BASIC SCIENCE: GYNECOLOGY

MAGE-A and NY-ESO-1 expression in cervical cancer: Prognostic factors and effects of chemotherapy

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OBJECTIVE: The aim of this study was to evaluate the prevalence of cancer testis tumor-associated antigens MAGE-A and NY-ESO-1 in cervical cancer and correlate expression patterns with clinicopathologic parameters and prognosis.

STUDY DESIGN: One hundred sixty-two cervical cancer samples from 109 patients who were treated with radical hysterectomy, neoadjuvant chemotherapy, or pelvic disease recurrence were analyzed by immunohistochemistry.

RESULTS: MAGE-A was expressed by 32/94 (34%) and 7/15 (47%) previously untreated and recurrent tumors, respectively. NY-ESO-1 was expressed by 46/94 (49%) and 6/15 (40%) previously untreated and recurrent tumors, respectively. MAGE-A in early stage tumors was correlated to tumor size and lymph node metastases ($P = .024$ and $P = .046$, respectively) whereas NY-ESO-1 to tumor grading ($P = .039$).

CONCLUSION: Cervical cancer frequently expresses cancer testis tumor-associated antigens. MAGE-A and NY-ESO-1 expression rates are not influenced by systemic therapies. Cancer testis tumor-associated antigens are correlated to common prognostic factors.

Key words: cancer testis tumor-associated antigens, cervical cancer, MAGE-A, neoadjuvant chemotherapy, NY-ESO-1

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Cervical cancer remains the second most common malignancy in women worldwide, with an estimated 493,000 new cases and 274,000 deaths in the year 2002.[1] The introduction of screening programs has noticeably reduced the incidence in developed countries, but most patients affected by invasive disease are still diagnosed with advanced tumors. Patients at an initial tumor stage can be treated with surgery or radiotherapy[2] and benefit from high cure rates. On the contrary, women with bulky or locally advanced disease require combined treatments such as concurrent chemoradiotherapy[3] or neoadjuvant chemotherapy followed by radical surgery.[4] Combined treatments are associated with high complication rates and poor survival rates. Survival progressively declines from 80-15% in patients affected by International Federation of Gynecology and Obstetrics (FIGO) stage Ib2-IVb, respectively.[5] New therapeutic strategies with limited side effects, such as immunotherapy, are constantly being investigated and have achieved promising results.[6]

Tumor-associated antigens (TAAs) coded by the cancer/testis (C/T) gene family are a group of antigens expressed by the trophoblast, germ line, and cancer cells.[7] To be designated to this family, these proteins must not be expressed by more than 2 nongerm line normal tissues.[8] The most-studied TAAs subgroup is the MAGE-A family. The extensive MAGE-A family of C/T antigens comprises more than 25 genes that are characterized by the presence of a large central region termed the MAGE homology domain (MHD).[9] The biologic function of TAAs in both germ lines and tumors has remained poorly understood. As far as cancerogenesis is concerned, scattered information has been gained. It has been demonstrated that MAGE-A1 represses genes that are necessary for differentiation.[10] Cell lines that express MAGE-A gene are associated with a high resistance to tumor necrosis factor-α (TNFα)–mediated cytotoxicity.[11] Finally, overexpression of MAGE-A genes is associated with resistance to doxorubicin and paclitaxel.[12] Several authors have attempted to correlate the expression of C/T TAAs with the prognosis of patients affected by various tumors. MAGE-A expression was examined in patients affected by se-

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rous ovarian neoplasms, and the results showed a significant correlation with poor prognosis.[13] In patients affected by non-small cell lung cancer (NSCLC), multitissue arrays identified the expression of MAGE-A as an independent negative prognostic factor.[14]

The primary objective of the current study was to analyze the expression of MAGE-A and NY-ESO-1 in cervical cancer patients and to evaluate their association with prognostic factors. Secondary endpoints were to evaluate modification of MAGE-A and NY-ESO-1 expression patterns after systemic treatment.

Materials and Methods

Patients

Institutional review board approval was granted by the ethical committee of the University of Rome "La Sapienza." Paraffin blocks from cervical cancer patients bearing at least 12 months of certified follow-up and treated at the Department of Gynecologic Oncology, University of Rome "La Sapienza" and Campus Bio-Medico were retrieved from the pathologic files. To examine whether expression pattern varied in different populations, patients were divided in 3 groups: patients affected by FIGO stage Ia1-Ib1 treated with primary radical surgery (group 1), patients affected by FIGO stage Ib2-IVa addressed to undergo platinum-based neoadjuvant chemotherapy, followed by radical surgery (group 2), and patients affected by recurrent cervical cancer (group 3). In particular, 34 and 15 samples were obtained from group 1 and group 3, respectively. In group 2, 60 patients were examined before and after chemotherapy. Seven patients benefited from complete pathologic response, and therefore postchemotherapy tumor specimens were unavailable for analysis. A total of 113 tumor samples were therefore available for group 2 analyses. Data including age, tumor size, FIGO stage, treatment protocol, histology, lymph node metastases, and surgical outcome were obtained from clinical and pathologic records. The duration of the follow-up was from time of pathologic diagnosis to death, dropout, or June 2006.

Immunohistochemistry

Serial formalin-fixed, paraffin-embedded cervical tumor samples were deparaffinized in xylene, followed by absolute ethanol, 95% ethanol, and distilled water. Before immunostaining procedures, sections were incubated for 5 minutes in citrate buffer (pH 6.0) in a pressure cooker at 112°C to enhance the immunoreactivity of samples. Endogenous peroxidase activity was quenched by treatment with 30% H_2O_2. After blocking of the unspecific sites, the sections were incubated at room temperature for 1 hour with mouse monoclonal α-NY-ESO-1 antibody (D8.38 clone; 1:5 dilution) and mouse monoclonal α-MAGE-A antibody (57B clone; 1:10 dilution). MoAb 57B recognizes multiple members of the MAGE-A family and predominantly MAGE-A4 in paraffin-embedded materials.[15-17] A biotin-labeled secondary antibody was used for detecting the primary antibody, followed by peroxidase-labeled streptavidin. The reaction was developed by adding 3-Amino-9-ethylcarbazole (AEC; ScyTek, Logan, UT) and the tissue sections were counterstained with hematoxylin. The number of stained tumor cells was graded as follows: focal or <5% –, negative; 5%-20% +, weak; 20%-50% ++, moderate; >50% +++, strong). Negative control slides were incubated with buffer instead of primary antibody. Two investigators blinded to the patient clinical information evaluated all specimens.

Statistical analysis

Analyses were carried out for the 3 different patient populations: patients affected by nonbulky tumors FIGO stage Ia1-Ib1 treated with radical surgery (group 1); patients affected by locally advanced disease treated with neoadjuvant chemotherapy, followed by radical surgery (group 2); and patients affected by recurrent cervical cancer (group 3). Statistical analyses were performed with the Fisher exact test or χ^2 test as appropriate. Patients were censored if lost to follow-up or alive at the end of the study period. Survival curves were plotted by means of Kaplan–Meier method and compared by using the log-rank test. A P value (2-tailed) <.05 was considered significant.

Results

The clinicopathologic characteristics of 109 patients with cervical cancer, treated between January 2000-December 2004 are summarized in Table 1. Briefly, the mean age of patients in group 1, group 2, and group 3 was 47 years (range, 31-77), 52 years (range, 29-78), and 49 years (range, 31-77), respectively. Most patients were affected by moderately or poorly differentiated squamous cell carcinoma. Patients in group 1 were mostly affected by FIGO stage Ib1 tumors, whereas patients in group 2 were affected by stage IIb-IIIb disease. Group 3 patients were all affected by local disease recurrence.

MAGE-A and NY-ESO-1 expression

In the 94 biopsy specimens from untreated patients (34 from group 1 and 60 from group 2 before chemotherapy) expression rates for MAGE-A and NY-ESO-1 were 34% (32/94) and 39% (46/94), respectively. In group 2 after chemotherapy, expression rates were 36% (19/53) and 40% (21/53) for MAGE-A and NY-ESO-1, respectively. In group 3, MAGE-A and NY-ESO-1 were expressed by 47% (7/15) and 40% (6/15) of patients, respectively.

One-hundred sixty-two tumor samples were obtained from 109 patients. One hundred thirty-one (80%) cervical cancer samples were immunopositive for at least 1 C/T-TAA. In particular 31 (19%) were positive for both MAGE-A and NY-ESO-1 and 100 (61%) were positive only for 1 antigen.

Fifty-eight (36%) cervical cancer samples were immunopositive on staining with anti-MAGE-A. Immunoreactivity for MAGE-A was strong, moderate, and weak in 17 (10%), 22 (14%), and 19