristine interventional therapy so as to diagnose the cases at an early stage and thus reduce the mortality rates.

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Conflicts of interest
There are no conflicts of interest.

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