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Expression of ezrin in oral squamous cell carcinoma: Prognostic impact and clinicopathological correlations

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ABSTRACT

Introduction: There have been few investigations into the effects of ezrin expression in oral squamous cell carcinoma (OSCC). The aim of this study was to analyze the influence of ezrin expression on the prognosis of patients with OSCC.
Materials and methods: Eighty patients were included in the retrospective study. Expression and localization of ezrin were evaluated using immunohistochemistry. Associations were identified using χ^2 tests. Prognostic factors were identified by univariate and multivariate analysis.
Results: Seventy-six (95%) patients showed ezrin expression. Ezrin expression had a significant impact on overall survival (OS) ($p < 0.001$). With increasing expression, the 5-year OS rate dropped from 100% for ezrin-negative patients to 47% for patients with high expression. Multivariate analysis confirmed the significant influence of ezrin expression on OS ($p = 0.011$). Cytoplasmic localization of ezrin led to a significantly lower survival rate in comparison with membranous expression.
Conclusions: Ezrin may serve as a biomarker that predicts biologically aggressive behavior of OSCC and hence improves therapeutic techniques and the prognosis of patients affected with the disease.

1. Introduction

Head and neck cancer is the sixth most common cancer worldwide (Vormittag et al., 2009; Kreppel et al., 2013). Almost 50% of the tumors occur in the oral cavity and 90% of them are diagnosed as oral squamous cell carcinoma (OSCC) (Jemal et al., 2009). The annual incidence of OSCC is more than 500,000 worldwide (Vormittag et al., 2009). In 2009 approximately 35,000 new cases in the USA and 40,000 new cases in the European Union were diagnosed (Jemal et al., 2009).

Despite many advances in diagnostic and therapeutic techniques (Bloebaum et al., 2014), the 5-year survival rate of patients with OSCC has not improved significantly over the last three decades (Bretscher et al., 2002) and still remains below 50% (Feller and Lemmer, 2012). One of the main reasons is the lack of biological markers that consider the molecular behavior of OSCC. Biological markers help to assess the aggressiveness of the disease and the prognosis more accurately (Blessmann et al., 2013), and thus may provide treatments targeted at individual patients, as the prognosis of patients is not just determined by the anatomic extent and the histopathological differentiation of the tumor but also by its molecular features (Eckert et al., 2012; Morandi et al., 2015). Molecular markers may improve our understanding of carcinogenesis in patients with different clinical courses of OSCC (Schliephake, 2003). Furthermore, therapy may be provided through gene therapy or antisense molecules along with adjuvant radiotherapy and/or chemotherapy (Schliephake, 2003).

In the past a variety of tumor suppressor genes, oncogenes, cell proliferation markers, angiogenic markers and cell adhesion molecules were presented as prognostic and predictive tools for OSCC

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(Schliephake, 2003; Lothaire et al., 2006). In particular, epithelial growth factor receptor (EGFR), matrix metalloproteinases (MMPs) and p53 were often considered as promising markers for prognosis in OSCC (Oliveira and Ribeiro-Silva, 2011). Studies showed that overexpression of EGFR, as part of the cell cycle acceleration and proliferation molecules, significantly correlated with poor prognosis in patients with OSCC. In other investigations these findings could not be confirmed (Lothaire et al., 2006). Matrix metalloproteinases modify cell adhesion and matrix degradation. Poor prognosis of OSCC correlated significantly with high expression of MMP-7, MMP-9, MMP-13 and MMP-14 (Lothaire et al., 2006; Oliveira and Ribeiro-Silva, 2011). Biomarker p53 belongs to the tumor suppression and apoptosis biomarker group and is one of the most studied biomarkers so far (Lothaire et al., 2006). In most studies a high expression, especially in combination with cyclin D1 and EGFR, was significantly associated with poor overall survival in patients with OSCC (Lothaire et al., 2006). To further clarify the role of p53 in the prognosis of OSCC, randomized prospective trials or meta-analysis using individual data is necessary (Oliveira and Ribeiro-Silva, 2011). Recent studies also indicated that markers associated with tumor hypoxia, for example HIF-1α, may be useful, as they promote chromosome instability, cell invasion and metastasis (Eckert et al., 2012). Nevertheless, several studies showed that not only the expression and quantitative analysis play an important role, but also the localization, particularly at the invasion front of the tumor (Oliveira and Ribeiro-Silva, 2011).

In 2011, Oliveira and Ribeiro-Silva showed that the results for the analyzed tumor markers were discrepant and concluded that it is necessary to identify better molecular biomarkers for OSCC.

Ezrin is a promising novel marker, a member of the ezrin/radixin/moesin (ERM) protein family (Hunter, 2004). It plays a key role in tumorigenesis and the metastatic cascade and has a significant influence on prognosis, which was shown for several different types of cancer, including carcinoma of head and neck region, esophagus, breast, endometrium, cutaneous and uveal melanoma and soft tissue sarcoma (Saito et al., 2013). Ezrin was initially identified as a substrate for tyrosine kinase to stimulate proliferation of intestinal microvilli (Mangeat et al., 1999). It is grouped together with radixin and moesin as an ERM protein (ezrin/radixin/moesin) because of their high homology (Mangeat et al., 1999). Furthermore, they belong to the superfamily of band 4.1 proteins, as they share a common ~300-amino-acid domain, named FERM (four-point one, ezrin/radixin/moesin) (Mangeat et al., 1999). The ERM proteins are characterized by two main functions. First, they link plasma membrane proteins and F-actin filaments of the cytoskeleton, and secondly they are part of various signal transduction pathways (including RhoA, Hedgehog, CD43/44 membrane receptor signaling) involving cytoskeletal remodeling and transcriptional regulation (Fehon et al., 2010). Therefore ERM proteins are involved in cell–cell as well as cell–matrix interactions and hence play an important role in the modification of cell shape, cell adhesion, cell motility, cytokinesis (Fehon et al., 2010) and phagocytosis as well as apoptosis (Bonilha, 2007). By intramolecular or intermolecular interaction between the N- and C-terminal end of ezrin, an active and inactive form is distinguished (Bonilha, 2007). The active form is physiologically localized in the membrane and the inactive form in the cytoplasm (Bretscher et al., 2002). Whereas an increased cytoplasmic ezrin expression was found in aggressive colorectal carcinomas (Patara et al., 2011) and lung adenocarcinomas (Tokunou et al., 2000), an increased membranous expression was detected in endometrioid adenocarcinoma (Ohtani et al., 1999). Tumor cells which had a strong ezrin expression concurrently showed an intensive loss of cell–cell contacts (Mangeat et al., 1999) and an increased expression of genes that promote cell migration, cell invasion and inhibition of apoptosis, thus facilitating carcinogenesis and metastasis (Curto and McClatchey, 2008). Yet the role of ezrin in OSCC is not clearly understood and therefore we examined the correlation between the expression and localization of ezrin with clinicopathological parameters and their influence on prognosis.

The impact of ezrin expression in head and neck squamous cell carcinoma has been analyzed in one study (Madan et al., 2006). However, this study comprised tumors from different regions of the head and neck area. These tumors have a different prognosis and exhibit different clinical behavior. So far there are no studies available which examine the impact of ezrin expression in oral squamous cell carcinoma specifically. The aim of this study was to investigate the influence of ezrin expression in OSCC and possible associations with clinicopathological factors.

2. Materials and methods

2.1. Patients and specimens

The retrospective study included resection specimens from 80 treatment-naive patients (51 male, 29 female; median age, 61.82 years; range, 30–91 years), diagnosed with primary OSCC, stages I–IVb, between 2002 and 2005. Treatment included radical surgery and neck dissection. None of the patients had received chemotherapy or radiation before surgery. Adjuvant radiochemotherapy was performed on patients with stage II–IVb. The radiation dose was 61–65 Gy. Carboplatin AUC 5 was administered in weeks 1 and 5. In order to get a homogeneous group of patients, all only patients receiving adjuvant RCT were included.

Table 1
Patient characteristics and univariate analysis of prognostic factors.

Parameter	*N* (%)	5-year OS	*p*-value	5-year DFS	*p*-value
T-classification					
T1	24 (30%)	87%	**<0.001**	62%	0.114
T2	27 (34%)	77%		56%	
T3	6 (8%)	63%		42%	
T4a	15 (19%)	52%		47%	
T4b	6 (8%)	25%		25%	
N-classification					
N0	49 (61%)	80%	**0.017**	61%	0.088
N1	9 (11%)	44%		44%	
N2	22 (28%)	57%		45%	
UICC stage					
I	23 (28%)	86%	**<0.001**	64%	0.060
II	27 (34%)	83%		65%	
III	6 (8%)	88%		60%	
IVa	18 (22%)	54%		41%	
IVb	6 (8%)	25%		25%	
Ezrin expression					
negative	4 (5%)	100%	**<0.001**	50%	**0.002**
weak	21 (26%)	95%		76%	
moderate	28 (35%)	66%		54%	
strong	27 (34%)	47%		31%	
Ezrin localization					
negative	4 (5%)	100%	**<0.001**	50%	**0.003**
cytoplasmic	25 (31%)	92%		79%	
membranous	24 (30%)	77%		57%	
combined	27 (34%)	37%		25%	
Resection margins					
R0	65 (81%)	70%	0.355	57%	**0.005**
R1	15 (19%)	62%		30%	
Extracapsular spread					
No	67 (84%)	72%	**0.045**	55%	0.152
Yes	13 (16%)	54%		39%	
Lymphangitic carcinomatosis					
No	68 (85%)	73%	**0.001**	54%	0.078
Yes	12 (15%)	46%		34%	
Histologic grade					
G1	5 (6%)	100%	0.210	80%	0.785
G2	62 (78%)	67%		48%	
G3	13 (16%)	66%		56%	