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Original contribution

Cancer-testis antigen expression in synovial sarcoma: NY-ESO-1, PRAME, MAGEA4, and MAGEA1☆,☆☆

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Summary Synovial sarcoma (SS) is regarded as a relatively chemosensitive sarcoma, but the prognosis of advanced SSs remains poor. Here we identified highly expressed cancer-testis antigens that could be promising immunotherapy targets for SS, using a previously conducted cDNA microarray, and we assessed the clinicopathological or prognostic relationships of these antigens in SS. We compared the gene expression profiles of 11 SSs with those of 3 normal adipose tissues. Among the up-regulated cancer-testis antigens, we analyzed PRAME, MAGEA1, and MAGEA4 and another cancer-testis antigen (NY-ESO-1) together, by immunohistochemistry and real-time polymerase chain reaction in 108 SSs. Immunohistochemically, NY-ESO-1, PRAME, MAGEA4, and MAGEA1 were positive in 66 (61%), 93 (86%), 89 (82%), and 16 (15%) of 108 SSs, respectively, and 104 (96%) of 108 SSs showed the immunohistochemical expression of at least 1 of NY-ESO-1, PRAME, and MAGEA4. Moreover, the high expression of at least 1 of these 3 antigens was observed in 83% of the SSs. High expression of NY-ESO-1 and MAGEA4 was significantly correlated with the presence of necrosis and advanced clinical stage. The immunohistochemical expression of these cancer-testis antigens was not correlated with prognosis, but the coexpression of NY-ESO-1, PRAME, and MAGEA4 was significantly associated with adverse prognosis. The real-time polymerase chain reaction results were closely related to the immunohistochemical results: NY-ESO-1 ($P = .0019$), PRAME ($P = .039$), MAGEA4 ($P = .0149$), and MAGEA1 ($P = .0766$). These data support the potential utility of NY-ESO-1, PRAME, and MAGEA4 as immunotherapy targets and ancillary prognostic parameters, suggesting the possible benefit of the combined use of these cancer-testis antigens as an SS immunotherapy target.

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1. Introduction

Synovial sarcoma (SS) has been classified as a tumor of uncertain differentiation, accounting for 5% to 10% of adult soft tissue sarcomas and affecting mainly young adults [1]. Cytogenetic studies frequently show chromosomal translocation t(X; 18) (p11.2; q11.2), which represents the fusion of *SS18* with either *SSX1* or *SSX2*, or rarely with *SSX4* [2].

SS is regarded as a relatively chemosensitive sarcoma, but when the disease is advanced, the prognosis is still poor. With respect to the morphology histologic grade, mitotic activity, age at diagnosis, and primary tumor size have been reported as prognostic factors [3,4]. The *SS18-SSX1* genotype, deletion of PTEN, and overexpression of p53, Ki-67, nuclear YB-1, or the Akt-mTOR pathway were reported as prognostic factors, but not critical [5-7]. Further studies of prognostic factors and therapeutic targets in SS are required.

Cancer-testis (CT) antigen has been described as a promising immunotherapy target because of its low or absent expression in normal tissue — except for the testes, which are immunoprivileged because of their lack of human leukocyte antigen class 1 [8]. CT antigens were reported to be expressed in various malignant tumors, and it was also reported that an increased expression of CT antigens is associated with high tumor grade or poor prognosis in various cancers [9-11].

The DNA microarray technique has been used to identify disease-associated biomarkers, create gene-based tumor classifications, distinguish tumor subclasses, and predict outcomes [12]. In the present study, we compared the gene expression profiles of SSs with those of normal adipose tissues by using cDNA microarray analysis data we obtained previously [13,14] (accession nos. GSE59568 and GSE65532) to detect up-regulated CT antigens that have potential as new therapeutic targets and prognostic markers.

Among the up-regulated CT antigen genes in SSs, we focused on *PRAME*, *MAGEA1*, and *MAGEA4,* for which several clinical trials of immunotherapy have been undertaken and are also currently underway [15-17]. Previous studies demonstrated that another CT antigen, NY-ESO-1 (*CTAG1B*), which is also reported as a promising target for immunotherapy [18], is highly expressed in SS [19-21]. Several clinical trials of immunotherapy targets for NY-ESO-1 were also undertaken and are currently underway [22]. We therefore investigated the expressions of PRAME, MAGEA1, MAGEA4, and NY-ESO-1 by immunohistochemistry and real-time polymerase chain reaction (PCR) in a large-scale investigation of SS.

2. Materials and methods

2.1. Patients and tissue samples

We retrieved the 108 paraffin-embedded primary SS specimens from the files of soft tissue tumors registered in the Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan, between 1985 and 2014. Each tumor was reclassified according to the most recent World Health Organization classification, by bone and soft tissue tumor pathologists. [1] Twenty frozen SS materials for real-time PCR were available. As controls for the real-time PCR, we examined 3 samples of surrounding nontumorous adipose tissue.

The specimens' histologic grades were evaluated according to the grading system of the French Federation of Cancer Centers [23]. The staging system described in the seventh edition of the American Joint Committee on Cancer (AJCC) manual was used [24]. The study was approved by the Ethics Committee of Kyushu University (No. 26-49) and conducted according to the Ethical Guidelines for Epidemiological Research enacted by the Japanese Government.

2.2. RNA preparation and gene microarray analysis

RNA was extracted from frozen samples as previously described [13]. The cDNA analysis was also conducted as described [13], using a 3D-Gene Human Oligo chip 25k (Toray Industries, Tokyo, Japan) with 25 370 distinct genes.

2.3. Fusion gene analysis

This assay was performed as described [13] and was based on the described primers [25] that specifically amplify the fusion gene transcripts of *SS18-SSX1* and *SS18-SSX2*.

2.4. Immunohistochemical staining and evaluation

Immunohistochemical staining was conducted as described [13]. Testis tissue was used as the positive control. Antigen retrieval, the primary antibodies, and dilutions are summarized in Supplementary Table 1. The immunohistochemical results were judged by 3 investigators who were blinded to the clinical data of the patients. A consensus judgment was adopted as the proper immunohistochemical result.

The percentage of immunoreactive cells and staining intensity were assessed in the most representative areas. The proportion of immunoreactive cells was scored as 0 to 4 (0, <5%; 1, 5% to <25%; 2, 25% to <50%; 3, 50% to <75%; 4, ≥ 75%), and the intensity was scored as 0 to 3 (0, negative; 1, weak staining; 2, moderate staining; 3, strong staining). The total score (proportion score + intensity score) was evaluated, and cases with a total score of 3 or higher were judged as positive, and a total score of 5 or higher was defined as high expression, as described [13].

2.5. Quantitative real-time reverse-transcription PCR

Quantitative real-time reverse-transcription PCR was conducted as previously described [13]. We purchased the TaqMan probes for *PRAME* (Hs01022301_m1), *CTAG1B* (Hs00265824_m1; which encodes for NY-ESO-1), *MAGEA1*