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의학박사학위논문

CIC-DUX4 또는 *BCOR-CCNB3* 유전자융합을 동반한 원형세포육종의 조직학적, 유전학적 특이적 소견과 유잉육종과의 비교

Distinct Histologic and Genetic Characteristics of Round Cell Sarcoma with *CIC-DUX4* or *BCOR-CCNB3* Fusion and Comparison with Ewing Sarcoma

울 산 대 학 교 대 학 원

의 학 과

송 민 정

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지도교수 조경자

이 논문을 의학박사학위 논문으로 제출함

2018 년 12 월

울 산 대 학 교 대 학 원

의 학 과

송 민 정

송민정의 의학박사학위 논문을 인준함

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2018 년 12 월

Abstract

Purpose: *CIC-DUX4* and *BCOR*-CCNB3 fusion gene associated sarcoma is a new emerging subgroup of round cell sarcoma with Ewing sarcoma-like morphology. Distinguishing these tumors from Ewing sarcoma family tumor (ESFT) is critical because of the clinical impact but is still challenging for overlapped histological and immunohistochemical phenotypes of each subtype.

The present study 1) investigated small round cell sarcoma to identify *CIC-DUX4* or *BCOR-CCNB3* fusion positive sarcoma, 2) examined clinical, histopathologic and immunohistochemical characteristics of *CIC-DUX4* or *BCOR-CCNB3* sarcoma, and 3) evaluated parameters to differentiate Ewing sarcoma family tumors.

Method and Materials: Seventy patients who were diagnosed with undifferentiated round cell sarcoma, Ewing–like sarcoma from Asan Medical Center were investigated. The inclusion criteria were as follow: 1) *EWSR1* translocation was negative or non-informative by molecular test and 2) available tumor tissue for molecular analysis. *EWSR1* FISH was performed to exclude ESFT. In addition, *CIC-DUX4* and *BCOR-CCNB3* gene fusion was investigated by RT-PCR. The clinical and histologic phenotypes were reviewed. CD99, NKX2.2, Caveolin-1, BCOR and NUT immunohistochemical stains were performed and assessed the diagnostic value to differentiate Ewing sarcoma from other round cell sarcomas.

Results: We identified six cases of *CIC-DUX4* sarcoma but *BCOR-CCNB3* sarcoma was not detected. Compared to ESFT, survival outcome was not significantly different (P=0.325) but they demonstrated short event-free survival (P=0.034) and poor response to treatment (P=0.007). Histologically, heterogeneous round, plasmacytoid, and spindle cells were observed. Unlike Ewing sarcoma, cytologic pleomorphism with bizarre nuclei and multinucleated cells was common. Myxoid stoma was also frequently identified. NUT immunohistochemistry was strong positive in one of ESFTs. By immunohistochemistry, CD99 and NKX2.2 combination test may be helpful to differentiate *CIC-DUX4 / BCOR-CCNB3* sarcoma from Ewing sarcoma.

Conclusion: We identified six of *CIC-DUX4* from undifferentiated round cell sarcomas. They had distinct histologic features and poor prognosis compared with ESFT. Therefore, molecular analysis to detect the distinctive genetic alteration is mandatory in tumors with atypical histologic, immunohistochemical and/or clinical presentation for accurate diagnosis

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Introduction

Small round cell sarcomas (SRCS) are heterogeneous group of aggressive soft tissue tumors that mainly affect children or young adults.[1,2] Histological and immunohistochemical phenotypes of each subtype significantly overlap. Therefore, the diagnosis depends on the integration of clinicoradiological, histological, immunohistochemical (IHC) and specific molecular findings.

A small portion of SRCS may not be classified based on clinical, morphologic, IHC features and negative or non-informative molecular results, leading to the diagnosis of undifferentiated round cell sarcoma (URCS). URCSs, which have Ewing sarcoma morphology and lack of *EWSR1*-rearrangement, are regarded as "Ewing sarcoma- like tumor" and often managed similarly to the Ewing sarcoma family tumor (ESFT). Recently, *CIC-DUX4* sarcoma or *BCOR-CCNB3* sarcoma have been described as a new emerging group in Ewing sarcoma- like tumor.[3-8] *CIC-DUX4* sarcoma is characterized by *CIC-DUX4* fusion resulting from either t(4;19)(q35;q13) or a t(10;19)(q26;q13) translocation[9,10] *BCOR-CCNB3* fusion, resulting from a paracentric inversion of chromosome X, is recently described sarcoma and considered a distinct bone tumor.[11,12] Both *CIC*-DUX4 sarcoma and *BCOR-CCNB3* sarcoma are known to show aggressive behavior. Ewing-type therapeutic regimen (vincristine, doxorubicin and cyclophosphamide with ifosfamide and etoposide) with surgical resection may be the only available treatment option. Although conventional Ewing sarcoma often showed sustained response to chemotherapy, *CIC-DUX4* sarcoma and *BCOR-CCNB3* sarcoma appears to show heterogeneous therapeutic response and quickly develop chemoresistance to Ewing-type therapeutic regimens.[4,13] Therefore, differentiating Ewing sarcoma from *CIC-DUX4* sarcoma or *BCOR-CCNB3* sarcoma is very important. However, it is difficult to differentiate these tumors because of their overlapped histologic feature and IHC phenotype. The diffuse strong membranous expression of CD99 appears to be sensitive in ESFT. Caveolin and NKX2.2 are also commonly expressed in ESFT. Despite high sensitivity, CD99, Caveolin-1 and NKX2.2 may also be expressed in other round cell sarcomas of bone and soft tissue.[14] Furthermore, BCOR is a recently introduced IHC marker reported to support the diagnosis of *BCOR*-associated sarcoma. However the diagnostic

value of BCOR IHC stain is unclear because it has not been examined in other round cell sarcoma including ESFT. In addition, the low incidence of *CIC-DUX4* sarcoma and *BCOR-CCNB3* sarcoma and limited documentation of characteristics for diagnosis make diagnosis more difficult.

The present study 1) investigated small round cell sarcoma to identify *CIC-DUX4* or *BCOR-CCNB3* fusion positive sarcoma, 2) examined clinical, histopathologic and immunohistochemical characteristics of *CIC-DUX4* or *BCOR-CCNB3* sarcoma, and 3) evaluated parameters to differentiate Ewing sarcoma family tumors.

Materials and Methods

Patient selection

Pathologic report of small round cell tumor registered from January, 2000 to June, 2018 of the archives of Asan Medical Center were reviewed. 138 patients with small round cell tumor were identified. The inclusion criteria were as follow; 1) diagnosed as undifferentiated small round cell sarcoma, Ewing sarcoma-like tumor 2) *EWSR1* translocation was negative by fluorescence *in situ* hybridization (FISH) or polymerase chain reaction (PCR) or molecular test were not performed, 3) diagnosed as Ewing sarcoma/Primitive neuroectodermal tumor (PNET), but *EWSR1* translocation was negative or not evaluated, and 4) available tumor tissue for molecular analysis. Ewing/ PNET confirmed by genetic study were excluded.

Tissue microarrays (TMAs) were constructed. Two representative 2.0mm cores were taken from each paraffin-embedded tumor. TMAs were subjected to IHC stains and fluorescence in situ hybridization. In addition, reverse transcriptase-polymerase chain reaction (RT-PCR) was performed to detect *CIC-DUX4* fusion and *BCOR-CCNB* fusion, respectively.

Clinicopathological and histological review

Clinical data including age, sex, tumor location, tumor size, treatment, metastasis, recurrence, overall survival were obtained from medical record. For each case, histologic features and IHC stains were reviewed.

Immunohistochemistry

IHC stains for CD99, NKX2.2, NUT, Caveolin-1, FLI-1 and BCOR were performed using BenchMark XT automated immunostaining system (Ventana Medical System, Tucson, AZ, USA). Briefly, 4-μm thick sections of TMAs were transferred onto adhesive slides and dried at 62℃ for 30 minutes. Following standard heat epitope retrieval for 60 minutes in ethylene diaminetetraacetic acid, pH 8.0, in the autostainer, the samples were incubated with antibodies to CD99 (clone 12E7, DAKO, Glostrup, Denmark, 1:200), NKX2.2 (clone 74.5A5, DSHB, MA, USA, 1:400), Cavolin-1 (clone 412M-15, Cell marque, CA, USA, 1:100), NUT (clone C52B1, Cell signaling, MA, USA, 1:100), FLI-1 (clone MRQ-1, Cell marque, CA, USA, 1:50), and BCOR (clone C-10, Santa Cruz Biotechnology, CA, USA, 1:50). After incubation with primary antibodies, the sections were incubated with an ultraView universal DAB kit (Ventana Medical Systems). CD99 expression pattern was record as membranous, cytoplasmic and perinuclear-dot. Cytoplasmic expression of Caveolin-1 was interpreted as positive result. NKX2.2, NUT and BCOR expression was evaluated at the nuclear level. The positive control of NKX2.2, NUT, and BCOR were *EWS-FLI1* fusion proven Ewing sarcoma, NUT carcinoma and clear cell sarcoma of kidney, respectively.

Fluorescence *in situ* hybridization

FISH to detect *EWSR1* rearrangement was performed on 2-μm thick sectioned TMA slides

using a dual-color break-apart probe (22q21; Abbott Molecular/Vysis, Des Plaines, IL) according to the manufacturer's instructions. The hybridized slides were viewed using an Olympus BX51 fluorescence microscope at 1000X magnification and DAPI/ Green/Orange triple band pass filter set. For the FISH break-apart approach, at least 60 tumor nuclei per specimen were counted and more than 15% of tumor cells had to show split signals (>1 signal diameter apart) to yield a positive result.[15]

Reverse transcriptase-polymerase chain reaction

Total RNA was extracted from formalin fixed, paraffin-embedded tissue block using commercially available kit (RNeasy FFPE kit, Qiagen, Cat.#73504) according to the manufacturer's instructions. Reverse transcriptase reaction was performed using AccuPower RocketScript Cycle RT PreMix(Bioneer, Cat.#K-2205). *CIC-DUX4* and *BCOR-CCNB3* fusion assays using AccuPower HotStart PCR PreMix (Bioneer, Cat. #K-5050) were performed with the primers (*CIC-DUX4*: forward primer, 5′-CTTCAGGACCATGGCTTCT-3′; reverse primer, 5′-CCAGGAAGAATGGCAG TTC-3′; *BCOR-CCNB3*: forward primer, 5′-GGCTCCACCCCAGTGATCT-3′; reverse primer, 5′-GGGTGTTTTGGAGGTGGTGG A-3′) as described by Yomada et al[16]. Each PCR products were loaded and onto 2.5% agarose gel with ethidium bromide and visualized under UV illumination.

Statistical Analysis

Statistical analysis was performed using IBM SPSS version 18.0 (IBM SPSS Inc.). The variables were expressed as median with interquartile range (IQR) or number (n) with percentage. The case and control groups were compared using Fisher's exact test for categorical variables and Mann-Whitney U-test for continuous variables. Cumulative survival was determined using the Kaplan-Meier method. The difference between the two

groups in terms of survival was assessed using the log-rank test. *P*-values less than 0.05 were considered to indicate statistical significance.

Results

Seventy patients were selected for molecular analysis. 63 cases were available for FISH analysis and 29 cases were available for RT-PCR. Among them, 22 cases were available for both FISH analysis and RT-PCR (Figure 1).

Molecular analysis

Thirty-seven (58.7%) out of 63 patients demonstrated *EWSR1*-rearrangement, thus they were classified as ESFT. In seventeen (27%) out of 63 patients, *EWSR1*-rearranement was negative by FISH. Twenty-nine patients were enrolled in RT-PCR. *CIC-DUX4* fusion was detected in six cases and *BCOR-CCNB3* fusion was negative in all cases (Figure 2). Three out of six *CIC-DUX4* fusion cases were *EWSR1*-rearrangement FISH negative. Three *CIC-DUX4* fusion cases could not be subjected to *EWSR1*-rearrangement FISH because the tissue was not enough for TMA construction.

Clinicopathologic findings

The clinical data of patients *CIC-DUX4* sarcoma was summarized in Table 1. *CIC-DUX4* sarcoma occurred in four females and two males with an age range of 16 to 67 years (median 40.5 years). Four cases (66.7%) of *CIC-DUX4* sarcoma occurred in intraabdominal cavity and two of them (50%) arose in visceral organ, stomach and uterus, respectively. Two *CIC-DUX4* sarcomas occurred in soft tissue of trunk. Distant metastasis was observed in four cases (66.7%) and the survival rate was 50%.

Compared with ESFT, *CIC-DUX4* sarcoma occurred in older age (28 years vs 40.5 years) and more frequently in intraabdominal cavity (27% vs 66.7%). CIC-DUX4 sarcoma showed more frequent bone (18.9% vs 28.6%) involvement and distant metastasis (51.4% vs 66.7%), but these results did not achieve statistical significance. Only one case reached to complete remission after initial treatment in *CIC-DUX4* sarcoma but 29 cases (78.4%) in ESFT (p=0.007). The median survival was 14 months in *CIC-DUX4* sarcoma and 33months in ESFT and the difference between two groups had statistically borderline significance (p=0.07). Five-year event-free survival rate were significantly short in *CIC-DUX4* sarcoma (p=0.034) but treatment response and survival rate were not significantly different between *CIC-DUX4* sarcoma and ESFT (Table 2, Figure 3).

Histological characteristics

Histologic findings are summarized in Table 3 with representative figures (Figure 4). Three of *CIC-DUX4* sarcoma cases were surgical resection specimen (Case #18, 23, and 28) and three cases were needle biopsy specimen (Case #2, 25, and 37). In *CIC*-DUX4 sarcomas, the tumor circumscription, and growth pattern were evaluated in resection specimen and cytologic features were evaluated in all cases. Two of them had nodular appearance with thick fibrous capsule and tumor cells were infiltrating into tumor capsule (Case #18 and 28). Fibrous septa dissecting tumor cells were observed (Case #18 and 23). Most cases of *CIC-DUX4* sarcomas had sheet-like growth pattern accompanied with cords (Case #28), or trabecular or nested pattern (Case #18, 28). The small to medium sized tumor cells had predominantly round to oval shape but short spindle (Case #23 and 28), or plasmacytoid (Case #23 and 28) phenotypes were also identified in parts. The majority of tumor cells had scanty to moderate amount of pale to eosinophilic cytoplasm, but clear cytoplasm with distinct cell border (Case #18) was also noted. The nuclei were irregular and hyperchromatic

with prominent but less commonly inconspicuous nucleoli (Case #25). Nuclear pleomorphism with bizarre nuclei was observed (Case #18, 23 and 28). Multinucleated cells were occasionally identified. The stroma was heterogeneously fibrotic, collagenous and myxoid with variable proportion (Case #18, 23 and 28). All of the cases had focal or extensive necrosis. The mitotic count varied from 3 to 25 per 10 HPFs.

Immunohistochemistry

CD99 was positive in three (50%) cases in *CIC-DUX4* sarcomas. Of them, two cases (33.3%) revealed typical diffuse membranous CD99 positivity. CD99 expression has been reported frequently in ESFT. In *CIC-DUX4* sarcomas, NKX2.2, Caveolin-1 and BCOR were expressed in 20%, 20% and 40%, respectively. On the other hands, 89.2% of 37 ESFT expressed CD99 in diffuse membranous pattern, and NKX2.2 and Caveolin-1 were expressed in 55.6% and 43.2% of ESFT, respectively. Only one case was positive for NUT IHC stain and this case had EWSR1 rearrangement (Table 4). In combination test for CD99 and NKX2.2, ESFT (55.5%) more frequently expressed both CD99 (diffuse, membranous) and NKX2.2 than *CIC-DUX4* sarcoma (20%) (P=0.004) (Table 5, Figure 5)

Discussion

Small round cell sarcoma is a heterogeneous group, characterized their histologic morphology. Because of their histological and immunohistochemical similarities, to make accurate diagnosis is often challenging. Arguably, pathologic subclassification of this heterogeneous group will have significant clinical impact because there are well-established and sensitive chemotherapy regimens for rhabdomyosarcoma and Ewing sarcoma, respectively.[17] The concomitant development of immunohistochemical markers and

molecular techniques facilitate to differentiate specific diagnosis from others. Recently, the *CIC* or *BCOR* rearrangement was detected in a subset of small cell sarcomas. *CIC-DUX4* gene fusion has been described frequently detected chromosomal alteration up to two-third of *EWSR1*-rearrangement negative round cell sarcoma.[16,18] Small round cell sarcoma, characterized by *BCOR- CCNB3* gene fusion, is currently considered a distinct type of bone sarcoma. In the literatures, *CIC*-associated sarcomas show more aggressive clinical behaviors; rapid growing, chemoresistant and early distant pulmonary metastasis.[2-4,7,16]

In Korea, presence of *CIC, BCOR*–associated sarcoma has not been studied. Present study investigated the frequency of *CIC-DUX4* and *BCOR-CCNB3* sarcomas. *CIC-DUX4* gene fusion was detected in 17.6% of non-ESFT round cell sarcoma. *CIC-DUX4* sarcomas frequently occurred in intraabdominal or thoracic cavity with or without visceral organ involvement. Unlike previously reported, *CIC-DUX4* sarcomas tended to affect older people than ESFT (median age: 40.5 vs 28, P=0.057). Even though we could not prove statistical significance, *CIC-DUX4* sarcoma patients had aggressive behaviors with frequent metastasis and poor treatment response. The 5 year event-free survival rate was significantly lower in *CIC*-DUX4 sarcoma than ESFT (P=0.034). In addition, they tended to show early mortality: 33.3% of them expired in a year and 2-year survival rate was 50%. Although *CIC-DUX4* sarcoma and ESFT shared histologic features (solid growth pattern, small to medium-sized small round cells), there were some distinct histologic features. In *CIC-DUX4* sarcomas, the histologic features were heterogeneous. Large-sized cells were frequently admixed with small cells. Cytoplasm was more abundant compared to Ewing sarcoma. Opposed to Ewing sarcoma, *CIC-DUX4* sarcomas often had irregular nuclei with prominent nucleoli. Nuclear pleomorphism was observed in more than half of the cases, bizarre nuclear and multinucleated cells were identified in some cases. In addition, myxoid stroma which is typically absent in Ewing sarcoma was commonly observed. Myxoid stroma could be

observed in focal area and was sometimes accompanied with fibrous or collagenous stroma.[19] For accurate diagnosis, ancillary tests including molecular analysis should be performed when we encounter mentioned atypical histologic features.

IHC stains were performed to differentiate ESFT from other round cell sarcoma and to identify distinct genetic abnormality in non-ESFT round cell sarcoma. CD99, NKX2.2, and Caveolin-1 were performed to differentiate ESFT from other round cell sarcoma and to assess the diagnostic value. Strong, diffuse membranous expression of CD99 is seen in most of Ewing sarcoma. However, CD99 expression has been reported in the large majority of *CIC-DUX4* sarcoma and other small round cell tumors.[5,19] Other immunohistochemical markers such as NKX2.2, Caveolin-1, FLI1, and their combination test were suggested to diagnose Ewing sarcoma from other small round cell sarcomas.[20-23] In present study, *CIC-DUX4* sarcoma also expressed CD99 (50%), NKX2.2 (20%), Caveolin-1 (20%). The combination of CD99 and NKX2.2 was significantly specific to differentiate ESFT and *CIC-DUX4* sarcoma. Strong, diffuse membranous expression of CD99 with NKX2.2 positivity was highly specific for ESFT (55.5% in ESFT), and negative expression of both markers favored non-ESFT round cell sarcoma (P=0.004). FLI1 was highly expressed in all of *CIC-DUX4* sarcoma, ESFT and other non-ESFT round cell sarcomas.

BCOR IHC staining has been suggested as a sensitive marker for *BCOR-CCNB3* sarcoma.[7,20] Matsuyama *et al*. investigated BCOR IHC expression in small round and spindle cell tumors and evaluated the diagnostic value for *BCOR*-associated sarcoma. In this study, BCOR IHC was positive in many tumors, including synovial sarcoma, solitary fibrous tumor, Ewing sarcoma and *BCOR-CCNB3* sarcoma. However, it was weakly positive and/or was expressed only focally in tumors other than *BCOR-CCNB3* sarcoma, in contrast to its diffuse and strong expression in *BCOR-CCNB3* sarcoma. Based on these results, BCOR IHC

staining might be assumed to be meaningful in diagnosing *BCOR-CCNB3* sarcoma when they were expressed in strong, diffuse pattern.[7] In present study, two of *CIC-DUX4* sarcoma and nine of ESFT cases expressed BCOR, but they were expressed weakly and/or were expressed in a small number of cells (less than 5%). Thus, BCOR expression might be non-specific. However, BCOR IHC stain was diffuse strong positive in one of non-ESFT round cell sarcoma. He was a 5-year old boy with sacral mass. The tumor was initially diagnosed as Ewing sarcoma but *EWSR1*-rearrangement FISH was negative. The tumor recurred a year after initial treatment (neoadjuvant chemotherapy and surgery) and he expired three years later. The tumor mainly composed of small round cells and short spindle cells with scanty eosinophilic cytoplasm. Curvilinear thin-walled vessels were observed. The tumor cells were positive for CD99 and NKX2.2 but negative for Caveolin-1, NUT, S100-protein, synaptophysin, chromogranin and myogenin. The clinical, histological and IHC staining results could support the diagnosis of *BCOR-CCNB3* sarcoma. Further molecular test for *BCOR-CCNB3* gene fusion would be necessary to completely exclude *BCOR-CCNB3* sarcoma.

NUT midline carcinoma is a clinically aggressive tumor. Although it is rare, over 80% of patients die within the first year of diagnosis of NUT carcinoma, and metastasis is common. Currently, there is limited therapeutic benefit from chemotherapy.[24] Recently, undifferentiated soft tissue tumor harboring *NUTM1*-rearrangement was identified. They shared some *NUTM1* fusion partner (*BRD4* and *BRD3*) to NUT carcinoma[25]. The present study performed NUT IHC stain to screen *NUTM1*-rearrangement. Only one case, 47-year old male with paranasal mass, was positive for NUT IHC stain. On IHC stains, it was negative for CK, CD99, NKX2.2, Caveolin-1, and BCOR. This tumor demonstrated not only *EWSR1* rearrangement, but also NUT rearrangement by FISH. Because this case did not express cytokeratin, a possibility undifferentiated soft tissue tumor harboring *NUTM1*-

rearrangement can be considered. This tumor is planned for gene sequencing to confirm the gene rearrangement in future.

One of the limitations of the present study is that *FUS* rearrangement, another rare genetic alteration of ESFT, was not evaluated. Furthermore, we could not evaluate *CIC-DUX4* and *BCOR-CCNB3* gene fusion in all of non-ESFT round cell sarcoma because of insufficient tissue for RT-PCR. Thus, prevalence of *CIC or BCOR*-associated sarcoma would vary with expansion of the study.

In conclusion, *CIC-DUX4* or *BCOR-CCNB3* sarcomas were first identified in Korea. We distinguished six of *CIC-DUX4* from round cell sarcomas without *EWSR1*-translocation. They had distinct histologic features and poor prognosis compared with ESFT. Therefore, molecular analysis to detect these genetic alterations in tumors with atypical histologic, immunohistochemical and/or clinical presentation would be mandatory for accurate diagnosis in future.

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Table 1. Clinicopathologic data of *CIC-DUX4* sarcomas

Case no.	Age /Sex	Primary site	Initial diagnosis	Tumor size (cm)	Distant metastasis	Treatment	Survival outcome (survival time, months)	EWSR1 rearrangement†
2	16/F	Chest wall	Ewing sarcoma	8.6	Absent	CTx only	Dead (3)	N.A
18	62/F	Stomach	Ewing sarcoma	7.0	Present	Surgery+ CTx	Alive (78)	Absent
23	32/F	Uterus	Ewing sarcoma	10.0	Present	Surgery+ CTx	Dead (4)	Absent
25	32/F	Perirenal soft tissue	Desmoplastic small round cell tumor	14.0	Present	CTx + RTx	Dead (14)	N.A
28	49/M	Suprapubic soft tissue	Small round cell tumor, NOS	3.8	Present	Surgery+ CTx	Alive (47)	Absent
37	67/M	Peripancreatic tissue	Small round cell tumor, NOS	3.5	Absent	CTx only	Dead (5)	N.A

M male, *F* female, *CTx* chemotherapy, *RTx* radiation therapy, *N.A* not available

† FISH analysis

Table 2. Clinical characteristics of *CIC-DUX4* sarcoma and ESFT groups

Parameters	*CIC-DUX4* ¶ (total n=6)	ESFT† (total n=37)	*P*-value
Sex (n, %)			0.309
Male	2 (33.3)	20 (54.1)	
Female	4 (66.7)	17 (45.9)	
Age (median, IQR)(years)	40.5 (35))	28 (25)	0.057
Tumor size (median, IQR) (cm)	7.8 (7)	6.7 (6)	0.343
Tumor location (n, %)			0.336
Head and neck	0 (0)	9 (24.3)	
Soft tissue (trunk and extremity)	2 (33.3)	17 (54.9)	
Bone	0 (0)	1 (2.7)	
Visceral soft tissue	4 (66.7)	10 (27)	
Bone involvement (n, %)			0.369
Not involved	4 (66.7)	30 (81.1)	
Involved	2 (33.3)	7 (18.9)	
CNS involvement (n, %)			0.860
Not involved	6 (100)	36 (97.3)	
Involved	0 (0)	1 (2.7)	
Metastasis (n, %)			0.403
No metastasis	2 (33.3)	18 (48.6)	
Metastasis	4 (66.7)	19(51.4)	
Treatment (n, %)			
Surgery + CTx	4 (57.1)	17 (45.9)	
Surgery+ CTx+RTx	0 (0)	13 (35.1)	
CTx only	2 (28.6)	0 (0)	
CTx+RTx	1 (14.3)	2 (5.4)	
Surgery only	0 (0)	4 (9.1)	

Parameters	CIC¶ (total n=6)	ESFT† (total n=37)	*P*-value
CR after initial Tx			0.007
CR	1 (16.7)	29 (78.4)	
No CR	5 (83.3)	8 (21.6)	
Response to neoCTx (n/total n, %)			0.853
CR	0/3 (0)	3/18 (16.7)	
PR	1/3 (33.3)	7/18 (38.9)	
SD	2/3 (66.7)	5/18 (27.8)	
PD	0/3 (0)	3/18 (16.7)	
Recurrence (n, %)			0.533
No recurrence	0(0)	14(48.3)	
Recurred	1(100)	15(51.7)	
Status (n, %)			0.645
Alive	3 (50.0)	18(48.6)	
Died	3 (50.0)	19(51.4)	
Overall survival (median, IQR)(months)	14 (48)	33 (80)	0.070

† FISH analysis

¶ RT-PCR analysis

IQR interquartile range, *CNS* central nerve system, *CTx* chemotherapy, *RTx* radiation therapy, *CR* complete remission, *PR* partial response, *SD* stable disease, *PD* progressive disease, *Neo CTx* neoadjuvant chemotherapy, *Tx* treatment, *ESFT* Ewing sarcoma family tumor

Table 3. Histopathologic features of *CIC-DUX4* sarcomas and ESFT

	CIC-DUX4 sarcoma	ESFT
Cell morphology		
Cell size	small to medium	small
Cytoplasm	scanty	
	occasionally clear, eosinophilic and plasmcytoid	scanty
Nuclei	irregular	round
	bizarre or multinucleated	monotonous
Nucleoli	conspicuous or inconspicuous	inconspicuous
Heterogeneous component	spindle	rare
Stroma	myxoid, collagenous or fibrotic	absent
Histologic pattern	solid, trabecular, cord or nested	solid
Mitosis	variable	Variable
	(3-25/10HPF)	(2-28/10HPF)
Necrosis	frequent	frequent

ESFT Ewing sarcoma family tumor

Table 4. Immunohistochemical findings of *CIC-DUX4* sarcoma and ESFT§

Makers	*CIC-DUX4*¶ (n=6)	*ESFT* † (n=37)	Other non-ESFT RCS (n=14)	*P*-value
CD99 (n, %)	3/6 (50.0)	36/37 (97.3)	9/14 (64.3)	0.002
Membranous	2/6 (33.3)	34/37(91.8)	4/14 (28.6)	
Cytoplasmic	0/6 (0)	2/37(5.4)	3/14 (21.4)	
Perinuclear-dot	1/6 (16.7)	0/37 (0)	2/14 (14.3)	
NKX2.2 (n, %)	1/5 (20)	20/36 (55.6)	3/14 (21.4)	0.052
Caveolin-1 (n, %)	1/5 (20)	16/37 (43.2)	1/14 (7.1)	0.042
BCOR (n, %)	2/5 (40)	9/32 (28.1)	3/14 (21.4)	0.724
NUT (n, %)	0/5 (0)	1/36 (2.8)	0/14 (0)	0.768
FLI-1 (n, %)	5/5 (100)	25/27 (92.6)	7/8 (87.5)	0.704

§ Cases which have non-informative *EWSR1* FISH result were excepted for analysis

† FISH analysis

¶ RT-PCR analysis

ESFT Ewing sarcoma family tumor, *RCS* round cell sarcoma

Table5. The combination of CD99 and NKX2.2 immunohistochemical stains in *CIC-DUX4* and ESFT§

Markers	ESFT (n=36)	CIC-DUX4 sarcoma (n=5)	
CD99 (+), NKX2.2 (+)*	20(55.5%)	1 (20%)	*P*=0.004
CD99 (-), NKX2.2 (-)	1 (2.8%)	3 (60%)	

§Calculated only available cases

*Strong, diffuse membraneous pattern is only counted as CD99 (+)

ESFT Ewing sarcoma family tumor

Figure legends

Figure 1. Flow chart of the study design and brief molecular test results.

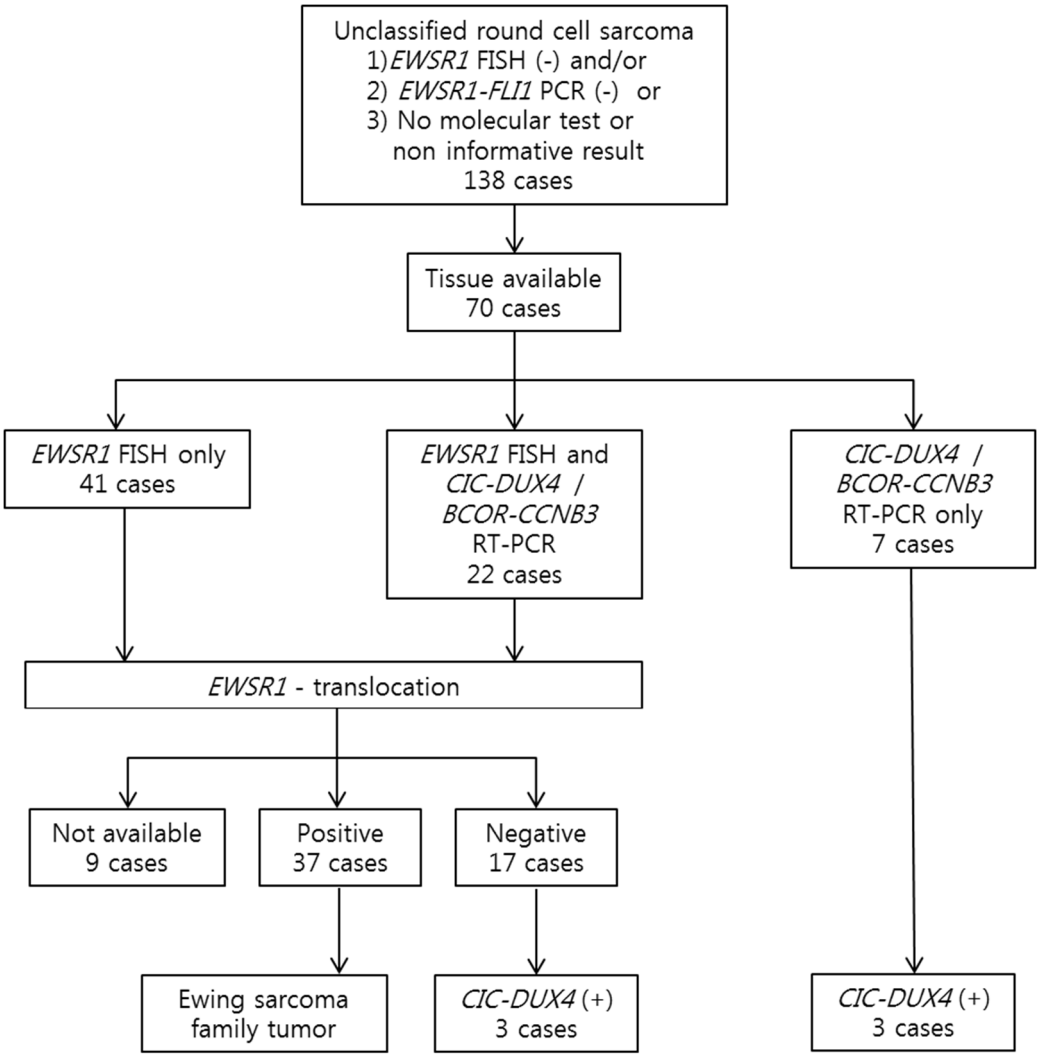


Figure 2. Result of *BCOR-CCNB3* fusion and *CIC*-DUX4 fusion RT-PCR. *BCOR-CCNB3* gene fusion is negative in all cases (A). *CIC-DUX4* gene fusion is positive for six patients (Case #2, 18, 23, 25, 28 and 37)

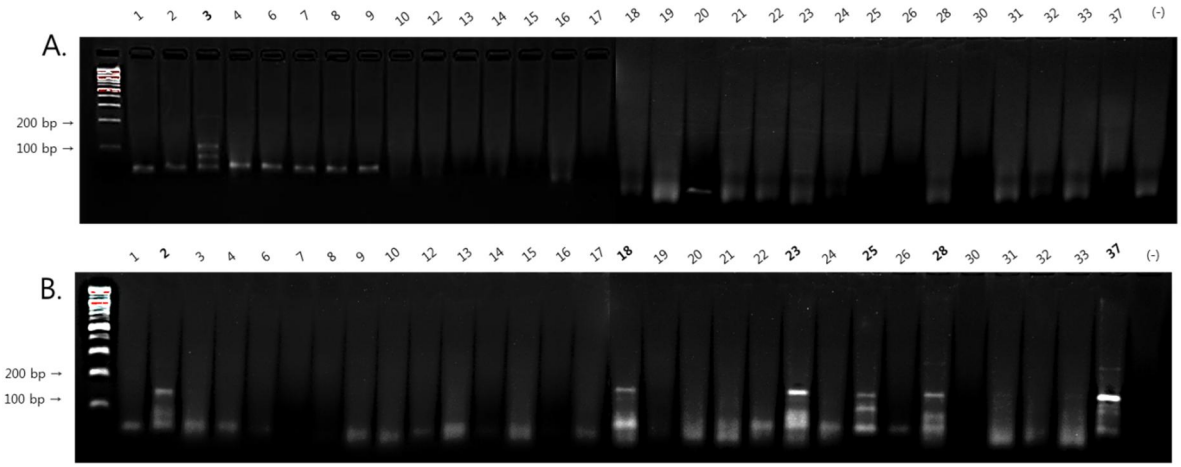


Figure 3. The comparison of 5-year survival and 5-year event-free survival between *CIC-DUX4* / *BCOR-CCNB3* sarcomas and Ewing sarcoma family tumors (ESFT). *CIC-DUX4 or BCOR-CCNB* sarcomas demonstrate poor 5-year survival outcome (A) and 5-year event-free survival (B) compared with ESFT.

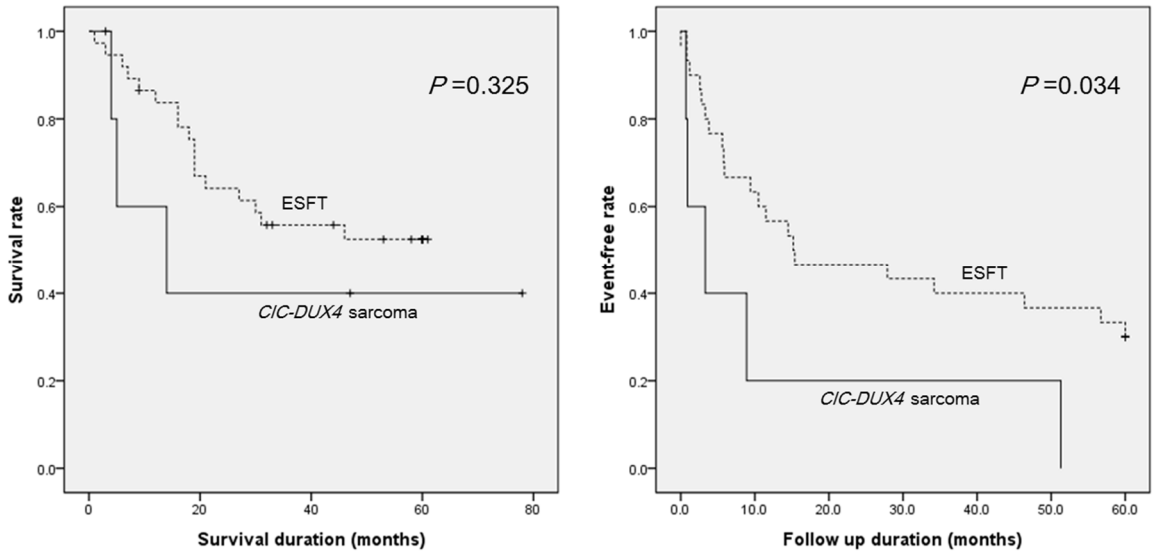


Figure 4. The histologic features of *CIC-DUX4* sarcomas (A, I: case #28, B, F, and G: case #23, C-E, H: case #18). In low magnification, the tumor has vaguely nodular and fibrous septa is common (A, B, and C, x40). The tumor cells are composed of small to medium cells (D, x200).They have relatively abundant clear to eosinophilic cytoplasm compared with Ewing sarcoma and they are sometimes plasmacytoid (E and F,x200). Spindle cell can be observed (G, x100). Bizarre pleomorphic nuclei and multinucleated cell are frequent (H, x200). Unlike typical Ewing sarcoma, myxoid stroma is common (I, x100).

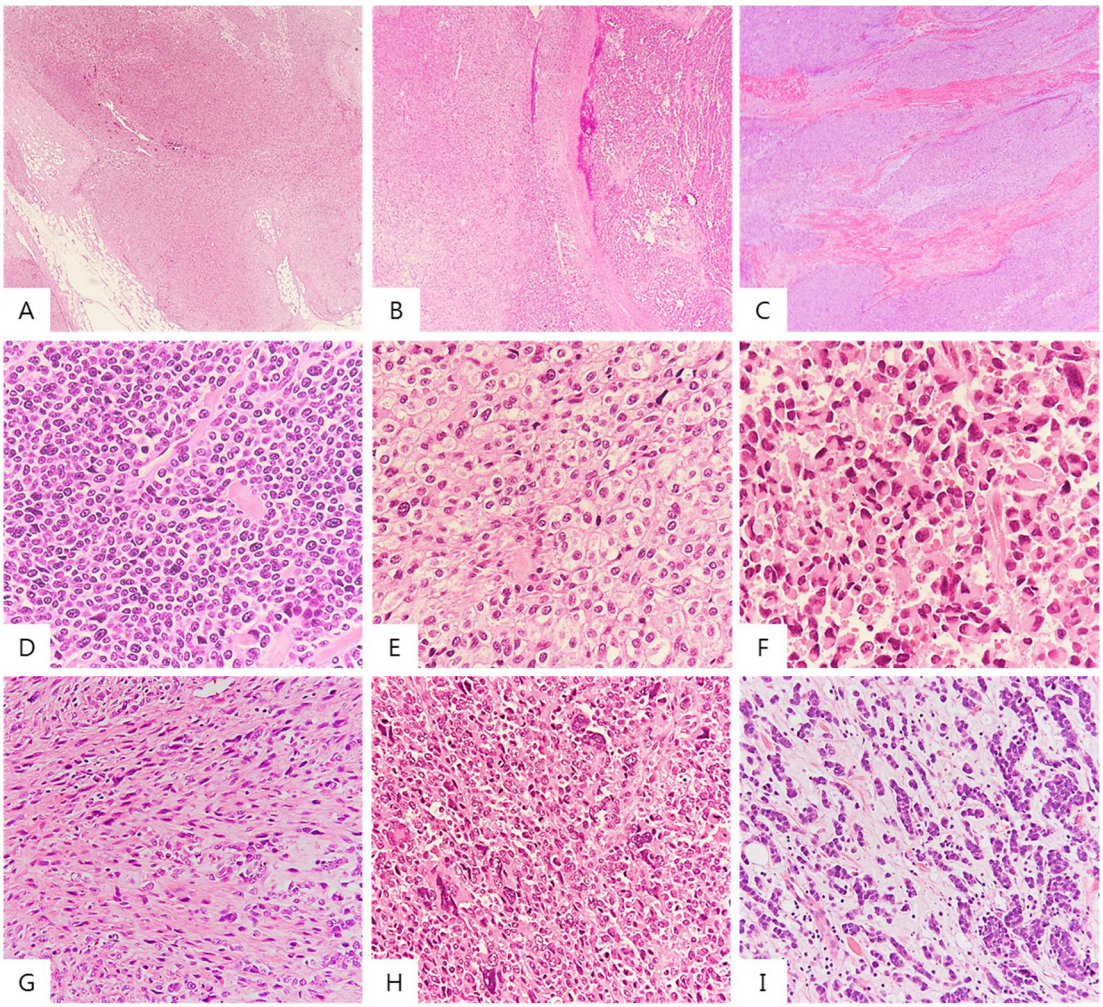
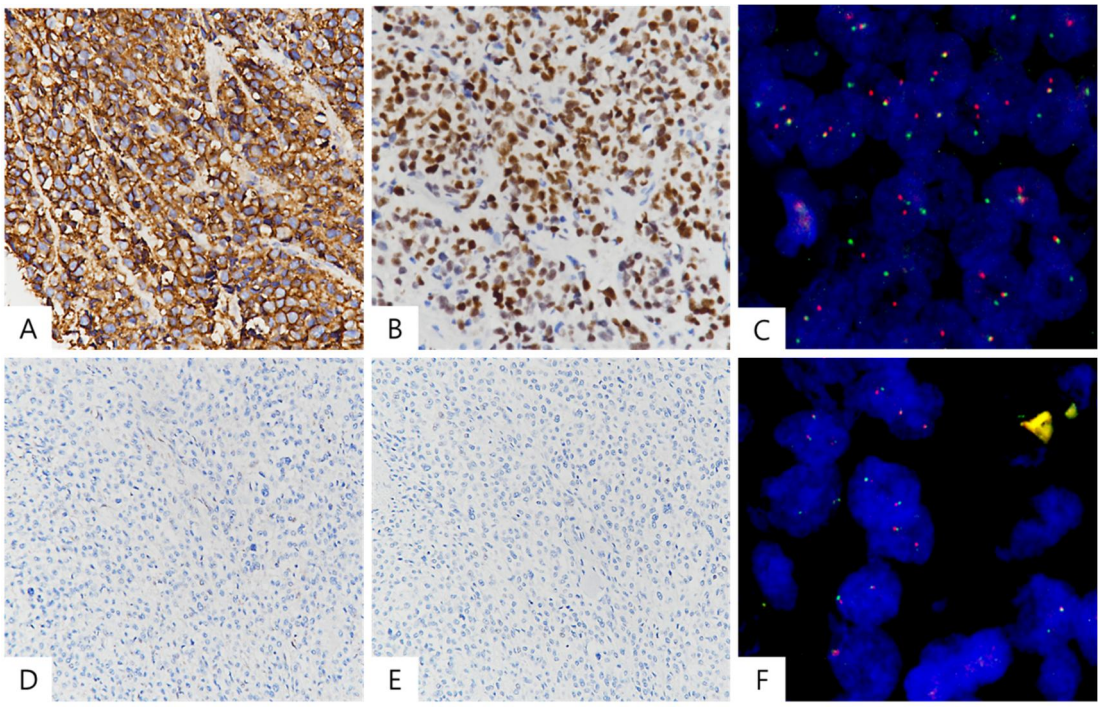


Figure 5. The combination of CD99 and NKX2.2 immunohistochemical stains in Ewing sarcoma family tumor (ESFT) and *CIC-DUX4* sarcoma. ESFT shows frequently positivity for both CD99 (strong, diffuse membranous) and NKX2.2(55.5%) (A and B, x200). *EWSR1* break apart FISH is positive (C).2.8% of ESFT were negative for both CD99and NKX2.2. However, 50% of *CIC-DUX4* sarcoma is negative for both CD99 and NKX2.2 IHC stains (C and D, x200). This case has no *EWSR1*-rearrangement (F).



국문요약

연구 필요성: 미분화환형세포육종 (undifferentiated small round cell sarcoma, USRCS)은 형태학적으로 작고, 원형이며 세포질이 거의 없는 세포로 구성이 되며, 세포의 분화를 특정할 수 있는 면역조직화학적 특징이나 분자유전학적인 특징을 가지지 않는 것으로 정의된다. 이들 가운데 일부는 유잉육종과 형태학적으로 매우 유사하여 Ewing sarcoma-like tumor로 불린다. *CIC-DUX4*와 *BCOR-CCNB3* 융합유전자와 관련된 육종은 비교적 최근에 알려진 미분화환형세포육종의 아형으로 유잉육종과는 달리 항암요법에 잘 반응하지 않고 예후가 더 나쁜 것으로 보고되었다. 그러나 *CIC-DUX4* 또는 *BCOR-CCNB3* 융합유전자와 연관된 육종은 형태학적, 면역조직화학적 소견이 유잉육종과 매우 유사하여 감별이 어렵다. 더불어, CIC 또는 BCOR gene fusion 과 관련된 소원형세포육종은 상대적으로 발병이 드물어 임상적 소견 및 진단에 유용한 특징적인 병리학적 소견 및 예후가 거의 밝혀지지 않았다. 또한 분자유전학적 검사의 고비용적 측면을 고려 할 때, 이를 대체할 수 있는 면역조직화학검사의 필요성이 높아지고 있으나, 그 유용성에 대한 연구는 거의 없는 실정이다. 저자는 본 연구를 통하여, 국내 최초로 1) *CIC-DUX4* 또는 *BCOR-CCNB3* 융합유전자 연관 육종의 발생률을 알아보고 2) 유잉육종과 차별되는 임상적, 조직학적 소견에 대해 조사하며, 3) 감별진단을 위한 면역학적 소견을 알아보고자 한다.

재료 및 방법: 2000년 1월부터 2018년 6월까지 서울아산병원에서 소형환형세포를 진단받은 사람을 대상으로 하였다. 그 중 유잉육종, 유잉유사육종, 미분화환형세포육종을 진단받은 환자 중 1)분자유전학적 방법으로 *EWSR1*유전자재배열이 음성이거나 2)분자유전학적 검사 결과가 진단적이지 못하거나 3) 검사를 시행하지 않은 군을 선정하여 검사를 진행했다. Tissue microarray를 제작하여 *EWSR1* FISH를 시행하고, 가능한 조직에서 역전사 중합효소 연쇄반응 (RT-PCR)을 통해*CIC-DUX4* 와 *BCOR-CCNB3* 융합유전자를 확인하였다. 이에 더불어 CD99, NKX2.2,

Caveolin-1 등의 면역조직화학염색을 시행하였다. *CIC-DUX4* 와 *BCOR-CCNB3* 융합유전자가 확인된 종양의 조직학적, 면역조직화학적 소견을 관찰하였고, FISH로 *EWSR1*-재배열이 확인된 종양과 임상적 소견을 비교하였다.

결론: 검사에 포함된 70 명의 환자 중에서 17명에서 *EWSR1* 유전자 재배열 음성이 확인되었고 그 중 3명에서 *CIC-DUX4* 융합유전자가 확인되었다. 또한 *EWSR1* FISH를 시행하지 않은 3명의 환자에서도 *CIC-DUX4* 융합유전자가 확인되었다. *BCOR-CCNB3* 융합유전자는 발견되지 않았다. 총 6명의 *CIC-DUX4* 융합유전자 연관 육종환자와 37명의 *EWSR1*-유전자 관련 육종 환자의 예후를 비교 한 결과, *CIC-DUX4* 융합유전자 연관 육종 환자에서 전체 생존기간이 더 짧고(14개월 vs 44개월), 원격전이, 재발률이 높았으나 통계학적 의의는 찾을 수 없었다. 그러나 초기 치료에 대한 반응은 *CIC-DUX4* 융합유전자 연관 육종에서 유의미하게 떨어지는 것을 알 수 있었다 (*P*=0.002*)*. 조직학적으로 *CIC-DUX4* 융합유전자 연관 육종에서는 유잉육종에서는 잘 보이지 않는 myxoid stroma가 흔하게 관찰되고, 붉거나 맑은 세포질이 풍부하고 일부에서는 형질세포와 같이 생긴 세포가 불균일하게 섞여있다. 또한 핵이 크고 핵인이 또렷하게 보이며, 다핵세포도 자주 동반되었다. 면역조직화학염색결과 진단에 특이적인 마커는 없었으나. 유잉육종의 진단에 널리 쓰이는 CD99와 NKX2.2 에서 모두 발현되는 경우 유잉육종의 진단에 도움이 된다는 것을 알 수 있었다 (*P*=0.004)

결론: *CIC-DUX4* 또는 *BCOR-CCNB3* 융합유전자 연관 육종은 환자의 치료와 예후에 유잉육종과 큰 차이를 보이므로 감별이 필요한 미분화소형세포육종의 특징적인 아형으로 비정형적 조직학적, 면역조직화학염색 소견, 임상적인 소견이 보이면 반드시 분자유전학적 검사를 통한 진단이 필요하다.