2.3. Immunohistochemical evaluation

A consensus judgment was adopted to establish the proper immunohistochemical scores for tumors, using a scoring system in a previous report [9] that was based on the strength of cytoplasmic expression of CXCR4, CXCR7, CCR6, CCR7, and VEGF: 0, negative; 1+, weak staining; 2+, moderate staining; 3+, strong staining. The distribution of positive cells was also recorded to impart the proportion of positive cells: sporadic (1% ≤ positive cells < 10%); focal (10% ≤ positive cells < 50%); diffuse (positive cells ≥ 50%). The immunohistochemical scores were defined as follows: score 0, no immunoreactivity; score 1, 1+ with sporadic or focal distribution; score 2, 1+ with diffuse distribution or 2+ or 3+ with sporadic distribution; score 3, 2+ with focal or diffuse distribution; score 4, 3+ with focal or diffuse distribution. Then, we considered immunohistochemical scores 0 to 2 as low protein expression and scores 3 and 4 as high protein expression according to our previous study [9].

The degree of angiogenesis was determined by the number of microvessels in defined areas as previously described [9]. The CD31-positive vessels were counted in 4 selected hot spots in a ×400 field (0.26-mm^2 field area). The mean of the 2 independent readings of each specimen was calculated, and microvessel density (MVD) was defined as the mean number of microvessels per 0.26-mm^2 field area.

The MIB-1 labeling index (MIB-1-LI) was estimated by counting the number of positive cells per 1000 tumor cells. Three independent pathologists (Y.O., H.Y., and K.K.), who were not aware of the clinical characteristics of the patients, judged the immunoreactivity. Then, MVD and MIB-1-LI were dichotomized as high or low, based on their median value.

2.4. TaqMan polymerase chain reaction to detect mRNA quantities of CXCR4, CXCR7, CCR6, CCR7, and VEGF

Total RNA was extracted from frozen samples, using Trizol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Quantitative real-time RT-PCR for these chemokine receptors and for *VEGF* was performed using an ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA) and predeveloped TaqMan assay reagents of human *CXCR4* (spanning exon 1/exon 2; ID: Hs00237052-m1), *CXCR7* (spanning exon 1/exon 2; ID: Hs00604567-m1), *CCR6* (spanning exon 1/exon 2; ID: Hs00171121-m1), *CCR7* (spanning exon 1/exon 2; ID: Hs00171054-m1), *VEGF* (spanning exon 1/exon 2; ID: Hs00173626-m1), and *GAPDH*. The polymerase chain reaction was carried out according to the manufacturer's protocol. The standard curve was constructed with serial dilutions of the *CXCR4*, *CXCR7*, and *VEGF* complementary DNA samples of MCF-7, a breast cancer cell line. As for *CCR6* and *CCR7*, standard curves were constructed using inflamed human tonsils. All reactions of the samples were triplicated, and the data were averaged from the values obtained in each reaction. The obtained data were standardized by using the internal housekeeping gene, *GAPDH*. The final mRNA expression index in each sample was calculated in arbitrary units (AU) as follows: mRNA expression index = *CXCR4*, *CXCR7*, *CCR6*, *CCR7*, or *VEGF* mRNA value/*GAPDH* mRNA value × 1000 AU.

2.5. Statistical analysis

Fisher exact test was used to evaluate the correlation between 2 dichotomous variables. The associations between immunohistochemical scores and mRNA expression were analyzed by the Mann-Whitney *U* test. For univariate and multivariate analyses of overall survival, the Kaplan-Meier method with the log-rank test and Cox proportional hazards model with stepwise procedure were used, respectively. A $P < .05$ was considered statistically significant.

3. Results

3.1. Patient characteristics

Table 1 summarizes the clinical and pathological characteristics of the 78 patients with RMS. The patients consisted of 38 males and 40 females, ranging in age from a month to 71 years old. Histologically, the 82 specimens

Table 1 Clinicopathological characteristics of 78 patients with primary RMS

Parameters	No. of cases (n = 78)
Age (y)	
≤15	53
>15	25
Sex	
Male	38
Female	40
Histology (primary)	
Embryonal	44
Alveolar	34
Stage (at diagnosis)	
1	22
2	5
3	41
4	5
Unknown	5
Location of primary tumor	
Favorable	24
Unfavorable	54
Tumor size (cm)	
≤5	27
>5	45
Unknown	6

Table 2 Results of immunostaining

	CXCR4 (n = 78)	CXCR7 (n = 77)	VEGF (n = 78)	CCR6 (n = 78)	CCR7 (n = 75)
ERMS					
High expression	18 (40.9%)	35 (79.5%)	22 (50.0%)	15 (34.1%)	30 (71.4%)
Low expression	26 (59.1%)	9 (20.5%)	22 (50.0%)	29 (65.9%)	12 (28.6%)
ARMS					
High expression	20 (58.8%)	29 (87.9%)	24 (70.6%)	25 (73.5%)	16 (48.5%)
Low expression	14 (41.2%)	4 (12.1%)	10 (29.4%)	9 (26.5%)	17 (51.5%)
P	.1703	.3760	.1036	.0007*	.0967

Abbreviations: ERMS, embryonal rhabdomyosarcoma; ARMS, alveolar rhabdomyosarcoma.
* $P < .05$.

included 34 primary ARMS, 44 primary ERMS, 3 metastatic ARMS, and 1 ARMS recurrence. *PAX3/PAX7-FKHR* fusion gene transcripts were examined in 21 cases of 34 ARMS and 15 cases of 44 ERMS. *PAX3-FKHR* fusion gene transcript was identified in 14 ARMS cases, and *PAX7-FKHR* fusion gene transcript was identified in 3 ARMS cases by RT-PCR and direct sequencing. None of *PAX3/PAX7-FKHR* fusion gene transcript was detected in ERMS. Survival data were available in 76 cases, with follow-up periods ranging from 1 to 223 months (median, 17 months). Of the 78 patients, 59 received combined modality therapy including chemotherapy using some of the standardized antitumor drugs (vincristine, actinomycin D, and cyclophosphamide), and 12 patients received surgical treatment and/or radiation therapy, whereas 7 patients had no therapeutic information.

3.2. Immunohistochemistry

Table 2 summarizes the results of immunostaining for CXCR4, CXCR7, VEGF, CCR6, and CCR7. *P* value

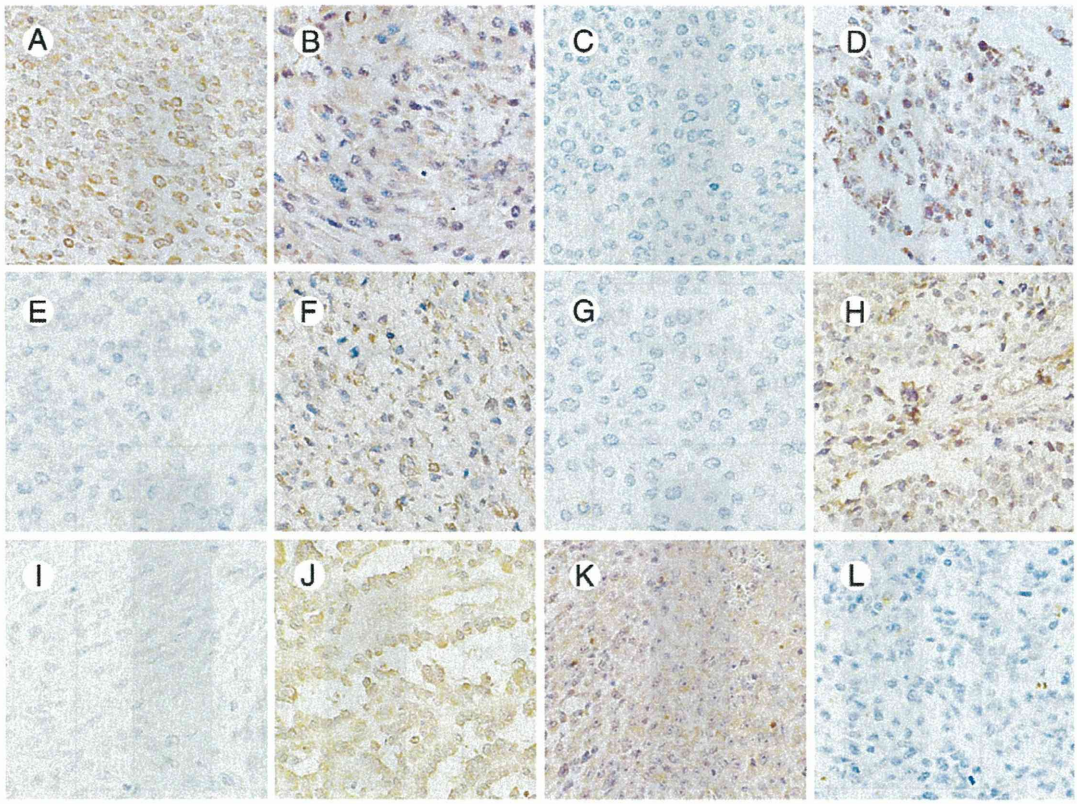


Fig. 1 Results of immunohistochemical expression of CXCR4 (A-C), CXCR7 (D, E), CCR6 (F, G), CCR7 (H, I), and VEGF (J-L) in primary site of RMS. A and B, ARMS (A) and ERMS (B) showing diffuse and strong immunoreactivity for CXCR4, evaluated as score 4. C, Negative staining of CXCR4. D, Diffuse and strong staining of CXCR7 in ARMS. E, Negative staining of CXCR7. F, Focal and strong cytoplasmic expression of CCR6 in ERMS. G, Negative staining of CCR6. H, Alveolar patterned tumor cells revealed diffuse and moderate CCR7 cytoplasmic immunostaining. I, Negative staining of CCR7. J and K, Cytoplasmic diffuse and strong (J) and diffuse and moderate (K) immunoreactivity for VEGF in ARMS (J) and ERMS (K). L, Negative staining of VEGF. Original magnification ×200.

represents the stastical association of the expression of each immunostaining factor between ERMS and ARMS. Positive staining for CXCR7, VEGF, and CCR6 was recognized mainly in the cytoplasm of the tumor cells or endothelium [26], whereas CXCR4 and CCR7 expression was seen in the nucleus as well as the cytoplasm shown in Fig. 1 [9,26].

3.3. CXCR4, CXCR7, and VEGF immunostaining

Of the 78 primary RMS, 38 (48.7%) showed high expression of cytoplasmic CXCR4. High CXCR4 expression was recognized in 20 (58.8%) of 34 in ARMS and 18 (40.9%) of 44 in ERMS. In the same manner, high VEGF expression was observed in 46 (58.9%) of 78 in all the RMS, 24 (70.6%) of 34 in ARMS, and 22 (50%) of 44 in ERMS. High CXCR7 expression was recognized in 64 (83.1%) of 77 in RMS, 29 (87.9%) of 33 in ARMS, and 35 (79.5%) of 44 in ERMS. RMS displayed high CXCR7 expression regardless of the histologic subtype (Table 2). None of these proteins showed expression patterns that differed significantly between the histologic subtypes.

Within the cases that showed high cytoplasmic CXCR4 expression, 19 (55.9%) of 34 ARMS and 14 (31.8%) of 44 ERMS showed high VEGF expression. This revealed a significant association between CXCR4 and VEGF expression (P = .0003 and P = .0051, respectively) as shown in Fig. 2. Immunohistochemical expression of CXCR7 showed no significant association with CXCR4 or VEGF expression in either ARMS or ERMS (CXCR7 versus CXCR4: ARMS P > .9999, ERMS P = .7161; CXCR7 versus VEGF: ARMS P = .2952, ERMS P > .9999; data not shown).

3.4. CCR6 and CCR7 immunostaining

Of 44 cases, 15 (34.1%) showed high CCR6 expression in ERMS, whereas 25 (73.5%) of 34 cases showed high expression in ARMS. High expression of CCR7 was recognized in 30 (71.4%) of 42 in ERMS and 16 (48.5%)

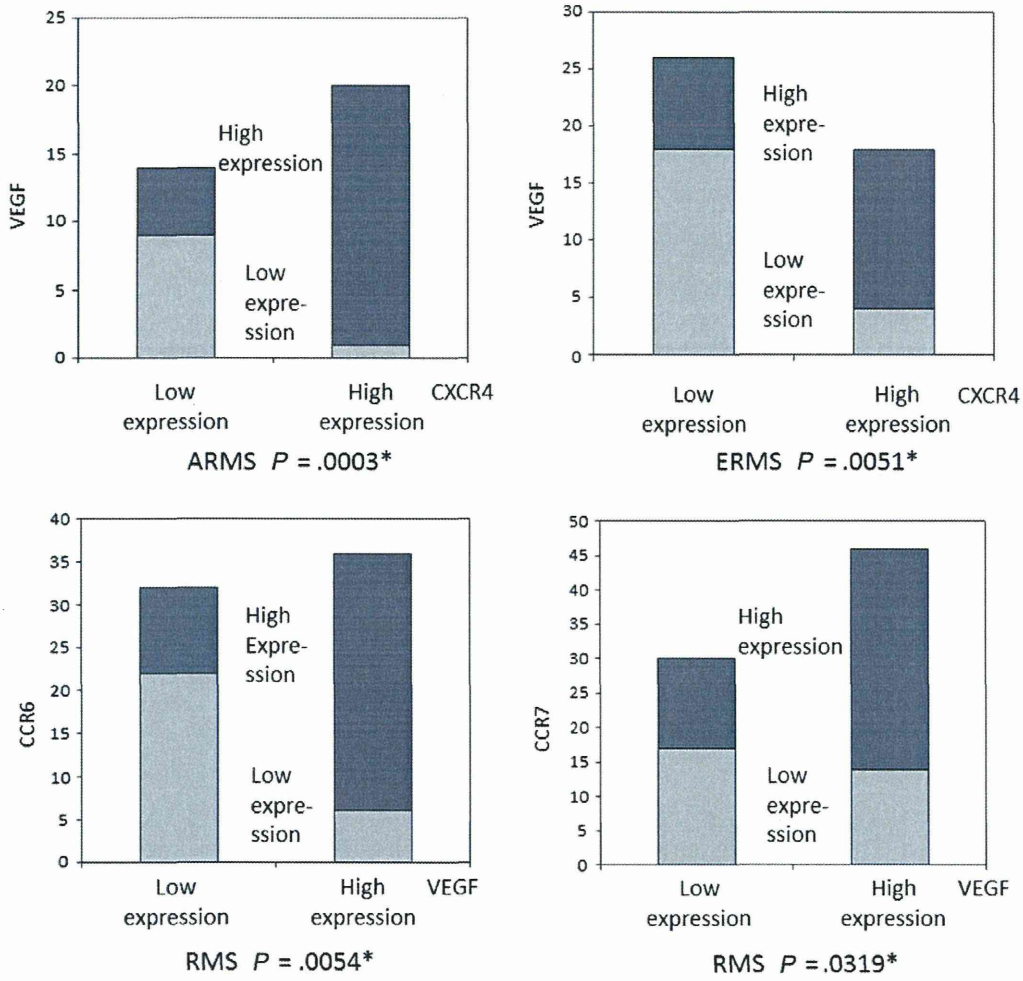


Fig. 2 Association between immunohistochemical score of CXCR4 and VEGF. There were significant positive associations in both ARMS and ERMS. There also are significant associations between VEGF and CCR6 as well as between VEGF and CCR7 in all RMS cases.

Table 3 Microvessel density and MIB-1-LI in RMS

		RMS (n = 78)	ERMS (n = 44)	ARMS (n = 34)	*P*
MVD	Mean ± SD	11.00 ± 6.61	11.13 ± 6.71	10.50 ± 6.46	.996
MIB-1-LI	Mean ± SD	14.15 ± 11.29	12.79 ± 11.85	11.85 ± 8.41	.2189

Abbreviations: MVD, microvessel density; MIB-1-LI, MIB-1 labeling index; RMS, rhabdomyosarcoma; ERMS, embryonal rhabdomyosarcoma; ARMS, alveolar rhabdomyosarcoma.

of 33 in ARMS (Table 2). ARMS cases revealed higher CCR6 expression than ERMS cases, with statistical significance ($P = .0007$), whereas CCR7 expression showed no significant difference between the 2 histologic types ($P = .0967$). CCR6 and CCR7 expression levels showed no association in RMS ($P = .6395$, data not shown). Neither CCR6 nor CCR7 showed an association with either CXCR4 or CXCR7 expression, whereas VEGF expression showed significant associations to both CCR6 expression and CCR7 expression ($P = .0054$ and $P = .0319$, respectively) in all RMS (Fig. 2).

3.5. Microvessel density

MVD was assessed by immunohistochemical staining of CD31. It ranged from 5.5 to 37.25 (median, 11.00 ± 6.61). Median of MVD did not show statistical difference between histologic subtypes (ERMS median, 11.13 ± 6.71; ARMS median, 10.50 ± 6.46; $P = .996$; Table 3). No significant relationship was observed between MVD and immunohistochemical expression of CXCR4, CXCR7, VEGF, CCR6, or CCR7 ($P = .7114$, $P = .1870$, $P = .3071$, $P = .8337$, and $P = .8733$, respectively; data not shown).

3.6. MIB-1 labeling index

The MIB-1-LI ranged from 2.2 to 65.38 (median, 14.15 ± 11.29), and its median of both subtypes showed no statistical difference (ERMS median, 12.79 ± 11.85; ARMS median, 11.85 ± 8.41; $P = .2189$; Table 3). No significant relationship was observed between MIB-1-LI and immunohistochemical expression of CXCR4, CXCR7, VEGF, CCR6, or CCR7 ($P = .0760$, $P = .9458$, $P = .7761$, $P = .4564$, and $P = .8985$, respectively; data not shown).

3.7. Quantitative mRNA expression of CXCR4, CXCR7, VEGF, CCR6, and CCR7 and their immunohistochemical expression

In comparison with the results of immunohistochemistry and quantitative real-time RT-PCR, a statistical association was found between immunohistochemical scores and mRNA expression levels in *CXCR4* and *VEGF* ($P = .0041$ and $P = .0235$, respectively; Fig. 3), whereas *CXCR7*, *CCR6*, and *CCR7* revealed no association ($P = .2706$, $P = .5067$, and $P = .1998$, respectively).

3.8. Survival analysis

Tables 4 and 5 summarize the results of survival analysis in all RMS cases and RMS groups separated by subtypes. By univariate analysis of ERMS cases, a poorer likelihood of survival has been revealed in the groups with high VEGF expression compared with the groups with low expression (Fig. 4, $P = .0017$). Especially about receptors expression, VEGF expression and CCR6 expression appeared to be an independent prognostic factor for ERMS ($P = .0008$ and $P = .042$, respectively; Table 5).

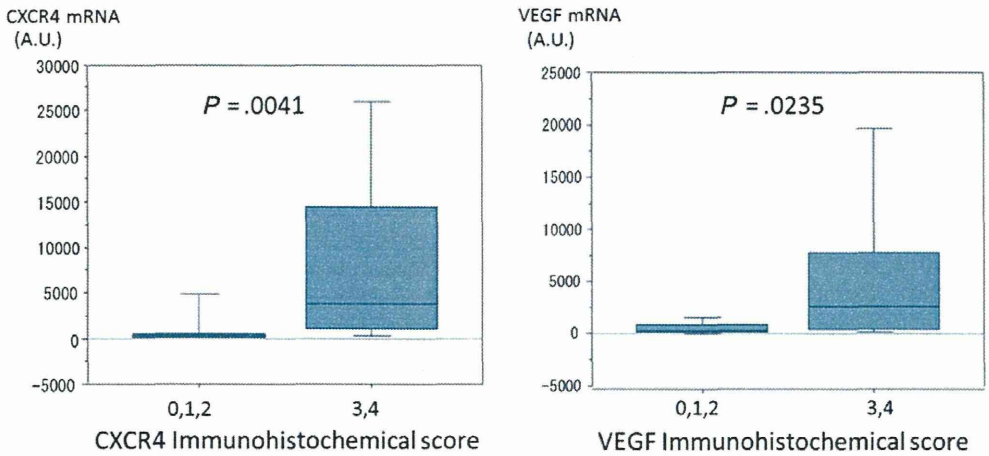


Fig. 3 Association between immunohistochemical expression status of CXCR4 and VEGF and their mRNA protein expression. The immunohistochemical status was associated with the corresponding mRNA expression.

Table 4 Univariate analysis for overall survival

Variables	RMS		ERMS		ARMS	
	No. of cases	*P*		*P*		*P*
Age		.0071*		.0375*		.0246*
<15	50		33		17	
≥15	22		5		17	
Sex		.7076		.6263		.6863
Male	34		17		17	
Female	38		21		17	
Histology		.0306*				
ERMS	38					
ARMS	34					
Tumor size		.0611		.0456*		.4301
≤5 cm	25		12		13	
5 cm	41		24		17	
Location		.0255*		.4496		.2066
Favorable	22		13		9	
Unfavorable	50		25		25	
Stage		.0102*		.1946		.2791
1,2	25		16		9	
3,4	42		20		22	
CXCR4		.2560		.3477		.7397
High	35		15		20	
Low	37		23		14	
CXCR7		.0414*		.0869		.1801
High	59		30		29	
Low	12		8		4	
VEGF		.0001*		.0010*		.1336
High	45		21		24	
Low	27		17		10	
CCR6		.3098		.9695		.4599
High	39		14		25	
Low	33		24		9	
CCR7		.6119		.3428		.6429
High	41		25		16	
Low	30		12		17	
MVD		.2707		.1892		.1933
≤11	36		19		17	
>11	36		19		17	
MIB-1-LI		.0480*		.0262*		.0258*
≤14.15	36		14		22	
>14.15	36		24		12	

Abbreviations: MVD, microvessel density; MIB-1-LI, MIB-1 labeling index; RMS, rhabdomyosarcoma; ERMS, embryonal rhabdomyosarcoma; ARMS, alveolar rhabdomyosarcoma.

* $P < .05$.

Only the VEGF expression came up as independent prognostic factors in ARMS ($P = .0353$; Table 5).

MVD did not affect survival in all RMS, whereas low MIB-1-LI in all RMS correlated significantly with poor survival in univariate analysis ($P = .048$; Table 4).

4. Discussion

CXCR4, a receptor for its sole ligand, SDF-1 (SDF-1/CXCL12), is known to be related to chemotaxis and homing, which are important steps in tumor metastasis [3,4]. Previous retrospective studies show that CXCR4 is highly expressed in various cancers and that its overexpression is closely correlated with lung, liver, and bone marrow metastasis as well as poor prognosis in several kinds of malignant solid tumors [4-7,9].

The important role of the CXCR4/SDF-1 pathway in tumor spread and metastasis has been demonstrated in RMS cell lines [6,7]. Especially, the cell lines derived from ARMS expressed higher levels of CXCR4 than cell lines derived from ERMS. A recent study found a significant correlation between high immunohistochemical expression of CXCR4 and poor prognosis in a clinical series of 40 RMS cases [27]. Those authors also noted significantly higher levels of CXCR4 expression in alveolar histology.

In the present study, there was no significant difference in the immunohistochemical expression of CXCR4 between the 2 histologic subtypes. However, almost half of RMS showed high CXCR4 expression. It is demonstrated that CXCR4 antagonists inhibit the primary tumor and metastasis in animal models of melanoma and osteosarcoma [5]. Therefore, CXCR4 could be a candidate for molecular target therapy in RMS with high CXCR4 expression.

We have also revealed significantly higher CCR6 expression in ARMS, and the present study is the first to investigate the CCR6 immunohistochemical expression status with RMS histology.

Recently, CXCR7 was identified as a receptor for SDF-1, and the SDF-1/CXCR7 axis was reported to regulate the metastatic potential of human RMS cells, similarly to SDF-1/CXCR4 [10]. Among RMS cells, ERMS cells express CXCR7 highly and express very low levels of CXCR4, whereas ARMS-like cells express CXCR4 highly and down-regulate CXCR7 expression [28]. In our study, no significant difference in the expression rate of CXCR7 was revealed, although CXCR7 showed consistently high expression in both histologic subtypes (ARMS: 87.9%, ERMS: 79.5%). It is reported that, in a hepatocellular carcinoma cell line, down-regulation of CXCR7 inhibits the growth and invasion of tumor cells, which indicates that CXCR7 may be a potential target for molecular targeted therapy [29]. Possibility for application of the CXCR7 antagonists to RMS, which widely expresses CXCR7, could be worth pursuing in the future.

Overexpression of VEGF has been reported in various epithelial malignancies and is thought to be a potent regulator of angiogenesis. Gee et al [30] have shown that the VEGF and VEGF family receptor mRNAs were expressed in RMS cell lines. However, we could not find any investigations into VEGF expression in large series of clinical RMS specimens. We demonstrated significantly more frequent VEGF expression in ARMS than in ERMS. Considering the statistical difference in prognosis between the subtypes, VEGF could still be a potential therapeutic target in ARMS, which is destined for poorer prognosis,

Bachelder et al [8] demonstrated that VEGF regulates CXCR4 expression in breast carcinoma cells. They also demonstrated that CXCR4 mediates the migration of breast

Table 5 Multivariate analysis in RMS

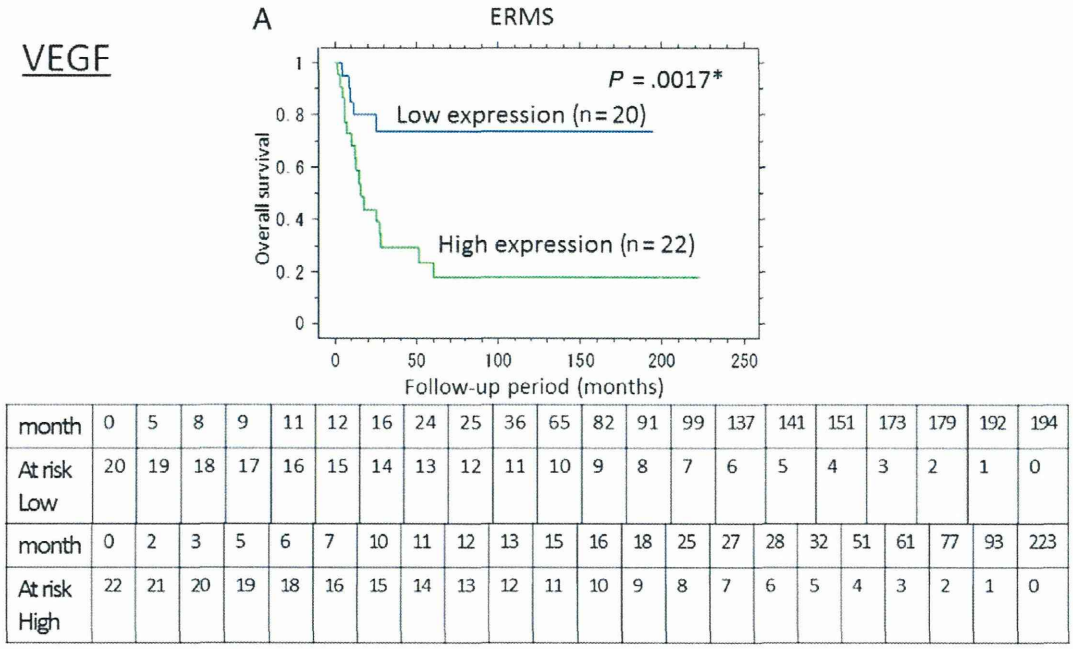
	RMS (n = 70)		ERMS (n = 40)		ARMS (n = 30)	
Variables	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)
Age	.0002*	0.221 (0.099-0.492)	.0019*	0.068 (0.012-0.369)	.0201*	0.345 (0.14-0.847)
Tumor size (≤5 cm *vs* >5 cm)	.0708	0.515 (0.251-1.058)	.0527*	0.312 (0.096-1.014)		
Location (favorable *vs* unfavorable)					.1005	0.387 (0.125-1.201)
CXCR4 (high *vs* low)	.0145*	2.824 (1.228-6.491)			.0716	3.287 (0.901-11.995)
CXCR7 (high *vs* low)						
VEGF (high *vs* low)	<.0001*	0.113 (0.04-0.313)	.0008*	0.124 (0.036-0.422)	.0353*	0.21 (0.049-0.898)
CCR6 (high *vs* low)	.0181*	2.403 (1.162-4.970)	.042*	3.778 (1.049-13.609)		
CCR7 (high *vs* low)						

Abbreviations: HR, hazard ratio; CI, confidence interval; RMS, rhabdomyosarcoma; ERMS, embryonal rhabdomyosarcoma; ARMS, alveolar rhabdomyosarcoma.

* $P < .05$.

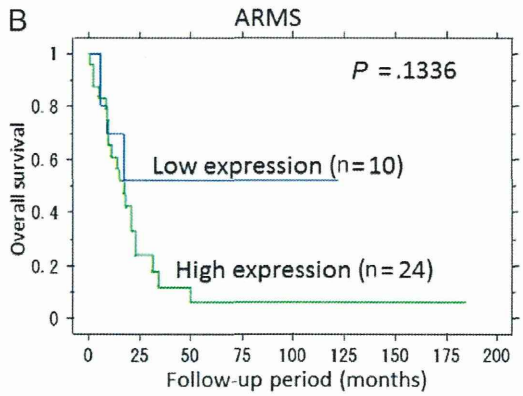
cancer cells, depending on autocrine VEGF. Since then, a close correlation between CXCR4 and VEGF expression has been demonstrated in several types of malignancies [21] and in osteosarcoma [31] in vitro and in vivo. In our previous study, we have investigated the CXCR4 and VEGF expression in soft tissue sarcoma. Nine primary RMS were included in the category of malignant round cell tumors and revealed higher mRNA levels of *CXCR4* and *VEGF* than the control skeletal muscle tissue. However, we could not detect significant association between mRNA expression levels for *CXCR4* and *VEGF* in a small number of malignant round cell tumor. In the present study, we confirmed the significant positive correlation between immunohistochemical CXCR4 and VEGF expression. To our knowledge, no other report refers to the correlation between VEGF and CXCR4 in a large series of clinical RMS.

The correlation between MVD and VEGF expression level and prognosis has been controversial, as some reports have failed to reveal a correlation in several solid tumors [17] or in soft tissue sarcomas [32], whereas other reports have questioned it in soft tissue sarcomas [33,34]. In the present study, we could not find correlation between MVD and VEGF or any other immunohistochemical factors in RMS. Moreover, MVD did not correlate with outcome. A similar result was reported in a study with soft tissue sarcomas that compared MVD and tissue VEGF concentration [33].



month	0	5	8	9	11	12	16	24	25	36	65	82	91	99	137	141	151	173	179	192	194
At risk Low	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
month	0	2	3	5	6	7	10	11	12	13	15	16	18	25	27	28	32	51	61	77	93	223
At risk High	22	21	20	19	18	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0

Fig. 4 Difference in overall survival between histologic subtypes of RMS. A high VEGF expression in ERMS (A) showed significantly poorer prognosis, but not in ARMS (B).



Month	0	6	9	10	11	14	17	28	102	122
At risk	10	9	7	6	5	4	3	2	1	0

month	0	1	2	5	8	9	10	11	14	15	17	18	21	23	28	31	34	50	184
At risk High	24	23	21	20	18	17	14	13	12	11	10	9	7	5	4	3	2	1	0

Fig. 4 (*continued*).

Other chemokine receptors, CCR6 and CCR7, have been revealed to bind to CCL20 and CCL 19/21 with involvement in liver metastases and lymph node metastases in gastrointestinal carcinoma [12,13]. CCL20 was originally identified in the liver and was the only chemokine known to interact with CCR6. Thus, the receptor-ligand pair CCR6-CCL20 plays an important role in the chemoattraction of T cells to the liver [35]. In our series of RMS, none of the patients developed liver metastasis, but ARMS showed frequently higher CCR6 expression than ERMS. The importance of chemokine receptors in metastasis is mostly related to CXCR4 and CCR7, aside from the correlation between CXCR4 and organ-specific metastases such as those of lung, liver, and bone marrow, CCR7 expression generally correlates with increased lymph node metastases [11,12]. In our study, high CCR7 expression showed no relation with other immunohistochemical factors.

In conclusion, both ARMS and ERMS displayed association between VEGF and CXCR4 expression. In addition, our results suggest that high VEGF expression may be predictive prognostic factors in RMS. Considering that RMS widely expresses CXCR4 and CXCR7, these chemokine receptors and VEGF may provide potential targets of molecular therapy as part of combined modality therapy in RMS.

Acknowledgment

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References

[1] Parham DM, Barr FG. Embryonal rhabdomyosarcoma and alveolar rhabdomyosarcoma. In: Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F, editors. World Health Organization Classification of Tumours, Pathology and Genetics of Tumours of Soft Tissue and Bone. 4th ed. Lyon: IARC Press; 2013. p. 127-35.

[2] Zlotnik A, Burkhardt AM, Homey B. Homeostatic chemokine receptors and organ-specific metastasis. Nat Rev Immunol 2011;11:597-606.

[3] Muller A, Homey B, Soto H, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature 2001;410:50-6.

[4] Taichman RS, Cooper C, Keller ET, et al. Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. Cancer Res 2002;62:1832-7.

[5] Kim SY, Lee CH, Midura BV, et al. Inhibition of the CXCR4/CXCL12 chemokine pathway reduces the development of murine pulmonary metastases. Clin Exp Metastasis 2008;25:201-11.

[6] Libura J, Drukala J, Majka M, et al. CXCR4-SDF-1 signaling is active in rhabdomyosarcoma cells and regulates locomotion, chemotaxis, and adhesion. Blood 2002;100:2597-606.

[7] Strahm B, Durbin AD, Sexsmith E, et al. The CXCR4-SDF1 axia is a critical mediator of rhabdomyosarcoma metastatic signaling induced by bone marrow stroma. Clin Exp Metastasis 2008;25:1-10.

[8] Bachelder RE, Wendt MA, Mercurio AM. Vascular endothelial growth factor promotes breast carcinoma invasion in an autocrine manner by regulating the chemokine receptor CXCR4. Cancer Res 2002;62:7203-6.

[9] Oda Y, Yamamoto H, Tamiya S, et al. CXCR4 and VEGF expression in the primary site and the metastatic site of human osteosarcoma: analysis within a group of patients, all of whom developed lung metastasis. Mod Pathol 2006;19:738-45.

[10] Balkwill F. The significance of cancer cell expression of CXCR4. Semin Cancer Biol 2004;14:171-9.

[11] Takanami I. Overexpression of CCR7 mRNA in nonsmall cell lung cancer: correlation with lymph node metastasis. Int J Cancer 2003;105:186-9.

[12] Günther K, Leier J, Henning G, et al. Prediction of lymph node metastasis in colorectal carcinoma by expression of chemokine receptor CCR7. Int J Cancer 2005;116:726-33.
[13] Ghadjar P, Coupland SE, Na IK, et al. Chemokine receptor CCR6 expression level and liver metastases in colorectal cancer. J Clin Oncol 2006;24:1910-6.
[14] Ghadjar P, Loddenkemper C, Coupland SE, et al. Chemokine receptor CCR6 expression level and aggressiveness of prostate cancer. J Cancer Res Clin Oncol 2008;134:1181-9.
[15] Dvorak HF. Vascular permeability factor/vascular endothelial growth factor: A critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. J Clin Oncol 2002;20:4368-80.
[16] Ferrara N, Hillan HP, Gerber W, et al. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. Nat Rev Drug Discov 2004;3:391-400.
[17] Ferrara N. VEGF as a therapeutic target in cancer. Oncology 2005;69:11-6.
[18] Semenza GL. A new weapon for attacking tumor blood vessels. N Engl J Med 2008;358:2066-7.
[19] Burns JM, Summers BC, Wang Y, et al. A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. J Exp Med 2006;203:2201-13.
[20] Grymula K, Tarnowski M, Wysoczynski M, et al. Overlapping and distinct role of CXCR7-SDF-1/ITAC and CXCR4-SDF-1 axes in regulating metastatic behavior of human rhabdomyosarcomas. Int J Cancer 2010;127:2554-68.
[21] Liang Z, Brooks J, Willard M, et al. CXCR4/CXCL12 axis promotes VEGF-mediated tumor angiogenesis through Akt signaling pathway. Biochem Biophys Res Commun 2007;359:716-22.
[22] Billadeau DD, Chatterjee S, Bramati P, et al. Characterization of CXCR4 signaling in pancreatic cancer cells. Int J Gastrointest Cancer 2006;37:110-9.
[23] Lawrence Jr W, Anderson JR, Gehan EA, et al. Pretreatment TNM staging of childhood rhabdomyosarcoma: a report of the Intergroup Rhabdomyosarcoma Study Group. Children's Cancer Study Group. Pediatric Oncology Group. Cancer 1997;80:1165-70.
[24] Oda Y, Tateishi N, Matono H, et al. Chemokine receptor CXCR4 expression is correlated with VEGF expression and poor survival in soft-tissue sarcoma. Int J Cancer 2009;124:1852-9.
[25] Wang L, Chen W, Gao L, et al. High expression of CXCR4, CXCR7 and SDF-1 predicts poor survival in renal cell carcinoma. World J Surg Oncol 2012;10:212.
[26] Toyozawa S, Yamamoto Y, Ishida Y, et al. Immunohistochemical analysis of CXCR4 expression in fibrohistiocytic tumors. Acta Histochem Cytochem 2010;43:45-50.
[27] Diomedi-Camassei F, McDowell HP, De Ioris MA, et al. Clinical significance of CXC chemokine receptor-4 and c-Met in childhood rhabdomyosarcoma. Clin Cancer Res 2008;14:4119-27.
[28] Tarnowski M, Grymula K, Reca R, et al. Regulation of expression of stromal-derived factor-1 receptors: CXCR4 and CXCR7 in human rhabdomyosarcomas. Mol Cancer Res 2010;8:1-14.
[29] Xue TC, Chen RX, Han D, et al. Down-regulation of CXCR7 inhibits the growth and lung metastasis of human hepatocellular carcinoma cells with highly metastatic potential. Exp Ther Med 2012;3:117-23.
[30] Gee MF, Tsuchida R, Eichler-Jonsson C, et al. Vascular endothelial growth factor acts in an autocrine manner in rhabdomyosarcoma cell lines and can be inhibited with all-trans-retinoic acid. Oncogene 2005;24:8025-37.
[31] de Nigris F, Rossiello R, Schiano C, et al. Deletion of Yin Yang 1 protein in osteosarcoma cells on cell invasion and CXCR4/angiogenesis and metastasis. Cancer Res 2008;68:1797-808.
[32] Pakos EE, Goussia AC, Tsekeris PG, et al. Expression of vascular endothelial growth factor and its receptor. KDR/Flk-1, in soft tissue sarcomas. Anticancer Res 2005;25:3591-6.
[33] Yudoh K, Kanamori M, Ohmori K, et al. Concentration of vascular endothelial growth factor in the tumour tissue as a prognostic factor of soft tissue sarcomas. Br J Cancer 2001;84:1610-5.
[34] West CC, Brown NJ, Mangham DC, et al. Microvessel density does not predict outcome in high grade soft tissue sarcoma. Eur J Surg Oncol 2005;31:1198-205.
[35] Schutyser E, Struyf S, Van Damme J. The CC chemokine CCL20 and its receptor CCR6. Cytokine Growth Factor Rev 2003;14:409-26.

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【第55回日本小児血液・がん学会学術集会】会長講演

小児血液・がんの治療としての外科的移植・再生医療

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要 旨

小児固形悪性腫瘍は腹部腫瘤として発見されるため診断時すでに進行性で切除不能なことが多い．かつては切除可能なだけ強引に切除し，その後に化学療法を行っていたがほぼ全滅であった．しかし小児白血病の治療の進歩により，造血幹細胞移植を含む大量化学療法が可能になり，またシスプラチンなどのすぐれた抗腫瘍剤の小児への導入により，術前化学療法により腫瘍をコントロールしてから完全切除にもっていくと生存可能になった．とくに肝芽腫や腎芽腫は切除可能な状態までもっていけば治癒が期待できる．

また肝芽腫では切除不能であっても肺などの遠隔転移がコントロールできれば肝移植により全摘が可能になった．肝芽腫の肝移植の成績はSIOPの147例では5年生存率が75%，10年生存率が66%であり，Primary TxのほうがSecondary Txよりも有意に成績がよかった．本邦では5年生存率も10年生存率も77.9%と良好な結果であり，Primary TxとSecondary Txでは差がなかった．

造血幹細胞や間葉系幹細胞の細胞移植や立体構造を有する再生臓器などの開発により臓器移植に代わる低侵襲治療の開発が期待される．脱落乳歯幹細胞は有力な幹細胞のソースである．

キーワード：外科切除，肝臓移植，化学療法，造血幹細胞移植，歯髄，脱落歯髄幹細胞，再生医療

Key words: surgical resection, liver transplantation, chemotherapy, hematopoietic stem cell transplantation, dental pulp, tissue engineering

はじめに

日本小児外科学会は今年で50周年を迎え，先人の意志と情熱を継志し大きく進歩した．この中で特記すべきことは，まず（1）新生児外科疾患はかつてほとんど救命できなかった病気が現在は救命できるようになったこと．（2）胆道閉鎖症は致死的な病気であったのが葛西手術により救命または延命できるようになり，さらに肝臓移植により治癒できる病気になったこと．（3）鏡視下手術や従来の鏃を利用した手術で腹部や胸部の傷がほとんど目立たない術式が標準術式になりつつあること．（4）小児がんは発見時に進行例が多く切除不能であれば救命困難であったが，造血幹細胞移植による大量化学療法や放射線療法など適切な集学的治療と根治手術の組み合わせにより，進行例でも長期生存できるようになったこと．（5）再生医療の開発である．

これらのなかでも小児がんと再生医療の開発について述べる．

小児がんの治療成績の向上

小児固形悪性腫瘍は腹部腫瘤として発見されるため診断時すでに進行性で切除不能なことが多い．かつては切除可能なだけ強引に切除し，その後に化学療法を行っていたがほぼ全滅であった．しかし小児白血病の治療の進歩により，造血幹細胞移植を含む大量化学療法が可能になり，またシスプラチンなどのすぐれた抗腫瘍剤の小児への導入により，術前化学療法により腫瘍をコントロールしてから完全切除にもっていくと生存可能になった．とくに肝芽腫や腎芽腫は切除可能な状態までもっていけば治癒が期待できる．さらに肝芽腫では切除不能であっても肺などの遠隔転移がコントロールできれば肝移植により全摘が可能になった．具体的な腎芽腫および肝芽腫の症例を提示する．

症例1

患者：2歳男児，下大静脈内伸展をともなう腎芽腫

主訴：腹部腫瘤

現病歴：感冒で前医受診．この際，腹部腫瘤を指摘され当院に入院．

入院時現症：腹右上腹部に7 cm大，表面平滑，弾性硬の腫瘤を触知

検査所見：尿所見では顕微鏡的血尿，Gaシンチで右腎に異常集積あり，骨シンチでは異常集積なく，CTでは右腎に巨大な充実性腫瘍があり右腎静脈および下大静脈内に進展がみられた（図1）．

治療経過：右腎静脈から連続的に下大静脈に腫瘍の伸展があるため，切除不能と判断し，術前化学療法を選択した（図2）．ICE療法2クールで効果をみとめ，画像にて原発

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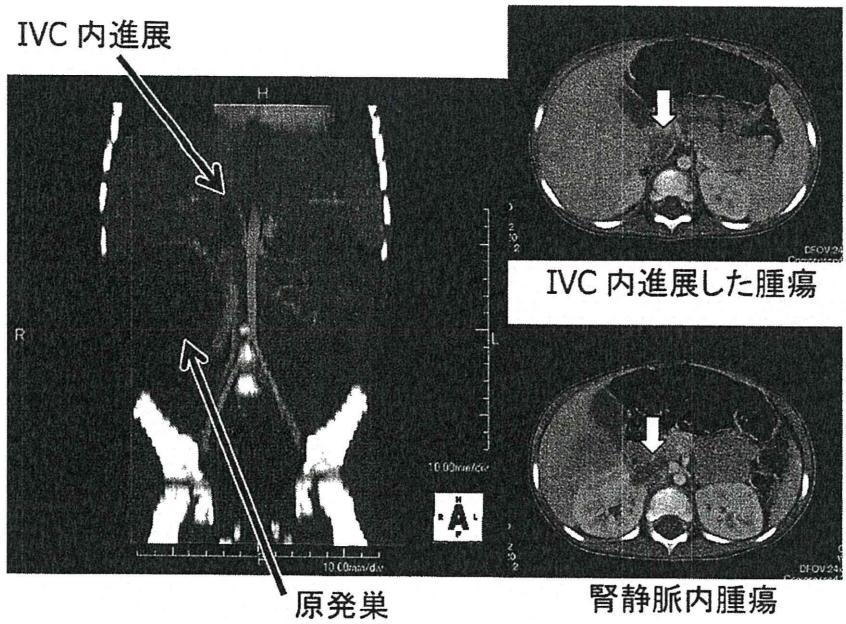
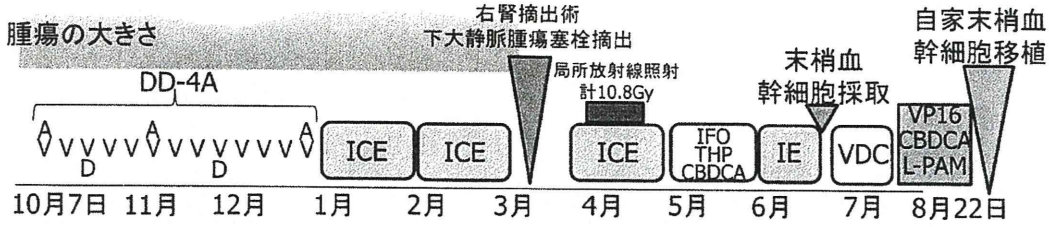


図1　症例1　初診時CT



ICE療法　Ifosfamide　3000mg/m²　day1～2
Carboplatin　350mg/m²　day5
Etoposide　75mg/m²　day1～5

IFO+THP+CBDCA
Ifosfamide 900mg/m² day1～5, Pirarubicin 40mg/m² day1, Carboplatin 400mg/m² day3

IE
Ifosfamide 1800mg/m² day1～3, Etoposide 100mg/m² day1～3

VDC
Vincristine 1.5mg/m² day1, Doxorubicin 37.5mg/m² day1～2 , Cyclophosphamide 1200mg/m² day1v

auto PBSCT regimen
Etoposide　200mg/m²　day-7～-4
Carboplatin　400mg/m²　day-7～-4
Melpharan　40mg/m²　day-3～-2

図2　症例1の経過

巣の縮小，下大静脈内の腫瘍が腎静脈からわずかに伸展するのみになったため，下大静脈一部合併切除で完全切除が可能と判断し，手術に踏み切った．手術は腫瘍を含めた右腎摘出および右腎静脈切除と下大静脈壁合併切除にて腫瘍全摘可能であった（図4）．病理はHE染色，免疫染色および遺伝子解析の結果MRTK，Stage IIIと診断した．術後は図に示すような集学的治療を行い，現在disease freeである．MRTKというunfavorable histologyであったが，手術