HIGH EXPRESSION OF CANCER TESTIS ANTIGENS MAGE-A, MAGE-C1/CT7, MAGE-C2/CT10, NY-ESO-1, AND GAGE IN ADVANCED SQUAMOUS CELL CARCINOMA OF THE LARYNX

David L. A. Figueiredo, MD, PhD,[1] Rui C. M. Mamede, MD, PhD,[1] Giulio C. Spagnoli, MD,[2] Wilson A. Silva, Jr, PhD,[3,5] Marco Zago, MD, PhD,[4,5] Luciano Neder, MD, PhD,[5] Achim A. Jungbluth, MD,[7] Fabiano P. Saggioro, MD, PhD[6]

[1] Department of Ophthalmology, Otorhinolaringology and Head and Neck Surgery, Faculty of Medicine, University of São Paulo, Ribeirão Preto, Brazil. E-mail: davidlaf@terra.com.br; davidlaf@usp.br
[2] Department of Research, Institute for Surgical Research and Hospital Management, University Hospital, Basel, Switzerland
[3] Department of Genetics, Faculty of Medicine, University of São Paulo, Ribeirão Preto, Brazil
[4] Department of Clinical Medicine, Faculty of Medicine, University of São Paulo, Ribeirão Preto, Brazil
[5] National Institute of Science and Technology in Stem Cell and Cell Therapy, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil
[6] Department of Pathology, Faculty of Medicine, University of São Paulo, Ribeirão Preto, Brazil
[7] Ludwig Institute for Cancer Research, New York Branch at Memorial Sloan–Kettering Cancer Center, New York, New York

Accepted 13 May 2010
Published online 30 September 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/hed.21522

Abstract: *Background.* Despite diagnostic and therapeutic advances in head and neck cancer, the 5-year survival of patients with laryngeal cancer has not improved in the last 30 years. Several recent studies indicate that specific targets for immunotherapeutic approaches can be useful in the control of cancer. There is considerable interest in the expression of cancer testis antigens in human cancers since they may serve as the basis for an immunologic approach to therapy.

Methods. We evaluated by immunohistochemical analysis the expression of cancer testis antigens MAGE-A4 (57B), MAGE-C1 (CT7-33), MAGE-A1 (MA454), MAGE-A3 (M3H67), MAGE-C2 (CT10.5), NY-ESO-1 (E978), and GAGE (GAGE) in squamous cell carcinoma (SCC) of the larynx.

Results. A total of 63 cases (57 men and 6 women) of laryngeal SCC were available for this study. The findings were correlated with the clinical course and laboratory data. Expression of at least 1 cancer testis antigen was detected in 42 of 63 of the laryngeal SCCs (67%). In 34 of 42 of the positive cases (81%) there was simultaneous expression of $\geq$2 cancer testis antigens. There was significant correlation between antigen expression and advanced tumor stage (stage III/IV) in cases with reactivity to only 1 antibody ($p = .01$) as well as in the cases with reactivity to $\geq$2 primary antibodies ($\geq$2 mAbs, $p = .04$). There was no association between survival and expression of any of the analyzed antigens.

Conclusions. We find a high incidence of cancer testis antigen expression in SCCs of the larynx, which was correlated with advanced clinical stage. Our data indicate that cancer testis antigens could be valuable vaccine targets in laryngeal tumors, especially in those with a worse prognosis. © 2010 Wiley Periodicals, Inc. *Head Neck* **33:** 702–707, 2011

Keywords: larynx cancer; tumor antigens; cancer testis antigens; squamous cell carcinoma; larynx

Despite diagnostic and therapeutic advances in head and neck cancer, the survival of patients with laryngeal cancer has not improved in the last 30 years.[1] Most patients can be cured only when treated at an early stage, and patients with advanced tumors usually succumb to the disease. Interestingly, recent studies reported decreased survival rates in larynx cancer.[2] Consequently, additional treatment options for patients with head and neck cancers are urgently needed.

Several recent studies indicate that specific targets for immunotherapeutic approaches can be useful in the control of cancer.[3,4] Surprisingly, there are a very few studies in head and neck cancer.

A significant advance was the identification of cancer testis antigens. As their name implies, cancer testis antigens are expressed in various types of malignant tumors, whereas in normal adult tissues their presence is restricted to testicular germ cells and occasionally placental trophoblast.[5] Based on their tumor-associated expression pattern, cancer testis antigens are regarded as potential targets for vaccine-based immunotherapy of cancer.[6]

Surprisingly, relatively little is known about the presence of cancer testis antigens in cancers of the head and neck and especially the larynx. Consequently, the

Additional Supporting Information may be found in the online version of this article.

Correspondence to: D. L. A. Figueiredo

Contract grant sponsor: Fundação de Amparo à Pesquisa do Estado de São Paulo.

aim of this study was to analyze the expression of cancer testis antigens in squamous cell carcinoma (SCC) of the larynx and to correlate their potential presence with pathomorphological as well as clinical data.

MATERIALS AND METHODS

The present study involved patients who were diagnosed with laryngeal SCC and surgically treated at the Hospital of the Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil, from January 1, 2001, through December 31, 2005. Corresponding archival tumor tissue was provided by the Department of Pathology of the same institution. The study was approved by the Research Ethics Committee of the University Hospital. All patients provided informed consent (Proc. No. 7235/2007).

The inclusion criteria were the patient's agreement to participate in the study including signed consent, absence of previous tumor treatment of the head and neck, and available representative tissue blocks.

Immunohistochemistry. The immunohistochemical analysis was performed on archival formalin-fixed, paraffin-embedded tissues. The serological reagents, corresponding antigens, and monoclonal antibody (mAb) concentrations for the detection of cancer testis antigen expression were previously described and are indicated in Table 1.[7–11]

Immunohistochemical staining and interpretation were done as previously described.[12] Appropriate primary antibody working concentrations were established by titration assays using testicular tissues with preserved spermatogenesis. Tissue sections (thickness, 4 microns) were deparaffinized in xylene and rehydrated in a series of graded alcohols. Endogenous peroxidase was blocked by incubating slides for 30 minutes at room temperature in 99.7% methanol containing 0.3% hydrogen peroxide. Slides were washed with Tris-buffered saline solution and blocked in 2% bovine serum albumin (BSA) at room temperature for 5 minutes to prevent unspecific protein interactions. A heat-based antigen retrieval technique using a commercial vegetable steamer was used by heating slides in a buffer solution (Table 1) for 30 minutes at approximately 96°C.

Tissue slides were incubated with primary antibodies overnight at 4°C in a wet chamber. Primary antibody detection was performed by use of a Novolink polymer detection kit (Leica Microsystems Inc., Bannockburn, IL) in accord with the manufacturer's instructions. 3,3-Diaminobenzidine (DAB) served as a chromogen and counterstains were done with Harris hematoxylin. Finally, slides were dehydrated in a series of graded ethanols, coverslipped, and examined under a light microscope.

For all assays, appropriate positive (normal testis with preserved spermiogenesis) and negative controls (omission of primary antibody and replacement with phosphate buffer saline, pH 7.4) were included.

Table 1. Primary antibodies, clones, dilution, and antigen retrieval buffers.

Antibody*	Clone	Dilution	Buffer
MAGE-A4	57B	1:4.000	EDTA 1 m*M*, pH 8.0
MAGE-C1	CT7.33	1:32.000	Citrate 10 m*M*, pH 6.0
MAGE-A1	MA454	1:200	EDTA 1 m*M*, pH 8.0
MAGE-A3	M3H67	1:80.000	EDTA 1 m*M*, pH 8.0
MAGE-C2	CT10.5	1:4.000	Tris-EDTA 20 m*M*, Tween 20 (0.0005%), pH 9.0
NY-ESO-1	E978	1:3.200	Tris-EDTA 20 m*M*, Tween 20 (0.0005%), pH 9.0
GAGE	#26	1:80.000	EDTA 1 m*M*, pH 8.0

**Source: Ludwig Institute for Cancer Research, New York, NY.*

Antigen expression was scored semiquantitatively in accord with the number of immunopositive tumor cells and graded as follows: 0 (zero) = <5% of tumor cells positive; 1+ = 5% to 25% of cells positive; 2+ = >25% to 50%; 3+ = >50% to 75%; 4+ = >75% of cells positive. It was considered negative expression when ≤5% of the tumor cells were stained and positive expression when >5% of the tumor cells were stained.

Finally, the immunohistochemical results were correlated with clinical data such as alcohol and tobacco abuse, tumor site, tumor recurrence, metastatic spread, clinical stage, and the patient's status in the last follow-up (alive without or with disease, death) as well as with histopathological tumor grade and lymph node status.

Statistical Analyses. Statistical analyses were performed using Fisher's exact tests for comparisons between immunohistochemical results and clinical data. The odds ratio (OR) and 95% confidence interval (CI) were calculated using an unconditional logistic regression model. A probability error of ≤5% was considered significant for all statistical analyses ($p < .05$). The survival curves were plotted in accord with the Kaplan–Meier method.

RESULTS

All clinical data and results of the immunohistochemcial antigen expression analyses for all the cases of our series are shown in Supplementary Table 1. In all, 63 cases of laryngeal SCC were available for this study: 57 patients were men, 6 were women. Age range was 27 to 78 years (mean, 57.2 years). In the majority of cases, the primary was supraglottic (37 of 63), 23 tumors were glottis, and 3 neoplasms were subglottic. On the basis of the TNM System of the Union Internationale Contre le Cancer (UICC), the tumors were classified as follows: T1, 8 cases; T2, 15 cases; T3, 27 cases; T4, 13 cases. Distribution by clinical stage was as follows: 7 cases were stage I, 8 cases stage II, 24 cases stage III, and 24 cases stage IV (2 of 24 IVa, 2 of 24 stage IVb). The diagnosis was

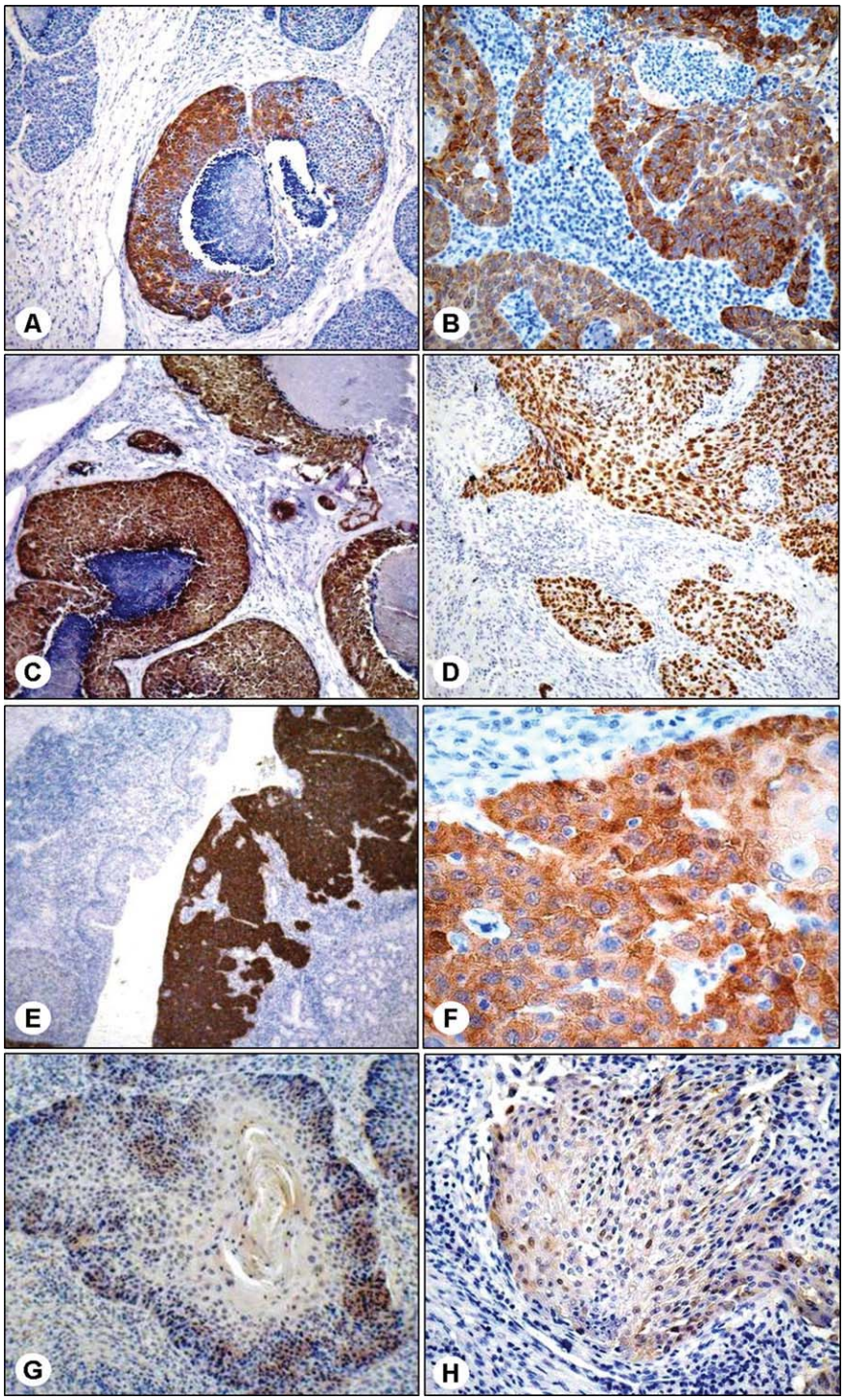

FIGURE 1. Immunohistochemical staining patterns for cancer testis antigens in larynx squamous cell carcinoma (SCC). **(A)** MAGE-A1 expression as detected by monoclonal antibody (mAb) MA454, heterogeneously present in approximately 50% (2+) of the invasive border area (original magnification ×50) and **(B)** homogeneously expressed in the cytoplasm of all tumor cells (original magnification ×100). **(C)** Strong and homogeneous (3+) MAGE-A4 (mAb 57B) expression in the invasive front of the tumor (original magnification ×50). **(D)** Strong nuclear expression pattern of MAGE-C2/CT10 (mAb CT10#5; original magnification ×100). **(E)** Immunoreactivity of mAb CT7-33 to MAGE-C1/CT7-33, displaying strong nuclear and cytoplasmic staining (original magnification ×50). **(F)** Cytoplasmic immunostaining of mAb E978 to NY-ESO-1 (original magnification ×400). **(G)** Weak expression of GAGE in the basally located tumor cells of laryngeal SCC at low (original magnification ×100) and medium **(H)** power (original magnification ×200).

Table 2. Frequency distribution of staining of the antigens.

Antigen	Antibody	Immunoreactivity (%)	0	1+	2+	3+	4+
MAGE-A1	MA454	30/63 (47.62)	33	10	9	11	0
MAGE-A3	M3H67	23/63 (36.51)	40	3	2	18	0
MAGE-A4	57B	38/63 (60.32)	25	9	4	25	0
MAGE-C1/CT7	CT7.33	14/63 (22.2)	49	9	4	1	0
MAGE-C2/CT10	CT10.5	5/63 (7.93)	58	3	1	1	0
GAGE	GAGE	13/63 (20.64)	50	7	5	1	0
NY-ESO-1	E978	6/63 (9.52)	57	5	0	1	0

morphologically confirmed by 2 pathologists (F.P.S., L.N.) and classified in accord with the World Health Organization (WHO) Classification of Tumors of the Head and Neck.[13] Tumors were histologically graded as follows: 15 of 63 well-differentiated (23.81%), 35 of 63 moderately differentiated (55.56%), and 13 of 63 poorly differentiated carcinomas (20.63%). A representative tumor block containing the invasion tumor front was selected for the immunohistochemical assays (see Figure 1).

For statistic analysis, the parameters tumor site, TNM classification, clinical stage, and tumor differentiation were grouped as follows: subglottic and glottic tumors versus supraglottic tumors; T1/T2 tumors versus T3/T4 tumors; clinical stage I/II versus stage III/IV tumors; moderately and poorly differentiated tumors versus well-differentiated tumors.

The result of the immunohistochemical staining is displayed in Table 2. The most prevalent immunoreactivity was seen with the serological reagents to MAGE-A antigens, with mAb 57B showing the highest incidence of staining being present in approximately 60% of tumors, followed by mAb MA454, which was positive in almost half of the tested cases. The lowest incidence of immunostaining with MAGE-A reagents was present with mAb M3H67 in approximately 1/3 of cases. Although most of the cases, which were positive for MAGE-A antigens, showed expression in >25% of the tumor cells, none of the cases was immunopositive in >75% to 100% of tumor area. The other antigens of our series were expressed in a much lower percentage, with MAGE-C1/CT7 and GAGE both being present in approximately 20% of the cases while MAGE-C2/CT10 and NY-ESO-1 were each expressed in only 10% of tumors. Different from MAGE-A antigens, the antigen expression was even more heterogeneous and present in most cases in only 5% to 25% of the tumor cells.

When looking at the overall cancer testis antigen expression, at least 1 cancer testis antigen was detected in 42 of the 63 laryngeal SCCs (67%). In 34 of 42 positive cases (81%) there was simultaneous expression of ≥2 cancer testis antigens. In 27 of 42 cases (64%) ≥3 antigens were present.

For the correlation between the antigen expression and clinical and histopathological characteristics, the cases were grouped as follows: no positive antigen versus at least 1 positive antigen; no positive antigen versus 1 positive antigen versus ≥2 positive antigens. The expression of each antigen was correlated with clinical and histopathological characteristics as well. There was no correlation between antigenic expression and alcohol and tobacco abuse, tumor site, histological differentiation grade, regional or distant metastasis, second primary tumor, or death.

Table 3 shows the percentage of expression of at least 1 of our tested antigens in accord with the different clinical stages and TNM classifications.

There was significant correlation between antigen expression and advanced tumors (stage III/IV) in cases with reactivity to only 1 antibody ($p = .01$) as well as in the cases with reactivity to ≥2 primary antibodies (≥2 mAbs, $p = .04$).

When antibody reactivity was individually considered, there was correlation of mAb 57B immunopositivity and tumors with advanced T classification (T3 and T4, $p = .03$).

Regional recurrence was associated with immunoreactivity for mAbs MA454 ($p = .02$) and M3H67 ($p = .04$).

The clinical follow-up varied between 1 month and 82 months (mean, 45.4 months). There was no association between survival and expression of any of the analyzed antigens.

DISCUSSION

The prognosis for patients with advanced laryngeal cancer is poor and little to no progress has been made in their treatment in the last few decades.[1,2] Thus, there is a great need for additional therapeutic options in this type of neoplasm. Recently, much hope has been put into new approaches such as antibody-based and vaccine-mediated immunotherapy.[3,5] Success of cancer immunotherapy depends on the identification of appropriate antigens, which can be used for immunization to trigger specific immune responses.

Table 3. Expression of at least 1 antigen in accord with TNM classification and clinical stage.

TNM classification	Expression, %	Clinical stage	Expression, %
T1	37.50	I	28.57
T2	60.00	II	50.00
T3	77.70	III	75.00
T4	69.23	IV	75.00

Because of their expression pattern with almost exclusive presence in malignant tumors, cancer testis antigens are regarded as almost ideal candidates for tumor vaccine targets. Moreover, it could be shown that cancer testis antigen can elicit specific autologous T-cell responses in patients with head and neck cancer, suggesting their usefulness as vaccine targets in this type of tumors.[14] In the present study, we analyzed the presence of several cancer testis antigens in carcinomas of the larynx and correlated them with various clinical parameters. Here, we focused our study on cancer testis antigens, analyzing tissue and clinical data from 63 patients (57 men and 6 women) with laryngeal SCC.

In the present series, we find expression of cancer testis antigens in 2/3 of the analyzed cases. Expression of cancer testis antigens can vary in different types of tumors. Although some neoplasms such as melanoma and non-small cell lung cancers show high cancer testis antigen expression, tumors such as renal cell carcinoma and colon carcinomas display a low frequency of expression.[10,15,16] Interestingly, discordant, high, low, and intermediate expression levels were found for cancer testis antigens in head and neck carcinomas previously.[7,17] However, some of these studies were hampered by low case numbers, and/or focused on particular antigens or comprised of a variety of different tumors. To our knowledge, laryngeal carcinomas have not been analyzed specifically in the past. In spite of a high number of positive cases, not all cancer testis antigens show a similar expression level. As seen in other malignant tumors, MAGE-A antigens were the most highly expressed proteins, whereas NY-ESO-1 showed comparably little expression.[18]

Also, most tumors of our analysis revealed a heterogeneous expression pattern. Heterogeneous expression has previously been seen for various cancer testis antigens in different tumors.[8–10,15,18] Heterogeneity of expression may explain the discrepancies between different methods, such as reverse transcription–polymerase chain reaction (RT-PCR) versus immunohistochemistry and between previous studies analyzing the presence of cancer testis antigens. For head and neck tumors, a lower incidence of cancer testis antigens has been reported in previous mRNA-based analysis. In the present analysis, we used immunohistochemistry of archival paraffin-embedded material. This enabled us to include a larger number of patients for which no suitable tissue for RT-PCR analysis was available. In our hands, immunohistochemistry has proven more sensitive than RT-PCR for the expression analysis of cancer testis antigens.[19,20]

Some authors argue that the heterogeneity of cancer testis antigen expression could be a limiting factor for immunotherapy, whereas others suggest that polyvalent vaccines will be able to overcome potential expression differences within the tumors.[21,22] Interestingly, we find a correlation between the cancer testis antigen expression and clinical stage. More advance tumors revealed a higher expression level. Since little is known about the biology of cancer testis antigen, an explanation for this phenomenon is purely speculative. It could indicate a causative role of these antigens in the progression of tumor or resemble a secondary phenomenon in advanced neoplasms. In either case, the increased expression of cancer testis antigens in clinically more advanced cases could indicate that tumors with worse prognosis might be more susceptible to cancer testis antigen–based immunotherapy. A similar correlation of stage of disease with cancer testis antigen expression has been postulated in the past for tumors such as bladder carcinoma,[23] myeloma,[24,25] ovarian carcinomas,[26] melanomas,[27] and other tumors.[22] However, some of these studies were based on RT-PCR analysis and did not study the actual presence of the protein. In our study, correlation of cancer testis antigens with TNM stage could be verified only for the immunoreactivity of mAb 57B. At this point a cautionary note should be made regarding the specificity of anti–cancer testis serological reagents. Whereas mAb #26 is a commercial reagent, which was generated to a consensus region of the GAGE family and consequently is reactive with several GAGE antigens, mAbs CT7-33, E978, CT10#5, and MA454 are considered specific reagents and little doubt has been raised as to their corresponding antigen. The immunoreactivity of mAbs 57B and M3H67, however, needs to be considered cautiously. This is best exemplified by mAb 57B, which was generated by an immunization procedure using a MAGE-A3 fusion protein.[28] However, subsequent analysis showed 57B-immunoreactivity with several MAGE-A antigens, and a more recent study indicated mAb 57B to be a MAGE-A4 reagent.[29,30] A similar situation appears to be present for mAb M3H67. Although MAGE-A3 has been identified to be among the most prevalent cancer testis antigen in various tumors on an mRNA level, at present there appears to be no MAGE-A3-specific reagent to verify these data on a protein level.[31] The difficulty of generating MAGE-A subtype-specific serological reagents is based on the high homology of the MAGE-A gene family members.

We also observed a frequent simultaneous expression of cancer testis antigens. Among the positive cases, 81% expressed $\geq$2 antigens and 64% expressed $\geq$3 cancer testis antigens. We found a similar coexpression pattern of mRNA levels in a previous study of head and neck carcinomas.[19] Demethylation appears to play a role in gene activation of cancer testis antigen and may be responsible for the simultaneous upregulation of cancer testis antigen genes in tumors.[32]

Several clinical trials using cancer testis antigens have been completed and/or initiated for different types of tumors and preliminary results as well as occasional observations of spectacular responses indicate that cancer testis antigens could, in fact, be effective vaccine targets for the treatment of malignant disease.[4,33,34]

In conclusion, in the present analysis of 63 laryngeal SCCs, we find a high expression of cancer testis antigens, particularly of the MAGE-A family, which are present in up to 60%, whereas MAGE-C1/CT7, MAGE-C2/CT10, NY-ESO-1, and GAGE showed a much lower incidence. The presence of cancer testis antigens was correlated with advanced clinical stage. Our data indicate that cancer testis antigens, especially those of the MAGE-A family, could be valuable vaccine targets in laryngeal tumors, especially in those with a worse prognosis.

Acknowledgments. The authors thank Patricia Alves Pontes Monteiro, Ana Maria Anselmi, and Maria Paula Scandar for excellent technical assistance.

REFERENCES

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2007.CA Cancer J Clin 2007;57:43–66.
2. Cosetti M, Yu G-P, Schantz PS. Five-year survival rates and time trends of laryngeal cancer in the US population. Arch Otolaryngol Head Neck Surg 2008;134:370–379.
3. Rosenberg SA, Dudley ME, Restifo NP. Cancer immunotherapy. N Engl J Med 2008;359:1072–1073.
4. Gnjatic S, Nishikawa H, Jungbluth AA, et al. NY-ESO-1: review of an immunogenic tumor antigen. Adv Cancer Res 2006;95:1–30.
5. Caballero OL, Chen YT. Cancer/testis (CT) antigens: potential targets for immunotherapy. Cancer Sci 2009;100:2014–2021.
6. Scanlan MJ, Simpson AJ, Old LJ. The cancer/testis genes: review, standardization, and commentary. Cancer Immun 2004; 4:1 (review).
7. Jungbluth AA, Chen YT, Busam KJ, et al. CT7 (MAGE-C1) antigen expression in normal and neoplastic tissues. Int J Cancer 2002;99:839–845.
8. Jungbluth AA, Chen YT, Stockert E, et al. Immunohistochemical analysis of NY-ESO-1 antigen expression in normal and malignant human tissues. Int J Cancer 2001;92:856–860.
9. Jungbluth AA, Stockert E, Chen YT, et al. Monoclonal antibody MA454 reveals a heterogeneous expression pattern of MAGE-1 antigen in formalin-fixed paraffin embedded lung tumours. Br J Cancer 2000;83:493–497.
10. Jungbluth AA, Busam KJ, Kolb D, et al. Expression of MAGE-antigens in normal tissues and cancer. Int J Cancer 2000;85: 460–465.
11. Zhuang R, Zhu Y, Fang L, et al. Generation of monoclonal antibodies to cancer/testis (CT) antigen CT10/MAGE-C2. Cancer Immun 2006;6:7 (abstract).
12. Neder L, Marie SK, Carlotti CG Jr, et al. Galectin-3 as an immunohistochemical tool to distinguish pilocytic astrocytomas from diffuse astrocytomas, and glioblastomas from anaplastic oligodendrogliomas. Brain Pathol 2004;14:399–405.
13. Solbin LH, Wittekind C. TNM classification of malignant tumours, 6th ed. Hoboken, NJ: Wiley–Liss; 2004. pp 21–61.
14. Filho PA, Lopez-Albaitero A, Xi L, Gooding W, Godfrey T, Ferris RL. Quantitative expression and immunogenicity of MAGE-3 and -6 in upper aerodigestive tract cancer. Int J Cancer 2009; 125:1912–1920.
15. Simpson AJG, Caballero OL, Jungbluth A, et al. Cancer/testis antigens, gametogenesis and cancer. Nat Rev Cancer 2005;5: 615–625.
16. Bolli M, Schultz-Thater E, Zajac P, et al. NY-ESO-1/LAGE-1 coexpression with MAGE-A cancer/testis antigens: a tissue microarray study. Int J Cancer 2005;115:960–966.
17. Prasad ML, Jungbluth AA, Patel SG, Iversen K, Hoshaw-Woodard S, Busam KJ. Expression and significance of cancer testis antigens in primary mucosal melanoma of the head and neck. Head Neck 2004;26:1053–1057.
18. Juretic A, Spagnoli GC, Schultz-Thater E, Sarcevic B. Cancer/testis tumour-associated antigens: immunohistochemical detection with monoclonal antibodies. Lancet Oncol 2003;4:104–109 (review).
19. Figueiredo DL, Mamede RC, Proto-Siqueira R, Neder L, Silva WA Jr, Zago MA. Expression of cancer testis antigens in head and neck squamous cell carcinomas. Head Neck 2006;28:614–619.
20. Kienstra MA, Neel HB, Strome SE, Roche P. Identification of NY-ESO-1, MAGE-1, and MAGE-3 in head and neck squamous cell carcinoma. Head Neck 2003;25:457–463.
21. Old JL. Cancer vaccine collaborative 2002. Cancer Immunity 2003;3 (Suppl 2):1–12.
22. Scanlan MJ, Gure AO, Jungbluth AA, et al. Cancer/testis antigens: an expanding family of targets for cancer immunotherapy. Immunol Rev 2002;188:22–32.
23. Patard JJ, Brasseur F, Gil-Diez S, et al. Expression of MAGE genes in transitional-cell carcinomas of the urinary bladder. Int J Cancer 1995;64:60–64.
24. van Baren N, Brasseur F, Godelaine D, et al. Genes encoding tumor-specific antigens are expressed in human myeloma cells. Blood 1999;94:1156–1164.
25. Jungbluth AA, Ely S, Diliberto M, et al. The cancer-testis antigens CT7 (MAGE-C1) and MAGE-A3/6 are commonly expressed in multiple myeloma and correlate with plasma cell proliferation. Blood 2005;106:167–174.
26. Russo V, Dalerba P, Bonazzi C, et al. MAGE, BAGE and GAGE genes expression in fresh epithelial ovarian carcinomas. Int J Cancer 1996;67:457–460.
27. Goydos JS, Patel M, Shih W. NY-ESO-1 and CTP11 expression may correlate with stage of progression in melanoma. J Surg Res 2001;98:76–80.
28. Kocher T, Schultz-Thater E, Gudat F, et al. Identification and intra cellular location of MAGE 3 gene product. Cancer Res 1995;55:2236–2239.
29. Rimoldi D, Salvi S, Schultz-Thater E, Spagnoli GC, Cerottini JC. Anti-MAGE-3 antibody 57B and anti-MAGE-1 antibody 6C1 can be used to study different proteins of the MAGE-A family. Int J Cancer 2000;86:749–751.
30. Landry C, Brasseur F, Spagnoli GC, et al. Monoclonal antibody 57B stains tumor tissues that express gene MAGE-A4. Int J Cancer 2000;86:835–841.
31. Dhodapkar MV, Osman K, Teruya-Feldstein J, et al. Expression of cancer/testis (CT) antigens MAGE-A1, MAGE-A3, MAGE-A4, CT-7, and NY-ESO-1 in malignant gammopathies is heterogeneous and correlates with site, stage and risk status of disease. Cancer Immun 2003;3:9 (review).
32. De Smet C, Lurquin C, Lethe B, Martelange V, Boon T. DNA methylation is the primary silencing mechanism for a set of germ line- and tumour-specific genes with a CpG-rich promoter. Mol Cell Biol 1999;19:7327–7333.
33. Brichard VG, Lejeune D. GSK's antigen-specific cancer immunotherapy programme: pilot results leading to Phase III clinical development. Vaccine 2007;25 (Suppl 2):B61–B71 (review).
34. Hunder NN, Wallen H, Cao J, et al. Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1. N Engl J Med 2008;358:2698–2703.