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Original Article

Clinicopathologic features and molecular spectrum of spindle cell and sclerosing rhabdomyosarcomas in the head and neck region

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Abstract: Recently, spindle cell/sclerosing rhabdomyosarcoma (SRMS/ScRMS) has been recognized as a stand-alone entity in the latest edition of *WHO Classification of Tumors of Soft Tissue and Bone*. As SRMS/ScRMS have a predilection for the head and neck, we evaluated the clinicopathologic and molecular features of 20 cases of SRMS/ScRMS (13 SRMS and 7 ScRMS) arising in the head and neck region. 10 patients were men, and 10 were women, and their ages ranged from 2 months to 57 years. Tumor size ranged from 1.5 to 20 cm. By immunohistochemistry, all tumors showed diffuse desmin expression, and *MYOD1* immunostaining was diffuse to multifocally positive: 16 cases showed myogenin positive immunostaining. 2 patients had local recurrences, and 5 patients developed distant metastases. So far, 10 patients have died of the disease. 7 of 13 SRMS and 4 of 7 ScRMS showed *PIK3CA* mutations, while 8 of 13 SRMS and all 7 ScRMS showed *MYOD1* mutations. A novel p.R524K hotspot mutation in 8 of 11 cases showed *PIK3CA* mutations. SRMS/ScRMS shares similar clinicopathological and molecular features. Diagnostic pitfalls from other spindle or sclerosing sarcomas should be avoided with the use of appropriate immunohistochemical stains and relevant clinical information. Co-occurrence of *PIK3CA* and *MYOD1* mutations are associated with unfavorable clinical outcomes.

Keywords: Sclerosing rhabdomyosarcoma, Spindle cell rhabdomyosarcoma, *PIK3CA*, *MYOD1*, mutation

Introduction

Rhabdomyosarcoma (RMS) is the most common malignant soft tissue tumor in children and adolescents [1]. Traditionally it has three morphologically and clinically distinct subtypes: embryonal RMS (ERMS), alveolar RMS (ARMS), and pleomorphic rhabdomyosarcoma (PRMS). Spindle cell RMS (SRMS) is a rare morphologic subtype of Rhabdomyosarcoma (RMS), which was initially grouped under ERMS. Sclerosing RMS (ScRMS) usually has clinical and pathological presentations that are similar to SRMS, so, recently, the World Health Organization (WHO) classification of Soft Tissue and Bone merged SRMS and ScRMS into a single pathologic entity.

Some recent studies have discussed the clinicopathological and genetic features of this rare tumor entity. They found that SRMS/ScRMS usually has a predilection for paratesticular and head and neck sites, and that children, were likely to have a more favorable prognosis. But some discrepancies still exist among research groups from different countries. Cavazzana et al. [2] from Italy first described SRMS as a variant of ERMS in children which had a relatively favorable prognosis, but Yasui et al. [3] from Japan suggested a worse prognosis for SRMS/ScRMS compared to the pediatric ERMS. And Owosho et al. [4] from the USA also reported that the 5-year overall survival rate for ERMS patients was significantly higher (82%) compared to SRMS/ScRMS (50%) [SRMS (75%); ScRMS (30%)]. Because of the rarity of SRMS/ScRMS, it is necessary to extend samples and focus on a detailed clinicopathologic and survival analysis of SRMS/ScRMS.

MyoD1, desmin and Myogenin et al. [5] are vital immunohistochemical markers to distinguish RMS from its mimics. *MyoD1*, as a myogenic

transcriptional regulatory protein which is expressed in early skeletal muscle differentiation, is rarely expressed in mature skeletal muscle tissue. The sustained expression of *MYOD1* in RMS might be caused by gene mutation or other mechanisms. However, its exact mechanism is still unknown. Previously, Van Antwerp et al. [6] described how the *MYOD1* p.L122R mutation occurs in the conserved DNA binding domain, leading to transactivation and *MYC*-like functions, which result in sustained proliferation and the blocking of the myogenic differentiation process. *MYOD1* p.L122R as a mutation hotspot can be detected in 38%-56% of SRMS/ScRMS and has never been detected in other types of RMS [7, 8]. But the mutation rate of *MYOD1* seems inconsistent with the positive expression rate of the *MYOD1* protein, which indicates that there are other factors influencing the expression level of *MYOD1* protein.

In addition to the above three markers, 15-18% [8, 9] of SRMS/ScRMS also showed phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) mutations, including *PIK3CA* p.E542V, p.E542K, p.E545K, p.H1047R and p.G1049R, along with *MYOD1* mutations, which may activate the PI3K/AKT signaling pathway and enhance proliferation. Remarkably, in previous reports, all the cases which harbored *MYOD1* and *PIK3CA* coexisting mutations showed sclerosing morphology. However, *PIK3CA* mutations can also been detected in ERMS and ARMS [10, 11]. Therefore, the exact role of *PIK3CA* mutations in SRMS/ScRMS still needs further research.

To sum up, in order to know more about this rare type of RMS, we present a clinicopathological retrospective study of SRMS/ScRMS in our institution during the period 1995-2016 to detect the mutation of *MYOD1* and *PIK3CA* and their correlation with the prognosis.

Materials and methods

Patient selection

We retrieved 166 cases diagnosed with primary head and neck RMS in the Department of Pathology, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, diagnosed between 1995 and 2016. The clinicopathological and follow up details were collected through the electronic medical records and telephonically. In each case, the diagnosis was confirmed by reviewing the hematoxylin-eosin (H&E) slides and the immunohistochemical stains. All the cases included in the study were reviewed by two independent pathologists, with the classification based on current WHO criteria as SRMS/ScRMS, which is characterized by a prominent fascicular spindle cell morphology and hyalinized stroma, and primitive-appearing tumor cells set in cords and nests. In several cases, the tumor displayed focal areas of sclerosing RMS and vice versa. The clinicopathologic feature data included: age at diagnosis, gender, primary site, tumor size, therapy method (surgical resection, chemotherapy, and/or radiation therapy) and pre-therapy stage, which was based on the Intergroup Rhabdomyosarcoma Study Group (IRSG) pretreatment staging system. The study procedures were approved by our hospital's ethical committee.

Immunohistochemistry of MYOD1, desmin and myogenin

After heating antigen retrieval (water bath/Tris-EDTA) on FFPE tissue sections, immunohistochemical studies were performed by the Envision™ method (Dako, Glostrup, Denmark) according to the manufacturer's protocol. Monoclonal antibodies including myogenin (Dako, cl: F5D; 1:300), *MYOD1* (Dako, cl: 5.8A; 1:40) and desmin (Dako, cl: D33; 1:200) were adopted. PBS was used to replace primary antibodies as a blank control. The number of immunoreactive cells in each section was semi quantitatively evaluated as follows: ++++, >75% positive cells; +++, 75-50% positive cells; ++, 50%-25% positive cells; +, <25% positive cells; and -, no positively stained cells.

PCR and sanger sequencing

Genomic DNA was isolated from FFPE samples using a TIANamp FFPE DNA Kit (TIANGEN, Beijing, China), following the manufacturer's instructions. An ultraviolet spectral photometer (NanoDrop 2000c, Thermo Scientific) was used to determine the concentration of DNA. The primer sequences for PCR amplifications of *PIK3CA* and *MYOD1* were based on the study of Agaram et al. [8]. Targeted PCR was performed for the known *MYOD1* exon 1 (122) hot spot mutation, *PIK3CA* exon 10 (542/545), and the exon 21 mutation (1047) by 2 μl primers in a 25 μl reaction volume on a CFX 96 Real-Time PCR detection system (Bio-Rad, USA). PCR conditions were used: initial hot-start denaturation step of 1 min at 96°C, followed by 30 cycles of

Table 1. Clinicopathologic features and follow-up

Case No.	Age/sex	Diagnosis	Primary site	Size (cm)	Therapy	Stage[a]	Follow-Up
1	18 y/male	ScRMS	Masseter region	1.5	Surgery	1	NED (205 mos)
2	22 y/female	ScRMS	Infratemporal fossa	4	Surgery	4	DM (7 mos); DOD (16 mos)
3	20 y/female	SRMS	Soft tissue mandible	20	Surgery	1	DM (10 mos); DOD (18 mos)
4	22 y/female	SRMS	Pterygomandibular space	4	Surgery, CT & RT	4	DM; DOD (4 mos)
5	14 y/female	SRMS	Parotid region	20	Surgery	1	
6	34 y/female	ScRMS	Infratemporal fossa	6	Surgery	1	DOD (24 mos)
7	15 y/female	ScRMS	Soft tissue maxilla	2.5	Surgery, CT & RT	4	LR, DM (14 mos); DOD (17 mos)
8	57 y/male	SRMS	Tongue	3.5	Surgery, CT & RT	1	NED (11 mos)
9	36 y/male	SRMS	Soft tissue maxilla	2	Surgery	1	NED (54 mos)
10	11 y/male	ScRMS	Neck	10	Surgery	1	DOD (80 mos)
11	4 y/male	SRMS	Lip	1.5	Surgery	1	NED (149 mos)
12	12 y/female	SRMS	Infratemporal fossa	2.5	Surgery	1	DOD (58 mos)
13	2 mos/male	SRMS	Tongue	2.5	Surgery	1	NED (82 mos)
14	7 y/female	SRMS	Infratemporal fossa	6.5	Surgery	1	DOD (63 mos)
15	29 y/male	SRMS	Infratemporal fossa	9	Surgery	1	DOD (46 mos)
16	47 y/male	ScRMS	Masseter region	4.5	Surgery, CT & RT	1	NED (41 mos)
17	13 y/female	ScRMS	Soft tissue mandible	2	Surgery, CT & RT	1	LR (24 mos); NED (39 mos)
18	8 y/female	SRMS	Nasolabial	2.5	Surgery	1	NED (259 mos)
19	33 y/male	SRMS	Soft tissue maxilla	2	Surgery	4	DM (33 mos); DOD (52 mos)
20	4 y/male	SRMS	Infratemporal fossa	1.5	Surgery	1	

CT-chemotherapy, RT-radiotherapy, LR-local recurrence after surgery, DM-distant metastasis after surgery, DOD-died of disease, NED-no evidence of disease. a-IRSG (Intergroup Rhabdomyosarcoma Study Group) indicates pretreatment staging system.

10 s at 96°C, 5s at 50°C, and 2 min at 60°C. Using the QIAquick PCR purification kit (Qiagen, Hilden, Germany), purified PCR products were sequenced on the ABI3500DX DNA sequencer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The data were further analyzed by using Chromas software v 2.6.4 (http://www.technelysium.com.au/chromas.html).

Statistical analysis

The data were analyzed using SPSS 20.0.0 (IBM Corp, NY, USA). The associations between the clinicopathologic features of SRMS/ScRMS with *PIK3CA* and the *MYOD1* mutation were calculated using Fisher's exact test. The relationship between the *MYOD1* mutation with its expression was performed using the Mann-Whitney U-test. Survival analyses were based on the Kaplan-Meier method and calculated using the log-rank test (calculated as the time of diagnosis). The threshold for statistical significance was set at $P \leq 0.05$.

Results

Clinicopathological features of SRMS and ScRMS

Twenty of 166 cases (13 SRMS and 7 ScRMS) were reclassified as SRMS/ScRMS. The clinicopathologic features and follow-up are summarized in **Table 1**. The cohort included 10 children (4 males and 6 females) and 10 adults (6 males and 4 females). Their ages ranged from 2 months to 57 years. The mean age was 20.3 years, and the median age was 16.5 years. The most common anatomic location of the neoplasms in our study was the infratemporal fossa (6), with rarer locations including the pterygomandibular space (1), the parotid region (1), the nasolabial area (1), the neck (1), and the lip (1). The tumor sizes ranged from 1.5 to 20 cm the mean size was 5.4 cm, and the median size was 3 cm. According to the IRSG pretreatment classification, 4 of the 20 cases were stage IV, and 16 of the 20 cases were stage I. All the patients underwent surgical resections, and only 5 patients chose chemotherapy and radiotherapy after surgery as adjuvant treatments.

Completed clinical follow-up information was available on 18 patients (**Table 1**), ranging from 4-259 months (average = 67.67 months, median = 49 months). 5 patients (33%, 6/18) developed recurrences at 4-33 months after surgery (2 SRMS and 3 ScRMS, **Table 1**). The regions of distant metastasis included bone and brain. Ten patients (53%, 10/19) died of disease at 4-80 months after surgery, including 4 adolescents and 5 adults. A Kaplan-Meier survival

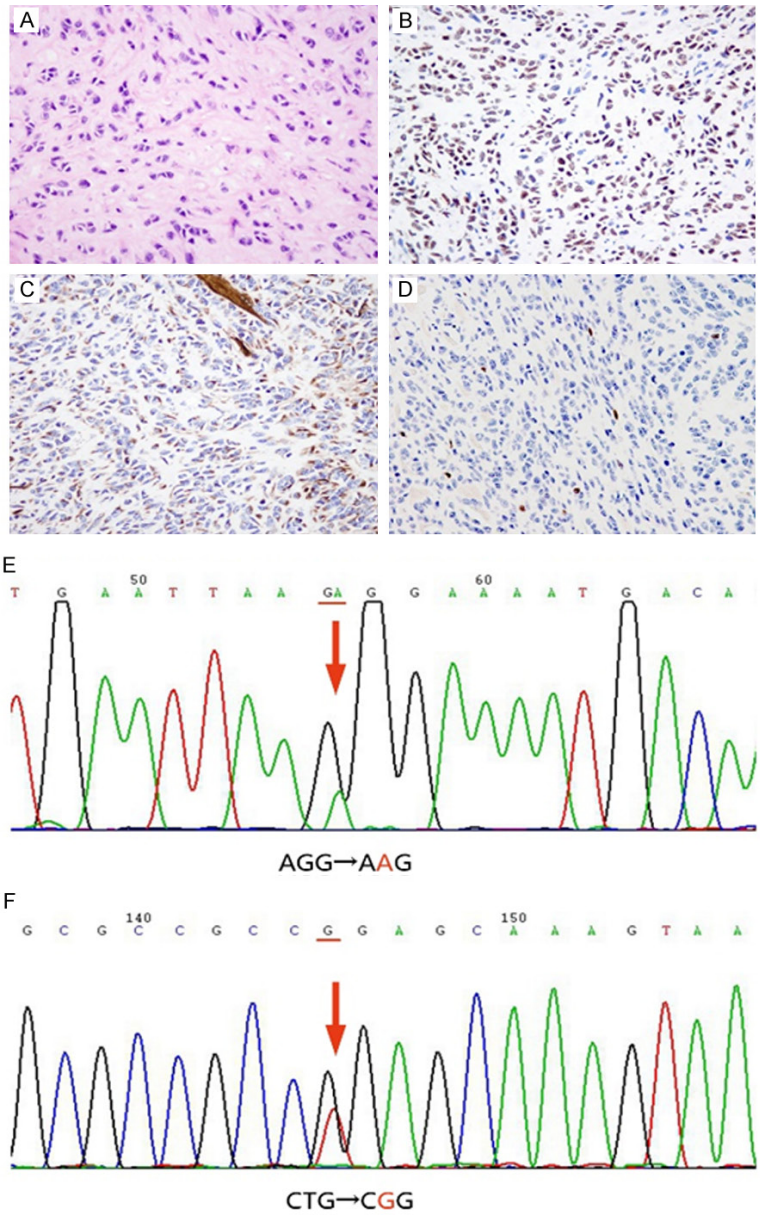

Figure 1. ScRMS of a 22 year-old woman (Case 2). (A) ScRMS characterized by stromal hyalinization with tumor cells arranged in nests, focal alveolar or psuedovascular and trabeculae appearance (H & E, 400×). (B) Immunohistochemical stains show diffuse positivity for MYOD1. (C) Cytoplasmic positive for desmin. (D) Focal myogenin positive immunostaining (400×). Sequencing showing a (E) heterozygous PIK3CA p.R524K mutation and (F) MYOD1 p.L122R mutation.

analysis showed that there was no statistical difference among the different age groups in overall survival (OS) (P>0.05, **Figure 4**). But the OS of patients in whom coexisting *PIK3CA* and *MYOD1* mutations were detected was significantly worse than those merely harboring the *MYOD1* mutation (P<0.05, **Figure 4**), which suggested that SRMS/ScRMS coexisting with the above mutations were associated with an aggressive clinical behavior and a poor outcome.

Immunohistochemical features

The immunohistochemical results are shown in **Table 2**. All of the cases showed *MYOD1* and desmin expression in varying degrees. But the *MYOD1* immunostaining did not show a statistically significant difference between the pediatric age group (<18 years) and the adult group (≥18 years) (P = 0.529). 16 of 20 cases showed positive myogenin staining, while most of the cases had a focal presentation and were rarely positive, and only one case showed 50%-75% positive cells.

Analysis of PIK3CA and MYOD1 mutations

PIK3CA mutations were found in 11 of 20 cases (55%). Unpredictably, 8 of 11 cases (4 SRMS cases and 4 ScRMS cases), were found that harbored a novelty p.R524K mutation, which had never been reported in SRMS/ScRMS (**Table 2**; **Figures 1** and **2**). Only one case showed the p.E545K mutation, which had previously been reported in SRMS/ScRMS [8]. The remaining two other cases showed the H1047R (**Figure 3**) and G1049D mutations, respectively. Recurrent *MYOD1* p.L122R mutations were detected in 15 of the 20 cases (75%). Of these 15 cases, two presented p.L122R homozygous mutations (**Table 2**), and one case appeared a p.121 nonsense mutation simultaneously (**Figure 3**). In addition, 8 of the 20 cases harbored both *PIK3CA* and *MYOD1* mutations (40%). But neither age group (P = 0.34), nor gender (P = 0.26)

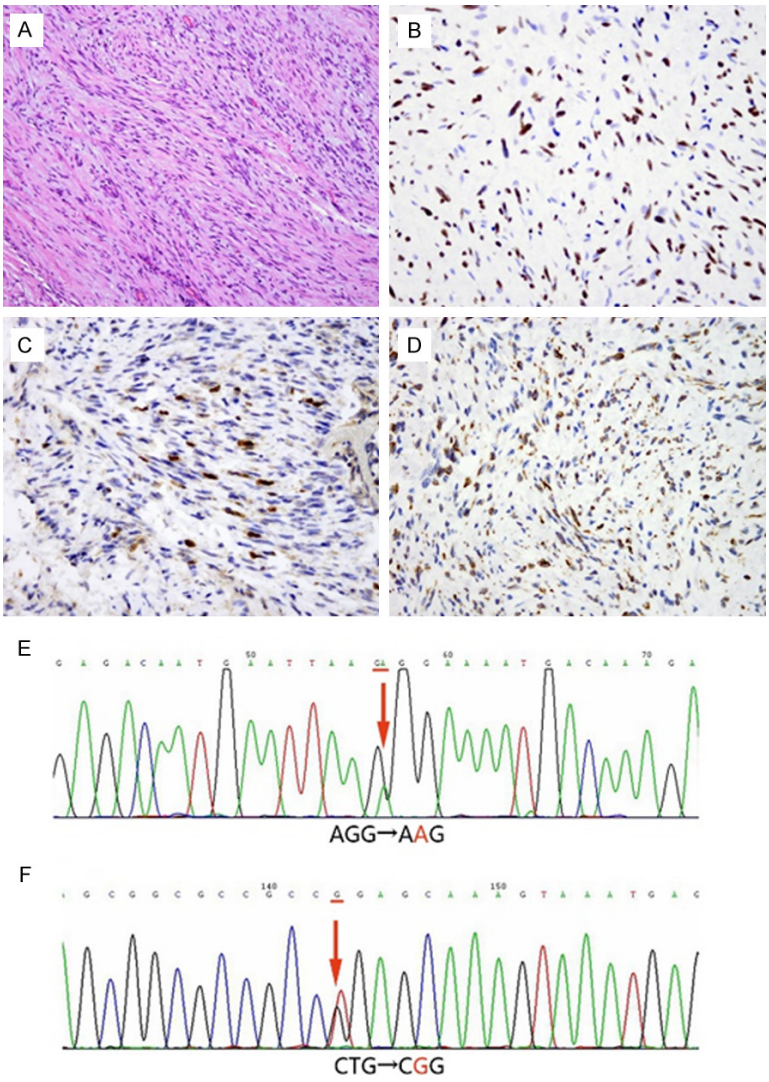

Figure 2. sRMS of a 15 year-old girl (Case 7). (A) Shows the tumor composed of neoplastic spindle cells arranged in elongated fascicles or storiform patterns (H & E, 400×). (B-D) Immunohistochemical stains show positivity for MYOD1, myogenin, desmin (400×). Sequencing showing a (E) heterozygous PIK3CA p.R524K mutation and (F) MYOD1 p. L122R mutation.

nor histopathologic subtype (P = 0.41) statistically correlated with the mutation status of above two genes (**Table 3**). The cases which harbored the *MYOD1* p.L122R mutations showed diffusely strong positive *MYOD1* staining (P<0.001, **Table 3**).

Discussion

We observed clinicopathologic features and the mutation spectrum of *PIK3CA* and *MYOD1* in a relatively large cohort of head and neck SRMS/ScRMS. In doing so, we identified a novel mutation hotspot of *PIK3CA* of SRMS/ScRMS in the head and neck. We also found that the *PIK3CA* mutation can exist independently without the *MYOD1* mutation in SRMS/ScRMS, a finding not previously described. Furthermore, the co-occurrence of the *PIK3CA* and *MYOD1* mutations in SRMS/ScRMS are associated with unfavorable clinical outcomes.

The spindle cell/sclerosing RMS (SRMS/ScRMS) is one of rare morphologic subtypes accounting for 5-10% of the RMS cases [3]. SRMS provides a diagnostic challenge by virtue of its similarity to other spindle cell neoplasms, including leiomyosarcoma, synovial sarcoma, malignant peripheral nerve sheath tumor, fibrosarcoma, sarcomatoid carcinoma, and spindle cell melanoma. Sometimes it is extremely difficult to diagnose ScRMS by histopathology alone. The differential diagnosis of ScRMS includes ARMS, sclerosing epithelioid fibrosarcoma, metastatic carcinoma, osteosarcoma, and angiosarcoma [15-17]. Therefore, immunohistochemistry plays a vital role in the diagnosis of SRMS/ScRMS.

SRMS/ScRMS tends to show a strong positivity for MYOD, a relatively weak positivity for myogenin, and a diffuse expression of desmin [3, 12]. Most of the cases in our study, like many other studies, have shown the typical immunohistochemical profile of SRMS/ScRMS. Interestingly, all of the cases in our cohort were positive for *MYOD1*. *MYOD1* showed a diffused expression in most ScRMS cases in our study, a finding which corresponded to previous observations [13, 14]. Recurring *MYOD1* p.L122R mutations in SRMS/ScRMS as well as in a subset of ERMS, which displayed frequent spindle cell morphology, have been reported [7, 8, 15]. This mutation has never been discovered in other types of RMS. And our study, similar to that of Agaram et al. [8], revealed cases of SRMS/ScRMS with heterozy-

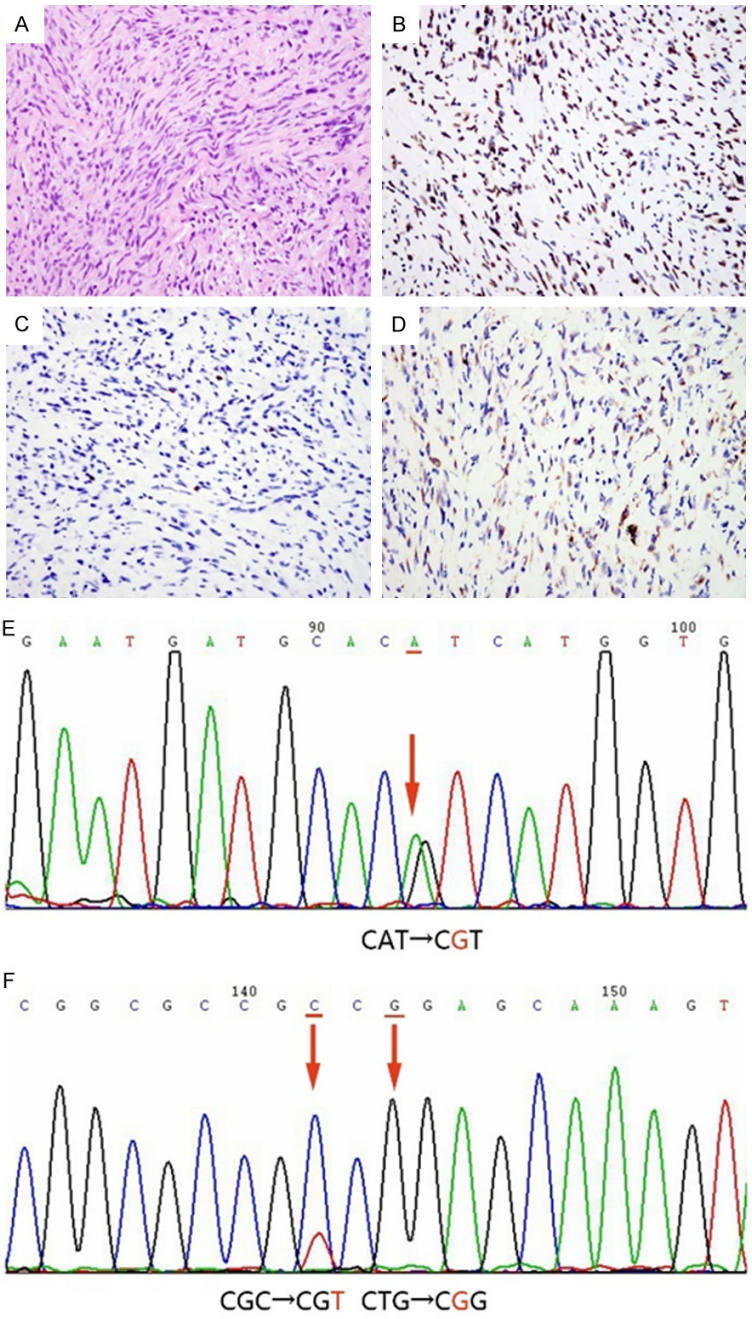

Figure 3. sRMS of a 7 year-old girl (Case 14). (A) Shows the pathologic features of *MYOD1*-mutant positive sRMS (H & E, 400×). (B-D) Immunohistochemical stains show positivity for *MYOD1*, myogenin, desmin (400×). Sequencing showing a heterozygous PIK3CA p.H1047R mutation (E). A MYOD1 p.121 nonsense mutation and a p.L122R homozygous mutation (F).

gous, as well as homozygous *MYOD1* p.L122R mutations. As a myogenic regulatory factor, the mutation of *MYOD1* might act as a key factor in skeletal muscle oncogenesis. Early studies showed the *MYOD1* protein with the p.L122R mutation could block wild-type *MYOD1* function and bind to *MYC* consensus sequences, then functionally substitute for *MYC* protein and stimulate proliferation through *MYC*-independent pathways. And *MYC* is one of the downstream target molecules of PI3K-AKT signaling [16]. Our present study also shows that the *MYOD1* p.L122R mutation is a common event in SRMS/ScRMS, which suggests that *MYOD1* p.L122R might be a specific mutation hotspot in SRMS/ScRMS. In addition, we observed cases which harbored *MYOD1* p.L122R mutations showing a strong positive *MYOD1* staining ($P<0.001$). A reasonable explanation for this finding is that the *MYOD1* p.L122R mutation may enhance the expression of *MYOD1*. However, wild-type *MYOD1* cases also show expression of *MYOD1* to a certain degree, which suggests that this mutation is not the initial cause of the *MYOD1* protein expression. And our study, like that of Agaram et al. [8], revealed cases of SRMS/ScRMS with heterozygous, as well as homozygous, *MYOD1* p.L122R mutations.

The previous molecular analysis also showed *PIK3CA* p.E542V, p.E542K, p.E545K, p.H1047R and p.G1049R as mutation hotspots in SRMS/ScRMS [8, 9, 15]. Many solid tumors showed that the *PIK3CA* missense mutations occur in all domains of p110α but are mainly concentrated on two hotspots, p.E542K and p.E545K in the helical domain and p.H1047R in the kinase domain [17]. Earlier studies described the presence of *PIK3CA* mutations only co-existing with the *MYOD1* mutations in ScRMS which occurred in older children or young adults [8]. In the present study, we

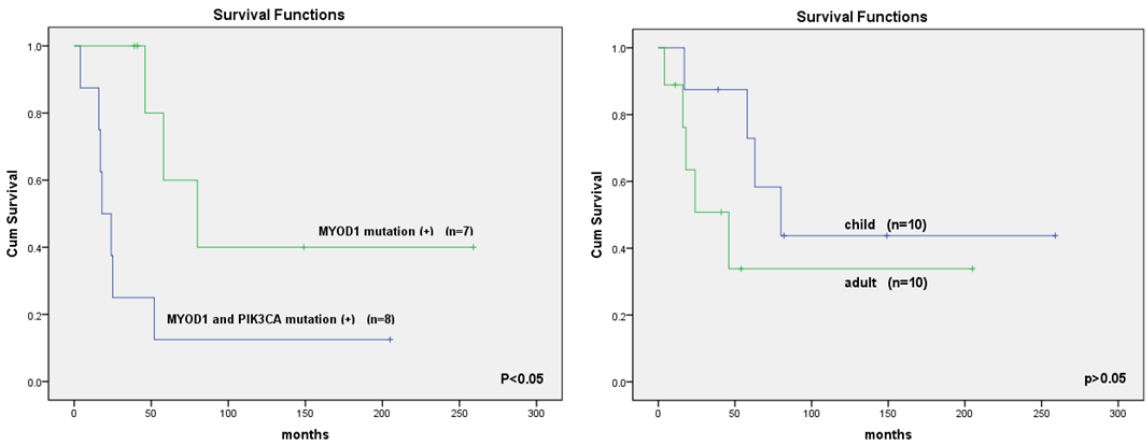

Figure 4. Kaplan-Meier survival analysis. A. Coexisting PIK3CA and MYOD1 mutation was significantly worse than the cases of merely harboring the MYOD1 mutation in the overall survival (OS). B. Shows the OS among the different age groups.

Table 2. Immunohistochemical and PIK3CA and MYOD1 mutation status of SRMS/ScRMS

Case No.	Immunohistochemical stains			Molecular		
				PIK3CA		*MYOD1*
	MYOD1	Myogenin	Desmin	Exon 10	Exon 21	Exon 1
1	++++	+++	++++	R524K	WT	L122R
2	++++	+	++++	R524K	WT	L122R
3	++++	+	++	R524K	WT	L122R
4	+++	+	++++	R524K	WT	L122R
5	+	+	++	R524K	WT	WT
6	++++	+	++++	R524K	WT	L122R
7	++++	++++	++	R524K	WT	L122R
8	++	-	++++	R524K	WT	WT
9	+++	+	++++	WT	WT	WT
10	++++	-	++++	WT	WT	L122R
11	++++	+	+++	WT	WT	L122R
12	++++	+	++++	WT	WT	L122R
13	++	+	++++	WT	WT	WT
14	++++	+	+	WT	H1047R	121* & L122R$^{\Delta}$
15	++++	-	++++	WT	WT	L122R$^{\Delta}$
16	++++	++	++++	WT	WT	L122R
17	++++	+++	+++	WT	WT	L122R
18	+++	+	++++	WT	WT	L122R
19	++++	-	++	E545K	WT	L122R
20	+	+	++	WT	G1049D	WT

(+) - Positive, (-) - negative, (f) - focal, WT-wild type, *nonsense mutation; $^{\Delta}$homozygous mutation.

observed a novel *PIK3CA* p.R524K heterozygous mutation hotspot in 8 of 11 SRMS/ScRMS cases which occurred in the head and neck region among the Chinese population. In addition, the *PIK3CA* mutation can exist independently without the *MYOD1* mutation in both SRMS and ScRMS. To our knowledge, it is the first study to report the *PIK3CA* p.R524K mutation in SRMS/ScRMS. A rare missense mutation in the helical domain of *PIK3CA*, the p.R524K mutation was detected in HER2-positive breast cancer patients [18]. A possible reason for the difference in the mutation location of *PIK3CA* between our study and others is the ethnicity.

Previous research shows pediatric SRMS/ScRMS cases seem to have a better prognosis than do pediatric ERMS cases, while in adult patients the disease may have a poor prognosis [19, 20]. But in our study, the OS among different age groups did not show any statistical significance (P>0.05, **Figure 4**). From the point of view of the mutation status, some studies showed SRMS/ScRMS which harbored *MYOD1* mutations with or without accompanying *PIK3CA* mutations, were highly associated with an

Table 3. Correlation between the outcome of SRMS/ScRMS with the PIK3CA and MYOD1 mutations; Correlation between the expression of MYOD1 with MYOD1 mutation

Clinicopathological features	Variable	Frequency (n = 20)	Mutation status of PIK3CA and MYOD1				Value	*P*-value$^{\Delta}$
			Wild	*PIK3CA* mutation	*MYOD1* mutation	Coexisted *PIK3CA* and *MYOD1* mutation		
Age	Pediatric (<18)	10	1	2	5	2		0.34$^{\Delta}$
	Adult (≥18)	10	1	1	2	6		
Gender	Male	10	2	2	4	2		0.26$^{\Delta}$
	Female	10	0	1	3	6		
Histopathologic subtype	Srms	13	2	3	4	4		0.41$^{\Delta}$
	Scrms	7	0	0	3	4		
	Mutation status of *MYOD1*							
	Positive	Wild	Mutant					
MYOD1 immunohistochemistry	+	2	0				74	<0.001†
	++	2	0					
	+++	1	2					
	++++	0	13					

$^{\Delta}$Fisher's Exact Test; †Mann-Whitney U test.

aggressive outcome such as early distant metastases and high mortality, despite multimodality therapy [4, 9, 21]. And in our study, the OS of patients in whom we detected a coexisting *PIK3CA* and *MYOD1* mutation was significantly worse than the cases in which patients had only the *MYOD1* mutation (P<0.05, **Figure 4**), which suggested that the cooccurrence of the *PIK3CA* and *MYOD1* mutations are associated with an aggressive clinical behavior and unfavorable clinical outcomes. Kohsaka et al [15] had indicated that either *MYOD1* p.L122R or *PIK3CA* p.H1047R increased the in vivo growth of tumor cells, while the combination of *PIK3CA* H1047R and *MYOD1* p.L122R resulted in more rapidly growing tumors, which suggests the coexisting mutations of *PIK3CA* and *MYOD1*, to some extent, prompt the progression of SRMS/ScRMS. As above, we suggest that the cooccurrence of *PIK3CA* and *MYOD1* mutations could be seen as an independent prognostic marker in SRMS/ScRMS.

In conclusion, *PIK3CA* and *MYOD1* mutations occur frequently with SRMS/ScRMS in the head and neck. *PIK3CA* p.R524K is a specific mutation hotspot of SRMS/ScRMS in the head and neck among the Chinese population. Coexisting mutations of *PIK3CA* and *MYOD1* are associated with a poor prognosis of SRMS/ScRMS and suggest that tumor progression may be influenced by activating the PI3K/AKT signaling pathways and by the blocking of myogenic genes' transcriptional differentiation. Morphologic observation and genetic testing may provide more comprehensive information in choosing the appropriate therapeutic regimen and in screening the targeted therapy of SRMS/ScRMS.

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Disclosure of conflict of interest

None.

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References

[1] Malempati S and Hawkins DS. Rhabdomyosarcoma: review of the Children's Oncology Group (COG) soft-tissue sarcoma committee experience and rationale for current COG studies. Pediatr Blood Cancer 2012; 59: 5-10.

[2] Cavazzana AO, Schmidt D, Ninfo V, Harms D, Tollot M, Carli M, Treuner J, Betto R and Salviati G. Spindle cell rhabdomyosarcoma. a prognostically favorable variant of rhabdomyosarcoma. Am J Surg Pathol 1992; 16: 229-235.

[3] Yasui N, Yoshida A, Kawamoto H, Yonemori K, Hosono A and Kawai A. Clinicopathologic anal-

ysis of spindle cell/sclerosing rhabdomyosarcoma. Pediatr Blood Cancer 2015; 62: 1011-1016.

[4] Owosho AA B Ch D, Huang SC Md, Chen S Mbbs, Kashikar S Dds, Estilo CL Dmd, Wolden SL Md, Wexler LH Md, Huryn JM Dds and Antonescu CRMd. A clinicopathologic study of head and neck rhabdomyosarcomas showing FOXO1 fusion-positive alveolar and *MYOD1*-mutant sclerosing are associated with unfavorable outcome. Oral Oncol 2016; 61: 89-97.

[5] Fletcher CD, Bridge JA, Hogendoorn P and Mertens F. Who classification of tumours of soft tissue and bone 2013. Lyon: IARC 2003; 305-310.

[6] Van Antwerp ME, Chen DG, Chang C and Prochownik EV. A point mutation in the MyoD basic domain imparts c-Myc-like properties. Proc Natl Acad Sci U S A 1992; 89: 9010-9014.

[7] Szuhai K, de Jong D, Leung WY, Fletcher CD and Hogendoorn PC. Transactivating mutation of the *MYOD1* gene is a frequent event in adult spindle cell rhabdomyosarcoma. J Pathol 2014; 232: 300-307.

[8] Agaram NP, Chen CL, Zhang L, LaQuaglia MP, Wexler L and Antonescu CR. Recurrent *MYOD1* mutations in pediatric and adult sclerosing and spindle cell rhabdomyosarcomas: evidence for a common pathogenesis. Genes Chromosomes Cancer 2014; 53: 779-787.

[9] Alaggio R, Zhang L, Sung YS, Huang SC, Chen CL, Bisogno G, Zin A, Agaram NP, LaQuaglia MP, Wexler LH and Antonescu CR. A molecular study of pediatric spindle and sclerosing rhabdomyosarcoma: identification of novel and recurrent VGLL2-related fusions in infantile cases. Am J Surg Pathol 2016; 40: 224-235.

[10] Liu CX, Li XY, Li CF, Chen YZ, Cui XB, Hu JM and Li F. Compound HRAS/PIK3CA mutations in Chinese patients with alveolar rhabdomyosarcomas. Asian Pac J Cancer Prev 2014; 15: 1771-1774.

[11] Shukla N, Ameur N, Yilmaz I, Nafa K, Lau CY, Marchetti A, Borsu L, Barr FG and Ladanyi M. Oncogene mutation profiling of pediatric solid tumors reveals significant subsets of embryonal rhabdomyosarcoma and neuroblastoma with mutated genes in growth signaling pathways. Clin Cancer Res 2012; 18: 748-757.

[12] Folpe AL, McKenney JK, Bridge JA and Weiss SW. Sclerosing rhabdomyosarcoma in adults: report of four cases of a hyalinizing, matrix-rich variant of rhabdomyosarcoma that may be confused with osteosarcoma, chondrosarcoma, or angiosarcoma. Am J Surg Pathol 2002; 26: 1175-1183.

[13] Rekhi B, Upadhyay P, Ramteke MP and Dutt A. *MYOD1* (L122R) mutations are associated with spindle cell and sclerosing rhabdomyosarcomas with aggressive clinical outcomes. Mod Pathol 2016; 29: 1532-1540.

[14] Rekhi B and Singhvi T. Histopathological, immunohistochemical and molecular cytogenetic analysis of 21 spindle cell/sclerosing rhabdomyosarcomas. APMIS 2014; 122: 1144-1152.

[15] Kohsaka S, Shukla N, Ameur N, Ito T, Ng CK, Wang L, Lim D, Marchetti A, Viale A, Pirun M, Socci ND, Qin LX, Sciot R, Bridge J, Singer S, Meyers P, Wexler LH, Barr FG, Dogan S, Fletcher JA, Reis-Filho JS and Ladanyi M. A recurrent neomorphic mutation in *MYOD1* defines a clinically aggressive subset of embryonal rhabdomyosarcoma associated with PI3K-AKT pathway mutations. Nat Genet 2014; 46: 595-600.

[16] Klempner SJ, Myers AP and Cantley LC. What a tangled web we weave: emerging resistance mechanisms to inhibition of the phosphoinositide 3-kinase pathway. Cancer Discov 2013; 3: 1345-1354.

[17] Thorpe LM, Yuzugullu H and Zhao JJ. PI3K in cancer: divergent roles of isoforms, modes of activation and therapeutic targeting. Nat Rev Cancer 2015; 15: 7-24.

[18] Elwy F, Helwa R, El Leithy AA, Shehab El din Z, Assem MM and Hassan NH. PIK3CA mutations in HER2-positive breast cancer patients; Frequency and clinicopathological perspective in egyptian patients. Asian Pac J Cancer Prev 2017; 18: 57-64.

[19] Sultan I, Qaddoumi I, Yaser S, Rodriguez-Galindo C and Ferrari A. Comparing adult and pediatric rhabdomyosarcoma in the surveillance, epidemiology and end results program, 1973 to 2005: an analysis of 2,600 patients. J Clin Oncol 2009; 27: 3391-3397.

[20] Nascimento AF and Fletcher CD. Spindle cell rhabdomyosarcoma in adults. Am J Surg Pathol 2005; 29: 1106-1113.

[21] Owosho AA, Chen S, Kashikar S, Zhang L, Chen CL, Wexler LH, Estilo CL, Huryn JM and Antonescu CR. Clinical and molecular heterogeneity of head and neck spindle cell and sclerosing rhabdomyosarcoma. Oral Oncol 2016; 58: e6-e11.