Journal of Cranio-Maxillo-Facial Surgery 43 (2015) 2214–2218

Contents lists available at ScienceDirect

Journal of Cranio-Maxillo-Facial Surgery

journal homepage: www.jcmfs.com

Oral brush biopsy and melanoma-associated antigens A (MAGE-A) staining in clinically suspicious lesions

Stefan Hartmann [a, *], Romy U.N. Kipke [a], Stephan Rauthe [b, d], Grit Mutzbauer [b, d], Roman C. Brands [a, d], Harald Ebhardt [c], Alexander C. Kübler [a], Urs D.A. Müller-Richter [a]

[a] Department of Oral and Maxillofacial Plastic Surgery (Head: A. C. Kübler), University Hospital Würzburg, Pleicherwall 2, 97070 Würzburg, Germany
[b] Institute of Pathology (Head: A. Rosenwald), University Würzburg, Josef-Schneider-Straße 2, 97080 Würzburg, Germany
[c] Center for Oral Pathology, Friedrich-Ebert-Str. 33-34, 14469 Potsdam, Germany
[d] Comprehensive Cancer Center Mainfranken (Head: R. Bargou), Josef-Schneider-Straße 6, 97080 Würzburg, Germany

ARTICLE INFO

Article history:
Paper received 26 August 2015
Accepted 8 October 2015
Available online 19 October 2015

Keywords:
Brush biopsy
MAGE-A
Melanoma-associated antigens
Oral cancer
HNSCC

ABSTRACT

Purpose: In oral cancer and in other tumor entities, melanoma-associated antigens are present. These antigens contribute to tumor progression and poor prognosis, and reduce the cytotoxicity of antineoplastic drugs. The aim of this study was to investigate the diagnostic potential of these antigens in combination with oral brush biopsies.

Material and methods: We analyzed 72 oral brush biopsy specimens for melanoma-associated antigens A (MAGE-A) expression by immunocytologic staining with the MAGE-A 57B antibody. A total of 24 healthy specimens, 15 lichen ruber cases, 18 leukoplakia cases, and 15 invasive carcinomas were studied. Incisional biopsy served as the gold standard.

Results: In total, 66 of 72 specimens (91.6%) could be assessed. Twelve of 15 (80%) carcinomas stained positive for MAGE-A. MAGE-A staining was detected in four of 51 nonmalignant specimens, resulting in a false-positive rate of 7.8%. However, MAGE-A positive staining was significantly correlated with oral squamous cell carcinoma ($p < 0.0005$). Sensitivity and specificity for MAGE-A staining and carcinoma were 80% and 92.2%, respectively. The diagnostic accuracy was 89.4%.

Conclusion: Our results indicate that oral brush biopsy with MAGE-A staining serves as an additional tool for use in oral cancer diagnosis. These findings might help to facilitate an easier and more representative surveillance of the mucosa, particularly for large areas of altered mucosa.

1. Introduction

Head and neck cancer, particularly oral squamous cell carcinoma, is a common tumor entity and is associated with a poor cumulative 5-year overall survival rate of only approximately 50% (Gupta et al., 2009). One major problem in follow-up is the adequate surveillance of the mucosa. Due to field cancerization, huge areas of the oral tissues might be altered, and the localization and timing of biopsy remain challenging (Simple et al., 2015). Based on sensitivity and specificity, the histological assessment of a scalpel biopsy is still the gold standard in the diagnosis of oral lesions (Richards, 2015); however, this method is invasive and burdensome, and the assessed tissue area is limited due to the specimen size. Based on these aspects, additional tools should be evaluated that could contribute to the accuracy of the surveillance of the oral mucosa. One potential method could be brush biopsy combined with additional workup, such as immunocytologic staining (Gupta et al., 2014).

In addition to several other tumor-specific proteins, melanoma-associated antigens A (MAGE-A) plays an important role in oral cancer (Laban et al., 2014). Notably, MAGE-A expression is correlated with poor prognosis in laryngeal cancer and is also associated with the minor efficacy of several drugs commonly used in head and neck cancer treatment (Hartmann et al., 2013; Han et al., 2014). Interestingly, MAGE-A expression is restricted to malignant transformed oral mucosa and is not found in healthy mucosa or inflammatory lesions (Krauss et al., 2011). Of note, MAGE-A proteins are recognized by T-cells and lead to immune

* Corresponding author. Tel.: +49 931 201 74868; fax: +49 931 201 72725.
E-mail address: hartmann_s2@ukw.de (S. Hartmann).

http://dx.doi.org/10.1016/j.jcms.2015.10.018
1010-5182/

activation. Based on this finding, MAGE-A3 vaccination was established in the treatment of non-small-cell lung cancer (NSCLC) (Vansteenkiste et al., 2013). This knowledge makes these markers attractive for the diagnosis and treatment of oral squamous cell carcinoma.

In the past, Metzler et al. reported on polymerase chain reaction (PCR)-based MAGE-A detection in an oral brush biopsy (Metzler et al., 2009). They could detect MAGE-A3 and -A4 expression in the suspicious lesion by oral brush biopsy, leading them to perform a larger incisional biopsy. Thereby, an invasive carcinoma was detected, underlining the potential of the combination of brush biopsy and MAGE-A detection. However, these findings are of only limited value because this was a case report.

The aim of our study was to investigate the usability, specificity, sensitivity, and diagnostic accuracy of oral brush biopsy combined with MAGE-A staining in the diagnosis of oral lesions in a larger cohort.

2. Material and methods

2.1. Patients

Patients enrolled in the study were treated in the Department of Oral and Maxillofacial Plastic Surgery of the University Hospital Würzburg between December 2010 and March 2014. Except for the healthy controls, all of the patients presented with a clinically suspicious lesion of the oral mucosa. The study was approved by the institutional review board of the University of Würzburg. A total of 72 patients were subdivided into four groups (Fig. 1). Group 1 consisted of 24 healthy volunteers (eight females and 16 males, mean age 41.6 years). Group 2 consisted of 15 patients with lichen ruber (four females and 11 males, mean age 60.3 years). Group 3 consisted of 18 patients with oral leukoplakia (eight females and 10 males, mean age 58.4 years). The leukoplakia group consisted of hyperkeratosis, hyperparakeratosis, subepithelial fibrosis, or acanthosis. Group 4 consisted of 15 patients with an oral squamous cell carcinoma (11 females and four males, mean age 65.7 years). TNM and UICC characteristics of the carcinoma patients are provided in Table 1.

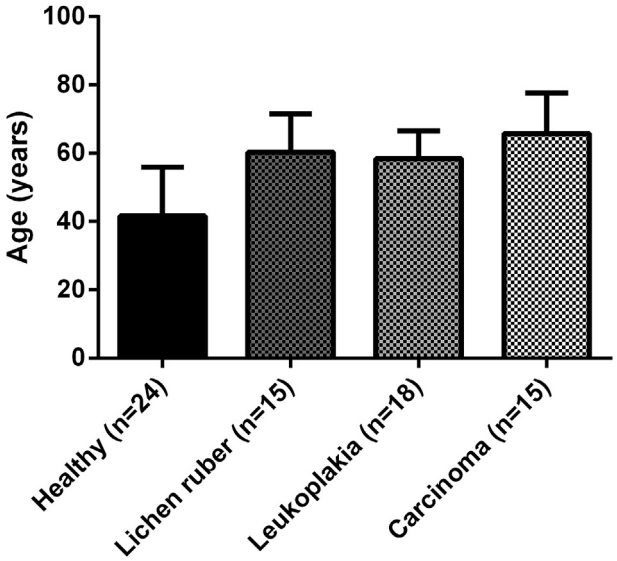

Fig. 1. Mean age of the four different groups included in the study. Error bars indicate standard deviation.

2.2. Oral brush biopsy

Oral brush biopsy specimens were taken in suspicious areas of the oral mucosa or, for the healthy controls, from the buccal planum. The suspicious areas comprised the buccal planum, the floor of the mouth, the tongue, and the gingiva. For adequate specimen harvesting, the brush was rotated until the area of interest started bleeding, indicating that the correct penetration depth was reached. We used an ORCA-Brush biopsy set (Microdent, Breckerfeld, Germany). The specimen was spread on a slide and fixed with the ORCA-Fixx spray set (Microdent, Breckerfeld, Germany). All of the brush biopsies were performed by the same examiner (R.U.N.K.). A conventional incisional biopsy served as the gold standard for brush biopsies in all of the investigated patients in groups 2, 3, and 4 and was used to calculate sensitivity and specificity of the brush biopsy method.

2.3. Staining and assessment

Assessment of the stained brush biopsy specimens was performed by an expert oral pathologist (H.E.). Assessment of the incisional biopsies (conventional hematoxylin/eosin staining) was performed by the Institute of Pathology of the University of Würzburg. A histologically confirmed invasive carcinoma served as a positive control for the MAGE-A staining. For MAGE-A staining, we used the MAGE-A 57B antibody, which was kindly provided by Prof. Spagnioli (Basel). This antibody is known for its affinity to MAGE-A subgroups A1, A2, A3, A4, A6, and A12 (Chambost et al., 2000). The immunohistochemical staining was performed by using an indirect three-step method (labeled streptavidin biotin [LASB]). The staining protocol is provided in Table 2.

2.4. Statistical analysis and graphical preparation

The usability of the method was calculated as the number of useful brush biopsy specimens divided by the number of brush biopsy specimens obtained. The sensitivity was represented by dividing the number of MAGE-A-stained carcinomas by the total number of carcinomas. The specificity was represented by the number of MAGE-A-stained nonmalignant specimens by the number of all of the specimens of groups 1, 2, and 3. Diagnostic accuracy was defined as the proportion of the correctly classified subjects among all of the subjects. To describe the association between two variables, we used a χ^2 test with the maximum-likelihood method (p_c). To compare groups without Gaussian normal distribution, we used a Mann–Whitney *U* test (p_u). To analyze MAGE-A staining as a predictor of carcinoma, a logistic regression model and a probit model were used. For statistical analysis, we used the MEDAS software (version 2014; Grund, Margetshöchheim, Germany). The statistical analysis was supported by Dr. Imme Haubitz, Würzburg, Germany.

To visualize the data, we used GraphPad Prism 6.04 (La Jolla, CA). The significance level was set at $p \leq 0.05$.

3. Results

3.1. Usability of the method

Six of 72 brush biopsy specimens could not be assessed. This resulted in a usability rate of 91.6%. The entire unusable specimen had to be discarded due to a lack of material. There was no significant correlation between unusable specimens and age, sex, or tumor staging. The following statistical analyses are based on the 66 usable specimens.