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The Cytologic Features of Desmoplastic Small Round Cell Tumor with Intranuclear Inclusions
– A Case Report –

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Desmoplastic small round cell tumor (DSRCT) is a rare neoplasm of young adults and it is characterized by polyphenotypic differentiation. We experienced a case of abdominal DSRCT that occurred in a 19-year-old female who presented with painful swelling of her right forearm. The tumor was cytokeratin-negative and it exhibited some tumor cells with intranuclear inclusions. Molecular demonstration of EWS-WT1 fusion transcripts is particularly useful to confirm the diagnosis of DSRCT without epithelial differentiation. We report here on a case of cytokeratin-negative DSRCT that showed an unusual feature of intranuclear inclusions.

Key Words : Desmoplastic small round cell tumor; Cytokeratin; EWS-WT1 fusion protein; Intranuclear Inclusions; Cytology

Desmoplastic small round cell tumor (DSRCT) is a highly aggressive malignant tumor that chiefly affects male adolescents and young adults, and it frequently presents as a huge paraserosal, intraabdominal mass.[1,2] However, DSRCT is not organ-specific. DSRCT is composed of nests of small, undifferentiated, round to oval hyperchromatic cells embedded in a desmoplastic stroma. It is characterized by a specific immunophenotype with the co-expression of proteins associated with epithelial (cytokeratin) and mesenchymal (desmin) markers.[3,4] In addition, DSRCT has a specific cytogenetic feature involving the reciprocal translocation of t(11;22) (p13;q12).[5] This translocation results in the fusion of exon 7 of the Ewing sarcoma gene (EWS) on 22q12 with exon 8 of the Wilms tumor gene (WT1) on 11p13 to produce a tumor-specific fusion protein.[6,7] This fusion protein activates the molecules that induce cell growth and the WT1 tumor suppressor gene normally suppresses these molecules.[4,8] Thus, detection of the EWS-WT1 fusion transcript serves as a specific and sensitive marker for DSRCT.[8-10]

We describe here a case of epithelial marker-negative DSRCT that showed an unusual feature of intranuclear inclusions. The demonstration of the EWS-WT1 fusion transcripts by reverse transcriptase polymerase chain reaction (RT-PCR) will allow physicians to make the correct diagnosis for the case of cytokeratin-negative DSRCT.

CASE REPORT

A 19-year-old female visited the outpatient clinic of the Department of Internal Medicine at the Chungbuk National University Hospital with a painful swelling of right forearm that she'd had for one month. She was admitted under the clinical impression of infectious myositis of the right forearm and she was immediately transferred to the Department of Orthopedic

Surgery for further evaluation and management. During the hospital course, she complained of palpitation and dypnea, and enlarged nodules of the neck were newly identified. Chest X-ray and CT revealed a huge axillary mass, multiple nodules of the neck, nodular pleural thickening and pleural effusion. Abdominal and pelvic CT also demonstrated multiple retroperitoneal nodules and a small quantity of ascites (Fig. 1). The radiologic diagnosis was malignant lymphoma. A pleural fluid cytologic examination was requested after chest tube insertion, and neck mass biopsy and cytology were then carried out. The patient expired six weeks after the biopsy because of treatment-associated complications such as uncontrolled hemothorax and the aggressiveness of the tumor.

The cytologic findings

The liquid-based cytologic smear of the Papanicolaou-stained pleural fluid contained some cohesive clusters of malignant small round cells. The tumor cells had relatively uniform, small, oval to spindled nuclei and scant cytoplasm. The nuclei showed some variations in size and shape, and nuclear molding was focally present. Finely dispersed chromatin and inconspicuous nucleoli that were similar to those of small cell carcinoma were also observed. Occasional mitoses were present, but no nuclear inclusion was identified. Other three dimensional structures such as gland or rosette-like formations were not observed. The cytologic diagnosis of the pleural fluid was malignant small round cell tumor that was suggestive of metastatic small cell carcinoma (Fig. 2A).

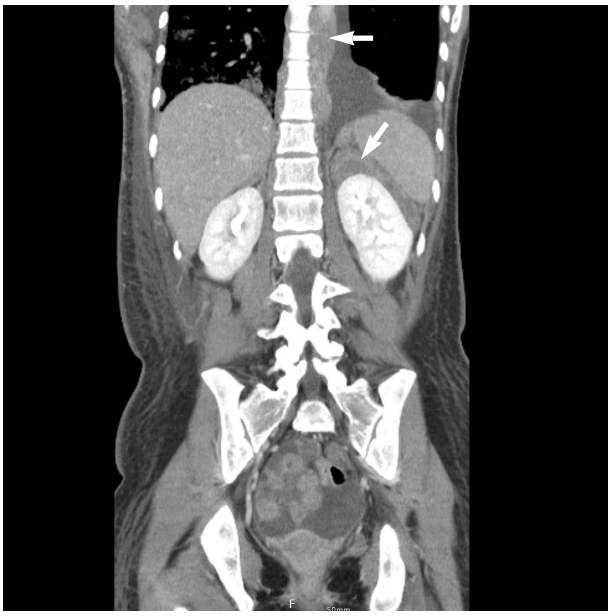

Fig. 1. Computed tomogram reveals retroperitoneal and pleural masses.

The Papanicolaou stained touch imprint of the neck mass biopsy specimen contained numerous cells arranged in cohesive small epithelial-like clusters and loosely scattered or isolated cells (Fig. 2B). The former were identical to those of the pleural fluid. The latter were cells with irregularly-folded and unevenly-contoured nuclei. They generally measured 15-50 μm in diameter and they exhibited round to mildly oval nuclei with condensed chromatin or inconspicuous nucleoli and scant cytoplasm. The nuclei were fairly monomorphic, but they were more pleomorphic than those nuclei of the epithelial-like cell clusters. Some of them showed intranuclear inclusions (Fig. 2C). The inclusions were round to slightly oval with mild variations in size and the inclusions were centrally or eccentrically placed within the nuclei. Frequent mitoses were also identified, but there was no evidence of necrosis. The cytologic impression was that the epithelial-like cell clusters resembled those of small cell carcinoma, while the loosely scattered cells were morphologically similar to those of malignant lymphoma or granulocytic sarcoma.

The histologic, immunohistochemical and molecular findings

The tumor revealed a biphasic pattern. One was the classic features of DSRCT composed of sharply outlined strands, nests and lobules of poorly differentiated small cells embedded in a desmoplastic stroma (Fig. 3A). The other was a plain sarcomatous pattern of the tumor cells with round to oval, reniform or indented nuclei and scant cytoplasm (Fig 3B). Both components progressively blended with each other. The nuclear inclusions of the tumor cells were more frequently observed in the sarcomatous area. Mitotic figures were common in both sides. Neither Homer-Wright rosettes nor rhabdomyoblasts were observed.

Immunohistochemistry was performed using a formalin-fixed, paraffin-embedded tissue block. The tumor cells were positive for vimentin (3B4, 1:100, DAKO, Denmark), desmin (DE-R-11, 1:50, Novocastra, UK), WT1 (6F-H2, 1:200, DAKO), smooth muscle actin (αsm-1, 1:100, Novocastra) and CD56 (1B6, 1:50, Novocastra), and the tumor cells were negative for pancytokeratin (AE1/AE3, 1:50, Novocastra), high molecular weight cytokeratin (34βE12, 1:50, NeoMarkers), epithelial membrane antigen (GP1.4, 1:50, Novocastra), CD45 (X16/99, 1:20, DiNonA, Korea), CD99 (YG32, 1:800, DiNonA), p63 (7JUL, 1:50, Novocastra), synaptophysin (SP11, 1:200, Neomarkers), neuron-specific enolase (5E2, 1:100, Novocastra), S-100 (polyclonal, 1:40, DAKO), HMB45 (HMB45, 1:50, Novocastra),

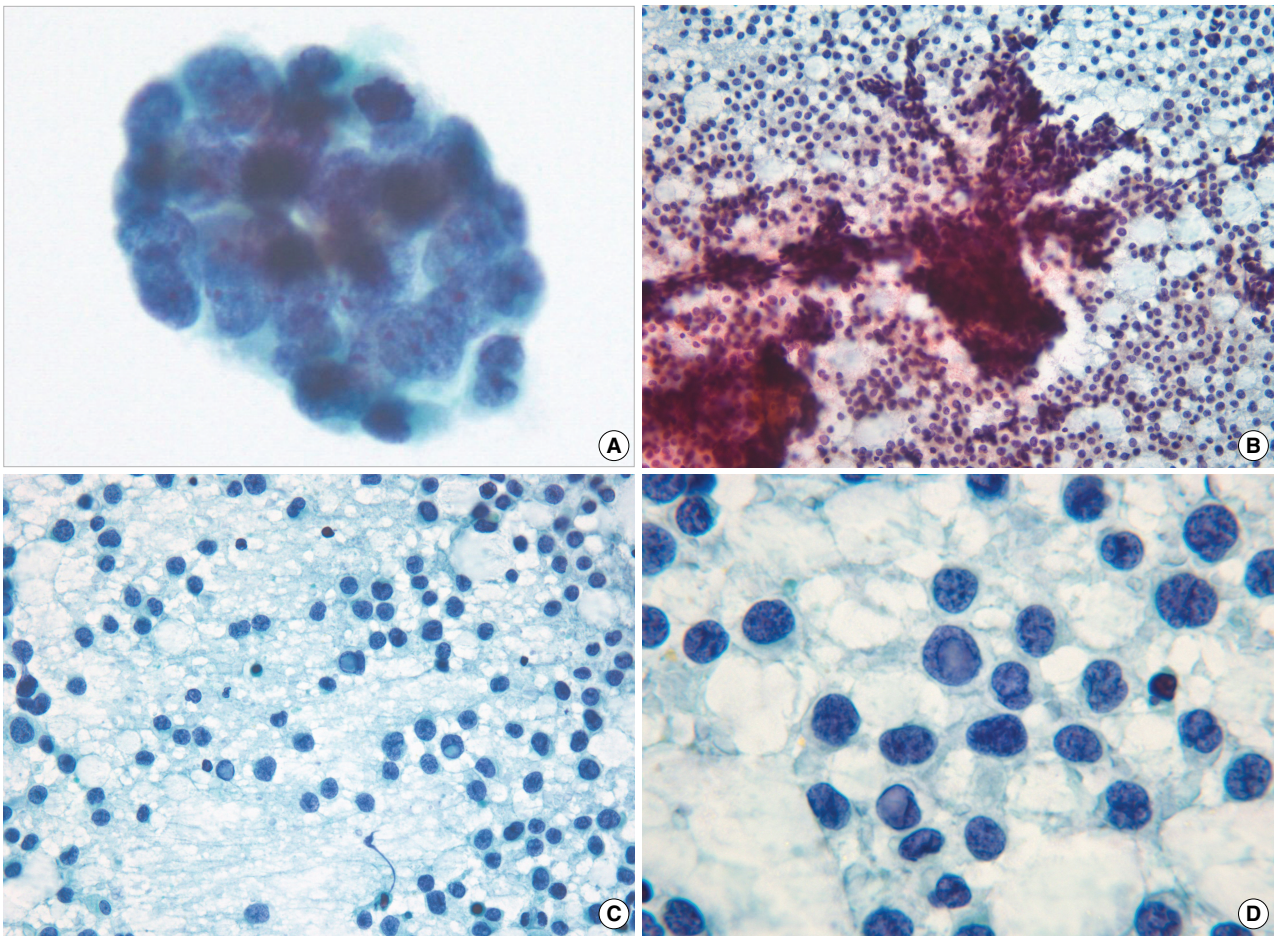

Fig. 2. (A) Pleural fluid cytology shows a cohesive cluster of malignant cells. The tumor cells have relatively uniform, small, oval nuclei and scant cytoplasm. (B) Touch imprint contains numerous cells composed of cohesive epithelial-like clusters and isolated cells. (C) Some of tumor cells show intranuclear inclusion. (D) High power view of intranuclear inclusions. (Papanicolaou stain).

myeloperoxidase (59A5, 1:50, Novocastra), CD68 (KP1, 1:200, DiNonA), CD117 (polyclonal, 1:400, DAKO) and CD138 (MI-15, 1:100, DAKO). The desmin and WT1 stained tumor cells showed a dot-like pattern of paranuclear cytoplasm and intranuclear inclusions (Fig. 3C). The tumor showed more than a 90% Ki-67 (MM1, 1:100, Novocastra) labeling index.

RT-PCR was performed to test for the presence of the EWS-WT1 fusion transcripts specific for DSRCT. The total RNA was extracted from a paraffin-embedded tumor block by using a TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Reverse transcription was done on 5 μg of total RNA with using Superscript II Reverse Transcriptase (Invitrogen) with random hexamers. Semi-nested PCR for the EWS-WT1 fusion transcripts was done with using the following primers: 1st primer 5′-TCCTACAGCCAAGCTCCAAG-3′ and 2nd primer 5′-TATAGCCAACAGAGCAGCAGC-3′ for EWS, and 5′-ACCTTCGTTCACAGTCCTTG-3′ for WT1. RT-PCR for the β-actin transcripts was performed as an internal control with using the primers 5′-GACACAACTGTGTTCACTAG-3′ and 5′-AGGGTAGACCACCAGCAGC-3′. The PCR products were analyzed by electrophoresis on a 2% agarose gel. A PCR product corresponding to the EWS-WT1 fusion transcripts in the tumor was demonstrated, and this was similar to that found in the positive control (Fig. 4).

DISCUSSION

This case has several unique features. First, the tumor was only positive for desmin and not for cytokeratin, while the classic immunophenotype of DSRCT is positive for both cytokeratin and desmin.[3,4] Ordonez *et al*.[3] stated that DSRCT with no immunohistochemical evidence of epithelial differentiation was very unusual. Only 10 of 107 (9%) reported cases of DSRCT were neg-

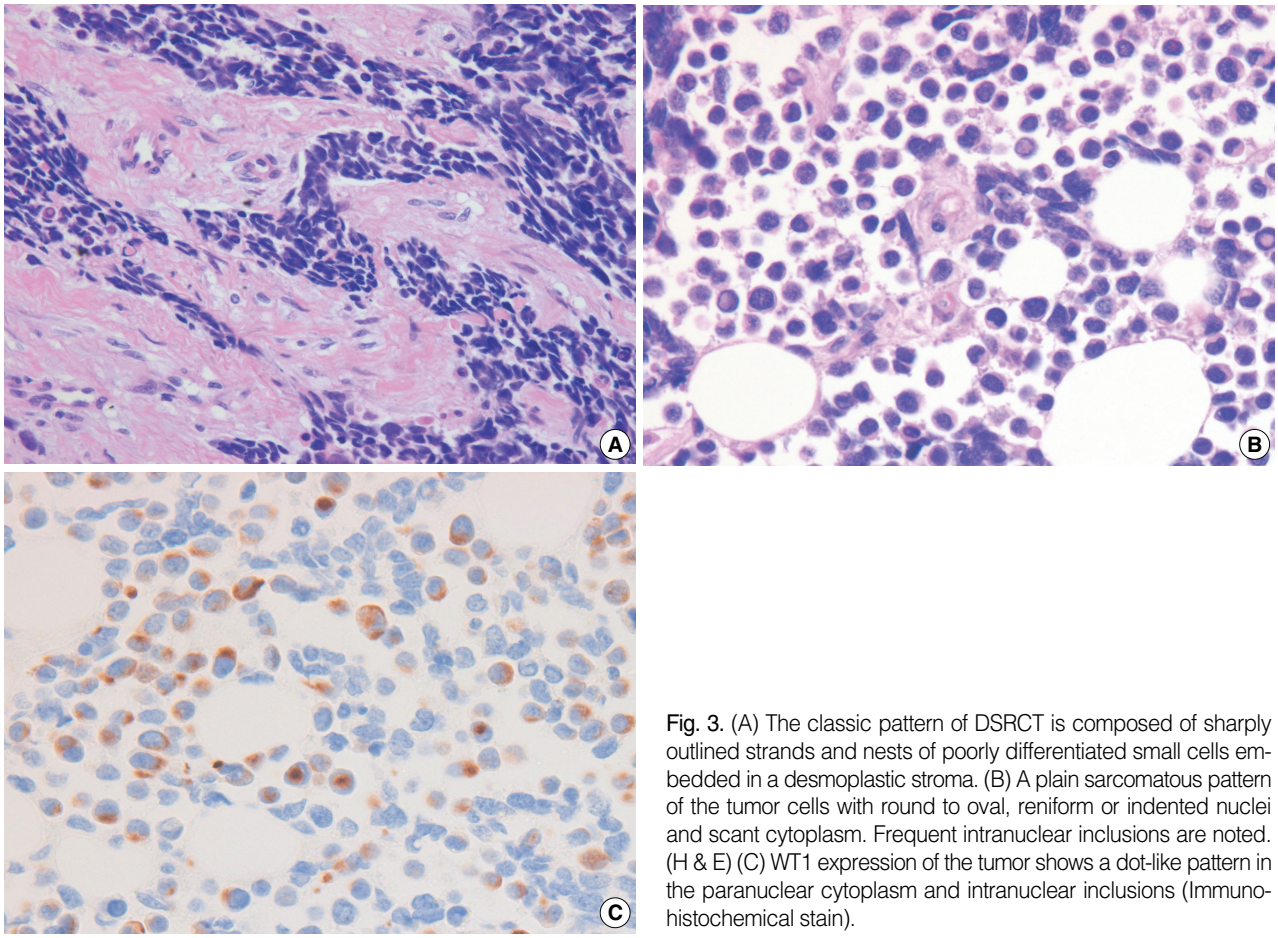

Fig. 3. (A) The classic pattern of DSRCT is composed of sharply outlined strands and nests of poorly differentiated small cells embedded in a desmoplastic stroma. (B) A plain sarcomatous pattern of the tumor cells with round to oval, reniform or indented nuclei and scant cytoplasm. Frequent intranuclear inclusions are noted. (H & E) (C) WT1 expression of the tumor shows a dot-like pattern in the paranuclear cytoplasm and intranuclear inclusions (Immunohistochemical stain).

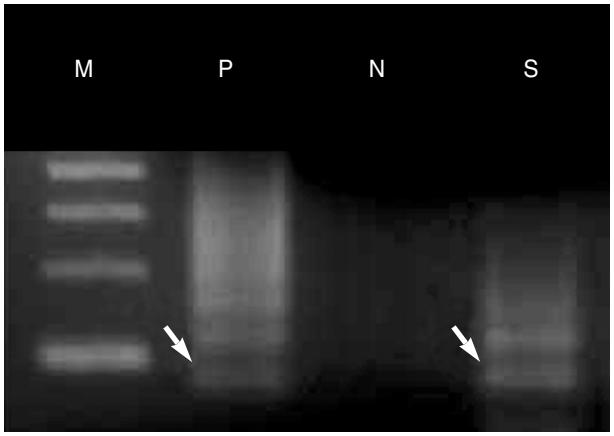

Fig. 4. RT-PCR assay for EWS-WT1 fusion transcript.
M, Marker; P, Positive control; N, Negatvie control; S, tumor specimen.

ative for cytokeratins, and 10 of 117 (9%) cases did not express desmin. Cytokeratin-negative DSRCT may represent one aspect of the diversity between these tumors. Second, the tumor cells had intranuclear pseudoinclusions, which have been observed in a variety of neoplasms, including papillary thyroid carcinoma, meningioma, paraganglioma, pheochromocytoma, lung adenocarcinoma and so on. These inclusions are generally accepted as being intranuclear cytoplasmic invaginations. Yet to the best of our knowledge, nuclear inclusions in DSRCT have never been reported. Furthermore, this tumor expressed desmin with a distinctive dot-like localization pattern in not only the paranuclear cytoplasm, but also in the intranuclear pseudoinclusions. The pattern of desmin expression of this tumor also supported the notion that these nuclear inclusions were the result of abnormal cytoplasmic invagination. Third, the present case showed a biphasic pattern composed of cohesive small epithelial-like clusters and loosely dispersed or isolated cells. Ricardo Drut described the fine-needle aspiration (FNA) cytology findings of biphasic DSRCT that occurred in a 10-year-old boy. One finding was a plain sarcomatous feature of an embryonal rhabdomyosarcoma, and the other was an epithelial morphology that resembled basal cell carcinoma.[11] The cytologic findings of finely dispersed chromatin and nuclear molding in DSRCT have been reported, and these cytologic features were similar to those of neuroendocrine tumors such as small cell carcinoma or carcinoid tumor.[12-14] Al-

though cytologic descriptions of the biphasic pattern of DSRCT are rare, it may be characteristic of DSRCT with polyphenotypic differentiation. Effusion cytology has also revealed three-dimensional clusters and single cells in a lymphocytic background.[12] However, when comparing effusion cytology with the FNA smear or touch imprint, epithelial-like clusters could become the focus of attention in effusion cytology, and DSRCT could be misinterpreted as metastatic small cell carcinoma, as happened in our case. Finally, the initial clinical presentation of the patient was not related to the primary tumor site, such as abdominal pain, distension, a palpable mass or ascites. She was admitted due to complaint of a painful swelling of her right forearm. On the retrospective view, the pain and swelling originated from metastatic nodules along the neurovascular bundles of the right arm, but at first, these nodules were mistaken for trauma-associated hematomas. We started to suspect the nature of her malignancy when the patient complained of respiratory difficulty, and a chest wall mass and pleural fluid were found later. The initial cytologic diagnosis of the pleural fluid was metastatic small cell carcinoma. After neck mass biopsy, the present case was finally diagnosed as DSRCT that first occurred in the retroperitoneum and multiple extra-abdominal metastases of the tumor were also recognized.

In terms of the cytokeratin-negative immunoreactivity of this tumor, DSRCT was not included in an initial differential diagnosis. Because cytokeratin-negative DSRCT is very unusual, a small round cell tumor showing only a desmin expression may lead to an erroneous diagnosis such as rhabdomyosarcoma. However, a characteristic paranuclear dot-like pattern of desmin and WT1 immunoreactivity is a strong diagnostic clue for DSRCT. The cytologic differential diagnoses of DSRCT included rhabdomyosarcoma, Ewing sarcoma/primitive neuroectodermal tumor, neuroblastoma, lymphoma, myeloid sarcoma and small cell carcinoma. Epithelial-like clusters are not typical of rhabdomyosarcoma because rhabdomyosarcoma appears less cohesive. Epithelial-like clusters simulating small cell carcinoma in the effusion cytology and a biphasic pattern in the FNA smear or touch imprint may represent the polyphenotypic differentiation of DSRCT, and so these findings could support the cytologic diagnosis of DSRCT. The microscopic features of rhabdomyoblasts, rosettes, neuropils and pseudoglandular structures and the ancillary studies such as immunohistochemistry, electron microscopy and molecular techniques are necessary to make the correct diagnosis.

We report here on a case of DSRCT that was cytokeratin-negative and the tumor showed intranuclear pseudoinclusions of the tumor cells. Making the diagnosis of DSRCT is not easy because of its rarity, so it could be potentially confused with other neoplastic conditions. When the morphology is consistent with DSRCT although the tumor is cytokeratin-negative, then the clinician should consider the possibility of DSRCT, and EWS-WT1 translocation assays are required to confirm the diagnosis.

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