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ORIGINAL ARTICLE

MAGE-A antigens in patients with primary oral squamous cell carcinoma

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Abstract MAGE-A antigens are only expressed on tumor cells. The aim of this study was to identify their expression in patients with oral squamous cell carcinoma (OSCC). Forty-seven patients with primary OSCC was selected retrospectively. Histo-pathological sections were stained immunohistochemically with MAGE-A antibody 57B. The results were evaluated regarding tumor size (T), lymph-node metastasis (N), blood vessel infiltration (V), lymph vessel infiltration (L), grading (G), and sex. MAGE-A antigens were expressed in 55% of all patients. Expression increased with tumor size (T1=56%; T2=44%; T3=67%; T4=71%). Lymph-node metastasis had no influence (N0 and N1 about 50%). Tumors with blood and lymph vessel infiltration had higher expression (V0=50%; V1=100%; L0=46%; L1=71%). Less-differentiated tumors showed higher rates (G1=50%; G2=45%; G3=83%). OSCC in men were positive in 62% and in women in 38%. MAGE-A antigens are frequently expressed in OSCC. Their expression seems to increase with tumor dedifferentiation.

Keywords MAGE-A antigens · Immunotherapy · Oral squamous cell carcinoma · Tumor antigens

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Introduction

Oral squamous cell carcinoma (OSCC) has still a poor prognosis [1]. Despite all improvements in cancer therapy within the last 30 years (modulated radiotherapy, chemotherapeutic drugs, and improved surgical procedures), about 50% of all patients diagnosed with OSCC do not survive more than 5 years [2, 3]. Therefore, strategies and techniques for early diagnosis and individualized prognosis and treatment are necessary. One of those strategies includes tumor specific antigens that are solely expressed on tumor cells but not on non-malignant cells [4]. An antigen class which represents these demands is the MAGE-A antigen class a subclass of cancer/testis antigens

[5]. MAGE-A subclass consists of 12 different MAGE-A antigens. Those subantigens are accused for different implications in tumor growth and response rate to chemotherapeutic drugs [6–8]. Those issues are subject of in vitro investigations today. MAGE-A antigens are not expressed in healthy tissues except from testes, placenta, and fetal tissues [5, 9]. In contrast, there are many reports on their expression in different tumor entities. Some of those tumors are melanoma, cervical cancer, non-small cell lung cancer, bladder cancer, and oral squamous cell carcinoma [10–16].

The restricted expression on tumor cells could make those antigens an ideal tool for assessing the individual risk, improving diagnosis and even give a target for selective immunotherapy. Studies investigating these issues in patients with OSCC are scarce.

This study investigates the frequency of expression in a cohort of patients with primary oral squamous cell carcinoma. For this purpose histo-pathologic specimens of 47 patients were selected and examined whether they express MAGE-A antigens or not by immunohistochemistry. The results were correlated with tumor size, lymph-node status, blood and lymph vessel infiltration, grading, and sex.

Materials and methods

Patients

Forty-seven patients, consecutively treated for primary oral squamous cell carcinoma, were selected for this study. The group consisted of 34 men and 13 women. T-stages were T1=28, T2=9, T3=3 and T4=7. Lymph-node-negative necks accounted for 29, lymph-node-positive necks for nine, and in nine patients, no neck dissection was performed (clinically no signs for metastasis, small tumor size). There were seven patients with lymphatic vessel infiltration (L1) and three patients with venous invasion (V1). The differentiation level was graded as G1=6, G2=33 and G3=6. In two patients, no grading was provided by the pathologists. No distant metastases were present in the study group.

As a positive control group, specimens of a testicular carcinoma (embryonal carcinoma) was used [Fig. 1].

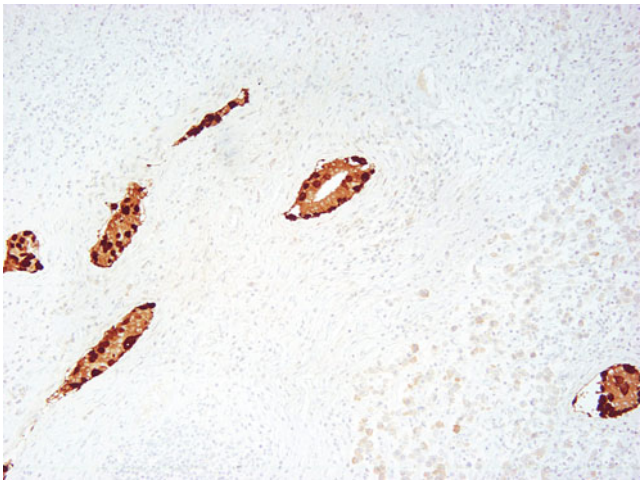

Fig. 1 The slide shows the positive control with the global MAGE-A antibody 57B of a testicular carcinoma (embryonal carcinoma; ×100)

Immunohistochemical staining

For immunocytochemistry, the monoclonal global MAGE-A antibody 57B was used (by 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 most common expressed epitopes MAGE-A1, -A2, -A3, -A4, -A6 and –A12 [17].

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. The slides were washed with the buffer and then peroxidase-blocking solution was applied. Again, the slides were washed with the buffer. MAGE-A antibody 57B was added. Then, another washing with buffer was done. Now, the Dual Link System was used and afterwards another washing with the buffer was performed. The chromogen with DAB+ was added and the slides were washed with aqua dest. The slides were now counterstained with hematoxylin and afterwards again washed with aqua dest.

Histo-pathological sections were stained with the MAGE-A antibody 57B and the results were grouped into a score (0=0%; 1=1–25%; 2=26–50%; 3=51–75%; 4=76–100%) referring to the stained cells.

Statistical analysis

Statistical analysis was performed by the Statistical Department of the Mathematical Branch of the University of Würzburg, Germany, using SPSS 15.0.1. Non-parametric tests were applied. For groups with two attributes, Mann–Whitney U and Wilcoxon tests were used (sex and N+/N−). In groups with more than two attributes the Kruskal–Wallis and chi-square tests were used (T, G, L, V). p values were calculated at a significance level of 0.05.

Results

T-stages

The overall staining for MAGE-A antigens were 55% (26/47) in all tumor sizes (T1–4) for all staining scores. Broken down to the single T-stages the results were as follows:

In T1 stage, 56% (15/28) of all tumors could be stained by the 57B antibody. The staining scores consisted of: score 1, 26% (four of 15); score 2, 20% (three of 15); score 3, 6% (one of 15); and score 4, 46% (seven of 15).

In T2 stage, 44% (four of nine) were positive for MAGE-A antigens. The single scores consisted of: score 1, 25% (one of four); score 2, 25% (one of four); score 3, 0% (zero of four); and score 4, 50% (two of four).

In T3 stage, 67% (five of seven) had a positive staining. The single scores were: score 1, 42% (three of five); score 2, 0% (zero of five); score 3, 28% (two of five); and score 4, 0% (zero of five).

In T4 stage, 71% (five of seven) of the tumors were stained by the 57B antibody. The scores were: score 1, 60% (three of five); score 2, 0% (zero of five); score 3, 40% (two of five); and score 4: 0 (zero of five).

The statistical analysis revealed no significant differences between the T-stages (p=0.889).

N-stages

In this group, the stages N2a and N3 were not assigned in the patients examined. In all N-stages, 55% (22/39; nine patients had no neck dissection) were positive for MAGE-A antigens. In N+ Neck the rate increased to 66% (six of nine). Because of the small numbers of patients in the single N-stages only the overall percentage of positive staining in each group is given: N0: 51% (15/29), N1: 100% (two of two), N2b: 60% (three of five), N2c: 50% (one of two). In the group with no Neck dissection, 55% (five of nine) were positive-stained for MAGE-A antigens and therefore in the same range as the whole N-stage group.

For statistical analysis, the N-stages were grouped to N+ and N−. The statistical analysis revealed no significant differences between N+ and N− specimens (p=0.432).

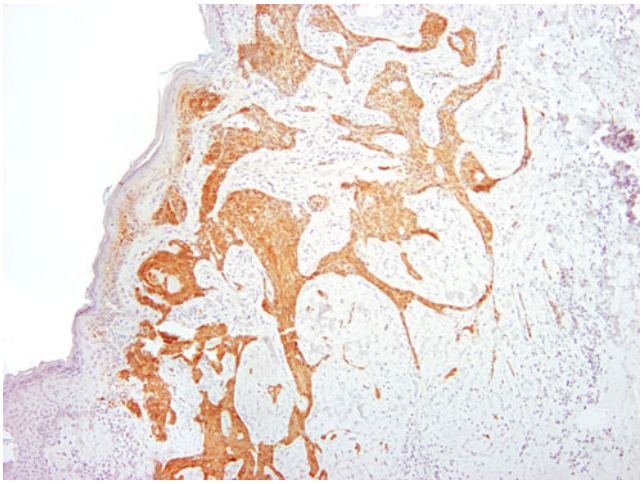

Fig. 3 Immunohistochemical staining of the cytoplasm with the MAGE-A antibody 57B of a pT1 G2 oral squamous cell carcinoma (×100)

Grading

Most tumors were graded as G2 70% (33/47). Two tumors were not graded. Because of the small numbers of G1 and G3 tumors, the count of the single scores will be omitted due to possible bias. In G1 tumors, 50% (3/6) were positive

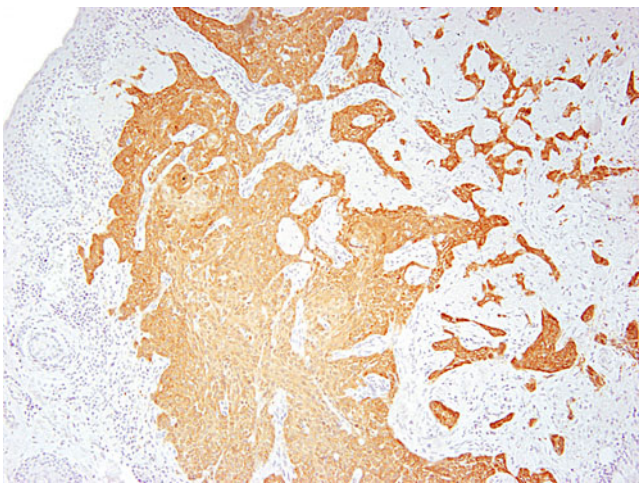

Fig. 2 Immunohistochemical staining of the cytoplasm with the MAGE-A antibody 57B of a pT1 G1 L0 V0 oral squamous cell carcinoma (×100)

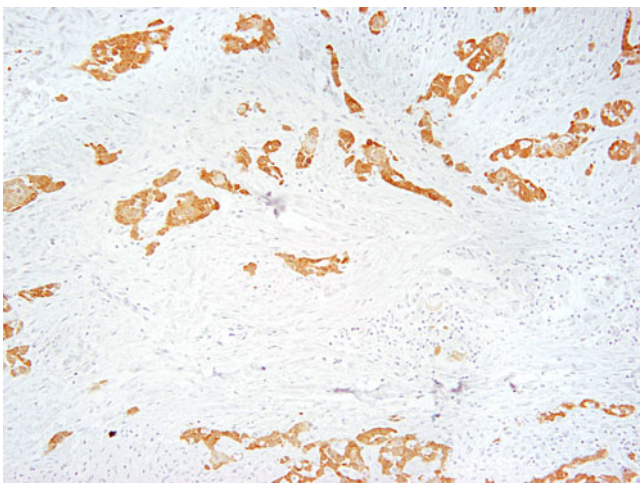

Fig. 4 Immunohistochemical staining of the cytoplasm with the MAGE-A antibody 57B of a pT1 G3 L1 V0 oral squamous cell carcinoma (×100)

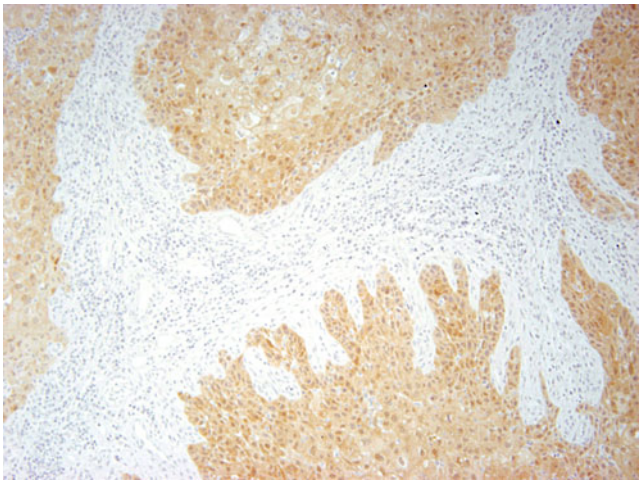

Fig. 5 Immunohistochemical staining of the cytoplasm with the MAGE-A antibody 57B of a pT3 G2 L0 V0 oral squamous cell carcinoma (×100)

for MAGE-A antigens. In G2 grading, 45% (15/33) were positive. They were grouped as: score 1, 6% (one of 15); score 2, 20% (three of 15); score 3, 13% (two of 15); and score 4: 60% (nine of 15). The G3 tumors were positive for MAGE-A in 83% (five of six). To show the tendency of expression, more MAGE-A antigens in poor differentiated tumors pictures of tumors with increasing gradings and tumor sizes are shown in Figs. 2, 3, 4, 5.

The statistical analysis revealed no significant differences between the grading levels (p=0.532).

Lymphatic vessel infiltration

Eighteen tumors were not classified by histo-pathological examination for L-stage. Those tumors that showed a lymphatic vessel infiltration 24% (seven of 29) were positive for MAGE-A antigens in 71% (five of seven; Fig. 4). Tumors with no lymphatic vessel infiltration 75% (22/29) were positive for MAGE-A antigens only in 45% (ten of 22).

The statistical analysis revealed no significant differences between the L-stages (p=0.365).

Venous invasion

Venous invasion was rare in the patients examined 10% (three of 29) but all those patients were positive for MAGE-A antigens. In the patients with no venous invasion, 89%

Table 1 Overview of the single groups expressing MAGE-A antigens

		n	score 0 (*n*)	score 1 (*n*)	score 2 (*n*)	score 3 (*n*)	score 4 (*n*)	% pos	*p* value
Sex	M	34	13	7	4	3	7	61	0.327
	F	13	8	1	1	0	3	38	
T-stage	1	28	13	4	3	1	7	53	0.889
	2	9	5	1	1	0	2	44	
	3	3	1	0	1	0	1	66	
	4	7	2	3	0	2	0	71	
Grading	1	6	3	2	0	0	1	50	0.532
	2	33	18	1	3	2	9	45	
	3	6	1	1	1	1	2	83	
	x	2	0	1	1	0	0	–	
L-stage	0	22	12	3	3	1	3	45	0.365
	1	7	2	2	0	0	3	71	
	x	18	7	4	1	2	4	–	
V-stage	0	26	13	3	3	1	6	50	0.609
	1	3	0	2	0	0	1	100	
	x	18	8	3	2	2	3	–	
N-stage	0	29	14	5	3	2	5	51	0.432
	1	2	0	0	1	0	1	100	
	2a	0	0	0	0	0	0	–	
	2b	5	2	3	0	0	0	60	
	2c	2	1	0	0	1	0	50	
	3	0	0	0	0	0	0	–	
	x	9	4	0	1	0	4	–	

X not classified. *Score 0*=0% positively stained cells (psc); *score 1*=1–24% psc; score 2=25–49% psc; *score 3*=50–74% psc; *score 4*=75–99% psc. *p* values were calculated for intragroup differences (Kruskal–Wallis test and chi-square test). In lymph-node stage, the *p* value was calculated for N+ and N− (Mann–Whitney *U* test and Wilcoxon test). The same tests were used in the variable "sex"

(26/29) one half 50% (13/26) were positively stained for MAGE-A. 18 tumors were not classified for venous invasion but were positive in 55% (ten of 18).

The statistical analysis revealed no significant differences between the V-stages (p=0.609).

An overview of the results is given in Table 1.

Discussion

In regard to the wide variety of the TNM-classification with further splitting up into grading, venous invasion or lymphatic vessel infiltration the single numbers in each group get very small. Comparing those small groups statistically results in p values that have to be handled with care. The authors are working on larger groups to confirm their findings. But there are some evident findings. One of these is that more than 55% of all primary squamous cell carcinomas were positively immunohistochemically stained for MAGE-A antigens. This is less than the positive rates reported by other groups [18, 19]. These findings may be explained by the lesser sensitivity of immunohistochemistry compared with PCR. Another finding is that in our study, the larger (T4=71%), the less differentiated (G3=83%), and the more aggressive the tumors were (N+=50–100%; L1=71%; V1=100%) they expressed more MAGE-A antigens compared to their "more benign" counterparts. This is in contrast to the findings of Ries et al. 2008 who reported similar levels in all tumor stages [19]. This might be caused by PCR amplification of MAGE-A antigens in subclones of the carcinomas that possibly would have had a low immunohistochemistry score in our study [9, 20]. But our findings correspond well with the findings of Figueiredo et al. 2006 who reported also an increasing number of MAGE-A expression in less-differentiated and aggressive oral squamous cell carcinomas [21]. This is also consistent with findings in other epithelial neoplasia [10, 12–16]. Regarding these results, MAGE-A antigens might be still an important aim for further studies in oral squamous cell carcinomas to improve the estimation of the individual risk of the patients. Our findings indicate that it will be worthwhile to explore these tumor antigens further. If more than 50% of all primary squamous cell carcinoma express those antigens and this number rises in less-differentiated tumors and if this finding will be substantiated by further research, MAGE-A antigens should be evaluated for diagnosis, assessing the individual risk, treatment options, and estimating the course of disease [13, 22, 23]. MAGE-A antigens are still a possible target for immunotherapy [23–25].

There is also a lack in studies comparing primary and recurrent oral squamous cell carcinoma regarding their expression profile of MAGE-A antigens. Such studies might help to enlighten further the influence of MAGE-A antigens on the course of the disease. This selective measurement of the MAGE-A subgroups is mandatory. It would be interesting to correlate histological and clinical parameters with the 12 MAGE-A subgroups. To prevent small group numbers in further studies selecting those patients with extremely poor or good course of the disease and evaluation of them for specific expression of the different MAGE-A subgroups seems more promising.

Conflict of interest The authors state that there is no conflict of interests.

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