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Original Contribution

NKX3.1 a useful marker for mesenchymal chondrosarcoma: An immunohistochemical study

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ABSTRACT

Introduction: Mesenchymal chondrosarcoma is a rare subtype of chondrosarcoma. The tumor has a characteristic bimorphic pattern with areas of poorly differentiated small round cell component and interspersed islands of well differentiated hyaline cartilage. Histological diagnosis of mesenchymal chondrosarcoma is very challenging especially in small biopsies when tumor presents with little cartilaginous component. In such cases, it is very difficult to distinguish mesenchymal chondrosarcoma from other round blue cell tumors like Ewing's sarcoma, rhabdomyosarcoma, small cell osteosarcoma and desmoplastic round blue cell tumor. Immunohistochemically, mesenchymal chondrosarcoma stains positive for NKX2.2, CD99, S100 and SOX9. This immunoprofile is non-specific and overlaps with other round blue cell tumors. Till recently, there was no reliable immunohistochemical marker to differentiate mesenchymal chondrosarcoma from other round blue cell tumors.
NKX3.1, though widely used as a diagnostic biomarker for prostatic adenocarcinoma, has been recently proposed by Yoshida et al. (2020) as a unique marker of mesenchymal chondrosarcoma and EWSR1-NFATC2 sarcoma.
Objective: The aim of our study was to further explore utility of NKX3.1 as a diagnostic marker of mesenchymal chondrosarcoma.
Material & methods: We applied NKX3.1 immunohistochemistry to 21 cases of mesenchymal chondrosarcoma and 32 cases of other round blue cell tumors.
Results: 14 out of 21 cases (66.7%) of mesenchymal chondrosarcoma stained positive for NKX3.1 with nuclear expression in small round component. Cartilaginous component was predominantly negative. All other round blue cell tumors showed negative results.
Conclusion: Based on our study results we suggest that NKX3.1 is a useful immunohistochemical marker in differentiating mesenchymal chondrosarcoma from its histological mimics.

1. Introduction

Mesenchymal chondrosarcoma is a rare high grade malignant tumor of bone and soft tissue first described by Lichtenstein and Bernstein in 1959 [2]. It represents <3% of all primary chondrosarcomas. The peak age incidence is second and third decade of life. Males and females are equally affected. Approximately two third cases of mesenchymal chondrosarcoma affect bones (craniofacial bones, ribs, ileum, vertebra) and one third cases present as extraskeletal soft tissue sarcoma (meninges is one of the most common sites) [3]. The tumor has poor prognosis with high rates of local recurrence and distant metastasis.

Mesenchymal chondrosarcomas have a characteristic biphasic morphology with area of well differentiated cartilaginous component, undifferentiated small round cell component and hemangiopericytoma like blood vessels [4,5] (Fig. 1A, B). The cartilaginous component shows strong positivity for S100 protein and small round cell component shows strong membranous staining for CD99 and nuclear positivity for NKX2.2. Both cartilaginous and undifferentiated round cell component show nuclear positivity for SOX9 [6]. These immunohistochemical stains are also expressed in other round blue cell tumors. NKX2.2 is co-expressed in Ewing's sarcoma and EWSR1-NFATC2 sarcomas. CD99 positivity is also seen lymphoblastic lymphoma and desmoplastic small round blue cell tumors. S100 is expressed in conventional chondrosarcomas. SOX9 (SRY-related high mobility group box 9) was once considered as a specific marker of mesenchymal chondrosarcoma. Studies have shown the expression of SOX-9 in other tumors as well like de-differentiated chondrosarcoma, Ewing's sarcoma and synovial sarcoma [7]. The small biopsies pose diagnostic dilemma as these may not

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show any cartilaginous component.

NKX3.1 (NK3homebox1) is a prostate specific gene located on chromosome 8p21 [8]. It encodes transcription factor that plays an important role in normal prostatic development and loss of expression leads to carcinogenesis [9]. In a recent study proposed by Yoshida et al., NKX3.1 has been proposed as a useful immunohistochemical marker of mesenchymal chondrosarcoma [10]. They tested 12 cases of mesenchymal chondrosarcoma for NKX3.1 and demonstrated immunoreactivity in 12/12 cases (100%). In addition to mesenchymal chondrosarcoma, NKX3.1 positivity was also seen in 9/11 (82%) cases of EWSR1-NFATC2 sarcomas. All other round blue cell tumors showed negative results. Our study is aimed to further explore diagnostic utility of NKX3.1 in mesenchymal chondrosarcoma.

2. Material and methods

The study was approved by institutional review board. All cases diagnosed as mesenchymal chondrosarcoma (21cases), Ewing's sarcoma (13 cases), alveolar rhabdomyosarcoma (13 cases) and desmoplastic small round blue cell tumor (6 cases) were retrieved through hospital archives from the year 2012–2020. These included both incisional biopsies and resections. H & E slides were reviewed and NKX3.1

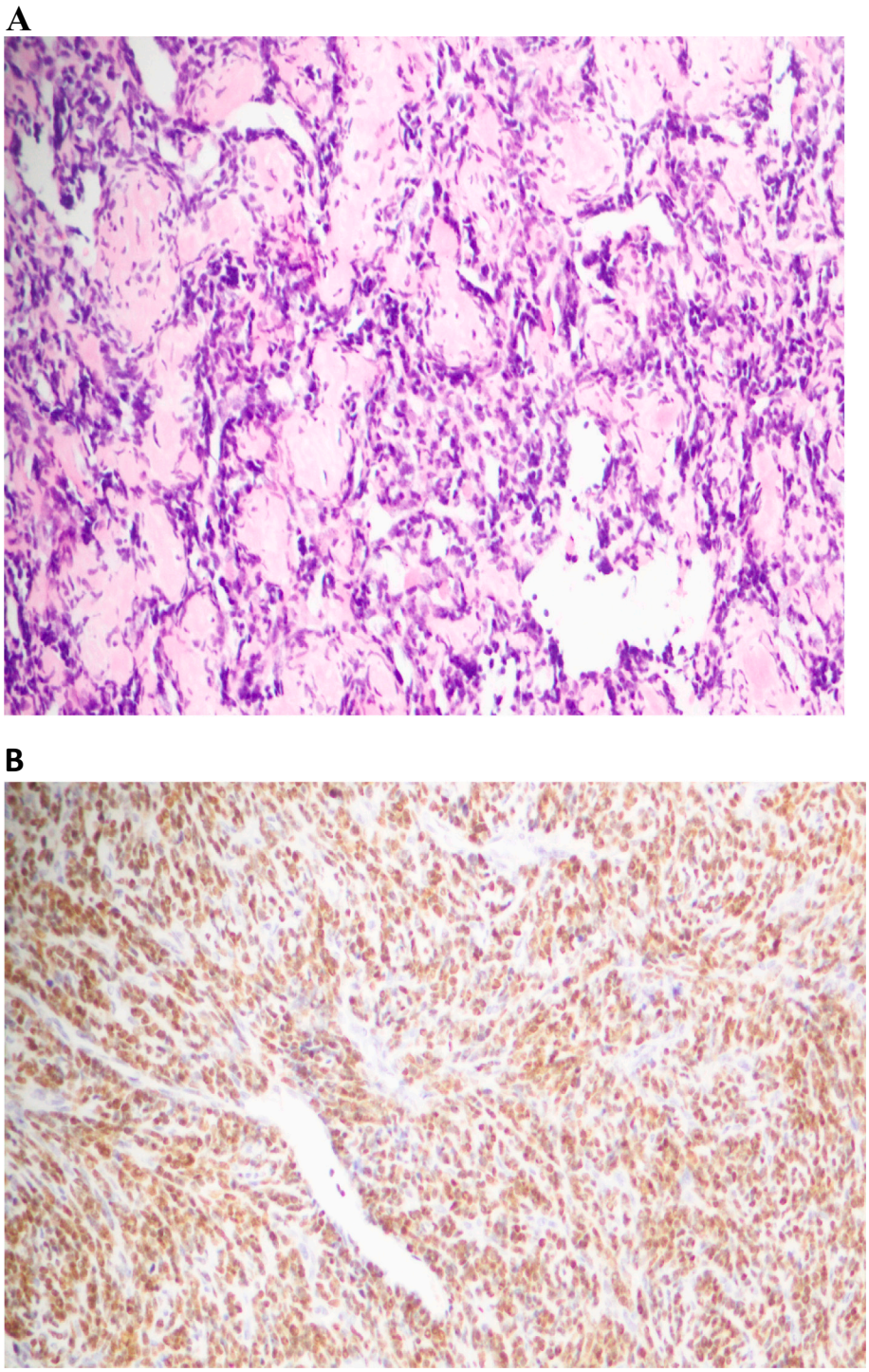

Fig. 1. A) Mesenchymal chondrosarcoma H&E stain, 40×. B) NKX3.1 monoclonal antibody diffuse positivity.

immunohistochemical stain was applied on the representative formalin-fixed, paraffin embedded tissue sections. We used NKX3.1 (EP 356) Rabbit Monoclonal ready to use Primary Antibody (CELL MARQUE). After baking and deparaffinization antigen retrieval was performed at 100 degree centigrade for 40 min. Slides were incubated with primary antibody NKX3.1 for 15 min. Positive and negative controls applied. Regarding immunohistochemical stain interpretation, nuclear staining was considered positive. The intensity and extent of staining was graded by criteria as proposed by Yoshida et al. [1]. The staining intensity was graded as weak, moderate and severe. The extent of staining was considered NEGATIVE (0% or <5% positive cells), FOCAL (5% to 50% positive cells) and DIFFUSE (>50% positive cells).

In all these cases information regarding management, follow up and outcome was obtained through direct communication with patients, family members and the treating physician.

3. Results

Out of 21 cases of mesenchymal chondrosarcoma there were 4 males (19%) and 17 females (80.9%) with age ranging from 7 to 65 years. Mean age for males was 35 years and for females 29 years. The patients were commonly affected in the second and third decades of life with female pre-dominance.

5 cases (23.8%) originated from bone, 14 cases (66.7%) from extra skeletal soft tissue and 2 cases (9.52%) had uncertain origin either bone or soft tissue. The tumor was located in lower limb in 8 cases, craniofacial region 6 cases, spine 5 cases, 1 in gluteal region and intra-abdominal in 1 case.

There were 9 incisional biopsies, 1 core biopsy, 9 excisions (7 fragmented, 2 intact excisions), 1 disarticulation and 1 enucleation of eye. The excisions were pre-dominantly received fragmented with a size range of 5.1 to 14.5 cm in aggregate. In intact excisions, enucleation and disarticulation tumor size range was 2.9 cm to 23 cm (Table 1).

Grossly, the tumor had grayish white soft to firm cut surface. Microscopically, the tumor had high grade small round cell component, hemangiopericytoma like blood vessels and areas of well differentiated hyaline cartilage. Incisional biopsies had pre-dominantly small round cell component with little cartilaginous component.

To explore diagnostic utility of NKX3.1 in mesenchymal chondrosarcomas, NKX3.1 immunohistochemical stain was applied on 21 cases of mesenchymal chondrosarcoma and its 32 histological mimics. 14 out of 21 cases (66.7%) of mesenchymal chondrosarcoma showed nuclear positivity for NKX3.1 in small round cell component. The cartilaginous component was pre-dominantly negative. 11 cases (52.3%) were diffusely positive (>50% positive cells) (Fig. 1B) and 3 cases (14.2%) were focal positive (5–50% positive cells) (Fig. 2B). 8 cases (38.05%) showed strong positivity, 5 cases (23.8%) were moderately positive and 1 case (4.76%) showed weak nuclear staining. All other round blue cell tumors Ewing's sarcoma 13 cases, alveolar rhabdomyosarcoma 13 cases and 6 cases of desmoplastic round blue cell tumor were negative for NKX3.1. The results of NKX3.1 immunoreactivity are summarized in Table 2.

Out of 21 cases, treatment details were available for 18 cases (85.7%) only. 3 cases (14.2%) were lost to follow up. 10/18 cases (55.5%) were

Table 1
Clinical presentation, treatment and outcome of patients with mesenchymal chondrosarcoma.

Case no.	Year of diagnosis	Age/ sex	Location	SPC.NATURE	Size of tumor	Treatment	Follow up	Outcome
1	2012	35/F	Nasal cavity	FE	6.5 cm in aggregate	SE	RE	Death (2017)
2	2012	50/F	Distal femur (B)	IB	2.5 cm in aggregate	LTFU	LTFU	LTFU
3	2012	26/M	Para-vertebral area neck	FE	14.5 cm in aggregate	SE, C, R	RE	Death (2015)
4	2012	24/F	L1 vertebra	IB	Review blocks	LTFU	LTFU	LTFU
5	2013	27/F	Left calf (ST)	E	8.0 cm	SE	LTFU	LTFU
6	2013	14/F	Right eye	Enucleation	2.9 cm	SE, R	NR	A
7	2013	65/M	Right thigh (ST)	E	6.0 cm	SE	RE	LTFU
8	2014	15/M	Right femur (B)	Right femur disarticulation	23 cm	R	LTFU	LTFU
9	2014	27/F	Spinal cord (intradural extra-medullary)	IB	1.5 cm in aggregate	SE	RE and brain met.	Death (2018)
10	2014	28/F	Right proximal tibia. (B)	IB	3.5 cm in aggregate	SE	_	Death (2014)
11	2016	32/F	Face (ST)	FE	8.5 cm in aggregate	SE	LTFU	LTFU
12	2016	29/F	Palate	IB	0.5 cm in aggregate	SE	RE	Death (2018)
13	2017	29/F	Face (ST)	FE	5.7 cm	SE	RE	AWD
14	2017	23/F	Iliac bone	IB	3.0 cm	SE, C, R	RE	Death (2020)
15	2017	40/F	Abdominal mass	FE	15.0 cm in aggregate	SE	_	Death (2017)
16	2017	34/M	Left gluteal region (ST)	IB	3.5 cm in aggregate	C, R	NR	Death (2019)
17	2019	33/F	Left thigh	CB	1.5 cm	LTFU	LTFU	LTFU
18	2020	36/F	Spine	IB	1.2 cm in aggregate	SE, C, R	_	AWD
19	2019	27/F	Sacral mass (B, ST)	IB	0.7 cm in aggregate	C, R	NR	AWD
20	2019	7/F	Nasal mass (ST)	FE	5.1 cm in aggregate	SE	NR	A
21	2020	35/F	Left thigh	FE	6 cm in aggregate	SE, C (ongoing)	RE	AWD

AWD, alive with disease; A, alive without disease; B, bone; C, chemotherapy; CB, core biopsy; E, excision; FE, fragmented excision; IB, incisional biopsy; LTFU, lost to follow up; NR, no recurrence; R, resection; R, radiotherapy; RE, recurrence; SE, surgical excision; ST, soft tissue.

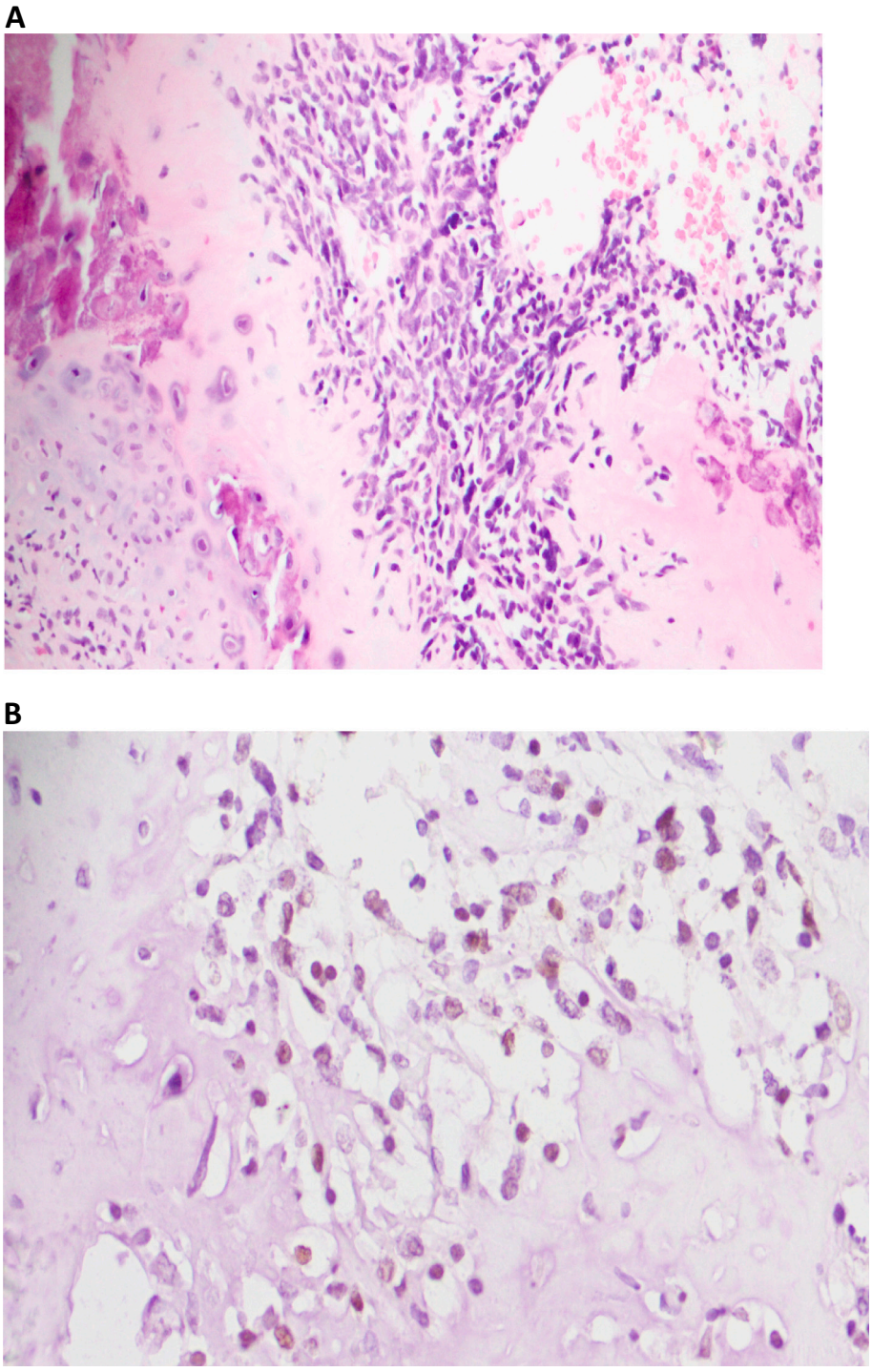

Fig. 2. A) Mesenchymal chondrosarcoma H&E stain, 40×. B) NKX3.1 monoclonal antibody focal positivity.

treated with surgery only, 1/18 (5.5%) with surgery and postoperative radiotherapy, 1/18 (5.5%) with surgery and postoperative chemotherapy, 3/18 (1.7%) with surgery and both postoperative chemotherapy and radiotherapy. 1/18 (5.5%) received radiotherapy only and 2/18 (11.1%) patients were treated with chemotherapy and radiotherapy alone without surgical excision.

Follow up and outcome details were available for 14 patients (66.7%) only. 7 patients (33.3%) were lost to follow up. The follow up duration varied from 6 months to 8 years.8/14 cases (57.1%) showed disease recurrence and died.4/14 (28.6%) patients remained alive with disease and 2/14 (1.42%) patients showed disease free survival. Only 1% patients survived after a follow up of 5 years.

Overall the disease showed poor prognosis with high rates of local recurrence and poor survival (<5 years). Clinicopathological analysis of mesenchymal chondrosarcoma is shown in Table 1 [3].

4. Discussion

The diagnosis of mesenchymal chondrosarcoma may be problematic, especially in small biopsies. Recently, NKX3.1 has been proposed as a novel marker of mesenchymal chondrosarcoma by Yoshida et al. [1]. The accumulating evidence has shown that this stain is negative in other

Table 2
Results of NKX3.1 immunoreactivity in mesenchymal chondrosarcomas.

Tumor type	Total cases	NKX3.1 immunoreactivity	Extent	Intensity
Mesenchymal chondrosarcoma	21	14/21 (66.7%)	D ($n = 11$, 52.3%), F ($n = 3$, 14.2%)	W (n = 1, 4.76%) M ($n = 5$, 23.8%) S ($n = 8$, 38.0%)
Ewing's sarcoma	13	0/13	–	–
Alveolar rhabdomyosarcoma	13	0/13	–	–
Desmoplastic small round blue cell tumor	06	0/6	–	–

D, diffuse; F, focal; M, moderate; S, strong; W, weak.

round blue cell tumors. The negativity of NKX3.1 in other round blue cell tumors can be of great help in differentiating mesenchymal chondrosarcoma from its multiple histological mimics.

Mesenchymal chondrosarcoma generally express CD99, NKX2.2, S100 and SOX9 immunohistochemical stains [10]. CD99 and NKX2.2 are co-expressed in Ewing's sarcoma and CD99 positivity is also seen in desmoplastic small round blue cell tumor, lymphoblastic lymphoma and few cases of rhabdomyosarcoma [11]. S100 is also expressed in conventional chondrosarcoma and SOX9 positivity is also observed in de-differentiated chondrosarcoma [7]. Based on above mentioned overlapping immunoprofile, it is very difficult to distinguish mesenchymal chondrosarcoma from its other strong differentials.

In our study, we performed NKX3.1 immunohistochemistry on 21 cases of mesenchymal chondrosarcoma. We used NKX3.1 (EP356) Rabbit Monoclonal ready to use Primary Antibody (CELL MARQUE) in contrast to study by Yoshida et al. [1] who used polyclonal antibody. 14 out of 21 cases (67%) of mesenchymal chondrosarcoma showed nuclear positivity in round cell component. The cartilaginous component was pre-dominantly negative. 11 cases showed diffuse positivity (>50% positive cells) with moderate to strong staining and 3 cases showed focal positivity (5–50% positive cells) with weak to moderate staining. NKX3.1 expression was not seen in any case of Ewing's sarcoma, desmoplastic small round blue cell tumor and alveolar rhabdomyosarcoma. This negative expression of NKX3.1 can be very useful in distinguishing mesenchymal chondrosarcoma from other round blue cell tumors. Till date, other than mesenchymal chondrosarcoma, NKX3.1 expression is only observed in EWSR1-NFATC2 sarcomas [1].

In the study proposed by Yoshida et al. 12/12 (100%) cases of mesenchymal chondrosarcoma showed positive staining for NKX3.1. In our study, 7/21 cases (33.3%) of mesenchymal chondrosarcoma showed negative expression for NKX3.1. One of the limiting factors is the use of monoclonal antibody which is produced by identical B cells and can recognize only a single epitope. In contrast, Yoshida et al. used polyclonal antibody which is produced by different B cells and has greater affinity for multiple epitopes [12]. Secondly varying proportion of small round cell component in different cases is another limiting factor.

NKX3.1 and NKX3.2 (*Bapx1*) are home box containing transcription factors that are expressed in early somites [13]. The somites form sclerotome which plays an important role in the development of axial skeleton through chondrogenic differentiation and endochondral ossification [14]. Mesenchymal chondrosarcoma is a tumor of chondroprogenitor cells [15,16]. Therefore, expression of NKX3.1 in mesenchymal chondrosarcoma may reflect its role in early chondrogenesis.

5. Conclusion

In our study NKX3.1 was expressed in 67% cases of mesenchymal chondrosarcoma and was negative in all other round blue cell tumors including Ewing's sarcoma, alveolar rhabdomyosarcoma and desmoplastic round blue cell tumor. NKX3.1 seems to be a useful marker for diagnosis of mesenchymal chondrosarcoma.

Declaration of competing interest

The authors declare no conflicts of interest or any financial association with any organization.

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