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Cytoplasmic accumulation of activated leukocyte cell adhesion molecule is a predictor of disease progression and reduced survival in oral cancer patients

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Activated leukocyte cell adhesion molecule (ALCAM) has been proposed to function as a cell surface sensor for cell density, controlling the transition between local cell proliferation and tissue invasion in cancer progression. Herein, we determined ALCAM expression in 107 oral squamous cell carcinomas (OSCCs), 78 oral lesions (58 hyperplasias and 20 dysplasias) and 30 histologically normal oral tissues using immunohistochemistry and correlated with clinicopathological parameters. Significant increase in ALCAM immunopositivity was observed from normal oral mucosa, hyperplasia, dysplasia to OSCCs ($p_{trend} < 0.001$). Increased ALCAM expression was observed in cytoplasm of epithelial cells as early as in hyperplasia ($p = 0.001$, OR = 3.8). Sixty-five of 107 (61%) OSCCs showed significant overexpression of ALCAM protein in cytoplasm/membrane of tumor cells ($p = 0.043$; OR = 3.3) in comparison with the normal oral tissues. Among OSCCs, cytoplasmic ALCAM was associated with advanced tumor size, tumor stage and tobacco consumption. Importantly, cytoplasmic ALCAM was an independent predictor of poor prognosis of OSCCs in multivariate analysis ($p = 0.012$, OR = 6.2). In an attempt to understand the molecular basis of cytoplasmic localization of ALCAM, 14-3-3ζ and 14-3-3σ were identified as its novel binding partners in oral cancer cells. In conclusion, increased expression of ALCAM is an early event in oral tumorigenesis; its cytoplasmic accumulation in tumor cells is a predictor of poor prognosis of OSCCs, underscoring its potential as a candidate prognostic marker for oral cancer.

Key words: ALCAM; 14-3-3; hyperplasia; dysplasia; oral cancer

Head and neck/oral squamous cell carcinoma (HNOSCC) is the sixth most common cause of cancer deaths in USA and remains a significant cause of cancer morbidity and mortality worldwide.[1] Head-and-neck cancer sites are readily amenable to clinical examination, yet a lack of suitable molecular markers for early detection and risk assessment is clearly reflected by the fact that more than 50% of all HNOSCC patients have advanced disease at the time of diagnosis.[2–6] Indeed, the 5-year survival rates of HNOSCC patients are in general poor (about 50% overall) and the prognosis of advanced HNOSCC cases has not improved much over the past 3 decades.[2,4] This limits treatment options and renders management of HNOSCC extremely challenging. Recurrence and formation of second primary tumors are frequent (10–25% of cases).[7,8] The clinical course of advanced disease is difficult to predict because the current clinicopathologic prognostic factors are not accurate predictors of clinical outcome. The heterogeneity of clinical outcomes in advanced stage HNOSCC patients emphasizes the need for accurate prognostic factors that can identify patients who are likely to develop recurrent tumors or second primary tumors, and thus might be candidates for novel therapeutics. The limited effectiveness of therapy for patients with advanced stage and recurrent disease is a reflection of an incomplete understanding of the molecular basis of head and neck carcinogenesis.[9,10] Conceivably, improvement in the ability to identify in an early stage and to predict malignant progression of HNOSCC lesions would lead to more effective treatment and reduction of morbidity and mortality.

Intense efforts are being directed toward developing accurate predictors of clinical outcome using high throughput techniques, such as differential display-reverse transcription PCR (DD), cDNA microarrays and proteomics, to assess global gene/protein expression patterns in head and neck cancer.[11–14] In search of such novel molecular targets, our laboratory reported increased levels of activated leukocyte cell adhesion molecule (ALCAM) transcripts in oral squamous cell carcinomas (OSCCs), that constitute majority of HNOSCCs in India, using DD in clinical specimens and cell lines.[15,16] Importantly, ALCAM mRNA up-regulation was also observed in cell cultures from a human oral hyperplasia (AMOL-III), exposed to smokeless tobacco extracts (ST) *in vitro*[16], providing the rationale for in-depth investigation of ALCAM expression in different stages of development and progression of OSCC.

ALCAM/melanoma metastasizing clone D (MEMD)/CD166 is a transmembrane glycoprotein of Ig superfamily that mediates cell–cell adhesion through both homophilic (ALCAM-ALCAM) and heterophilic (ALCAM-CD6) interactions.[17] ALCAM, first identified as a CD6 ligand,[18] is involved in hematopoiesis,[19,20] neurite extension,[21] osteogenesis[22] and embryonal implantation in the uterus.[23] The stage-specific expression of ALCAM during fetal development resembles distinct steps of tumor metastasis. Expression of ALCAM correlates with the aggregation and metastatic potential of few human tumors including melanoma, prostate, ovarian, breast, lung, colorectal, esophageal, pancreatic cancer and hepatocellular carcinoma.[24–36] In recent years, ALCAM has become one of the frequently applied cell surface markers to select pluripotent cells from mesenchymal progenitor populations by flow cytometry.[30,32]

Herein, we investigated the clinical significance of ALCAM expression in different stages of oral tumorigenesis and determined its correlation with clinicopathologic factors and disease prognosis, with the aim of exploring its association with biological

Abbreviations: ALCAM, activated leukocyte cell adhesion molecule; CD, cluster of differentiation; CLSM, confocal laser scan microscopy; DD, differential display-reverse transcription PCR; ESCC, esophageal squamous cell carcinoma; HNOSCC, head and neck/oral squamous cell carcinoma; MDSCC, moderately differentiated squamous cell carcinoma; MEMD, melanoma metastasizing molecule D; PDSCC, poorly differentiated squamous cell carcinoma; ST, smokeless tobacco extract; WDSCC, well differentiated squamous cell carcinoma.

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