Human Pathology (2014) **45**, 259–267

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Human PATHOLOGY

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Original contribution

Expression of New York esophageal squamous cell carcinoma-1 in primary and metastatic melanoma ☆

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Received 16 January 2013; revised 21 May 2013; accepted 31 May 2013

Keywords:
NY-ESO-1;
Melanoma;
Cancer testis antigen;
Immunotherapy

Summary New York esophageal squamous cell carcinoma-1 (NY-ESO-1), a cancer testis antigen, is an ideal target for adoptive cell transfer immunotherapy. Evidence from several clinical trials in melanoma and other malignancies shows the potential value of targeting the NY-ESO-1 antigen in immune-based therapy of metastatic tumors. However, the incidence of NY-ESO-1 expression in metastatic melanoma is unknown, and thus, it is unclear how many patients might benefit from this therapy. In this study, we analyzed NY-ESO-1 expression in 222 melanoma specimens, including 16 primary and 206 metastatic tumors. Our results support previous findings showing higher expression of NY-ESO-1 in metastatic (58/206; 28.2%) versus primary (0/16) tumors. In addition, our results show that the epithelioid subtype of melanoma has the highest incidence of NY-ESO-1 expression. These findings provide evidence of the value of this specific adoptive cell transfer therapy for the treatment of metastatic melanoma.
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1. Introduction

The diagnosis of melanocytic lesions is challenging because of their diverse morphology and similarity to many poorly differentiated metastatic malignancies. Diagnosis is primarily based on histopathologic analysis and ancillary studies such as immunohistochemistry of melanocytic markers [1]. New York esophageal squamous cell carcinoma-1 (NY-ESO-1), a member of the cancer testis antigen (CTA) family, has been suggested as a prognostic marker of advanced-stage disease because of its higher expression in metastatic than in primary melanomas [2].

Studies examining the diagnostic role of CTAs are ongoing, so far with discordant data. However, it has been suggested that expression of CTAs may be useful in differentiating between melanoma and benign melanocytic lesions with atypical features [3].

Immunotherapy is a promising therapeutic option for patients with metastatic melanoma. CTAs have been studied as potential therapeutic targets and/or diagnostic/prognostic markers in melanoma and other malignant lesions [2-6]. Humoral and cellular responses to NY-ESO-1 have been analyzed extensively in patients with various cancers, such as gastrointestinal, hematogenous, and skin malignancies as well as ovarian and urothelial carcinomas [7-10].

The adoptive transfer of cultured melanoma-reactive T cells, isolated from autologous tumor-infiltrating lymphocytes after

☆ Disclosure: The authors declare that they have no conflicting financial interests.

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0046-8177/$ – see front matter. Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.humpath.2013.05.029

lymphodepletion chemotherapy, mediates objective tumor regression in 49% to 72% of patients with metastatic melanoma [11,12]. By using genetically modified lymphocytes targeting a specific tumor marker, adoptive cell transfer has proved a highly effective treatment for patients with metastatic melanomas [13]. Rosenberg et al from our institution [4,14] have also published findings that T-cell receptor–based gene therapies targeting NY-ESO-1 represent a new and effective therapeutic approach for patients with metastatic melanoma. This study confirms NY-ESO-1 as an immunogenic tumor marker that could play an important role in the treatment of metastatic melanoma. The immunohistochemical staining profiling of these tumors is critical for patient selection and potential enrollment in targeted adoptive cell transfer immunotherapy clinical trials.

In this study, we characterized the expression of NY-ESO-1 in primary and metastatic melanomas, including melanomas of various morphologic subtypes. To our knowledge, this study is one of the largest evaluating NY-ESO-1 expression in metastatic melanomas of different morphologies.

2. Materials and methods

2.1. Case selection

A series of 16 primary and 206 metastatic melanoma specimens from 186 patients were retrieved from the archives of the Laboratory of Pathology at the National Cancer Institute between April 2008 and April 2009. All studies were carried out in accordance with the approved guidelines of the National Cancer Institute Institutional Review Board. Cases with available paraffin blocks or unstained slides were selected, and all were reviewed histologically to confirm the diagnosis. The available clinicopathologic information of the primary melanomas is summarized in Table 1. For the 16 primary cancers, the median thickness was 3.12 mm (mean, 4.93 mm; range, 0.0-25.0 mm). Of these tumors, 2 (12.5%) were less than 1.0 mm in Breslow thickness (defined as thin), 8 (50.0%) were 1.01 to 4.0 mm thick (intermediate), and 6 (37.5%) were greater than 4.0 mm thick. Ten tumors (62.5%) were axial, and 6 (37.5%) were on the extremities. The average patient age was 53.1 years with a male predominance (male-to-female ratio, 2.2:1.0). Histologically, 5 (31.3%) of these primary melanomas were of the superficial spreading type; 7 (43.8%) were nodular; and 1 (6.3%) each were acral lentiginous, mucosal lentiginous, and lentigo maligna type. There was 1 case classified as T_0 (malignant melanoma in situ) and 1 case as T_{1b}. Five cases were T_{3a}, 3 cases T_{3b}, and 6 T_{4b}. The distribution of tumor stage among the cases was as follows: stage 0 (n = 1), stage II (n = 6), stage III (n = 3), and stage IV (n = 6).

2.2. Classification of the lesions

All cases were classified into morphologic subtypes, namely, epithelioid, spindle cell, and mixed (epithelioid and spindle). Tumors with anaplastic, balloon cell, or desmoplastic features were specially annotated. Lesions had to have more than 50% of the tumor cells of either epithelioid or spindle cell morphology to be classified into either category. Lesions without predominant cell morphology were classified as mixed (epithelioid and spindle). The classification of cell morphology subtype was performed and agreed on by 2 pathologists (Y. C. L. and C. C. L.).

2.3. Immunohistochemical staining analysis

The primary antibodies, their sources, and the dilutions used were as follows:

- NY-ESO-1 (1:100; Invitrogen, Carlsbad, CA);
- MART1 (no. CMC756, 1:200; Cell Marque, Rocklin, CA);
- Tyrosinase (no. NCL-TYROS, 1:20; Novocastra Division, Leica Microsystems, Buffalo Grove, IL);
- HMB45 (no. 30930, 1:4; Enzo Life Sciences, Farmingdale, NY);
- S-100 (1:8000; BioGenex, Fremont, CA).

Paraffin-embedded tissue sections of 5 mm were deparaffinized through xylene and graded alcohols. Immunohistochemical staining was performed following heat-induced epitope retrieval with target retrieval solution (low pH; DAKO, Carpinteria, CA). Slides were incubated in Tris with 3% goat serum for 15 minutes and then incubated at room temperature with primary antibody for 1 to 2 hours. Detection was carried out using an automated slide stainer (Autostainer; DAKO) with a horseradish peroxidase/3,3′-diaminobenzidine polymer-based detection system (Envision+; DAKO). The immunohistochemical staining was classified as positive or negative by 2 pathologists (Y. C. L. and C. L. L.). Red chromogen was used in heavily pigmented melanomas. For cases with limited unstained material, the missing results were classified as unknown.

2.4. Statistics

The JMP 5.1 software (SAS, Cary, NC) was used for the statistical analyses. The χ^2 test was used to characterize the immunohistochemical results. $P < .05$ was considered statistically significant.

3. Results

3.1. Melanoma-associated marker expression in primary and metastatic melanoma

The NY-ESO-1 stain was negative in all the primary melanomas and positive in 58 (28.2%) of the metastatic melanomas (Table 2). In comparison, expression of S-100