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ORIGINAL ARTICLE

Comparision of New Diagnostic Tools for Malignant Peripheral Nerve Sheath Tumors

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Introduction

Malignant peripheral nerve sheath tumors (MPNST) may show spindle cell or epithelioid morphology, differentiate along Schwann cell or perineurial lines, and often arise in association with a peripheral nerve or a pre-existing neurofibroma [1]. Morphologically, MPNSTs are typically composed of fascicles of alternating cellularity, whorls, palisades or rosette-like arrangements, subendothelial accumulation of tumor cells, and large areas of geographic necrosis [2]. Rare MPNST may show heterologous, glandular or rhabdomyoblastic differentiation [3]. A malignant spindle cell tumor arising from a pre-existing benign nerve sheath tumor or one seen in a patient with Neurofibromatosis Type 1 Syndrome (NF1), is generally considered to represent MPNST until proven otherwise [2, 4]. Tumors arising within a nerve also raise suspicion for MPNST, although other types of sarcomas may occur in this location as well [5]. There are a variety of sarcomas including adult-type fibrosarcoma, rhabdomyosarcoma, leiomyosarcoma, Ewing sarcoma, clear cell sarcoma and benign peripheral nerve sheath tumors, especially atypical neurofibromas and cellular schwannomas in the differential diagnosis [2]. Another critically important differential diagnosis is with malignant melanoma, in particular desmoplastic or spindle cell types [6] Some immunohistochemical (IHC) markers such as S100 protein (often in a patchy and weak pattern) or CD57 may support the diagnosis but some MPNSTs may also be entirely negative for these antibodies [7]. Thus there continues to be considerable interest in the development of new immunohistochemical reagents that will assist in these sometimes-difficult differential diagnoses [5, 8–11].

SOX2, SOX10 and p75 neurotrophin receptor (p75NTR) are neuroectodermal stem cell/progenitor markers, and may potentially be useful for the diagnosis of MPNST. Prior studies reported variable results regarding the expression of these markers in MPNSTs [9, 12–14]. SOX10 appears to be positive in all developmental stages of Schwann cells [15]. It is also expressed in benign tumors like schwannoma [16]. SOX10 expression in MPNST has been reported [5, 9]. p75NTR is another marker of neural crest origin [17]. İnitially, Bonetti et al. reported p75NTR expression to be a feature of schwannomas, but not non-neoplastic tissues and malignant peripheral nerve sheath tumors [12]. More recent studies, however, have showed high rates of expression in both schwannomas and MPNST [13, 14]. SOX2, an inhibitor of Schwann cell differentiation, has been identified as a potential marker of immature Schwann cells. SOX2 expression is seen in schwannomas, including cellular schwannomas, and a percentage of MPNST, but not in non-neoplastic neural lesions such as traumatic neuroma [18] [9]. More recently, studies have demonstrated loss of trimethylation at lysine 27 of histone 3 in a subset of MPNSTs reflecting loss-of-function somatic alterations in the Polycomb Repressive Complex 2 (PRC2) core components [10, 11, 19, 20]. Therefore H3K27me3 seems to be a promising IHC marker for the diagnosis of MPNST.

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Table 1 Types, dilutions and sources for antibodies

Antigen	Dilution	Source
p75NTR	1:100	Abcam
SOX2	1:200	Millipore
SOX10	1:50	Santa Cruz
H3K27M	1:100	Millipore

The purpose of this study was to determine the clinical utility of a panel composed of these relatively novel markers in the diagnosis of MPNSTs.

Materials & Methods

Patients

We electronically searched the archives of the Division of Anatomic Pathology, Mayo Clinic, Rochester MN for all cases coded as "malignant peripheral nerve sheath tumor" or "neurofibrosarcoma" for the period 1988–2011. Three neuropathologists (AE, BWS, TT) reviewed all available pathology material to confirm the diagnosis. The malignant tumors with spindle cell morphology were included with at least two of the following criteria: 1) partial expression of S100, 2) arising within a nerve, 3) pre-existing neurofibroma. We also included the NF1 associated malignant tumors exhibiting spindle cell morphology. Cases which were not uniformly felt to represent MPNST were excluded. We obtained the clinical information, including NF1 status, patient age and gender and tumor site and size from the medical records, including pathology reports.

Immunohistochemistry

Representative formalin-fixed, paraffin-embedded tissue blocks were selected from primary tumors and recurrences (when available), and 4-μm slides were immunostained for SOX10, SOX2, p75NTR, and H3K27me3. Table 1 lists the primary antibodies, resources, and final dilutions used for each antibody.

Assessment of Immunohistochemical Expression

Immunohistochemistry slides were independently reviewed by at least one of the authors (AE, TT MP) and the results were categorized as "positive" and "negative" for SOX10, SOX2 and p75NTR (expression in <5 % of cells was considered "negative"). H3K27me3 expression results were categorized into 3 groups: "positive" (diffuse and strong expression), "partial loss" (focal or patchy expression) and "complete loss" (negative in every tumor cell).

Statistical Analysis

Statistical analyses were performed using the SPSS 22.0.0 software package (SPSS, Chicago, IL, USA). Correlation between IHC results were analyzed by the Kendall's Tau test and the results are reported as correlation coefficients. Comparisons among categorical variables were performed with Pearson test, and comparisons of continuous variables were performed with the Mann–Whitney *U* test and t-test. *p* values of less than 0.05 were accepted as significant.

Results

A total of 137 cases were initially identified from the electronical search. Of these, 45 were excluded after re-review, leaving a final study population of 92 cases. A subset of these cases were included in a prior study from our group [5].

The median patient age 38 years (range 11 to 85 years); nine cases occurred in patients less than 18 years of age. The male to female ratio was 1.4:1. Most of the tumors occurred in a non-extremity location (71 %). A history of NF1 was available for 30 patients; 3 tumors arose following previous therapeutic irradiation. Histopathologically, 8 cases were epithelioid MPNST, 2 showed glandular differentiation, 2 contained rhabdomyoblastic elements (malignant Triton tumor) and 1 showed heterologous cartilaginous differentiation. The IHC results are shown in Tables 2, 3, and 4. Briefly; 75 % of the cases showed expression of p75NTR, and approximately 50 % were

Table 2 The results of immunohistochemical stains

	MPNST cohort (n)	NF1 cases (n)
H3K27 negativity (complete loss)	41 % (38)	47 % (14)
H3K27 negativity (complete/partial loss)	68 % (63)	90 % (27)
SOX2 positivitiy	46 % (38)	46 % (14)
P75NTR positivitiy	78 % (72)	83 % (25)
SOX10 positivitiy	54 % (50)	66 % (20)

Table 3 The correlation of immunohistochemical stains

	SOX2 positive	P75NTR positive	SOX10 positive	H3K27me3 loss**
SOX2 positive				
P75NTR positive	$p = 0.706$ (cc: −0.40)			
SOX10 positive	$p = 0.569$ (cc: 0.60)	$p = 0.003$* (cc: 0.31)		
H3K27me3 loss**	$p = 0.171$ (cc: −0.14)	$p = 0.212$ (cc: −0.131)	$p = 0.733$ (cc: 0.36)	

*$p < 0,05$, statistically significant

**H3K27me3 loss is described as either complete or partial loss

positive for SOX10 and SOX2 (Figs. 1,2 and 3). Thirty eight tumors (42 %) showed complete loss of H3K27me3 expression, with only 31 % (n = 29) showing diffuse expression (Fig. 4). Of the cases showing diffuse H3K27me3 expression, 8 were epithelioid MPNST and 2 showed rhabdomyoblastic differentiation (Fig. 5). In the tumors showing rhabdomyoblastic differentiation, both the conventional and rhabdomyoblastic components were strongly H3K27me3-positive. MPNSTs with glandular components expressed H3K27me3 only in the glandular component. Interestingly, diffuse H3K27me3 positivity was seen in only 1 of 9 pediatric MPNSTs and in none of the radiation-associated case.

There was a statistically significant positive correlation between SOX10 and p75NTR expression for all MPNSTs (correlation coefficient: 0.311, $p = 0.003$). Other pairwise correlations using SOX10 (positive), SOX2 (positive), p75NTR (positive), and H3K27me3 (complete or partial loss) did not show significant correlations (Table 3). The clinical and histological features of MPNSTs with negative or positive results for SOX2, SOX10 and p75NTR were similar ($p > 0.05$ for all). There was a statistical difference between NF1-associated MPNSTs and non-NF1-associated MPNSTs with regards to partial or complete loss of H3K27me3. Partial or complete loss of H3K27me3 expression was seen in 27 of 30 NF1-associated cases, a significantly greater percentage than

Table 4 H3K27me staining results

	SOX2 +	SOX2 −	p	p75ntr+	p75ntr−	p	SOX10 +	SOX10−	p	H3K27 loss *** (n)	H3K27 + (n)	p
Cohort	38	54		72	20		50	42		63	29	
Histologic variants												
Conventional	32	47	0.702	64	15	0.115	44	35	0.522	54	25	0,950
Non-conventional	6	7		8	5		6	7		9	4	
Histologic grade												
Low	5	12	0.270	14	3	0.651	9	8	0.897	13	4	0,432
High	33	42		58	17		41	34		50	25	
NF status												
NF1 +	14	16	0.467	25	5	0.412	20	10	0.099	27	3	0,002*
NF1 -	24	38		47	15		30	32		36	26	
Tumor Localization												
Extremities	15	12	0.074	22	5	0.629	16	11	0.542	15	12	0,086
Non-extremities	23	42		50	15		34	31		48	17	
Gender												
Male	20	32	0.528	43	9	0.240	26	26	0.340	37	15	0,529
Female	18	22		29	11		24	16		26	11	
Age Mean +/− SD (n) **	41+/−17	43+/−17	0.489	43+/−18	39+/−14	0.529	42+/−19	42+/−16	0.914	41+/− 17	45 +/− 17	0,241

*p < 0,05, statistically significant

**Mann-Whitney U test and t-test have been performed

***H3K27me3 loss is described as either complete or partial loss

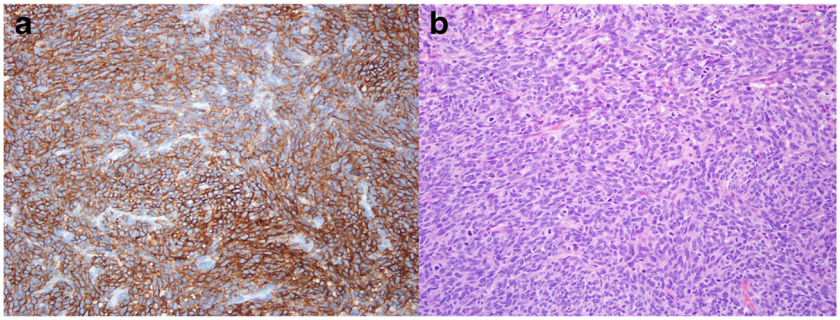

Fig. 1 **a** p75NTR, diffuse and strong staining pattern **b** corresponding morphology on H&E

in non-NF1-associated MPNSTs ($p = 0.002$). There were no differences between MPNSTs with and without H3K27me3 loss with regards to patient gender or age, tumor location and histologic grade ($p > 0.05$ for all, Table 4).

Discussion

In the present study, we have identified SOX10 expression in 54 % of MPNST, a somewhat higher percentage of cases than has been previously reported by Karamchandani et al. (27 %) [8] and Pekmezci et al. (18 %) [9]. The higher sensitivity of SOX10 for MPNST in the current study likely reflects technical differences between these studies, although differences in the relative percentage of NF-1 associated cases may also be relevant. In our previous study we found SOX10 expression to be 67 % sensitive and 93 % specific in the distinction of NF1-associated MPNST from monophasic synovial sarcoma, and to be more specific in this differential diagnosis than was S100 protein [5]. The sensitivity of SOX10 for MPNST is lower than for other neural crest-related neoplasms, such as schwannoma, neurofibroma and melanoma [8]. Reduced expression of SOX10 in MPNSTs likely reflects down-regulation of Schwann cell differentiation markers in these tumors [21]. SOX10 expression has also been shown in other potential mimics of MPNST, such as cellular blue nevus, clear cell sarcoma, granular cell tumor, melanoma, neuroblastoma and paraganglioma [22].

We found p75NTR expression in 78 % of MPNSTs, including 83 % of NF1-associated cases, consistently with previously published report [9]. It has been suggested that expression of p75NTR may occasionally be useful in the differential diagnosis of MPNST and cellular schwannoma [9]. It is well recognized, however, that p75NTR expression is by no means specific to nerve sheath tumors, and may be seen in a variety of other tumors including (but not limited to) synovial sarcoma and Ewing sarcoma [13].

The rate of SOX2 expression (46 %) in MPNST in the present series is lower than in the study of Pekmezci et al. (83 %) [9], likely reflecting our inclusion of a greater number of cases without known NF1. SOX2 is known to be expressed by nearly all cellular schwannomas, and it is possible that the finding of diminished SOX2 expression may be useful in this sometimes challenging differential diagnosis. Overall, however, data about the potential utility (if any) of SOX2 is limited, and it is difficult to recommend this marker for the differential diagnosis of MPNST until additional studies are completed.

Most MPNSTs, whether NF1-associated or sporadic, show mutations or deletions in the *NF1* gene [23]. MPNST also frequently show *CDKN2A* alterations, and in combination these genetic events may lead to inactivation of the polycomb repressive complex (PRC2), with subsequent loss of H3K27me3 expression in tumor cells [24]. It has recently been shown that partial or complete loss of HK27me3 is a frequent event in MPNSTs, in particular NF1-associated tumors, and that immunohistochemistry for H3K27me3 may be helpful in the distinction of MPNST from cellular schwannoma and non-

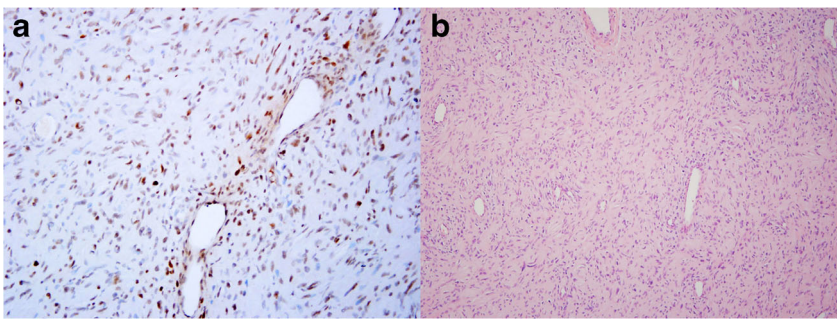

Fig. 2 **a** SOX2 nuclear staining pattern **b** corresponding H&E morphology

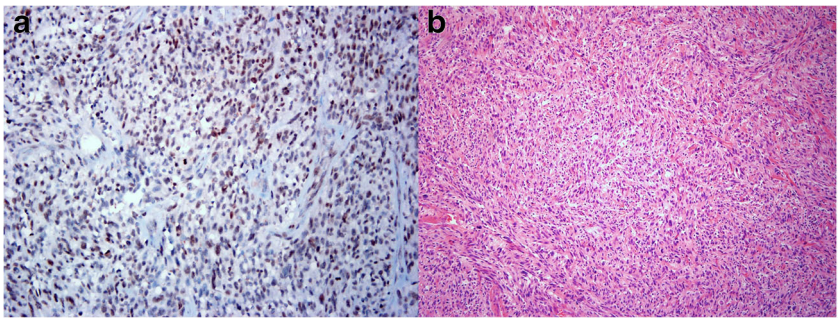

Fig. 3 **a** SOX10 nuclear staining pattern **b** corresponding H&E morphology

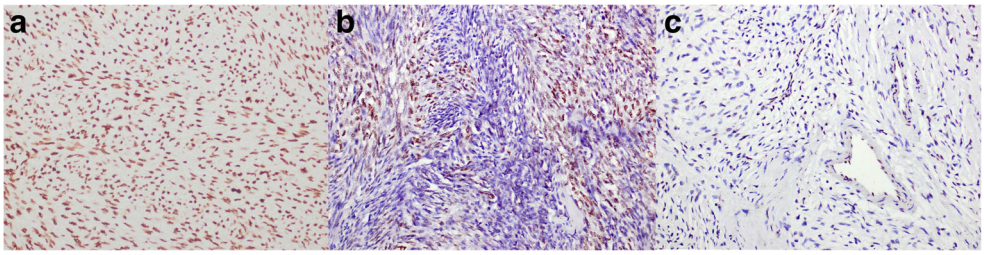

Fig. 4 Different staining patterns of H3K27me3 **a** Diffuse positivity, **b** partial loss of staining **c** complete loss of staining

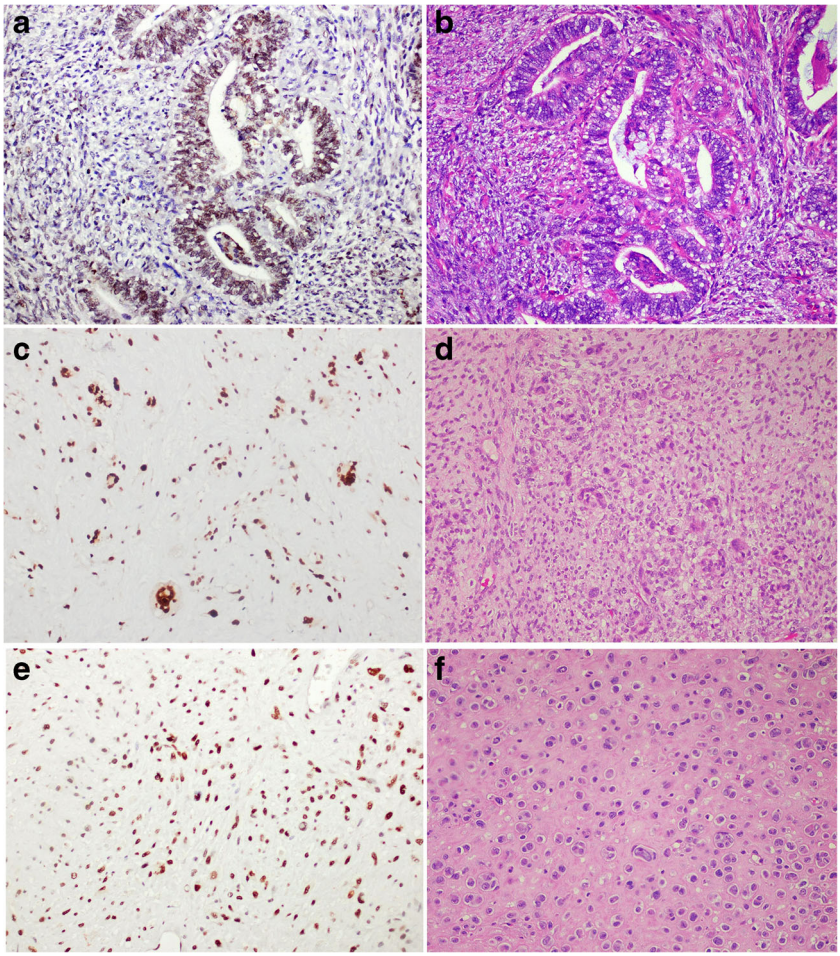

Fig. 5 H3K27me3 positive cases with non-conventional morphology **a**-**b** MPNST with glandular differentiation, **c**-**d** MPNST with rhabdomyoblastic differantation, **e**-**f** Epithelioid MPNST

MPNST sarcomas [19] [11]. It is unclear, however, whether H3K27me3 loss is specific for MPNST, as loss of this protein has also recently been demonstrated in some synovial sarcomas [10]. In the present series, 41 % of MPNST showed complete loss of H3K27me3 expression with at least partial loss in 68 % of tested cases. In agreement with prior studies, we found H3K27me3 loss to be more frequent in NF1 associated tumors as compared with sporadic MPNST, commonly present in radiation-associated MPNST, and absent in epithelioid MPNST [9] [20]. It is unclear why H3K27me3 loss was not a feature of MPNST with rhabdomyoblastic differentiation, as such lesions are frequently NF1-associated [25]. Interestingly, H3K27me3 loss was seen only in the spindled component of MPNSTs showing glandular differentiation, with retained expression in the malignant glandular component.

In summary, we have evaluated the utility of a panel of immunohistochemical markers, including SOX10, SOX2, p75NTR and H3K27me3 in a large series of NF1-associated, radiation-associated and sporadic MPNST. Our study suggests that antibodies to SOX10, p75NTR and H3K27me3 have clinical utility, and should be included in the routine evaluation of spindle cell neoplasms where the differential diagnosis includes MPNST. It must be emphasized, however, that none of these markers shows perfect sensitivity or specificity for MPNST, and this diagnosis must continue to rely on comprehensive integration of all available clinical, morphological, immunohistochemical and genetic data.

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