

Evaluation of Immune-Biomarker Expression in High Grade Soft Tissue Sarcoma : Emphasis of importance of HLA-DQA1 expression as a prognostic marker

Jung Yun Bae

Pusan National University Yangsan Hospital

Kyung Un Choi

Pusan National University Hospital

Ahrong Kim

Pusan National University Hospital

So Jung Lee

Pusan National University Hospital

Kyungbin Kim

Pusan National University Hospital

Jee Yeon Kim

Pusan National University Yangsan Hospital

In Sook Lee

Pusan National University Hospital

So Hak Chung

Kosin University Gospel Hospital

Jeung Il Kim (✉ dlsgo90@hanmail.net)

Pusan National University School of Medicine

Research

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Abstract

Background: High-grade soft tissue sarcoma (STS) is a highly malignant neoplasm having an overall poor prognosis. Numerous prognostic factors determine tumor progression and patient outcome. Many immune-related cells identified in the tumor microenvironment play important roles in various tumor types. This study was undertaken to evaluate the expression of immune-related genes and to elucidate the correlation between these genes and prognosis in high grade STS.

Methods: Twelve cases of formalin-fixed paraffin embedded tissue samples of high grade STS were included for gene expression analysis using the NanoString nCounter® System and 35 cases were used for immunohistochemistry. For comparison analysis, the patients were divided into two groups, depending on the overall survival (OS). Expressions of 770 Genes were analyzed using the nCounter® PanCancer Immune Profiling Panel of the NanoString nCounter® Analysis System. Immunohistochemistry for the most significantly altered genes was additionally performed. The correlation between gene expression and prognosis of high grade STS was then evaluated.

Results: Of the 770 immune-related genes analyzed by NanoString nCounter® Analysis, several genes were identified as differentially expressed between the two groups. Based on the gene expression level and fold change, representative 13 genes were identified: 7 of the 13 candidate genes (C3, CD36, DOCK9, FCER2, FOS, HLA-DRB4, and NCAM1) were found to be significantly overexpressed in the poor prognostic group. In contrast, expressions of the other 6 immune-related genes (BIRC5, DUSP4, FOXP3, HLA-DQA1, HLA-DQB1, and LAG3) were increased in the good prognostic group. Positive expressions of HLA-DQA1, HLA-DQB1, and HLA-DRB4 were obtained in 74.3%, 34.3% and 48.6% tumors, respectively. The positive expression of HLA-DQA1 was associated with a significantly longer OS ($p=0.028$). .

Conclusions: Analysis of gene expression determined that expressions of 13 immune-related genes were significantly different between two groups. HLA-DQA1 and HLA-DQB1 were significantly decreased, whereas HLA-DRB4 was significantly increased in the poor prognostic group. HLA-DQA1 expression was significantly correlated with long-term survival, and may therefore be an immune-biomarker for good prognosis of high grade STS.

Background

Soft tissue sarcomas (STS) are rare malignant tumors that account for 1% of adult cancers. They are a heterogeneous group, comprising more than 50 different types [1]. The diagnosis of STS is based on the morphologic pattern and immunohistochemistry (IHC), in addition to the traditional hematoxylin-eosin (H-E) staining. An increasing number of specific genetic aberrations, including chromosomal translocations, gene amplification, and mutation have been identified in various types of sarcoma. These findings led to the recommendation of conducting molecular tests such as reverse transcription polymerase chain reaction (RT-PCR), fluorescence in situ hybridization (FISH), and next generation sequencing (NGS) [2, 3]. Histologic diagnosis is important in predicting the STS outcome. Sarcoma grading is also widely used as

a predictor for outcome of the majority STS and several different grading systems have been developed [4, 5]. Irrespective of the system used, high grade STS has a higher rate of metastasis and lower survival rate, and their identification may influence clinical decisions made during primary tumor treatment [6]. Although surgical resection is the primary treatment method, conventional chemotherapy is generally used in patients with high grade and/or advanced stage STS. In high-risk patients, the tumor is not completely resected and the prognosis is poor, despite additional treatment. Patients are also reported to suffer from treatment toxicity [7]. These data emphasize the urgent need for new therapies [8].

Many cells related to the immune system detected in the tumor microenvironment show genetic mutations that are essential for prognosis or treatment of the different tumor types [9]. Recent studies show that immunotherapy is widely used to effectively treat solid tumors. The FDA has also recently approved pazopanib, a multi-tyrosine kinase receptor inhibitor, after a study demonstrated that the treated group for STS showed an increased progression-free survival compared to the placebo group [8]. Immune checkpoint inhibitors targeting the PD-1/PD-L1 signaling axis are being explored as a new treatment modality in STS, and the evaluation of PD1- and PD-L1-positivity in STS could be possible criteria for selecting suitability of patients for PD1-based immunotherapy [10, 11].

Analyzing immune-related genes of STS in the tumor microenvironment could consequently be helpful in identifying useful validated markers for predicting the prognosis and management of STS. The present study evaluated the expressions of immune-related genes in high grade STS using the NanoString nCounter® PanCancer Immune Profiling Panel, that is a novel multiplex gene expression panel designed to quantitate 770 genes from different immune cell types and populations covering both adaptive and innate immune responses, common checkpoint inhibitors, tumor-specific antigens, and housekeeping genes and applied to identify tumor-specific immune targets [12]. The selected genes were subsequently evaluated for clinicopathological significance through additional immunohistochemistry using paraffin blocks of the high grade STS.

Materials And Methods

Patients and tissue samples

High grade STS tissue samples were obtained by surgical resection between January 1998 and December 2013, performed at the Pusan National University Hospital, Busan, Korea. Twelve formalin-fixed paraffin embedded blocks were selected for gene expression analysis using the NanoString nCounter® System and 35 cases were subjected to immunohistochemistry. Diagnoses were confirmed by pathological analysis using the diagnostic criteria defined in the World Health Organization (WHO) classification and histologic grade was evaluated according to the French Federation of Cancer Centers (FNCLCC) sarcoma group grading system. All cases showed morphologically pleomorphic tumor cells, and were determined to be FNCLCC grade 2 or 3 high grade STS. The 35 cases were analyzed and identified as 27 undifferentiated pleomorphic sarcoma (UPS), 5 high grade myxofibrosarcoma (MFS), 2 leiomyosarcoma (LMS), and 1 rhabdomyosarcoma (RMS). Clinical information was obtained from the

medical records. The overall survival (OS) was calculated from the date of surgery to the date of mortality or last followup visit. For comparative analysis, patients were divided into two categories: good prognosis group versus poor prognosis group depending on the survival during the follow-up period.

Written informed consent from the patients, and approval from the Institutional Ethics of Pusan National University Hospital, were obtained prior to use of these materials. The biospecimens and data used for this study were provided by the Biobank of Pusan National University Hospital, a member of the Korea Biobank Network.

NanoString nCounter® PanCancer Immune Profiling Panel for gene expression analysis

Formalin-fixed paraffin blocks were selected from 12 high grade STS cases for gene expression analysis using the nCounter® PanCancer Immune Profiling Panel (Nanostring Technologies, Inc., Seattle, WA, USA), a unique 770-multiplex gene expression panel that determines the human immune response in all cancer types. The 12 high grade STS cases had 7 cases belonging to the good prognostic group with survival, and 5 cases of death during the follow-up period.

Total RNA was extracted using the RNeasy mini prep kit (Qiagen, Valencia, CA, USA). The RNA yield and purity were assessed using a DS 11 Spectrophotometer (Denovix Inc, DE, USA) and the RNA quality check was performed using Fragment Analyzer™ (Advanced Analytical Technologies, IA, USA). Total RNA (100 ng) was assayed on the nCounter Digital Analyzer (Nanostring Technologies), according to the manufacturer's protocol. Total RNA was processed using the digital multiplexed nCounter® human mRNA expression assay and the Human Pancancer Immune Profiling Panel Kit. Hybridizations were carried out by combining 5 µl of each RNA sample with 8 µl of nCounter® Reporter probes in the hybridization buffer and 2 µl of nCounter® Capture probes (for a total reaction volume of 15 µl) overnight at 65 °C for 18 hr. Excess probes were removed by a two-step magnetic bead based purification of the nCounter® Prep Station. Specific target molecules were then quantified on the nCounter® Digital Analyzer by counting the individual fluorescent barcodes and assessing the target molecules. For each assay, a high-density scan encompassing 280 fields of view was performed. The data was collected using the nCounter® Digital Analyzer after taking images of the immobilized fluorescent reporters in the sample cartridge with a charge-coupled device (CCD) camera.

The mRNA data analysis was performed using the freely available nSolver™ analysis software (NanoString Technology), with the mRNA profiling data normalized using housekeeping genes. R software was used for the analysis. Changes greater than 2-fold upregulation or downregulation were considered significant. The genes greater than 2 fold change and p-value < 0.05 between the two groups were selected. The heat map of gene expression for differently expressed genes between the good and poor prognostic group was then plotted.

Immunohistochemical Staining And Analysis

Sections were transferred to poly-L-lysine-coated glass slides, dewaxed in xylene, and rehydrated in ethanol. Staining was performed using the BondMax autostainer and reagents (Vision Biosystems). Deparaffinization was performed automatically in the autostainer with BondWash solution at 72 °C for 30 minutes. Slides were then incubated sequentially with the Epitope Retrieval Solution 1 (Leica Microsystems, Wetzlar, Germany) for 20 minutes at 100 °C, peroxide block for 5 minutes, primary monoclonal antibody for 15 minutes, post primary reagent for 8 minutes, and polymer for 8 minutes. HLA-DQA1 (rabbit monoclonal, ab128959, Abcam, Cambridge, UK), HLA-DQB1 (rabbit polyclonal, ab224600, Abcam, Cambridge, UK), and HLA-DRB4 (rabbit monoclonal, ab140612, Abcam, Cambridge, UK) were used as primary antibodies. Human tonsil tissue was used as positive control, due to the known expression of these markers.

For all primary antibodies, the cases were considered positive if cells showed cytoplasmic and/or membranous staining. A combined positive score (CPS) was used for the immunohistochemical scoring method. The CPS was estimated by summing the number of positively stained cells (tumor cells, lymphocytes, macrophages) and dividing the result by total number of viable tumor cells, multiplied by 100. Immunohistochemical results were considered positive expression of HLA-DQA1, HLA-DQB1, and HLA-DRB4 if CPS was 1 or more, and was considered as negatively expressed when was less than 1 [13].

$$\text{CPS} = \frac{\text{Number of Positively stained cells (tumor cells, lymphocytes, macrophages)}}{\text{Total number of viable tumor cells}} \times 100$$

Statistical analysis was conducted using the SPSS 22.0 software (SPSS, Chicago, IL, USA). The associations between overall survival and expressions of HLA-DQA1, HLA-DQB1, and HLA-DRB4 were assessed using Pearson's χ^2 test, and calculated using the KaplanMeier logrank test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

NanoString nCounter® PanCancer Immune Profiling

The 12 patients selected for gene expression analysis were aged 50 to 86 years (average 56 years). Patient follow-up was done from the date of surgery until death or last visit to the hospital. The follow-up period ranged from 4 to 122 months (mean 68.3 months). Totally, 7 patients survived during the follow-up period and were classified as the good prognostic group, while 5 died of disease and were classified as the poor prognosis group.

Nanostring nCounter® Analysis was performed for all samples, and gene expressions were compared between the two groups. In the 770 immune-related genes panel of the nCounter® Analysis, a number of genes were found differentially expressed in the two groups, and 13 representative genes were selected based on gene expression levels and fold changes (Fig. 1). Of the 13 candidate genes, 7 genes (HLA-

DRB4, NCAM1, CD36, CD3, FOS, FCER2, DOCK9) showed a significant increase in the poor prognostic group (Table 1). In contrast, the expressions of 6 immune-related genes (HLA-DQA1, HLA-DQB1, LAG3, FOXP3, BIRC5, DUSP4) were increased in the good prognostic group (Table 2). The associated molecules are classified according to the primary function of each gene: antigen processing and presentation (HLA-DRB4, HLA-DQA1, HLA-DQB1), adhesion molecule (NCAM1), transporter function (CD36), regulation of immune response (C3, FCER2, LAG3), cytokine (FOXP3), cell cycle (BIRC5) and others (FOS, DOCK9, DUSP4).

Table 1
Seven genes showing increased expression in the poor prognostic group by the NanoString nCounter® analysis.

Gene Name	mRNA/probe ID	Fold Change	Gene Annotation	p-value
HLA-DRB4	NM_021983.4:194	56.93	Antigen processing and presentation	0.011
NCAM1	NM_000615.5:1620	6.12	Adhesion molecules	0.046
CD36	NM_001001548.2:705	5.24	Transporter function	0.016
C3	NM_000064.2:4396	3.61	Immune response regulation	0.046
FOS	NM_005252.2:1475	3.51	Innate immune response	0.010
FCER2	NM_002002.4:420	3.06	Immune response regulation	0.043
DOCK9	NM_001130048.1:1020	2.17	Immune cell function	0.016

Table 2
Six genes showing significant expression in the good prognostic group by the NanoString nCounter® analysis.

Gene Name	mRNA/probe ID	Fold Change	Gene Annotation	p-value
HLA-DQA1	NM_002122.3:261	77.78	Antigen processing and presentation	0.026
HLA-DQB1	NM_002123.3:384	28.94	Antigen processing and presentation	0.022
LAG3	NM_002286.5:1735	2.91	Immune response regulation T-cell function	0.015
FOXP3	NM_014009.3:1230	2.41	Cytokines and receptors T-cell function	0.020
BIRC5	NM_001168.2:1215	2.38	G2 phase and G2/M transition	0.001
DUSP4	NM_057158.2:3115	2.18	Innate immune response	0.043

Immunohistochemical Analysis

Study subjects included 35 high grade STS patients, including 19 males and 16 females with an average age of 66.2 years (range: 39–86 years). Anatomical locations of the tumors were 25 patients in a lower limb, 8 in an upper limb, and 2 in the trunk. Of these, 26 patients had tumor sizes less than 10 cm, while the other patients had tumor sizes greater than 10 cm. The average follow-up period was 69.1 months (range: 4–150 months). As the clinical information was obtained from medical records, the overall survival (OS) was calculated from the date of surgery to the date of mortality or last follow up visit. Totally, 15 patients survived during the follow up period and were classified as the good prognostic group, while 20 patients who died of the disease were classified as the poor prognosis group. Clinicopathological data are summarized in Table 3.

Table 3

Clinicopathological features of 35 high grade STS patients for immunohisto-chemical study.

	All	Good prognostic group	Poor prognostic group
	n = 35	n = 15	n = 20
Age	66.2	62.3	67.9
Gender			
Male	16	6	13
Female	19	9	7
Tumor location			
Upper extremity	8	0	8
Lower extremity	25	14	11
Trunk	2	1	1
Tumor size (cm)			
≤10 cm	26	13	13
>10 cm	9	2	7
AJCC stage			
I	23	11	12
II	11	4	7
IV	1	0	1
Histological diagnosis			
UPS	27	11	16
High grade myxofibrosarcoma	5	3	2
Leiomyosarcoma	2	1	1
Rhabdomyosarcoma	1	0	1
UPS: undifferentiated pleomorphic sarcoma			

Based on data obtained from the Nanostring nCounter® Analysis, HLA-DQA1, HLA-DQB1, and HLA-DRB4 showed greatest difference in fold change, and were selected for further immunohistochemical studies using paraffin embedded blocks of the 35 patients. The data analyzed from immunohistochemistry were read as positive if the cells were either cytoplasmic or membranous stained; such results were analyzed through the combined positive score (CPS) (12) (Fig. 2). Positive expressions of HLA-DQA1, HLA-DQB1,

and HLA-DRB4 were obtained in 74.3% (26/35), 34.3% (12/35) and 48.6% (17/35) tumors, respectively (Table 4). For HLA-DQA1, 26 (74.3%) of patients showed positive expression, comprising of 14 cases (53.9%) in the good prognostic group and 12 cases (46.1%) in the poor prognostic group (46.1%), whereas only 1 (11.1%) survived and 8 (88.9%) died of disease; a statistically significant difference between the two groups, with an increase of survival rate in the positive expression group ($p = 0.028$). HLA-DQB1 showed positive expression in 12 (34.3%) patients, with 4 (33.3%) in the good prognostic group and 8 (66.7%) in the poor prognostic group, but showing no significant difference ($p = 0.489$). HLA-DRB4 positivity was observed in 17 (48.6%) patients, with no statistically significant difference between 6 (35.3%) in the good prognostic group and 11 (64.7%) of the poor prognostic group ($p = 0.500$). The positive expression of HLA-DQA1 was associated with a significantly longer OS ($p = 0.028$) (Fig. 3).

Table 4
Survival analysis of results of immunohistochemistry for HLA - DQA1, HLA-DQB1, HLA-DRB4.

	Survival (n = 15) (%)	DOD (n = 20) (%)	Total (n = 35)	p-value
HLA DQA1				
CPS (-)	1 (11.1)	8 (88.9)	9	0.028
CPS (+)	14 (53.9)	12 (46.1)	26	
HLA DQB1				
CPS (-)	11 (47.8)	12 (52.2)	23	0.489
CPS (+)	4 (33.3)	8 (66.7)	12	
HLA DRB4				
CPS (-)	9 (50)	9 (50)	18	0.500
CPS (+)	6 (35.3)	11 (64.7)	17	
DOD: die of disease				
CPS: combined positive score				

Discussion

STs have a mortality rate ranging from 40–60% for high-grade lesions. The predictors of survival time in patients with high grade STS include tumor size, histology, grading, margin status at resection, and the presence of pre-surgical metastasis [4]. Standard treatment of high grade STS includes surgical resection, with radiation therapy as a supplementary treatment and chemotherapy, if required. Based on the detected genetic mutations, target therapy may also be performed [14, 15]. A recent study reports that co-administration of the monoclonal antibody Olaratumab, which acts against the platelet-derived growth

factor receptor alpha (PDGFR α), and the traditional chemotherapy agent doxorubicin, increases the overall long-term survival rate [16]. A better understanding of prognostic factors is required to apprise patients for prognosis and treatment regimens.

A few studies have reported immune-response and action of immune checkpoint inhibitors in STS [8–11]. Recent years has seen the emergence of programmed death-1 (PD-1) and programmed death-ligand 1 (PD-L1) as new targets in cancer immunotherapy. PD-1 and PD-L1 expressions were detected in the tumor tissue and microenvironment in some types of STS. The association of their expressions and clinical outcomes provided the importance of immune checkpoint inhibitions in patients with high grade STS [10].

The current study investigated expressions of diverse immune-related genes in high grade STS according to the prognosis, and further examined the correlation between immune-related genes and survival using formalin-fixed, paraffin embedded material. This was achieved on the fully automated and highly precise Nanostring nCounter $\text{\textcircled{R}}$ system used for gene expression analysis, which accurately detects and counts 770 transcripts from 24 different immune cells, including adaptive immune response, innate immune response, common checkpoint inhibitors, tumor-specific antigens, and housekeeping genes, using a small amount of mRNA based on the digital color-coded barcode technology [12]. The Nanostring nCounter $\text{\textcircled{R}}$ Sarcoma Fusion CodeSet has been recently introduced in the STS research area to assess fusion transcripts using formalin-fixed, paraffin-embedded material [17]. The custom-designed NanoString nCounter $\text{\textcircled{R}}$ based sarcoma assay is highly sensitive and specific in a clinical setting for sarcoma molecular diagnosis.

Our results reveal significant genetic variations between the survival group (good prognosis group) and DOD group (poor prognosis group). The criteria were applied to select an important gene, having a p value < 0.05 and a 2- or more fold change between the two groups. In all, 13 genes were selected; 7 immune-related genes (C3, CD36, DOCK9, FCER2, FOS, HLA-DRB4, and NCAM1) were significantly increased in the poor prognosis group. In contrast, the expression of 6 immune-related genes (BIRC5, DUSP4, FOXP3, HLA-DQA1, HLA-DQB1, and LAG3) were increased in the good prognosis group.

NCAM1 (neural cell adhesion molecule 1) encodes a cell adhesion protein which is a member of the immunoglobulin superfamily. The encoded protein is reported to be involved in the expansion of T cells and dendritic cells, which play an important role in immune surveillance. Some reports that NCAM1 is related to therapeutic resistance in cancer [18, 19]. CD36 plays a role in immune signaling as well as a scavenger receptor for fatty acid uptake that modulates cell-to-extracellular matrix attachment and TGF β activation. CD36 has increasingly emerged as a prognostic marker associated with the metastatic process [20]. Complement C3 is useful in the development of novel strategies to improve the effectiveness of cancer immunotherapy [21]. FCER2 (Fc fragment of IgE receptor II) encoded by this gene is a B-cell specific antigen, and has essential roles in B cell growth and differentiation, and the regulation of IgE production, although it remains unknown how this gene plays a role in the development or treatment of cancer [22]. DOCK (decicator of cytokines) are a family of guanine-nucleotide exchange

factors (GEF) for Rho GTPases and FOS is a subunit of AP-1 transcription factor. Their functions are not well understood in the function of immune system and cancer.

BIRC5, also called survivin, is a well-known cancer therapeutic target. BIRC5 covers a numerous mechanisms of action in immune responses as well as in molecular cancer diagnostics and therapeutics [23]. Survivin peptide immunogen-reactive antibodies are considered to exert an additional advantage for survivin immunotherapy. Survivin-specific T-cell reactivity strongly correlates with tumor response and patient survival [24]. This study revealed reduced levels of survivin in the poor prognosis group, hence, the possibility as a therapeutic target need to be considered in cases of high grade STS. FOXP3 (forkhead box P3) is a member of the forkhead/winged-helix family of transcriptional regulators and is associated with T cell function. Regulatory T cells expressing the transcription factor FOXP3 have a critical role in the maintenance of immune homeostasis and prevention of autoimmunity and cancer pathogenesis [25]. Due to their ability to suppress self-antigen responses, regulatory T cells with FOXP3 may have anti-tumor immune function, whereas increased FOXP3 expression in tumor tissue is reported to be associated with a better prognosis in some cancers. This discrepancy was identified particularly in colorectal cancers [26, 27]. In the current study, FOXP3 expression was increased in the good prognosis group and decreased in the poor prognosis of high grade STS. These findings underline the need for accurate assessment of FOXP3 expression in tumor immunity. DUSP4 (dual specificity phosphatase 4) negatively regulate members of the mitogen-activated protein (MAP) kinase superfamily which are associated with cellular proliferation and differentiation. DUSP4 promotes doxorubicin resistance in gastric cancer through epithelial-mesenchymal transition [28].

The proteins encoded by HLA-DRB4, HLA-DQA1, and HLA-DQB1 are associated with antigen processing and presentation. HLA, the human leukocyte antigen, is a cell membrane glycoprotein expressed on the surface of human nucleated cells, and induces an adaptive immune response against invading antigens by presenting an antigen to T lymphocytes, and protecting normal cells from the apoptotic function of natural killer cells. HLA class II gene locus includes 9 types (HLA-DRA, -DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, -DPA1, -DPB1), and the HLA class II DR, DQ, and DP molecules are expressed by antigen-presenting cells (APCs). HLA genes have been reported over a period of several decades: HLA-DQA1, HLA-DQB1 and DRB4 are related to numerous inflammatory and autoimmune diseases, such as Celiac disease, Addison disease, idiopathic inflammatory myopathy, Hashimoto's thyroiditis, and so on. Some previous studies on the association of HLA class II and human cancers have shown diverse results. Mahmoodi M et al. reported that HLA-DQA1*0301 allele is mainly associated with increased risk of breast cancer development, however, HLA-DQB1*0602 appears to protect against the early-onset of breast cancer [29]. Shen FF et al. recently demonstrated that HLA-DQA1 plays an important role in esophageal squamous cell carcinoma (ESCC) progression and may be a biomarker for ESCC diagnosis and prognosis, as well as a potential target for the treatment of patients with ESCC [30].

To validate the correlation between alterations of immune-related genes and patient prognosis, we selected cases of high grade STS and performed immunohistochemistry for HLA-DQA1, HLA-DQB1, and HLA-DRB4 genes, which showed the maximum fold change difference between the two groups. The

Nanostring nCounter® system analysis revealed that the expression of HLA-DRB4 was greatly increased in the poor prognosis group, whereas the expressions of HLA-DQA1 and HLA-DQB1 were increased in the good prognosis group. Immunohistochemistry further demonstrated a positive expression of HLA-DQA1, HLA-DQB1, and HLA-DRB4 in the frequency of 74.3% (26/35 cases), 34.3% (12/35 cases), and 48.6% (17/35 cases), respectively. However, only the expression of HLA-DQA1 was significantly correlated with survival ($p = 0.028$), suggesting the possibility of its application as a good prognostic factor for high grade STS.

Conclusion

We believe that this study is meaningful in research areas of immune-biomarker expressions of high grade STS. In spite of using formalin-fixed, paraffin embedded materials, good results were obtained from the Nanostring nCounter® system. Some immune-related genes were identified, and immunohistochemistry was carried out to validate the prognostic significance of HLA-DQA1, HLA-DQB1, and HLA-DRB4. Our results indicate that the expression of HLA-DQA1 is significantly related to long-term survival, suggesting the potential to be used as an immune biomarker of good prognosis in high grade STS.

Declarations

Acknowledgement

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Authors' contributions

JYB and KUC contributed to the conception of the study. AK and SJL collected the patients' clinical data. JYK and ISK contributed significantly to the data analysis and study preparation. SHC helped performing the analysis with constructive discussions. JIK wrote the study and made supervision of the study. All authors have read and approved the final study.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Approval for the study was obtained from the Institutional Ethics of Pusan National University Hospital. Informed consent was obtained from all the patients whose tissues were used in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Orthopedic Surgery, Pusan National University Yangsan Hospital, Gyeongsangnam-do, Republic of Korea. ²Department of Pathology, Pusan National University Hospital, Busan, Republic of Korea. ³Medical Research Institute, Pusan National University Hospital, Busan, Republic of Korea. ⁴Department of Pathology, Pusan National University Yangsan Hospital, Gyeongsangnam-do, Republic of Korea. ⁵Department of Radiology, Pusan National University Hospital, Busan, Republic of Korea. ⁶Department of Orthopedic Surgery, Kosin University Gospel Hospital, Collge of Medicine, Kosin University, Busan, Republic of Korea. ⁷Department of Orthopedic Surgery, Pusan National University Hospital, Busan, Republic of Korea.

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Figures

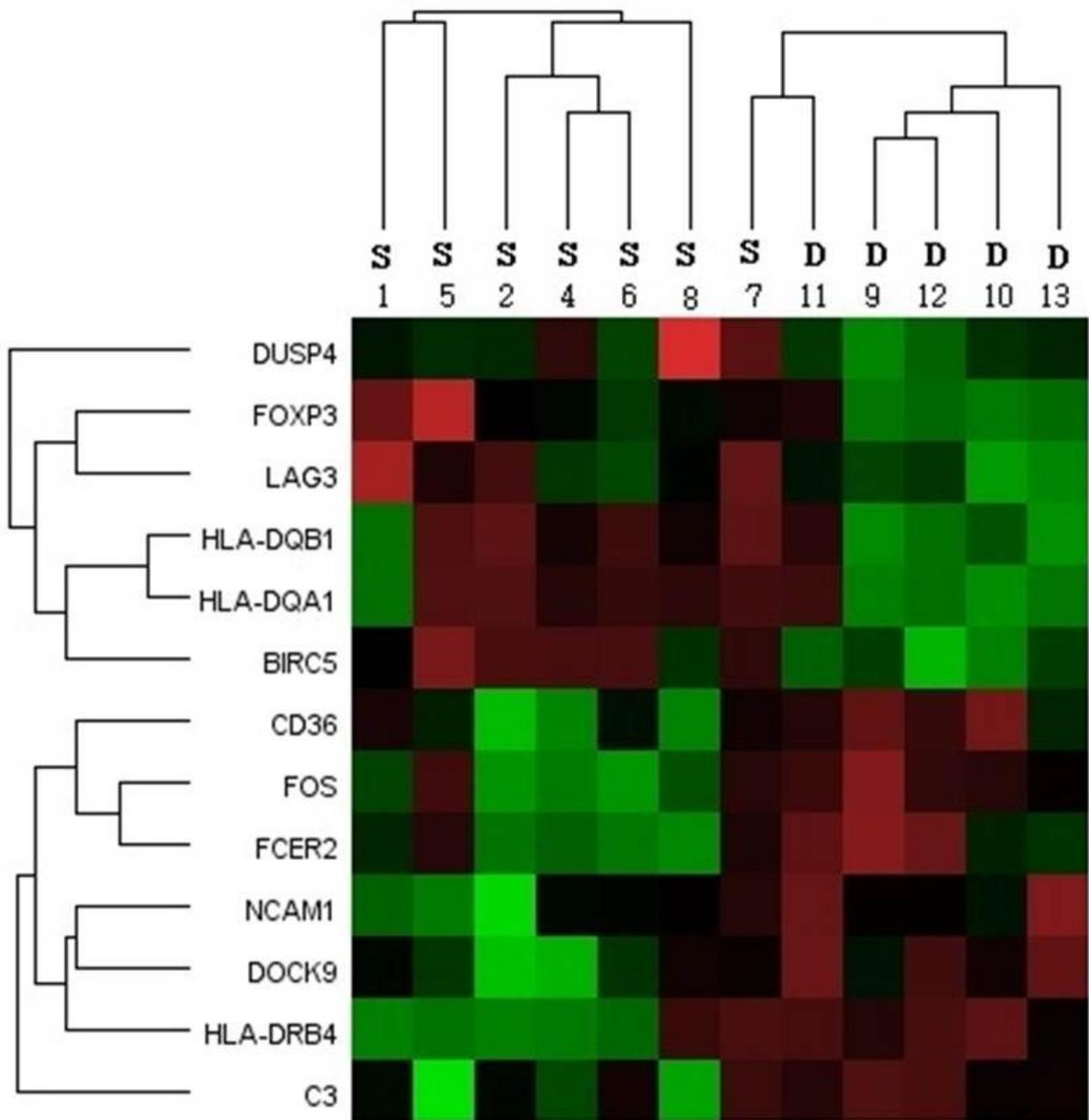


Figure 1

Heat map generated from mRNA data reflecting representative 13 genes with differential expression between the good (S) and the poor prognostic group (D). Color scale with red indicates highly expressed genes.

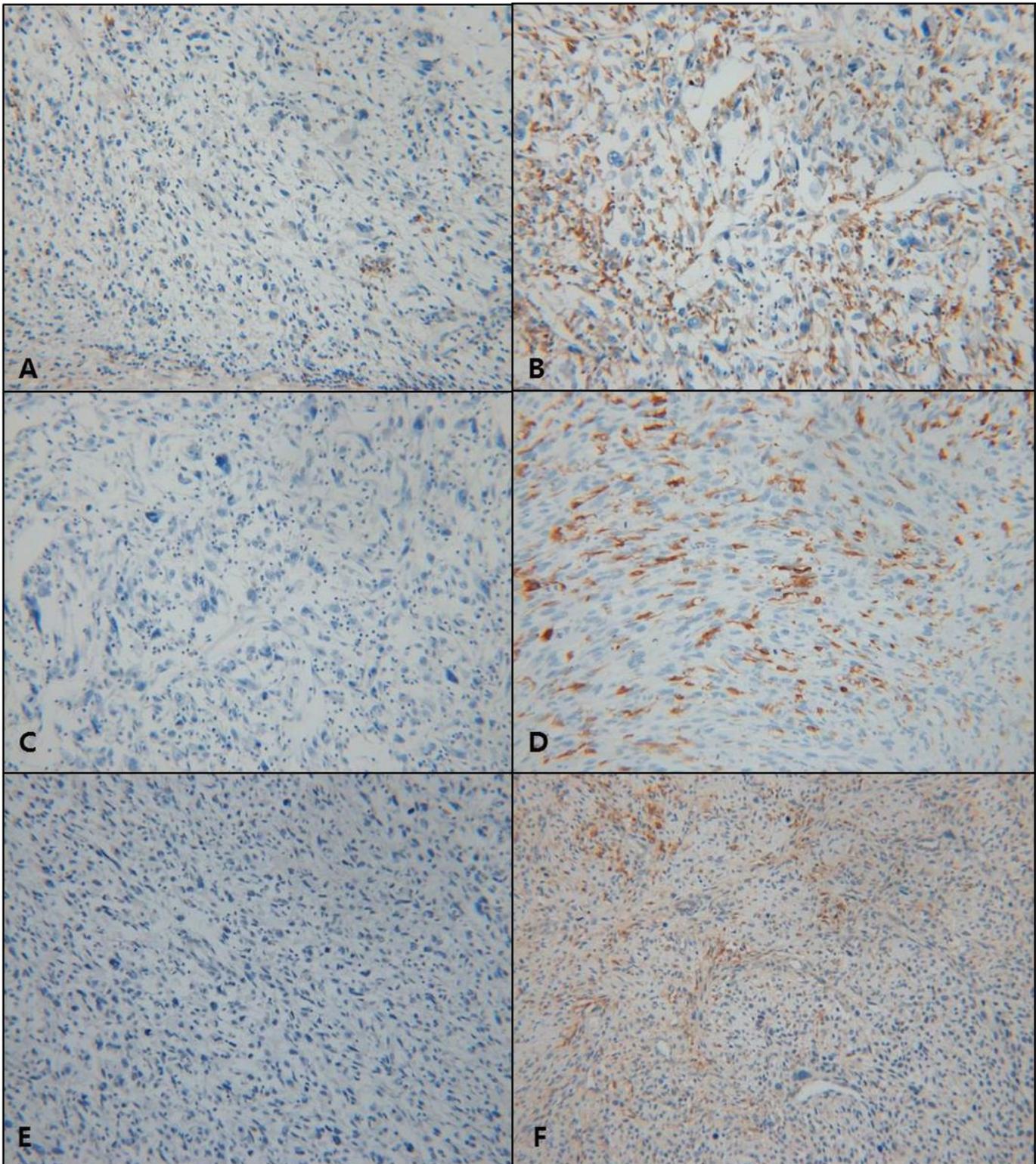


Figure 2

Representative cases of immunohistochemical staining in high grade STS. Brown-stained cells indicated positively stained cells and the combined positive score (CPS) was used to analyze the results. [A] Immunostaining for HLA-DRB (CPS < 1) shows negative expression in the good prognosis group. [B] Immunostaining for HLA- DRB4 (CPS \geq 1) shows positive expression in the poor prognosis group. [C] Immunostaining for HLA- DQA1 (CPS < 1) shows negative expression in the poor prognosis group. [D]

Immunostaining for HLA- DQA1 (CPS ≥ 1) shows positive expression in the good prognosis group. [E]
 Immunostaining for HLA- DQB1 (CPS < 1) shows negative expression in the poor grognosis group. [F]
 Immunostaining for HLA- DQB1 (CPS ≥ 1) shows positive expression in the good prognosis group.

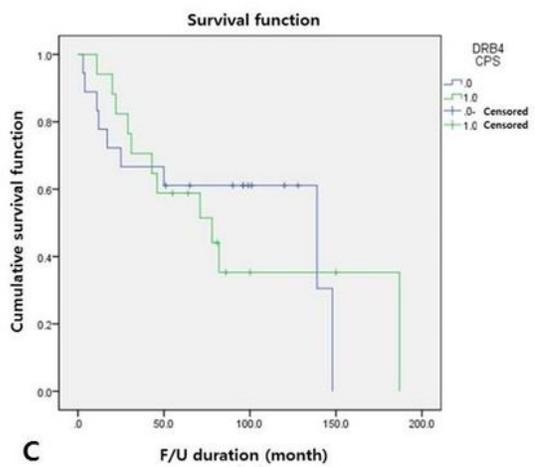
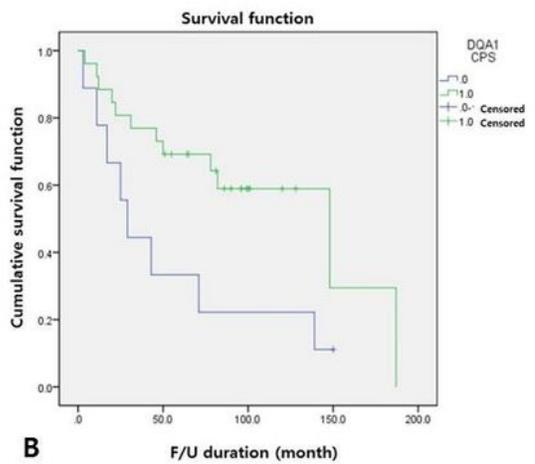
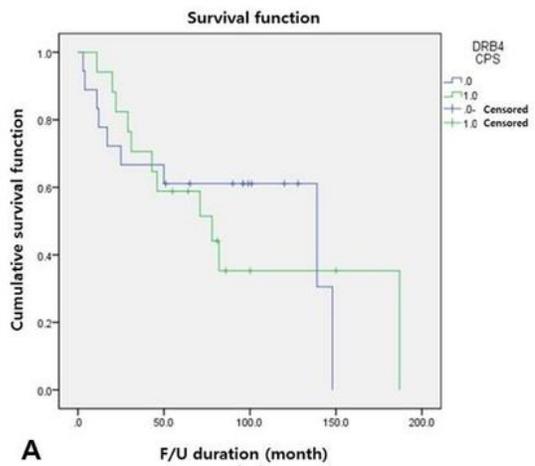


Figure 3

Survival analysis for immuno-expression of HLA-DRB4, HLA-DQA1, and HLA-DQB1. [A] The survival curve of HLA-DRB4 expression was not related to prognosis ($p=0.489$). [B] HLA-DQA1 expression was

significantly associated with increase of the survival rate ($p=0.028$). [C] The survival curve of HLA-DRB4 expression was not related to prognosis ($p=0.500$). CPS : combined positive score Blue line: CPS negative expression group Green line: CPS positive expression group