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ORIGINAL ARTICLE

MAGE-A antigens in lesions of the oral mucosa

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Abstract Oral squamous cell carcinoma develops continuously out of predamaged oral mucosa. For the physician and pathologist, difficulties arise in distinguishing precancerous from cancerous lesions. MAGE-A antigens are tumor antigens that are found solely in malignant transformed cells. These antigens might be useful in distinguishing precancerous from cancerous lesions. The aim of this study was to verify this assumption by comparing MAGE-A expression in benign, precancerous, and cancerous lesions of the oral mucosa. Retrospectively, biopsies of different oral lesions were randomly selected. The lesions that were included are 64 benign oral lesions (25 traumatic lesions (oral ulcers), 13 dental follicles, and 26 epulis), 26 oral lichen planus, 123 epithelial precursor lesions (32 epithelial hyperplasia found in leukoplakias, 24 epithelial dysplasia found in leukoplakias, 26 erythroplasia with oral epithelial dysplasia, and 41 carcinomas in situ in erythroleukoplakias). The lesions were immunohistochemically stained with the poly-MAGE-A antibody 57B, and the results were compared. Biopsies of oral lichen planus, oral ulcers, dental follicles, epulis, and leukoplakia without dysplasia showed no positive staining for MAGE-A antigens. Leukoplakia with dysplasia, dysplasia, and carcinomata in situ displayed positive staining in 33%, 65%, and 56% of the cases, respectively. MAGE-A antigens were not detectable via immunohistochemistry in benign lesions of the oral mucosa. The staining rate of dysplastic precancerous lesions or malignant lesions ranged from 33% to 65%. The MAGE-A antigens might facilitate better differentiation between precancerous and cancerous lesions of the oral mucosa.

Keywords MAGE-A antigens · Cancer/testis antigens · Precancerous lesions · Early diagnosis · Oral lesions · Oral squamous cell carcinoma

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Introduction

Oral squamous cell carcinoma is the sixth most common cancer in the Western world [1–4]. Due to this high incidence, it has significant socioeconomical implications. Despite all the improvements in surgical and conservative treatment regimes (radiation and chemotherapy), prognosis has not improved in recent decades [5, 6]. In fact, the mean survival after 5 years is still only about 50%. In the advanced tumor stages, at which two thirds of all patients present, survival drops to just a little more than 30% [4, 7, 8]. Oral squamous cell carcinomas do not normally develop de novo, but rather result from transformation of several oral precancerous lesions [9–12]. During a regular schedule of follow-up examinations, these lesions might be diagnosed at the right time using sufficient diagnostic tools. Continuous damage of the mucosa is widely caused by smoking and alcohol (ab)use. This leads to the damage of the whole oro-esophago-tracheal mucosa (field cancerization) [13–20]. At this point, the clinician and pathologist face the difficult task of distinguishing benign from precancerous and cancerous lesions. The most common lesions examined are oral lichen planus and epithelial

precursor lesions (erythroleukoplakia) [12, 21, 22]. In addition to the clinical examination, scalpel biopsies are the gold standard of diagnosis. Another less invasive method of harvesting mucosal cells is the brush cytology that has currently become popular [23]. In both cases, the pathologist often faces difficult decisions regarding whether the examined lesion is still benign or where the borders of a malignant transformed lesion are present [24]. Molecular markers with high specificity and sensitivity might help to answer these questions. For such markers, the following characteristics are mandatory: first, the marker should only be found on malignant cells in a sufficient amount, and second, the marker should be easily detectable via screening methods. MAGE-A antigens, a subgroup of cancer/testis antigens, could be such a marker. MAGE-A antigens are not found on healthy tissue (except from testes or placenta) [25–27]. In previous studies, they were found on many solid tumors, as well as on oral squamous cell carcinomas [25, 28, 29]. Until now, a question that has been ignored is whether those antigens might be helpful in distinguishing between benign, precancerous, and cancerous lesions of the oral mucosa. For this purpose, different oral lesions (benign, precancerous, carcinoma in situ) were tested for the expression of MAGE-A antigens.

Materials and methods

Patients

Retrospectively, formalin-fixed specimens from biopsies of different oral lesions were included. The lesions that were included are 64 benign oral lesions (25 traumatic lesions (oral ulcers), 13 dental follicles, and 26 epulis), 26 oral lichen planus, 123 epithelial precursor lesions (32 epithelial hyperplasia found in leukoplakias, 24 epithelial dysplasia found in leukoplakias, 26 erythroplasia with oral epithelial dysplasia, and 41 carcinomas in situ in erythroleukoplakias).

Immunohistochemical staining

For immunohistochemistry, the monoclonal global MAGE-A antibody 57B was used (courtesy of Prof. Giulio C. Spagnoli, Onkologische Chirurgie, Institute for Surgical Research and Hospital Management, University Hospital Basel, 4031 Basel, Switzerland). This monoclonal antibody binds to a common epitope of MAGE-A antigens and facilitates simultaneous detection of the most MAGE-A antigen subgroups (MAGE-A1 to MAGE-A8 and MAGE-A12) [30].

After fixation, the slides were stained using DakoCytomation EnVision + Dual Link System-HRP (DakoCytomation Inc., 6392 Via Real, Carpinteria, California, 93013, USA) according to the manufacturer's instructions and the MAGE-A antibody 57B (Dilution 1:100). The slides were washed with Tris–HCl buffer, and then peroxidase blocking solution was applied. The slides were again washed with Tris–HCl buffer, and MAGE-A antibody 57B was added. Another wash with Tris–HCl buffer was then performed. Next, the Dual link system with the secondary antibody was used, followed by another washing with Tris–HCl buffer. The chromogen with DAB+ was added, and the slides were washed with distilled water. The slides were then counterstained with hematoxylin and again washed with distilled water.

Results

Benign lesions and oral lichen planus

Oral lichen planus None of the 26 examined specimens had dysplasia. No lesion was positively stained immunohistochemically for MAGE-A antigens.

Oral traumatic lesions (ulcers) Dysplasias were not found in the 25 specimens, and MAGE-A antigens were not detected.

Dental follicles All 13 specimens were free of dysplasias, and MAGE-A antigens were not found by immunohistochemical staining.

Epulis The 26 epulis examined were free of dysplasia, and MAGE-A antigens were not detected in the specimens (Fig. 1).

Epithelial precursor lesions (leukoplakias)

Overall, 56 epithelial precursor lesions found in leukoplakias were tested. In 24 leukoplakias, dysplastic regions were present. The immunohistochemical staining with 57B for MAGE-A was positive in eight cases of leukoplakias with dysplasias. Sixteen dysplastic lesions were negative for MAGE-A antigens. This results in a positive staining rate of 33%. All leukoplakias without dysplasia were also negative for MAGE-A antigens (Fig. 2).

Erythroplasia with epithelial dysplasias

In the 26 specimens examined, 17 showed positive staining with antibody 57B for MAGE-A antigens. Nine specimens showed no staining. The staining rate was 65% (Fig. 3).

Carcinoma in situ found in erythroleukoplakias

Forty-one of these cancerous lesions were tested for MAGE-A antigens. In 23 specimens, positive immunohistochemical

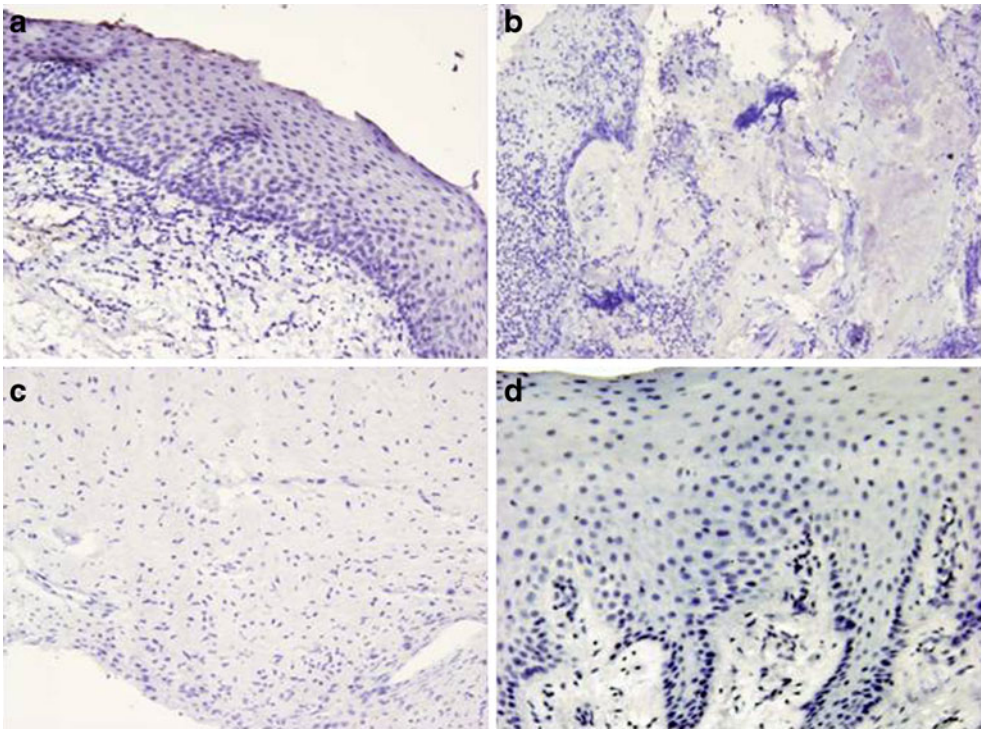

Fig. 1 Immunohistochemical staining (HE and monoclonal poly-MAGE-A antibody 57B, ×200) of oral lichen planus (**a**), oral ulcer (**b**), dental follicle (**c**), and epulis (**d**). No immunohistochemical staining for MAGE-A antigens was observed

staining for MAGE-A was detected. Eighteen lesions did not reveal any staining with the 57B antibody. The staining rate was thus 56% (Fig. 4).

In summary, we found that no benign lesion showed immunohistochemical staining for MAGE-A antigens with the 57B antibody. This represents a specificity of 100%. In epithelial precursor lesions with dysplastic cells or carcinomas in situ, the staining rate ranged from 33% to 65%. When combining these lesions (24 leukoplakias, 26 dysplasias, and 41 carcinomas in situ), the mean staining rate was 44% (48/91). This result means that the technique is adequately sensitive.

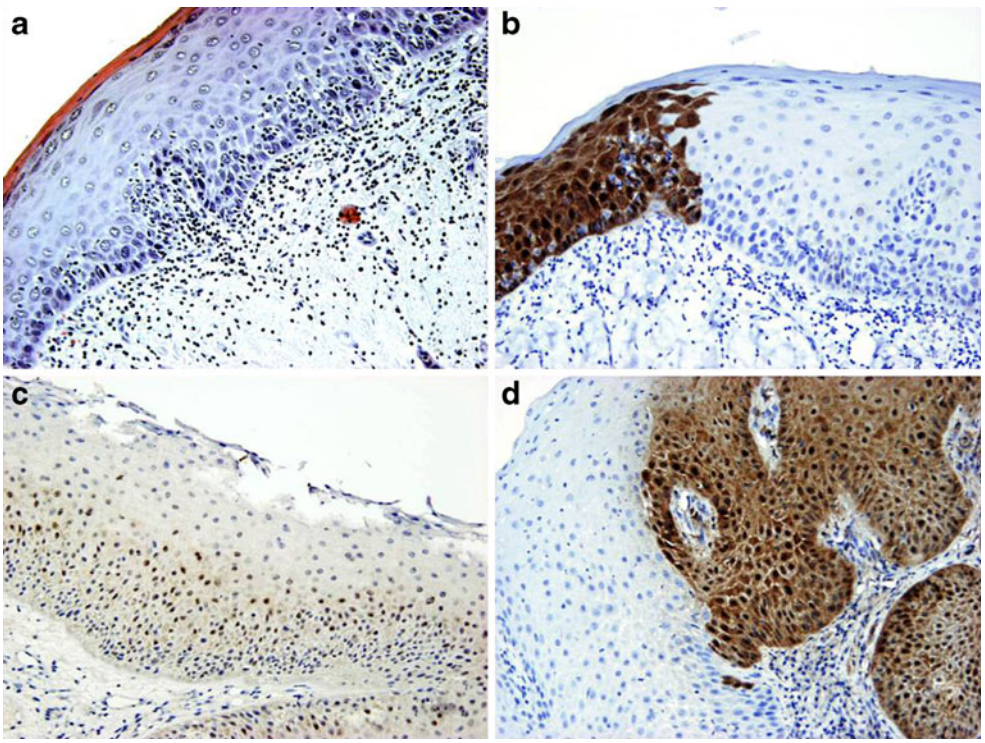

Fig. 2 Immunohistochemical staining (HE and monoclonal poly-MAGE-A antibody 57B, ×200) of different oral leukoplakias with dysplastic areas. **a**, **b** From the same lesion. **c** A leukoplakia with dysplasia that was negative for MAGE-A antigens. **d** Another leukoplakia with MAGE-A antigens is shown

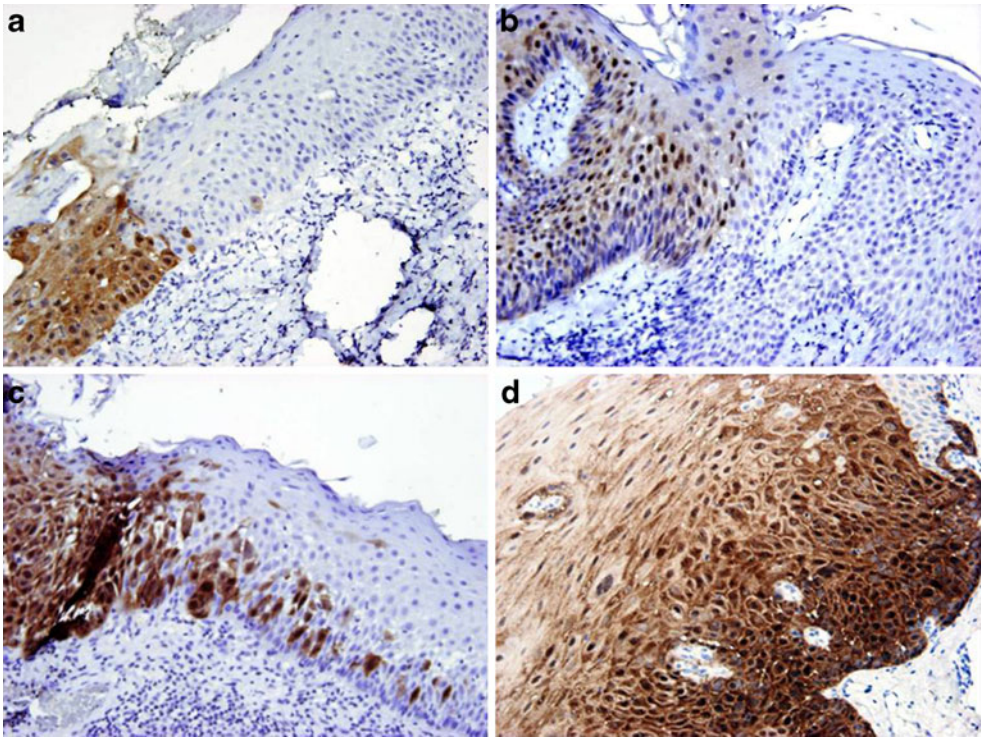

Fig. 3 **a**–**d** Four different oral dysplasias (HE and monoclonal poly-MAGE-A antibody 57B, ×200) are depicted. All panels show the epithelial cells near the basement membrane as the origin of the dysplasia and the ascending of the epithelial cells to the surface. Especially noticeable in (**a**) and (**c**) are the sharp borders and the staining of single, basement membrane near detached dysplastic cells

Discussion

For the first time, this study addresses the incidence of MAGE-A antigens in benign and precancerous lesions of the oral mucosa. In daily clinical work, the differentiation between benign and malignant tissue is crucial. In our study, no benign lesion showed immunohistochemical staining for MAGE-A antigens. This means that the 57B antibody is able to distinguish benign from malignant transformed cells. Healthy or benign oral lesions do not have immunohistochemically detectable MAGE-A antigens.

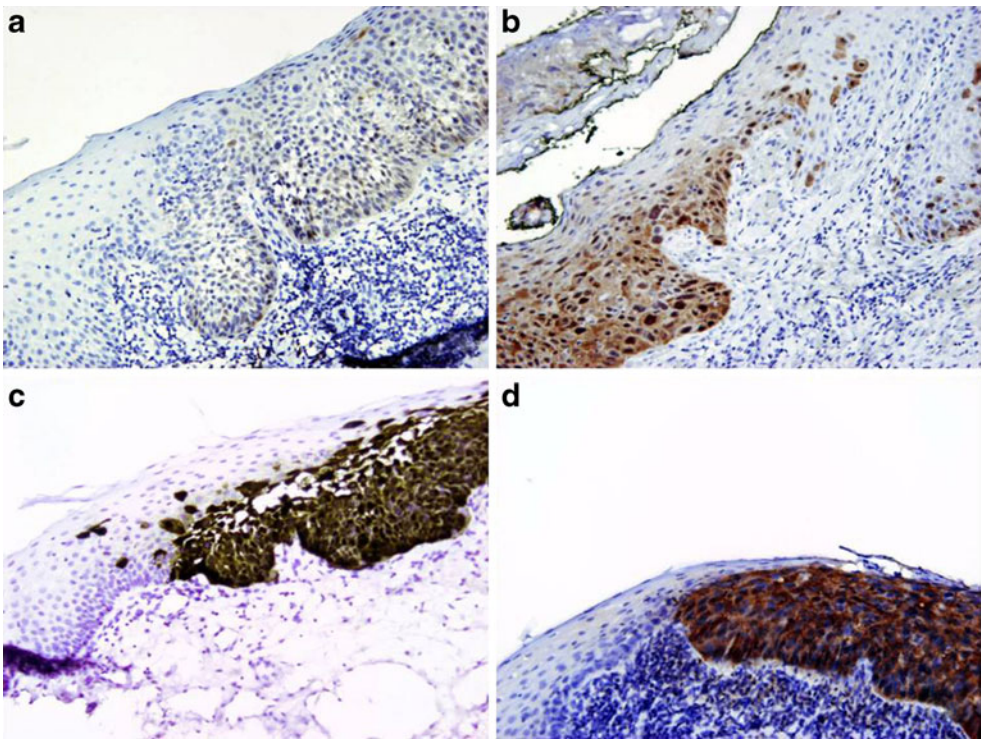

Fig. 4 Immunohistochemical staining (HE and monoclonal poly-MAGE-A antibody 57B, ×200) of four different carcinomas in situ. Here, the basement membrane near cells as the origin can also be seen very well. The extension of the lesions under a healthy layer of epithelial cells is striking (especially in **c** and **d**)

Unfortunately, the sensitivity does not reach the level of specificity. The immunohistochemical method used achieved a maximum staining rate of 65% and, in the worse case, a staining rate of 33%. The staining rate was higher in more malignant transformed lesions than in leukoplakias with dysplasia. Overall, the staining rate is too low for daily clinical use. However, if MAGE-A antigens are present, a benign lesion can be clearly distinguished from a malignant lesion with the appropriate technology. Until now, this opportunity has not been addressed. This is applicable not only to oral mucosa but also to other tissues. The only other study that addresses benign oral lesions was performed by Lee et al. and could also not find MAGE-A antigens in a small sample of benign oral lesions. MAGE-A antigens were found only in a specimen of one dysplasia [31]. Other solid tumors have also not been addressed. A study by Hudolin et al. examined prostatic tissue [32]. The rate of false positive results ranged from 5% to 15%. A study on gastric carcinoma that addressed tumor antigens in precancerous and cancerous lesions simply states that MAGE-A antigens occur with DNA hypomethylation and might be useful in diagnosis and prognosis. Concrete numbers, however, are not given [33].

Why MAGE-A antigens cannot be found in all dysplasias or carcinomas has not yet been elucidated. It might depend upon the methods used. Studies using polymerase chain reaction (PCR) for the detection of MAGE-A antigens in oral squamous cell carcinomas achieve positive rates of 85% in all the specimens examined [28]. Our own data from a comparable collection show a positive immunohistochemical staining rate of 65% in oral squamous cell carcinoma [34]. There is a problem with PCR detection of MAGE-A antigens. Own tests in oral squamous cell carcinoma cell lines revealed that a positive real-time (RT)-PCR result does not always correspond to a significant quantitative real-time (QRT)-PCR result (reference: adult keratinocyte cell line) [35]. Therefore, to avoid false positive results, a quick examination at a favorable price like immunohistochemistry seems adequate for screening purposes.

In our study, extremely sharp borders between immunohistochemically stained, MAGE-A-positive areas and the surrounding benign tissues can be seen. This might be of extraordinary interest because oral squamous cell carcinomas develop mainly from mucosa damaged by smoking and alcohol (ab)use [14, 17, 20]. These substances cause field cancerization of the upper aerodigestive tract. This complicates the correct definition of the borders of oral lesions [23]. In these cases, MAGE-A staining might help (Figs. 2, 3, and 4). Testing for MAGE-A antigens might also increase the sensitivity and specificity of other diagnosis tools like oral brush cytology. This has already been demonstrated with other markers [36].

In summary, we found that MAGE-A antigens might significantly improve the diagnosis and differentiation of oral lesions. Thus far, the sensitivity of the 57B antibody is not sufficient for daily clinical use. Their real value in early diagnosis of dysplastic oral lesions has to be validated by clinical follow-up studies. Thus, developing more sensitive antigen sensors should be a task for further studies. Other antibodies and methods should be examined for their utility in the diagnosis of oral lesions.

Conflict of interests The authors declare that they have no conflict of interest.

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