Expression of Cancer-testis Antigens in Adenoid Cystic Carcinoma of the Salivary Glands Correlates with Clinical Outcomes

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Abstract: This study aimed to explore the expression and clinicopathological significance of CTAs MAGE-1, NY-ESO-1, MAGE-C2 and SCP-1 in adenoid cystic carcinoma (ACC). Immunohistochemistry was used to detect their expressions in 70 cases of ACC, and in 6 healthy tumor-adjacent salivary glands. The correlation between the expressions of the four CTAs, clinical and pathological features, and patients’ overall survival (OS) were analyzed. Of the 70 ACC cases, strong staining was observed in 43 (61.4%) for MAGE-1, 14 (20%) for NY-ESO-1, 9 (12.9%) for SCP-1, and 6 (8.6%) for MAGE-C2. We also found some significant correlations between the CTAs expression and clinicopathological parameters, for example, MAGE-1 and tumor size, NY-ESO-1 and distant metastasis, MAGE-C2 and tumor site, SCP-1 and age, SCP-1 and histopathological types (P < 0.05). Patients with any single CTAs positive staining showed a similar OS compared to those with negative staining, however patients with strong expression (score 6-7) of MAGE-C2 showed a significantly reduced OS compared to those scored 0-5 (P < 0.05). There was no OS difference between patients expressing simultaneously any 2 of the 4 CTAs and those with negative expression or those expressing only one of the 2 CTAs. Similar results were found in patients expressing at least 3 CTAs compared with patients expressing less than 3 CTAs. However, patients with the four CTAs co-expression had a substantially reduced mean survival time of 131.8 months compared with 176.5 months in patients with at least one CTA negative (P < 0.05). In conclusion, a significant fraction of patients with ACC showed expression of MAGE-1, NY-ESO-1, MAGE-C2 and SCP-1, indicating these CTAs might represent potential antigens for cancer vaccines. In addition, MAGE-C2 may be an important prognostic marker of ACC.

Key words: Cancer/testis antigens (CTAs), Adenoid cystic carcinoma (ACC), Prognosis

Introduction

Adenoid cystic carcinoma (ACC) is a relatively rare type of epithelial tumor that primarily originates from major salivary glands. ACC accounts for approximately 5% – 10% of all salivary gland neoplasms. Due to the high potential of local recurrences and distant metastases, ACC significantly affects patient long-term survival. Although surgery continues to represent the most dominant treatment approach in association with radiation therapy, chemotherapy, and gene therapy, there still remains a large proportion of ACC patients who do not respond to standard systemic therapy or relapse following treatment. As a consequence of recent advances in combination treatment modalities, immunogenic treatment, through acting on specific immune targets, represents a neo-adjuvant therapy for ACC patients with durable clinical responses.

Cancer testis antigens (CTAs) represent a family of tumor-associated immunogenic proteins expressed in certain malignant tumors, with selective expression in normal healthy tissues. Several CTAs have been found to induce a spontaneous immune response, among which Melanoma-Associated-antiGen homolog (MAGE-1), Cancer/testis antigen 1B (NY-ESO-1, also known as CTAG1B), MAGE family member C2 (MAGE-C2), and Synaptonemal complex protein 1 (SCP-1) are of particular interest. MAGE-1 was the first isolated antigen to be recognized by cytotoxic T lymphocytes in human melanoma, and was subsequently renamed melanoma antigen A1 (MAGE-A1). The SCP-1 antigen is a synaptonemal complex protein involved in chromosomal reduction during meiosis, and its role in gamete development has been verified. In addition, CTAs of the MAGE-family and NY-ESO-1 have been identified as independent markers for poor overall survival (OS) in cancer patients. Preliminary analyses indicated that MAGE-A1 and NY-ESO-1 were frequently expressed in ACC cases, and that patients expressing both NY-ESO-1 and pan-MAGE displayed significantly reduced OS compared with negative patients. Few studies have simultaneously investigated the association between OS and the expression of MAGE-1, NY-ESO-1, MAGE-C2, and SCP-1 in ACC.

In this study, we collected 70 cases of ACCs and 6 tumor-adjacent normal salivary glands to detect the expression of the four CTAs, and analyze the relationship between their expressions and clinical outcomes.
Immunohistochemistry

The classification of ACC histological types was based on the WHO 1991 classification criteria. The tumor-node-metastasis (TNM) clinical staging criteria were derived from the sixth edition of American Joint Committee on Cancer (AJCC) and International Union Against Cancer (UICC)\(^\text{11}\). The follow-up period after surgery was longer than 2 years until September 2007 (mean: 92 months). Lymph node metastasis, distant metastasis, and recurrence after surgery were confirmed by clinical examination, radiograph, B type supersonic and pathologic biopsy. Perineural invasion was analyzed by hematoxylin-eosin staining and clinical symptoms. Vascular invasion was detected by hematoxylin-eosin staining.

As a positive control, normal testis tissue known to express MAGE-1, NY-ESO-1, MAGE-C2 and SCP-1 was provided by the Department of Urinary Surgery at the Renmin Hospital of Wuhan University with the approval of the Wuhan University Medical Ethics Committee.

Antibodies

The following antibodies were used for immunohistochemistry (IHC): polyclonal rabbit anti-human MAGE-1 (dilution 1:200, Lab Vision, USA); monoclonal mouse anti-NY-ESO-1; Clone E978 (dilution 1:100, Zymed, Ltd. South San Francisco, CA); polyclonal goat anti-human SCP-1 (dilution 1:300, Santa Cruz, CA); and LX-CT10.9/MAGE-C2 (working concentration: 2 μg/ml) which was kindly provided by Professor Boquan Jin (Department of Immunology, Fourth Military Medical University, Xi’an, China). An SP Histostain™-plus kit (ZSGB-BIO Ltd. Beijing, China) and a DAB (3,3’-diaminobenzidine) chromogen kit (Maixin-Bio Ltd. Fu Zhou, China) were used in accordance with the manufacturers’ instructions.

Immunohistochemistry

Paraffin-embedded 5-μm-thick tissue sections from 70 ACC samples, 6 tumor-adjacent normal salivary glands tissues, and 1 normal testis tissues were deparaffinized through a series of xylene baths for 15 min each, and the sections were rehydrated in graded alcohol for 1-2 min. All sections were placed into 3% hydrogen peroxide for 20 min to block endogenous peroxidase activity, and the tissues were subsequently boiled in 10 mM freshly prepared citrate buffer (pH 6.0 for MAGE-1, MAGE-C2 and SCP-1) or in 1 mM EDTA (pH 8.0 for NY-ESO-1) followed by cooling at room temperature for antigen retrieval. After the sections were incubated in a working dilution of normal goat serum (MAGE-1, MAGE-C2, and NY-ESO-1) or normal rabbit serum (for SCP-1) for 20 min, the sections were incubated with primary antibodies overnight at 4°C. The avidin-biotin-complex peroxidase systems with biotinylated anti-goat, anti-rabbit, or anti-mouse secondary antibodies (working dilution) were used for 20 min, and the sections were rinsed with Tris-buffered saline after each step. DAB as a chromogen was applied to detect primary antibodies, after which the slides were rinsed completely in tap water and counterstained with Mayer’s hematoxylin.

Evaluation of staining

Nuclear and/or cytoplasm immunoreactivity of the tumor cells were both considered to indicate positive staining. Each case was scored according to the intensity of staining (viewed at a magnification of 200×) and the extension of stained area (viewed at a magnification of 40×).

The intensity of staining was evaluated as follows: 0, no staining; 1+, mild staining; 2+, moderate staining; 3+, intense staining. The extension of stained area was: 0, no staining of cells in any microscopic fields; 1+, ≤ 25% of positively-stained tissue; 2+, < 25% and ≤ 50% stained positive; 3+, < 50% and ≤ 75% stained positive; and 4+, > 75% stained positive. The final staining score was obtained by adding the intensity and extension scores. The combined staining score of 0 was considered as negative staining; a score of 1-3 was weak staining; a score of 4-5 was moderate staining; and a score of 6-7 was strong staining\(^\text{12}\).

Statistical analysis

Statistical analysis was performed using SPSS22.0 software (IBM, New York, USA). A chi-squared test (Fisher’s exact test) was performed to explore the association between the expression of these four CTAs and clinical variables, including gender, age, tumor size, site, TNM stage, perineural and vascular invasion, tumor recurrence, lymph and distant metastasis. Survival rates were obtained using the Kaplan-Meier method and the significance levels were tested by log-rank tests. Death caused by ACC was considered to be the outcome. Death caused by other causes was censored and the missing values were replaced by the series mean method. A univariate analysis with a Cox’s proportional hazards model was applied to determine each of the identified prognostic factors. A multivariate analysis with a Cox’s proportional hazards model was applied to detect the combined effects. The threshold of \(P < 0.05\) indicated a significant difference.

Results

Immunohistochemical evaluation of CTAs expression in ACC

Weak MAGE-1 staining was observed in 4 (5.7%) cases, moderate staining in 19 (27.1%) cases, and strong staining in 43 (61.4%) cases. NY-ESO-1 was found to be expressed weakly in 12 (17.1%) cases, moderately in 38 (54.3%) cases, and strongly in 14 (20%) cases. MAGE-C2 expression was observed to be weak in 13 (18.6%) cases, moderate in 30 (42.9%) cases, and strong in 6 (8.6%) cases. SCP-1 expression was weak in 7 (10%) cases, moderate in 34 (48.6%) cases, and strong in 9 (12.9%) cases.

In addition, 49 (70%) of 70 patients exhibited strong expression of at least one of the four CTAs, among which 14 (20%), 3 (3%), and 2 (2.9%) strongly expressed any two, three, or four of these CTAs proteins, respectively.

Regarding the location of the 4 CTAs, we found that MAGE-1 and NY-ESO-1 were detected in the cytoplasm and nuclear region of intratubular germ cells of normal testis samples. MAGE-C2 was selectively expressed in the nuclei of intratubular germ cells. SCP-1 was selectively expressed in the nuclei of testicular germ cells. All of the samples were negative in the interstitial tissue and intratubular sertoli cells (Fig. 1A-D).

MAGE-1, NY-ESO-1, MAGE-C2 and SCP-1 were detected in the
cytoplasm and/or nuclei of the tumor cells (Figs. 1 and 2). In cribriform type, the stained cells were primarily located among the ductal lining cells (Fig. 1E-H). The similar result was also observed in tubular type (Fig. 1I-L). In solid type, strong staining of MAGE-1 was observed (Fig. 1M), however NY-ESO-1, MAGE-C2 and SCP-1 showed weak to moderate staining (Fig. 1N-P).

In tumor-adjacent normal salivary glands, the 4 CTAs were observed in the cytoplasm of tubular cells, rather than in the acinar cells (Fig. 1Q-T). Especially, nucleus-positive staining and perineural invasion were found for MAGE-C2 in some cases (Fig. 2).

**Patient characteristics and the clinicopathological significance of CTA expression in ACC**

The clinical and pathological parameters of the 70 ACC patients, and the expressions of the 4 CTAs were presented in Table 1. MAGE-1 expression was significantly correlated with tumor size, and patients with tumor size > 2cm showed more moderate to strong staining than those with tumor size ≤ 2cm ($P < 0.05$). NY-ESO-1 expression was significantly associated with distant metastasis ($P < 0.05$). MAGE-C2 ex-

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**Figure 1.** Immunohistochemical expression of MAGE-1, NY-ESO-1, MAGE-C2 and SCP-1 in healthy testis tissue, cribriform type of ACC, tubular type of ACC, solid type of ACC and normal salivary gland. (bar size = 50 µm)

**Figure 2.** Positive staining for MAGE-C2 in one case of ACC showed tumor nest with perineural invasion. (dark arrow: positive cells; bar size = 50 µm)
expression was significantly correlated with tumor site \(P < 0.05\). SCP-1 expression was correlated with age and histopathological type \(P < 0.05\). No other clinical factors achieved statistical significance.

**Prognostic impact of cancer-testis antigen expression**

Among the 70 total patients, 2 (2.9%) lost contact, 1 (1.4%) died of unrelated reason, 16 (22.9%) died from ACC, 51 (72.9%) were still alive, and 31 (44.3%) had recurrent ACC. The mean follow-up period was 92.07 months and the standard deviation (SD) was 54.95 months. The results of the univariate analysis (Cox’s proportional hazards model) were presented in Table 2. Age > 50, vascular invasion, distant metastasis, recurrence, and histopathologic type (solid) were found to be significantly associated with a worse prognosis \(P < 0.05\). However, the expression of MAGE-1, NY-ESO-1, MAGE-C2 and SCP-1 were not significantly related to disease prognosis. According to the results of a multivariate analysis (Cox’s proportional hazards model), patients with disease recurrence or strong expression (score 6-7) of MAGE-C2 showed a significantly reduced OS \(P < 0.05\). Table 3).

Survival curves were calculated using the Kaplan-Meier method and a log-rank test. There was no survival difference between patients with MAGE-1 positive staining and those with negative staining (Fig. 3A). The same findings were also observed for the other 3 CTAs (Fig. 3B-D).

It seemed that patients expressing simultaneously any 2 of the 4 CTAs were inclined to had a reduced survival compared to those with negative expression or those expressing only one of the 2 CTAs. How-ever, there was no significant differences (Fig. 4A-F). Patients expressing simultaneously at least 3 CTAs had similar survival compared with those expressing less than 3 CTAs (Fig. 5A). Whereas, patients with the

### Table 1. The correlation between the expression of MAGE-1, NY-ESO-1, MAGE-C2, SCP-1 and clinical features

| Clinical parameters | MAGE-1 Scores | NY-ESO-1 Scores | MAGE-C2 Scores | SCP-1 Scores |
|---------------------|---------------|----------------|---------------|--------------|
|                     | 0-1            | 2-3            | 4-5           | 6-7          |
|                     | 0-1            | 2-3            | 4-5           | 6-7          |
|                     | 0-1            | 2-3            | 4-5           | 6-7          |
|                     | 0-1            | 2-3            | 4-5           | 6-7          |

**Table 2. Prognostic factors by univariate analysis (Cox’s proportional hazards model)**

| Variables                  | Hazards ratio (95% confidence interval) | P value |
|----------------------------|----------------------------------------|---------|
| Age (≤50/>50)              | 3.605 (1.145-11.350)                   | 0.028   |
| Gender (M/f)               | 0.614 (0.221-1.707)                    | 0.350   |
| Site (major/minor)         | 0.683 (0.246-1.894)                    | 0.463   |
| Size (≤2cm/>2cm)           | 3.029 (0.398-23.059)                   | 0.285   |
| Lymph metastasis (N/P)     | 0.045 (0.324-3.75)                     | 0.493   |
| Perineural invasion (N/P)  | 1.957 (0.724-5.287)                    | 0.186   |
| Vascular invasion (N/P)    | 6.991 (2.243-21.793)                   | 0.001   |
| Distant metastasis (I+II/III+IV) | 3.684 (1.334-10.172)                | 0.012 |
| Recurrence (N/P)           | 1.074 (2.436-47.372)                   | 0.002   |
| Histotypes (solid/cribriform) | 0.521 (0.174-1.554)                 | 0.242   |
| Histotypes (solid/tubular) | 0.226 (0.056-0.908)                    | 0.036   |
| MAGE-1 Scores (0-5/6-7)     | 1.605 (0.551-4.681)                    | 0.386   |
| NY-ESO-1 Scores (0-5/6-7)   | 0.896 (0.198-4.047)                    | 0.887   |
| MAGE-C2 Scores (0-5/6-7)    | 2.631 (0.749-3.354)                    | 0.135   |
| SCP-1 Scores (0-5/6-7)      | 0.975 (0.221-4.296)                    | 0.973   |

**Table 3. Significant prognostic factors by multivariate analysis (Cox’s proportional hazards model)**

| Variables                  | Hazards ratio (95% confidence interval) | P value |
|----------------------------|----------------------------------------|---------|
| age (≤50/>50)              | 2.659 (0.777-9.103)                    | 0.119   |
| Vascular invasion (N/P)    | 3.577 (0.730-17.151)                   | 0.116   |
| Recurrence (N/P)           | 6.967 (1.367-35.495)                   | 0.019   |
| MAGE-C2 Scores (0-5/6-7)   | 4.824 (1.034-22.493)                   | 0.045   |
| Histotypes (solid/cribriform) | 0.591 (0.183-1.912)                 | 0.380   |
| Histotypes (solid/tubular) | 0.463 (0.072-2.960)                    | 0.416   |

N/P: negative / positive; histotypes: histopathologic types. Bold values indicate \(P < 0.05\).
Figure 3. Overall Survival (OS) analysis of patients with ACC by the Kaplan-Meier method based on staining scores. (A) OS of MAGE-1 positive versus negative patients. (B) OS of NY-ESO-1 positive versus negative patients. (C) OS of MAGE-C2 positive versus negative patients. (D) OS of SCP-1 positive versus negative patients.

Figure 4. Overall Survival (OS) analysis of patients with ACC by the Kaplan-Meier method based on staining scores. (A) OS of MAGE-1 and NY-ESO-1 co-expressing versus MAGE-1 or NY-ESO-1 negative versus both negative patients. (B) OS of MAGE-1 and MAGE-C2 co-expressing versus MAGE-1 or MAGE-C2 negative versus both negative patients. (C) OS of MAGE-1 and SCP-1 co-expressing versus MAGE-1 or SCP-1 negative versus both negative patients. (D) NY-ESO-1 and MAGE-C2 co-expressing versus NY-ESO-1 or MAGE-C2 negative versus both negative patients. (E) NY-ESO-1 and SCP-1 co-expressing versus NY-ESO-1 or SCP-1 negative versus both negative patients. (F) OS of MAGE-C2 and SCP-1 co-expressing versus MAGE-C2 or SCP-1 negative versus both negative patients.
four CTAs co-expression had a substantially reduced mean survival time of 131.8 months compared with 176.5 months in patients with at least one CTA negative (Fig. 5B; \( P < 0.05 \)).

**Discussion**

Although multiple treatment approaches have been reported to prevent distant metastases and reduce recurrence in ACC patients, the clinical and survival data that can provide indications for better therapy remain limited due to the low incidence of ACC. Since CTAs are selectively expressed in a broad spectrum of malignant tumors and have not been found in normal tissues, with the exception of the testis and fetal placenta\(^8,13\), there is an urgent need for the discovery and validation of specific CTA targets for targeted cancer immunotherapy. MAGE-1, NY-ESO-1, MAGE-C2 and SCP-1 are expressed in a variable pattern in multiple human malignant tumors\(^3\). In this study, we analyzed the expression of these four CTA proteins in 70 cases of ACCs and 6 cases of tumor-adjacent normal salivary glands, as well as their relationship with clinical prognosis. To our knowledge, this is the largest study to simultaneously examine the immunohistochemical reaction and prognostic significance of MAGE-1, NY-ESO-1, MAGE-C2 and SCP-1 in ACC.

To date, 12 subtypes are known to comprise the MAGE-A family\(^6\). MAGE-1 has been reported to be strongly expressed in small hepatorenal carcinoma (HCC) and exclusively in the HCC nodule\(^14\). Moreover, expression of the MAGE-A family in head and neck carcinoma is highly variable\(^3\). In our 70 cases, 43 (61.4%) strongly expressed MAGE-1 and 14 (20%) strongly expressed NY-ESO-1. The study by Veit et al. reported that 31.2% out of 84 ACC patients expressed pan-MAGE and 57.1% expressed NY-ESO-1\(^16\). Beppu et al. detected MAGE-A expression in 62% out of 21 ACC patients\(^9\). Each study has demonstrated a different trend in expression. We determined that this discrepancy may be considered to be due to various factors, such as sample capacity, staining reagents and protocol, scoring method, and ethnicity. First, Veit et al. used an anti-pan-MAGE antibody that could recognize MAGE-1, MAGE-3, MAGE-4, MAGE-8, MAGE-10, MAGE-B1, and MAGE-C2\(^16\). In addition, Beppu et al. used an anti-MAGE-A antibody that recognized MAGE-1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A10, and MAGE-A12\(^9\). Both of these studies included several MAGE subtypes, both of which failed to recognize which subtype contributes to the strong expression in ACC. In contrast, our study determined that MAGE-1 is the precise MAGE-A subtype that is strongly expressed in ACC patients. Furthermore, Veit et al. reported that pan-MAGE expression was associated with positive lymph nodes\(^16\). Beppu et al. suggested that MAGE-A expression, along with the tumor site, tumor size, clinical stage, and histological grade were risk factors for poor prognosis\(^8\). In our study, MAGE1 expression is only associated with tumor size but not significant for ACC patient poor prognosis or other clinicopathological characters. These findings indicate that different MAGE-A subtypes are related to various clinicopathological features and contribute to reduced OS. Aside from different antibodies we used, each study has applied different methods to calculate and categorize the level of staining may be another important reason. In addition, the patient sample capacities are also unique, as Veit’s study included 84 patients from Germany, Beppu’s included 21 patients from Japan, and ours included 70 patients from China. The patients’ genetic inheritance, dietary structures, living conditions, psychosocial stress, and other environmental factors varied between each other. The distinct medical level is another non-negligible uncertainty that lead to the discordance in the three studies.

Some MAGE-A subtypes (e.g., MAGE-A2 and MAGE-A10) have been reported to be predictive factors associated with markedly poorer survival in head and neck carcinoma\(^8,16,17\). NY-ESO-1 expression was found to be correlated with a higher differentiation grade, lymph node metastasis, clinical stage, and recurrence across tumor types\(^15,19\). MAGE-C2 expression is a predictor of lymph node metastasis and recurrence in primary melanoma\(^20\) and prostate cancer\(^21\). Studies have also reported that patients with SCP-1-positive epithelial ovarian cancer tended to have a higher grade and a significantly lower survival time compared to those who were negative\(^9\). Some reports suggest that SCP-1 expression is correlated with lymph node metastasis and participates in liver metastasis\(^22,23\). Based on our results, age, vascular invasion, distant metastasis, recurrence, and histopathologic type (solid/tubular) represent important prognostic features in ACCs by a univariable survival analysis. At the same time, the level of MAGE-1 expression was significantly correlated with tumor size \((P < 0.05)\). NY-ESO-1 was significantly associated with distant metastasis \((P < 0.05)\). MAGE-C2 expression was significantly correlated with tumor site \((P < 0.05)\). SCP-1 was also significantly correlated with age and histopathological type \((P < 0.05)\). SCP-1 was strongly expressed in 5/29 of the cribriform type, 4/26 of the tubular type, and 0/15 of the solid type, indicating that SCP-1 is a potential tumor-specific ACC marker. In addition, perineural invasion was found to be an adverse factor in several studies\(^9\). Although we did not show significant evidence between perineural invasion and CTAs expression, Fig. 2 showed obvious perineural invasion related to MAGE-C2. This phenomenon has also been suggested in some other
cases. Therefore we predicted MAGE-C2 may be an important prognostic marker of ACC. Perineural invasion was present 34.3% of patients in our study, and varied widely in previous studies\(^9\), \(^25\), \(^26\). The study by Chen et al. found that perineural invasion cutaneous squamous cell carcinoma was associated with increased MAGE-A3 expression\(^27\). The results of our study suggest that larger samples are required to interpret the role of perineural invasion in ACC. Since ACC is a carcinoma with relatively low lymph node metastasis, we did not obtain optimal results.

Despite the fact that no significant correlation was identified between MAGE-A1, NY-ESO-1, MAGE-C2 and SCP-1 protein expression and the OS in our ACC patients. One novel finding was that four CTAs co-expression was related to a worse clinical outcome. ACC patients co-expressing four CTAs were associated with a reduced survival time compared with those expressing less than 4 CTAs. There were also some studies reported the co-expression of some CTA genes in triple-negative invasive bone and soft tissue tumors, as well as non-small cell lung cancer\(^20\), \(^29\). A prior study also showed that the simultaneous expression of MAGE-A and NY-ESO-1 was associated with a reduced OS\(^20\); however, the study by Beppu et al. did not obtain similar results\(^9\). This discordance may also be due to specific ethnicity, sample numbers, heterogeneity in the stages of evaluable tumors, and different immuno-histochemical methods between studies. On the other hand, previous research found that the use of the combination of MAGE-A4 and NY-ESO-1 expression was associated with an increase in the sensitivity, specificity, positive predictive values, and negative predictive values for differentiating tumors\(^29\). In our study, combined CTA expression lead to a shorter OS, which may be due to the synergistic effect between four CTAs.

Recently, CTAs have drawn attention due to their irreplaceable role in tumorigenesis. Therapeutically, since antigens derived from CTAs are highly recognizable by T lymphocytes, they are capable of generating a potent antitumor immune response\(^28\). In addition, MAGE-1, NY-ESO-1, and MAGE-C2 have been show to induce a spontaneous immune response in an autologous host\(^30\), \(^31\). Several investigations have demonstrated that MAGE-C2 displays oncogenic properties that can promote cancer cell viability, proliferation, and tumor formation\(^32\), \(^33\). We also found that MAGE-C2 may get involved in tumorigenesis and cancer progression due to its close relationship with the hallmarks of aggressive cancers. In several trials, NY-ESO-1-specific engineered T cell receptor (TCR) T cell therapy for myeloma\(^33\) and MAGE-A3-specific engineered TCR T cell immunotherapy for melanomas, cervical, esophageal, and urothelial cancer\(^34\), \(^35\) have been shown to be effective. In this study, we demonstrated that the frequent expression of MAGE-1, NY-ESO1, MAGE-C2 and SCP-1 CTAs represent potential immunological targets in ACC patients. It is clear that combined CTA-targeted immunotherapy represents the most promising method for the synergistic effects and reduction of T cell exhaustion and counter regulation\(^36\). Further verification with additional experiments and clinical trials are required to explore optional combination therapy.

In conclusion, a significant fraction of patients with ACC showed expression of MAGE-1, NY-ESO-1, MAGE-C2 and SCP-1, indicating these CTAs might represent potential antigens for cancer vaccines. In addition, MAGE-C2 may be an important prognostic marker of ACC. Further studies are urgently required to investigate the potential functions of CTAs in the salivary glands. There is also a need to elucidate the relationship between those clinicopathological factors and the survival rate at both the gene and protein level.

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**Conflict of Interest**

The authors declare that they have no conflict of interest.

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