

Frequent expression of human leukocyte antigen class I and the status of intratumoral immune cells in alveolar soft part sarcoma

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Abstract. The prognosis of alveolar soft part sarcoma is poor, despite the slow growth of the tumor. A number of cases with spontaneous regression of this rare tumor have been reported. Although the mechanisms underlying spontaneous regression remain uncertain, local immune reaction may be a possible contributing factor. Immunohistochemical expression of human leukocyte antigen (HLA) class I, cluster of differentiation (CD) 3, CD4, CD8, CD20, CD45, CD56, CD68, CD138 and CD163 were assessed in a series of 10 alveolar soft part sarcomas, and the expression profiles were associated with patients' clinicopathological parameters. Expression of HLA class I was observed in almost all the tumor cells of all cases. CD8(+) cells were identified in all tumors with varying densities. Moderate infiltration of CD8(+) cells was detected in three patients; one of these patients survived with long-term tumor remission. Infiltration of CD10(+), CD20(+), CD56(+) or CD138(+) cells was not revealed in all tumors. Moderate-diffuse infiltration of CD163(+) cells was observed in all tumors. To the best of our knowledge, the present study represents the first report of intratumoral immune cells in alveolar soft part sarcoma. Frequent expression of HLA class I in tumor cells was observed. CD8(+) cells were identified at various densities and CD163(+) cells were observed in alveolar soft part

sarcoma. Moderate infiltration of CD8(+) cells in patients with a good prognosis may indicate the antitumor effects of immune cells in alveolar soft part sarcoma.

Introduction

Alveolar soft part sarcoma is a morphologically and clinically distinct neoplasm, that was first termed and defined by Christopherson *et al* (1), in 1952. The name 'alveolar' was derived from its pseudo alveolar appearance, with clustered polygonal cells with eosinophilic cytoplasm (1). Although the tissue origin of alveolar soft part sarcoma still remains unclear, molecular studies have revealed chromosomal rearrangements at translocation (X;17)(p11;q25), resulting in an alveolar soft part sarcoma critical region 1 (*ASPSCR1*) and transcription factor E3 (*TFE3*) gene fusion (2-4). The alveolar soft part sarcoma tumor usually presents as a painless, slow growing mass without functional impairment (1). Typically, there is a lack of symptoms, and it is easily overlooked, with metastasis to the lung or brain often the first signs of manifestation of the disease (5-12). Prognosis is poor despite the slow growth of the tumor (5-12). There has been partial initial success with tyrosine kinase inhibitors (13-15), however, active systemic therapy has not been successful for the majority of patients with metastatic diseases (9,15,16). This emphasizes the requirement for alternative treatments such as immunotherapy.

Cases with spontaneous regression of alveolar soft part sarcoma have previously been reported (17-19). However, the mechanisms underlying the spontaneous regression of this type of tumor remain uncertain, and the local immune reaction is considered to be a possible causative factor (20).

Previous studies have demonstrated efficacy of immunotherapeutic strategies against cancer, where antitumor cytotoxic T lymphocytes are induced by cancer vaccination (21-24). Antitumor responses are modulated by the recognition of immunogenic epitopes in the context of human leukocyte antigen (HLA) class I in the tumors (21). Therefore,

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it is important to evaluate the infiltrations of immune cells and the presence of HLA class I in the tumors. Using immunohistochemistry, the present study evaluated the expression profiles of HLA class I and the presence of intratumoral immune cells in alveolar soft-part sarcoma.

Materials and methods

Patients. The present study was approved by the Institutional Review Boards of the Niigata University Hospital (Niigata, Japan) and Niigata Cancer Hospital (Niigata, Japan), and complies with the Declaration of Helsinki. Written informed consent was obtained from all patients and/or their families prior to enrollment in the present study. Between January, 1985 and December, 2014, 10 patients with alveolar soft part sarcoma were treated at the Niigata University Hospital and Niigata Cancer Hospital. Data from these patients were reviewed retrospectively. The demographic data for the patients are summarized in Table I. The average age at the time of primary treatment was 32 years (range, 12-75 years). The patient group was comprised of six females and four males. The tumor diameter ranged between 30 and 200 mm (average 103 mm).

Immunohistochemistry. The surgically excised tumor samples were routinely fixed in 10% buffered formalin for 18-20 h at room temperature, prior to being embedded in paraffin. A single representative section of each sample (4 μ m thick) was cut and attached to amino silane (APS) coated glass slides (SFRC11; Matsunami Glass Ind., Ltd., Osaka, Japan.). Hematoxylin (Vector Laboratories, Inc. Burlingame, CA, USA) and eosin was used for routine staining. The tissue sections were deparaffinized in xylene and rehydrated in graded ethanol. The slides were steamed for antigen retrieval in 10 mM citrate buffer at pH 6.0 (Dako North America, Inc., Carpinteria, CA, USA) at 85-100°C for 40 min. Parts of each sample were treated with 0.2% trypsin solution at 37°C for 20 min, for antigen retrieval. Following cooling for 15 min, the slides were immersed in methanol with 0.3% hydrogen peroxide for 20 min at room temperature, in order to block the endogenous peroxidase activity. Subsequently, the slides were incubated with PBS supplemented with 10% goat serum (Dako North America, Inc.) for 20 min at room temperature, to reduce the occurrence of nonspecific reactions. The slides were then incubated with the primary antibodies at 4°C overnight, followed by incubation with a universal immunoperoxidase polymer, including anti-mouse and anti-rabbit antibodies (Histofine Simple Stain MAX PO [MULTI]; dilution 1:1; cat. no. 424141, Nichirei Biosciences, Inc., Tokyo, Japan) for 30 min at room temperature. The peroxidase activity was detected with 3'-diaminobenzidine tetrahydrochloride (Nichirei). The slides were counterstained with hematoxylin. The following primary antibodies were used: Anti-CD3 (rabbit monoclonal antibody; clone: SP7; dilution 1:1; cat. no. 713591; Nichirei), anti-CD4 (mouse monoclonal antibody; clone: 1F6; dilution 1:1; cat. no. 713181; Nichirei), anti-CD8 (mouse monoclonal antibody; clone: C8/144B; dilution 1:1; cat. no. 713201; Nichirei) anti-CD56 (monoclonal mouse anti-human CD56; clone: 1B6; dilution 1:1; cat. no. 713331; Nichirei), anti-CD20 (mouse monoclonal

antibody; clone: L26; dilution 1:1,000; cat. no. SL26; Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan), anti-CD45 (monoclonal mouse anti-human antibody; clone: 2B11; dilution 1:200, cat. no. 2B11; Kyowa Hakko Kirin) anti-CD68 (monoclonal mouse anti-human antibody; clone: KP1; dilution 1:5,000; cat. no. Nr.M0814; Dako North America, Inc.) anti-CD138 (monoclonal mouse anti-human CD138; clone: MI15; dilution 1:50; cat. no. Nr.M722; Dako North America, Inc.), anti-CD163 (monoclonal mouse anti-human antibody; clone: 10D6; dilution 1:500; cat. no. NCL-CD163; Leica Microsystems, Inc., Buffalo Grove, IL, USA) and anti HLA class I (mouse monoclonal antibody; clone: EMR8-5; dilution 1:100; cat. no. AB-46; Hokudo Co., Ltd., Sapporo, Japan).

A total of 10 fields of view from each section were assessed by two researchers by light microscopy, and cells were counted manually. The immunohistochemical results were evaluated by semi-quantitative scoring, according to a previous report (23) as follows: +++, high density of positive cells in the fields; ++, moderate density and +, low density.

Result

Clinical course of the patients. At initial presentation, 7/10 patients had metastatic disease and 3/10 patients had developed metastasis following initial treatment. A thigh tumor with eight metastatic lung-nodules at presentation was identified in patient 10. This patient underwent wide local excision of the thigh tumor, but chemotherapy was not administered, as the patient's parents did not approve the additional cytotoxic treatment. For one year following excision of the primary tumor, the patient received monthly intravenous infusions containing 1×10^{10} *ex vivo* expanded and interferon (IFN) α -treated peripheral blood mononuclear cells at another hospital (21). All but one of the lung nodules slowly diminished in size in the six months following the initial surgery. Only one lung-nodule was detected by computed tomography, 24 months following surgery. The size of the lung tumor was stable, and endoscopic resection of the tumor was carried out nine years subsequent to the initial presentation. The patient was free of metastatic disease two years following the metastasectomy (Fig. 1). In total, during the present study, seven patients succumbed to alveolar soft-part sarcoma, two patients survived with metastatic disease, and one patient survived and was free from cancer.

Immunohistochemistry. As presented in Figs. 2-4 almost all tumor cells of the tissue samples stained with the antibody against HLA class I. The expression of HLA class I in soft tissue tumors was examined and the level of expression differed from case to case (data not presented). Infiltration of CD3(+) T cells was demonstrated in all tumors. Scattered-moderate infiltration of CD4(+) cells was observed in 8/10 of the tumors. CD8(+) cells were revealed in all tissue samples with varying densities. Moderate infiltration of CD8(+) cells was demonstrated in tissue samples 1, 6 and 10 (Figs. 2-4). Intratumoral infiltration of CD10(+), CD20(+), CD56(+) or CD138(+) cells was not revealed in the tumor samples. Moderate-diffuse infiltration of CD163(+) cells was observed in all tumor samples (Figs. 2-4); these cells were interspersed within nests of tumor cells and the perivascular region. CD68 levels were

Table I. Clinical characteristics of 10 cases with alveolar soft-part sarcoma.

Patient	Age (years)	Gender	Site	Maximum diameter (mm)	Metastasis at presentation	Prognosis	Survival (months)
1	28	M	Leg	70	Yes	SOD	124
2	61	M	Thigh	80	Yes	SOD	120
3	21	F	Buttock	200	Yes	SOD	12
4	25	F	Retroperitoneum	100	No	SOD	66
5	75	F	Foot	70	No	SOD	42
6	24	F	Axilla	120	Yes	SOD	10
7	14	M	Leg	30	Yes	AWD	61
8	21	F	Retroperitoneum	150	No	SOD	19
9	36	F	Thigh	160	Yes	AWD	16
10	12	M	Thigh	55	Yes	NED	138

M, male; F, female; SOD, succumbed to disease; AWD, alive with disease; NED, no evidence of disease.

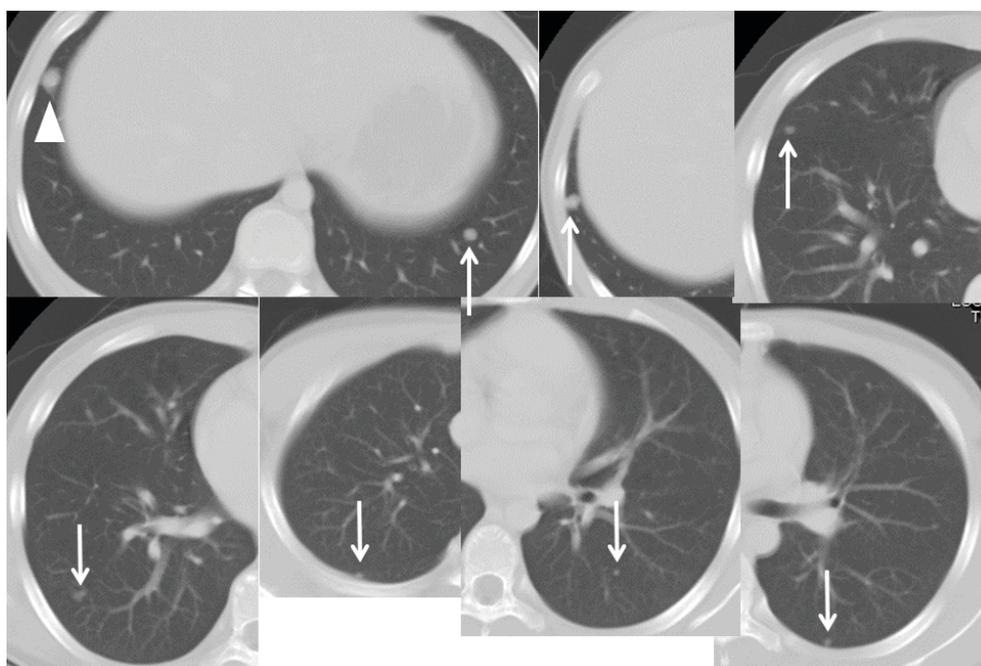


Figure 1. Multiple lung metastases in case 10. Arrows show the regressing nodules following initial the surgery and the arrowhead shows the persistent nodule that was resected seven years later.

analyzed in all tissue samples; however, precise measurements were difficult to obtain as inflammatory cells and numerous tumor cells were positive for this antigen. In case 10, the metastatic lung tumor cells were also positive for HLA class I, and moderate infiltration of CD8(+) cells was revealed in the metastatic tumor tissues (Fig. 5; Table II).

Discussion

Previous studies have demonstrated five cases of spontaneous regression of alveolar soft part sarcoma (Table III), in which three of the five patients are Japanese (17-19,25,26) The high incidence of Japanese cases may due to language bias during literature review. All three Japanese patients remain alive

following an extended period of remission, as determined by verbal communication with the patients. The spontaneous regression of sarcomas is rare and the actual incidence has not been reported (20). Although the underlying mechanism of regression is not fully known, immune mechanisms have been demonstrated in certain types of cancer (20).

The present study revealed moderate infiltration of CD8(+) lymphocytes in patient 10, who had long-term remission of multiple lung metastases, which suggests an anticancer immune response in this tumor. The specific immunotherapy treatment of this case is not known; however, diffuse infiltration of CD8(+) lymphocytes in the primary tumor prior to immunotherapy suggests specific immune components were activated. CD8(+) cells in malignant tumors have

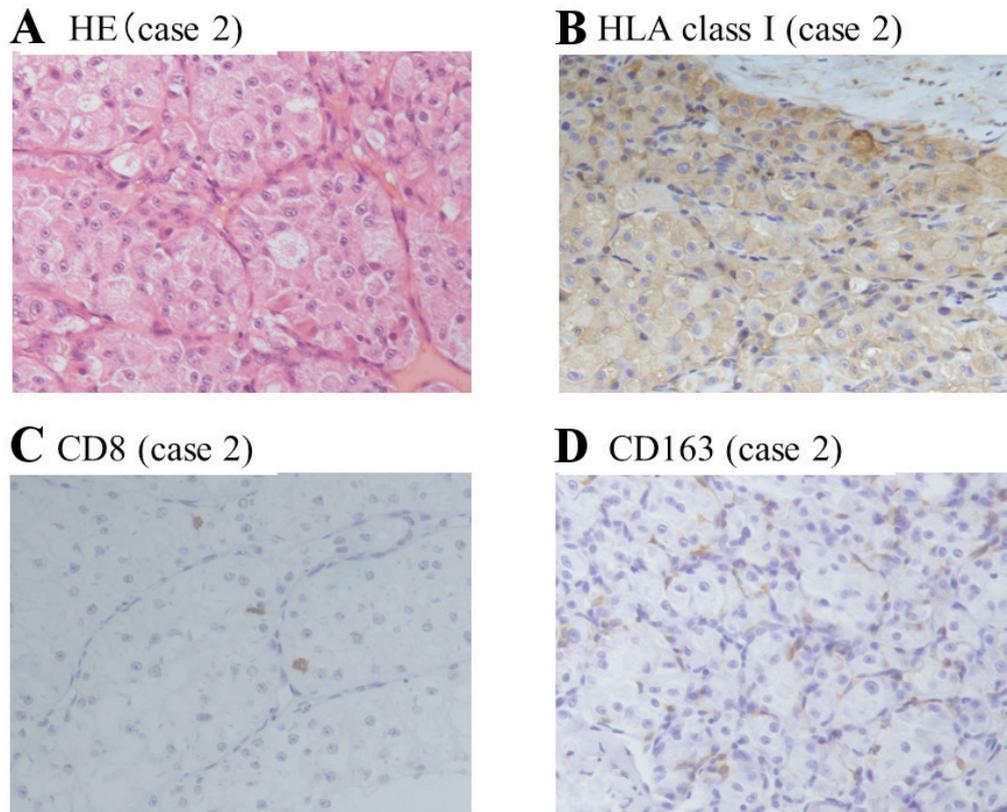


Figure 2. Representative immunohistochemical reactions for HLA class I, CD8, and CD163 in case 2. (A) HE staining; (B) diffusely positive tumor cells for HLA class I; (C) scattered infiltration of CD8(+) immune cells; (D) diffuse infiltration of CD163(+) cells. HLA, human leukocyte antigen; CD, cluster of differentiation; HE, hematoxylin and eosin.

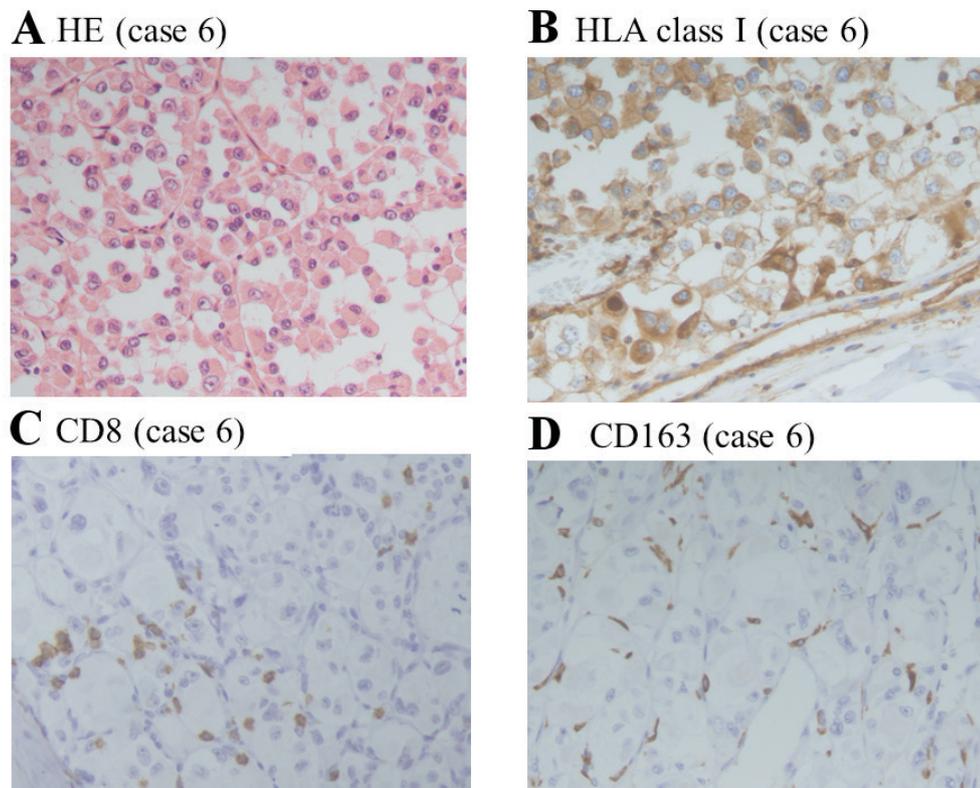


Figure 3. Representative immunohistochemical reactions for HLA class I, CD8 and CD163 in case 6 (A) HE staining; (B) diffusely positive tumor cells for HLA class I; (C) moderate infiltration of CD8(+) immune cells; (D) diffuse infiltration of CD163(+) cells. HLA, human leukocyte antigen; CD, cluster of differentiation. HE, hematoxylin and eosin.

Table II. Results of immunohistochemically analysis of 10 cases of alveolar soft part sarcoma.

Patient	HLA class I	CD3	CD4	CD8	CD10	CD20	CD45	CD56	CD138	CD163
1	+++	++	-	++	-	-	++	-	-	+++
2	+++	+	+	+	-	-	+	-	-	+++
3	+++	+	-	+	-	-	+	-	-	++
4	+++	+	-	+	-	-	+	-	-	+++
5	+++	+	-	+	-	-	+	-	-	++
6	+++	++	+	++	-	-	++	-	-	+++
7	+++	+	+	+	-	-	+	-	-	++
8	+++	+	+	+	-	-	+	-	-	+++
9	+++	+	+	+	-	-	+	-	-	++
10	+++	++	+	++	-	-	++	-	-	+++

+++ , high density; ++ , moderate density; + , low density; - , Negative; HLA, human leukocyte antigen; CD, cluster of differentiation.

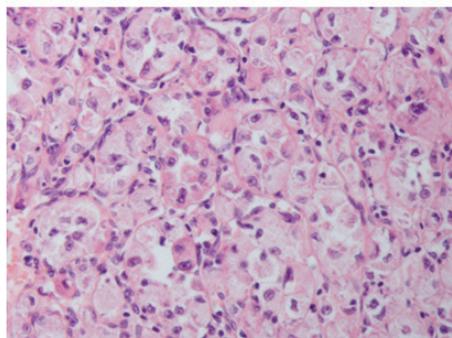
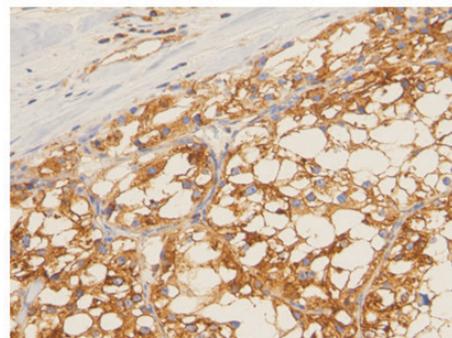
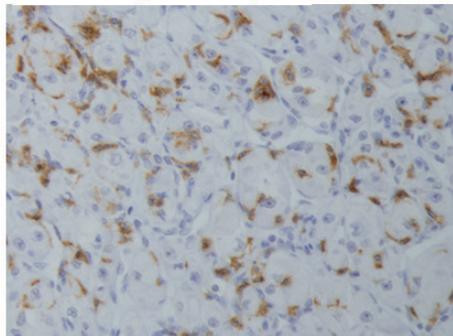
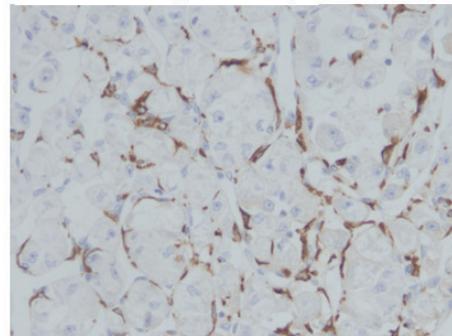
A HE (case 10)**B** HLA class I (case 10)**C** CD8 (case 10)**D** CD163 (case 10)

Figure 4. Representative immunohistochemical reactions for HLA class I, CD8 and CD163 in Case 10. (A) HE; (B) diffusely positive tumor cells for HLA class I; (C) moderate infiltration of CD8(+) immune cells; (D) diffuse infiltration of CD163(+) cells. HLA, human leukocyte antigen; CD, cluster of differentiation. HE, hematoxylin and eosin.

been associated with an improved survival rate for patients with various types of cancer, including non-small cell lung carcinoma, endometrial carcinoma, melanoma and Ewing sarcoma (20). In the present study, the infiltration of CD10(+), CD20(+), CD56(+), or CD138(+) cells was not identified in all tissue samples. Therefore, B or natural killer cells do not dominate the immune response in alveolar soft-part sarcoma.

The present study was designed to determine the expression levels of HLA class I and infiltration levels of immune cells in alveolar soft part sarcoma. It was revealed that the expression levels of HLA class I were preserved in all cases investigated

in the current study. Previously, Tsukahara *et al* (27) investigated expression levels of HLA class I in bone and soft tissue tumors. Tsukahara *et al* (27) revealed that HLA class I was not expressed or was downregulated in 63% of bone and soft tissue tumor samples. Although the sample size in the current study is small, preservation of HLA class I expression and infiltration of CD8(+) lymphocytes in the tumor cells demonstrates noteworthy results in all cases. The results were concordant with those of previous studies, confirming the presence of an immune response in alveolar soft part sarcoma (28). In addition, the diffuse infiltrations of CD8(+) lymphocytes in

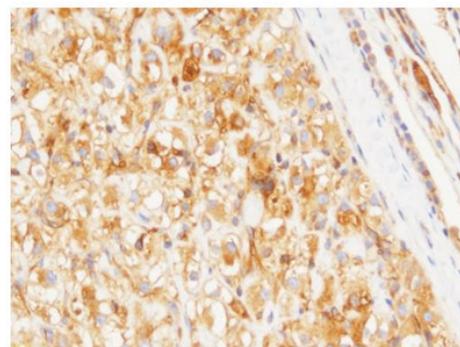
Table III. Cases of regression without cytotoxic therapy in alveolar soft part sarcoma.

Age	Gender	Location	Tumor size	Metastasis at presentation	Preoperative treatment	Surgery for the primary tumor	Postoperative course	Outcome	Reference
20	Female	Buttock	16 cm	Yes	None	Resection	No effect of cytotoxic chemotherapy just following the surgery Start of oral and topical paste of Chinese herb four years following the surgery Regression of the lung tumors 6 years after the surgery Progression of the tumor following interruption of the treatment eight years following the surgery	Succumbed to disease (10 years)	(18)
14	Female	Retropertoneum	Unknown	None	Radiotherapy (50 Gy) Embolization	Total resection	Detection of lung metastasis two years following the surgery Soft tissue metastasis and multiple lung metastasis six years following the surgery Diminishing the lung metastasis seven years following the surgery	Alive without disease (15 years; personal communication)	(25)
14	Male	Thigh	4 cm	None	Hyperthermia Radiotherapy (30 Gy) Chemotherapy (Cisplatin Pirarubicin)	Wide resection Progression of the disease	Detection of lung metastases four months following the surgery following chemotherapy (Ifosfamide) Decreasing in size and number of metastases following three years	Alive without disease (21 years; personal communication)	(26)
12	Male	Thigh	Unknown	Yes	None	Wide resection	Regression of multiple lung metastases seven years following the surgery	Alive without disease (12 years) (personal communication)	(19)

Table III. Continued.

Age	Gender	Location	Tumor size	Metastasis at presentation	Preoperative treatment	Surgery for the primary tumor	Postoperative course	Outcome	Reference
38	Female	Abdominal cavity	16 cm	None	None	Biopsy only	Detection of lung metastases seven months following the biopsy Disappearance of lung metastases 12 months following the biopsy Brain metastases 14 months after the biopsy	Succumbed to disease (20 months)	(17)
12	Male	Thigh	5.5 cm	Yes	None	Wide resection	Autologous lymphocyte infusion (11 cycles for one year) Diminishing the number of the lung metastases Metastectomy for one lung tumor nine years following initial treatment	Alive without disease (11 years following initial treatment)	The present study

A HLA class1(lung metastasis in case 10)



B CD8 (lung metastasis in case 10)

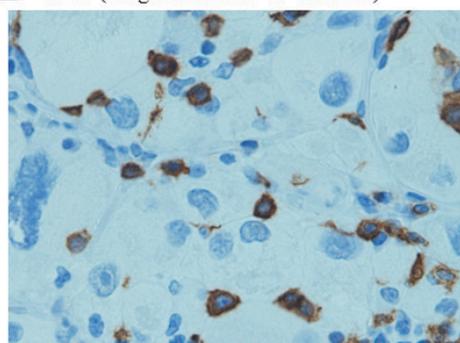


Figure 5. Representative immunohistochemical reactions for HLA class 1 and CD8 (A) diffusely positive tumor cells for HLA class 1; (B) moderate infiltration of CD8(+) immune cells. HLA, human leukocyte antigen; CD, cluster of differentiation. HE, hematoxylin and eosin.

case 10 indicates that the immune response may contribute to improved prognosis of the tumor.

Tumor-associated macrophages are stromal cells that are known to promote tumor invasion, metastasis and angiogenesis in numerous types of cancer (27). The macrophages are able to produce and secrete growth factors, cytokines and other inflammatory mediators that may have important roles in tumor progression (29). These tumor-activating functions are consistent with the results of previous clinical studies, demonstrating that the high macrophage density of various types of cancer is associated with a poor prognosis (29). In addition, a previous study also demonstrated that macrophage infiltration is associated with poor prognosis in Ewing sarcoma (30). However, a study of osteosarcoma revealed that macrophages were associated with a good clinical outcome (31). The role of macrophages in sarcoma differs depending on the tumor subtype (31). In the present study, CD163 macrophages infiltrated diffusely in all alveolar soft tissue tumor samples, similar to a previous study (28). The prognostic value of macrophages in alveolar soft part sarcoma remains controversial. Additional studies are required to improve understanding of the role of macrophages in soft tissue tumors.

In conclusion, to the best of our knowledge, the present study represents the first report of intratumoral immune cell presence in alveolar soft part sarcoma. Frequent expression of HLA class I was revealed in the tumor cells. CD8(+) cells were identified at varying densities and CD163(+) cells were present in alveolar soft part sarcoma. Moderate infiltration of CD8(+) cells was observed in the tumors of patients with a

good prognosis, which may indicate the antitumor effect of immune cells in alveolar soft-part sarcoma.

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