HHS Public Access

Author manuscript

Histopathology. Author manuscript; available in PMC 2023 May 01.

Published in final edited form as:

Histopathology. 2022 May ; 80(6): 995–1000. doi:10.1111/his.14626.

CDX2 expression in malignant peripheral nerve sheath tumor: A potential diagnostic pitfall associated with PRC2 inactivation

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Abstract

Aims: Malignant peripheral nerve sheath tumor (MPNST) is a soft tissue sarcoma that exhibits features of Schwann cell differentiation. Heterologous, often mesenchymal-type differentiation occurs in a subset of MPNST, while glandular morphology also is encountered in rare cases. We observed in MPNST unanticipated expression of CDX2, a transcription factor that regulates intestinal epithelial differentiation, and aimed to further characterize this phenomenon.

Methods/Results: Expression of CDX2 was assessed by immunohistochemistry in a total of 32 high-grade MPNSTs lacking morphological evidence of epithelial differentiation, including twelve tumors (38%) that developed in the setting of neurofibromatosis and four (13%) in the setting of prior radiation therapy. CDX2 was expressed by 14 of 32 MPNSTs (44%), wherein immunoreactivity, varying from weak to strong, was present in 2–95% of neoplastic spindle cells (median 10%, mean 23%). Notably, CDX2 expression was limited to tumors with PRC2 inactivation (22/32; 69%), as evidenced immunohistochemically by diffuse loss of trimethylated histone H3K27. Analyzing publicly available RNA-sequencing data from twelve MPNST cell lines, two of which are clonally related, we observed *CDX2* expression in all six PRC2-inactivated cell lines, while *CDX2* expression was negligible in six cell lines with intact PRC2, amounting to a 58-fold increase in *CDX2* expression on average with PRC2 inactivation.

Conclusions: CDX2 is expressed in a subset of MPNSTs, even in the absence of morphological evidence of epithelial differentiation. CDX2 expression in MPNST is strongly associated with underlying PRC2 inactivation.

Keywords

CDX2; malignant peripheral nerve sheath tumor; PRC2; sarcoma; gene expression

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AUTHOR CONTRIBUTIONS

GWC conceived the study. All authors contributed to study design, performed components of the research, and analyzed data. OOO and GWC wrote the paper. All authors edited the manuscript.

Declaration of conflicting interests: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics declarations: The manuscript is an original work of all authors. All authors made a significant contribution to this study. All authors have read and approved the final version of the manuscript. This study was performed in accordance with research policies approved by the Stanford University Institutional Review Board.

INTRODUCTION

Malignant peripheral nerve sheath tumor (MPNST) is a clinically aggressive soft tissue neoplasm characterized by features of Schwannian differentiation.[1] Histologically, conventional MPNST exhibits fascicles of spindle cells that express S100 protein in 30–64% of cases and SOX10 in 22–49% of cases, often in a patchy or focal distribution.[2–5] In about 15% of tumors, there is morphological evidence of heterologous differentiation in the form of bone, cartilage, blood vessels, or skeletal muscle (malignant triton tumor).[1] Less frequently, there can be heterologous glandular differentiation with expression of keratin and, in rare cases, neuroendocrine markers.[6–10]

The relatively high frequency of heterologous differentiation observed in MPNST may be related, at least in part, to recurrent pathogenic loss of function mutations affecting core components of the polycomb repressor complex 2 (PRC2), namely EED or SUZ12.[11,12] PRC2 is a chromatin-modifying enzymatic complex responsible for trimethylation of lysine 27 of histone H3 (H3K27me3) with resulting widespread effects on gene expression and, in turn, cytodifferentiation.[13] As many as 80% of high-grade MPNSTs show complete loss of H3K27me3 by immunohistochemistry (IHC) as a surrogate marker of EED or SUZ12 alterations leading to PRC2 dysfunction.[14–19]

Despite the insight that PRC2 inactivation may influence cellular plasticity in MPNST,[20] the effect of PRC2 inactivation on histopathologic variability in MPNST remains incompletely characterized. Having observed prominent CDX2 expression that caused diagnostic confusion in cases of MPNST referred to us for evaluation, we aimed to further explore this phenomenon and its relationship to PRC2 inactivation.

MATERIALS AND METHODS

Cases were retrieved from the surgical pathology archives of Stanford Health Care under an Institutional Review Board-approved protocol (#42887, most recent review 4 January 2022). Representative hematoxylin and eosin-stained slides were reviewed. In total, whole-tissue sections of 33 consecutive MPNSTs were evaluated for expression of CDX2 and H3K27me3.

Immunohistochemical detection of CDX2 was performed with a monoclonal antibody raised in mouse (Clone CDX2-88) purchased from Biocare Medical (Pacheco, CA, USA). Anti-CDX2 antibody was used at a dilution of 1:40 (by volume) following heat-induced antigen retrieval using Leica Epitope Retrieval Solution 2 on the Leica BOND-III platform. Anti-CDX2 immunoreactivity was assessed as a percentage of neoplastic cells exhibiting nuclear chromogen using a single, whole representative histologic section of tumor. Cases were considered positive for CDX2 expression if more than 1% of neoplastic cells exhibited nuclear anti-CDX2 immunoreactivity; the intensity of staining was graded as weak (1+), moderate (2+), or strong (3+).

The presence of PRC2 inactivation was assessed by immunohistochemistry for trimethylated histone H3K27. Immunohistochemical detection of H3K27me3 was performed with a monoclonal antibody raised in rabbit (Clone C36B11) purchased from Cell Signaling

Technology (Danvers, MA, USA). Anti-H3K27me3 antibody was used at a dilution of 1:50 (by volume) following heat-induced antigen retrieval using Leica Epitope Retrieval Solution 2 on the Leica BOND-III platform. Complete absence of anti-H3K27me3 immunoreactivity in all tumor nuclei was interpreted as H3K27me3 loss, a surrogate marker of PRC2 inactivation; any amount of nuclear anti-H3K27me3 staining was interpreted as evidence of intact/wild-type PRC2.

Immunohistochemical detection of cytokeratin 7 was performed with a monoclonal antibody raised in mouse (Clone OV-TL 12/30) purchased from Agilent Dako (Santa Clara, CA, USA). Anti-cytokeratin 7 antibody was used at a dilution of 1:800 (by volume) following enzymatic antigen retrieval on the Leica BOND-III platform. Immunohistochemical detection of cytokeratin 20 was performed with a monoclonal antibody raised in mouse (Clone Ks20.8) purchased from Cell Marque (Rocklin, CA, USA). Anti-cytokeratin 20 antibody was used at a dilution of 1:100 (by volume) following heat-induced antigen retrieval using Leica Epitope Retrieval Solution 2 on the Leica BOND-III platform.

Previously published RNA-sequencing data (GSE141439) from 12 cultured MPNST cell lines (6 PRC2-mutant, 6 PRC2-wild-type) were accessed between 1 October 2021 and 27 December 2021.[20] Ten cell lines were derived from NF1-associated MPNST (MPNST007, MPNST181, SNF02.2, MPNST4970, ST88, S462, MPNST642, MPNST3813E, T265, SNF96.2); two cell lines were derived from sporadic MPNST (STS26T, MPNST724). It has been reported that T265 was contaminated by ST88 at very early passage, so these cell lines may be clonally related, although they appear to exhibit non-identical genomic and transcriptomic features at current passage.[20,21] Fisher's exact test was used to assess the association between CDX2 expression and PRC2 inactivation among cases analyzed by immunohistochemistry. Welch's t-test was used to compare average Log2-transformed normalized RNA-sequencing read counts between PRC2-mutant and PRC2-intact MPNST cell lines.

RESULTS

CDX2 expression was analyzed by immunohistochemistry in 32 MPNSTs from patients ranging in age from 6 to 82 years (median 39 years). Twelve patients (12/32; 38%) were female. Twelve tumors (12/32; 38%) developed in the setting of neurofibromatosis and four (4/32; 13%) in the setting of prior radiation therapy. Tumors arose in the head/neck (n=7), extremities (n=6), brachial plexus (n=5), spine or paraspinal soft tissue (n=5), chest or abdominal wall (n=4), retroperitoneum (n=3), and mediastinum (n=2). Tissue was sampled from resection specimens in 26 cases (26/32; 81%); the remaining cases were biopsies.

CDX2 was expressed by immunohistochemistry in 14 tumors overall (14/32; 44%; Figure 1, Table 1). Among CDX2-positive cases, the frequency of CDX2-expressing tumor cells varied from 2% to 95% (median 10%, mean 23%) and the intensity of expression varied from weak (1+) to strong (3+). Six cases (6/32; 19%) showed expression in greater than 20% of tumor cells. All thirty-two tumors were morphologically high-grade when applying a two-tier grading system.[1] Eighteen cases (18/32; 56%) were characterized by at least focal expression of S100 protein; six of these (6/18; 33%) were CDX2-positive. All 32 cases were

negative for both cytokeratin 7 and cytokeratin 20. There was CDX2 expression in three of twelve (3/12; 25%) neurofibromatosis-associated tumors and in three of four (3/4; 75%) radiation-associated tumors. Two malignant triton tumors both showed CDX2 expression, in addition to expression of desmin, myogenin, and MyoD1. These two malignant triton tumors were the only cases in this cohort exhibiting heterologous differentiation.

Twenty-two tumors (22/32; 69%) demonstrated PRC2 complex inactivation, as evidenced immunohistochemically by complete loss of H3K27me3 in tumor cells (Figure 1). Targeted next generation sequencing was performed in three cases with loss of H3K27me3; pathogenic PRC2 subunit alterations were detected in all three tumors (*EED* nonsense mutation, p.E90*; *SUZ12* splice site mutation, c.917+2T>G; *SUZ12* deletion, involving exons 1–3). Both malignant triton tumors were characterized by PRC2 inactivation. Strikingly, CDX2 expression was seen in the majority of tumors with PRC2 inactivation (14/22, 64%), while no tumors with intact PRC2 were CDX2-positive (0/10, 0%; $p < 0.001$).

To confirm that the anti-CDX2 immunoreactivity observed in MPNSTs with PRC2 inactivation reflects expression of the *CDX2* locus itself, we analyzed the association between *CDX2* expression and PRC2 inactivation in an independent RNA-sequencing dataset. *CDX2* expression was analyzed in publicly available RNA-sequencing data (GSE141439) from 6 PRC2-mutant MPNST cell lines and 6 PRC2-intact MPNST cell lines.[20] In all cell lines, the status of PRC2 function was previously confirmed by next-generation sequencing: *SUZ12* mutation was present in 4 cell lines, *EED* mutation in 1 cell line, and combined *SUZ12/EED* mutation in 1 cell line. Consistent with our immunohistochemical findings, *CDX2* expression was observed by RNA-sequencing in all PRC2-inactivated MPNST cell lines, whereas all cell lines with wild-type PRC2 exhibited negligible *CDX2* expression (Figure 1). The Log2-transformed normalized RNA-sequencing read counts for *CDX2* were 58-fold greater on average in PRC2-mutant MPNST cell lines relative to PRC2-intact MPNST cell lines ($p < 0.001$), underscoring the strong association between *CDX2* expression and PRC2 inactivation in MPNST.

DISCUSSION

The finding of CDX2 expression in MPNST may prompt consideration of sarcomatoid gastrointestinal carcinoma in exceptional cases. Indeed, we initially observed this phenomenon in cases referred to us with a differential diagnosis of sarcomatoid carcinoma owing in part to CDX2 expression. Despite these overlapping features between MPNST and sarcomatoid carcinoma, careful histological examination remains essential to avoiding the diagnostic pitfall posed by CDX2 expression in MPNST. In particular, the spindle cells of sarcomatoid carcinoma are ordinarily more cytologically atypical than those of conventional MPNST. Additionally, keratin expression in MPNST is usually focal and weak, whereas keratin expression in sarcomatoid carcinoma is often stronger and more diffuse.[22] On the other hand, the finding of S100 protein or SOX10 expression, even if focal, would suggest Schwann cell-type differentiation, favoring MPNST over sarcomatoid gastrointestinal carcinoma. Our findings indicate that H3K27me3 would be a particularly useful immunohistochemical marker in addressing a CDX2-expressing spindle cell neoplasm, as all CDX2-positive MPNSTs in our cohort were H3K27me3-negative.

In cases of a CDX2-expressing spindle cell tumor where there is a previous history of gastrointestinal carcinoma, genomic comparisons using next generation sequencing could be employed to assess clonality. Overall, it must be emphasized that staining solely for transcription factors is not advisable if tumors with extensive epigenetic dysregulation, such as MPNST, are among the differential diagnosis.

Conversely, whether CDX2 immunohistochemistry could be used to support the diagnosis of MPNST when faced with a spindle cell sarcoma remains an open question. Previous studies indicate that CDX2 expression outside of the gastrointestinal tract and its associated epithelial neoplasms is very rare; to our knowledge, anti-CDX2 immunoreactivity has not be seen in previously studied soft issue tumors.[23,24] While these data would suggest that CDX2 expression may be relatively specific for MPNST in the differential diagnosis of a spindle cell tumor, systematic studies of additional cases are needed. Moreover, our data estimate that the sensitivity of CDX2 immunohistochemistry in MPNST is only about 42% overall and about 64% in PRC2-mutant tumors. The relatively limited extent and weak intensity of CDX2 expression typically observed in MPNST, as compared with colorectal adenocarcinoma, magnifies the risk of “false-negative” staining in MPNST, particularly if assessed on a single histological section. We can only speculate that the relatively limited nature of the CDX2 expression in MPNST reflects limited gastrointestinal-type differentiation from a developmentally primitive epigenetic state in PRC2-deficient tumor cells. Given that all CDX2-expressing MPNSTs in our cohort exhibit PRC2 inactivation as assessed by H3K27me3 immunohistochemistry, H3K27me3 loss itself appears to be a more sensitive marker of MPNST relative to CDX2.

ACKNOWLEDGEMENTS

GWC acknowledges the support of the Stanford University School of Medicine Clinical and Translational Science Award Program (National Center for Advancing Translational Sciences, KL2TR003143).

Funding:

GWC is supported in part by the Stanford University School of Medicine Clinical and Translational Science Award Program (National Center for Advancing Translational Sciences, KL2TR003143).

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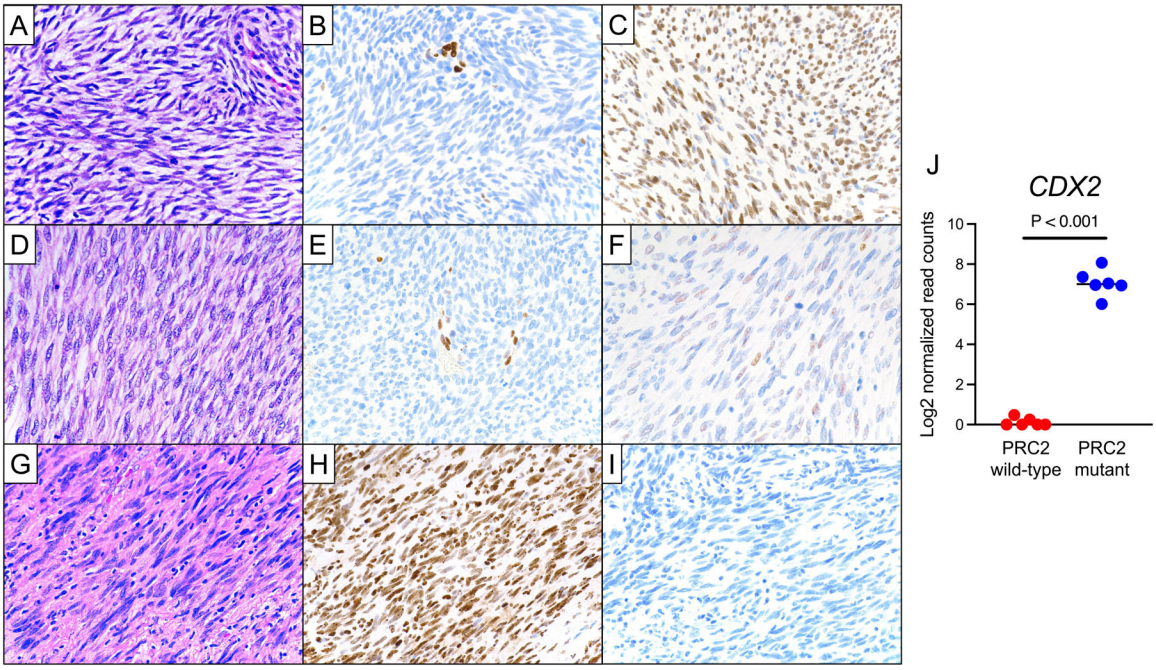

Figure 1.

Representative photomicrographs of H&E stain (A, D, G) and immunohistochemical stains for trimethylated histone H3K27 (B, E, H) and CDX2 (C, F, I) in MPNST with diffuse CDX2 expression (A-C), heterogeneous/weak CDX2 expression (D-F), and no CDX2 expression (G-I). (J) Average Log2-transformed normalized RNA-sequencing read counts (GSE141439) for *CDX2* from six MPNST cell lines with wild-type PRC2 (Red; MPNST007, MPNST181, SNF02.2, MPNST4970, STS26T, MPNST724) and six MPNST cell lines with mutant PRC2 (Blue; ST88, S462, MPNST642, MPNST3813E, T265, SNF96.2). Each dot represents expression in a distinct cell line. It has been reported that ST88 and T265 may be clonally related owing to contamination of T265 by ST88 at early passage.

Table 1.

Clinical and immunohistochemical features of the study cohort.

Case ID	Age (y)	Sex	Radiation	NF1	Triton	S100 protein	H3K27me3	CDX2 (extent)	CDX2 (intensity)
1	61	M	N	N	Y	−	Lost	95%	2–3+
2	30	M	N	N	N	+	Lost	50%	2–3+
3	41	F	Y	N	N	−	Lost	2%	1–2+
4	55	M	Y	N	N	−	Lost	5%	1+
5	48	F	Y	N	N	+	Lost	5%	1+
6	36	M	N	Y	N	+	Lost	5%	1+
7	36	M	N	N	Y	−	Lost	20%	1–2+
8	63	M	N	N	N	+	Lost	20%	1–2+
9	30	F	N	N	N	−	Lost	10%	1–2+
10	46	M	N	Y	N	−	Lost	3%	1–2+
11	65	F	N	N	N	+	Lost	10%	1–2+
12	40	M	N	N	N	−	Lost	5%	1–2+
13	27	F	N	Y	N	+	Lost	25%	1–2+
14	66	M	N	N	N	−	Lost	70%	1–2+
15	46	F	N	Y	N	+	Retained	-	-
16	27	M	N	Y	N	+	Lost	-	-
17	79	M	N	N	N	+	Retained	-	-
18	23	M	N	N	N	+	Lost	-	-
19	27	M	N	Y	N	+	Lost	-	-
20	26	M	Y	N	N	−	Lost	-	-
21	38	F	N	Y	N	−	Retained	-	-
22	49	F	N	Y	N	−	Lost	-	-
23	7	M	N	N	N	+	Retained	-	-
24	53	F	N	N	N	+	Retained	-	-
25	29	M	N	Y	N	−	Lost	-	-
26	56	M	N	N	N	+	Retained	-	-
27	6	F	N	Y	N	−	Lost	-	-
28	18	F	N	Y	N	+	Retained	-	-
29	53	F	N	Y	N	+	Retained	-	-
30	18	M	N	N	N	+	Retained	-	-
31	82	M	N	N	N	+	Retained	-	-
32	25	M	N	N	N	−	Lost	-	-

NF1, neurofibromatosis type 1; M, male; F, female; Y, yes; N, no