**"Revealing *RB1* loss in an emerging entity: report of two cases of PRRX1-rearranged mesenchymal tumors"**

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Abstract:

**Aims:**
*PRRX1*-rearranged mesenchymal tumors are a recently identified and rare subgroup of soft tissue neoplasms with distinct morphological features and genetic alterations. This study aims to further investigate the immunohistochemical profile and underlying genetic alterations in these tumors in order to get more insight on their underlying biology and the unique profile of these tumors.

**Methods:**
Two new molecular confirmed cases of *PRRX1*-rearranged mesenchymal tumors were thoroughly studied with immunohistochemical stainings (RB1, CD34, ALK, and pan-TRK), fluorescence in situ hybridization (FISH) *RB1/13q12* and RNA-based next-generation sequencing (NGS).

**Results:**
Both cases exhibited typical morphological and molecular features, confirming the diagnosis of *PRRX1*-rearranged mesenchymal tumors. Immunohistochemistry revealed RB1 loss in both cases, which was subsequently confirmed through FISH analysis. Additionally, one case showed focal positivity for CD34, ALK, and pan-TRK on immunohistochemistry.

**Conclusions:**
We identified loss of *RB1* in two cases of PRRX1-rearranged mesenchymal tumors. This could suggest a potential association with *RB1*-deficient soft tissue tumors, although further research is necessary. Furthermore, the finding of focal positivity for CD34, ALK, and pan-TRK on immunohistochemistry, enriches the immunohistochemical profile of these tumors.

**What is already known?**
*PRRX1*-rearranged mesenchymal tumors are newly identified soft tissue neoplasms with distinct morphological characteristics and genetic alterations. Only, limited case reports and case series have been published.

**What this study adds?**
Previous research on *PRRX1*-rearranged mesenchymal tumors lacked clarity regarding *RB1* loss and specific immunohistochemical markers such as ALK, CD34, and pan-TRK. Concurrent *RB1* loss has not been described in *PRRX1*-rearranged mesenchymal tumors before.

**How this study might affect research, practice or policy?**
The identification of concurrent *RB1* loss and immunohistochemical positivity for ALK, CD34, and pan-TRK provides new insights into *PRRX1*-rearranged mesenchymal tumors. This new knowledge could significantly influence future research directions. In clinical practice, our findings can improve accurate diagnoses and guide treatment decisions.

**Introduction:**

*PRRX1*-rearranged mesenchymal tumorsrepresent a rare subset of soft tissue neoplasms, initially described by Lacambra et al. in 2019.(1) To date, only 20 cases of these fibroblastic tumors harboring *PRRX1* gene translocations have been documented in literature. While these cases can occur in diverse anatomical regions, they predominantly manifest in the subcutis. We hereby describe two additional cases of *PRRX1*-rearranged mesenchymal tumors, both occurring in the subcutaneous tissue of the shoulder, bringing the overall total of cases to twenty-two documented cases. Notably, we detected loss of *Retinoblastoma 1 (RB1)* in both cases, a finding not documented in literature so far. In addition, focal expression for CD34, ALK and pan-TRK was observed in one case. Using this case study, we try to shed more light on the emerging entity of *PRRX1*-rearranged mesenchymal tumors, contributing to our understanding of their morphological, immunohistochemical and molecular aspects within the broader landscape of fibroblastic soft tissue tumors.

**Materials and methods**

***Immunohistochemistry***Immunohistochemistry was performed on 4 μm-thick sections of formalin-fixed, paraffin-embedded (FFPE) material with a Benchmark XT immunostainer (Ventana Medical Systems, Tucson, AZ, USA). Primary monoclonal antibodies were CD34 (1:100; QBEnd10; Dako, Glostrup, Denmark), Pankeratin (ready-to-use; AE1/AE3/PCK26; Roche, Tucson, AZ, USA), EMA (1:50; E29; Agilent, Santa Clara, CA, USA), ERG (ready-to-use; EPR3864; Roche, Tucson, AZ, USA), SOX10 (ready-to-use; SP3864; Roche), SMA (1:200; EP188; Cell Marque, Rocklin, CA, USA); desmin (1:200; D33; Agilent, Santa Clara, CA, USA), STAT6 (1:50; EP325; Cell Marque, Rocklin, CA, USA), S100 (ready-to-use; 4C4.9; Roche, Tucson, AZ, USA), MUC4 (1:50; 8G7; Santa Cruz); p63 (ready-to-use; 4A4; Roche, Tucson, AZ, USA), p40 (ready-to-use; BC28; Roche, Tucson, AZ, USA), SMARCB1 (INI1) (ready-to-use; MRQ-27; Roche, Tucson, AZ, USA), SMARCA4 (BRG1) (1:50; ZR390; Zeta Corporation, Arcadia, CA, USA), RB1 (1:50; G3-245; BD Pharmingen, San Diego, CA, USA), p16 (ready-to-use; E6H4; CINtec MTM laboratories-Roche, Tucson, AZ, USA), and MDM2 (1:10; IF2; Invitrogen, Carlsbad, CA, USA). Heat-induced epitope retrieval was performed for CD34, RB1, p16 and MDM2 using Cell Conditioning 1. Visualization was achieved with the Ultraview Universal DAB Detection kit (Ventana Medical Systems, Tucson, AZ, USA). Throughout the process, appropriate positive and negative controls were employed. RB1 nuclear immunoreactivity was assessed by an experienced soft tissue pathologist (DC) and classified as deficient (<10% of tumor cells with nuclear staining), heterogeneous (10–80% of tumor cells with nuclear staining), or intact (>80% of tumor cells with nuclear staining).(2)

***Molecular testing***

FISH was performed on FFPE tissue using the FISH *RB1*/13q12 Zytovision detection kit, containing two labeled DNA probes. The RB1 probe spans the entire *RB1* gene (13q) and is labeled in spectrum orange. The 13q12 probe is labeled in spectrum green and serves as a control probe. Possible deletion patterns are mono-allelic deletion (1 red/2 green signals), bi-allelic deletion (2 green signals) or monosomy of chromosome 13q (1 red/1 green signal). The patterns are interpreted according to the criteria of Agaimy et al.(3)

Furthermore, RNA extraction from FFPE was performed using the Maxwell(R) RSC RNA FFPE kit. RNA-based next-generation sequencing (NGS) was performed with the Archer FusionPlex Expanded Sarcoma panel (55 genes).

**Results
*Case histories***

***CASE 1***

A man in his 50s, with an unremarkable medical, history noticed a lesion (2.7 cm) on his left shoulder, clinically suggestive of a sebaceous cyst. Macroscopic examination showed a well-defined, regular, firm white-tan lesion with an almost sharp boundary. Following the initial surgery a wide resection was performed.

***CASE 2***

A woman in her late 40s presented with a clinically cystic lesion on the right shoulder. After the initial surgery a wider resection was performed.

***Pathological findings***

***CASE 1***

Microscopic evaluation revealed a non-encapsulated lesion predominantly located in the subcutaneous tissue. The lesion showed a variable cellular proliferation composed of spindle- to star-shaped cells arranged individually or in ill-defined fascicular bundles or forming solid nests (Figure 1A). The cells had bland, vesicular nuclei, which demonstrated only slight polymorphism. There was no high-grade cytonuclear atypia or pleomorphism. Mitotic activity or necrosis were not observed. The underlying stroma was predominantly collagenous, showing ropey-type or sclerotic collagen with hyalinization and presence of hyalinized giant rosette-like structures. Focally a more myxoid background was seen with scattered mast cells, ropey-type collagen and adipocytes (Figure 1B). At the periphery of the lesion, there was a notable presence of hemangiopericytoma-like vessels (Figure 1C). Pre-existing adnexal structures were entrapped by the lesion (Figure 1D).

The spindle cells displayed strong positivity for S100 (Figure 2) and p16, while being negative for pancytokeratin AE1/AE3, EMA, p63, p40, CD34, ERG, SOX10, SMA, desmin, STAT6, MUC4 and MDM2. There was a preserved nuclear expression of INI1 (SMARCB1) and BRG1 (SMARCA4). RB1 staining exhibited a heterogenous pattern, with areas showing loss of nuclear expression (Figure 3A). This loss of RB1 expression was subsequently verified through fluorescence in situ hybridization (FISH), demonstrating a loss of *RB1* in 30% of the tumor cells, primarily characterized by a monosomy 13 pattern (Figure 4). Particularly striking in the nested areas was, in addition to expression of CD34 (Figure 3B), a remarkably strong diffuse cytoplasmatic positivity for pan-TRK (Figure 3C) and ALK (Figure 3D). Notably, no *ALK* and *NTRK* rearrangements were detected by FISH analysis and/or targeted RNA NGS.

***CASE 2***

Histological examination revealed a relatively well-circumscribed mesenchymal lesion (Figure 5A) that was not fully encapsulated. The lesion was composed of spindle- to star-shaped cells arranged in a vaguely bundled or fascicular growth pattern (Figure 5B). These spindle cells had oval to elongated nuclei with open chromatin and a small rim of eosinophilic cytoplasm. The nuclei showed moderate atypia without prominent high-grade cytonuclear atypia or pleomorphism. There was no brisk mitotic activity or necrosis. The background of the lesion exhibited prominent collagenous stroma with ropey-type collagen and hemangiopericytoma-like vessels (Figure 5C). Towards the periphery of the lesion, there was a decrease in cellularity and a more myxoid background, accompanied by the presence of mast cells and ropey-type collagen (Figure 5D).

On immunohistochemistry, the spindle cells were negative for pancytokeratin AE1/AE3, EMA, p63, p40 CD34 (Figure 6A), ERG, SOX10, SMA, desmin, STAT6, S100 (Figure 6B), MUC4 and MDM2. Nuclear expression of INI1 (SMARCB1) and BRG1 (SMARCA4) was preserved in the tumor cells. RB1 staining revealed a heterogenous staining showing areas with loss of nuclear expression (Figure 6C). There was diffuse positivity for p16 (Figure 6C). Additionally, FISH showed a loss of *RB1* in 23% of the tumor cells (also with predominantly monosomy 13 pattern). No ALK or pan-TRK expression was observed with immunohistochemistry.

***Molecular findings***

In both cases, targeted RNA NGS revealed the presence of a gene rearrangement between exon 1 of the *paired related homeobox 1 (PRRX1)* gene and exon 13 of the *nuclear receptor coactivator 1 (NCOA1)* gene (NM\_006902.4/NM\_003743.4)(Figure 7). No *ALK* or *NTRK* gene rearrangements were identified.

**Discussion**

Fibroblastic soft tissue tumors constitute a heterogeneous group of tumors that arise from (myo)fibroblasts. The identification of molecular alterations has enabled further subclassification of this tumor group. Recently, Lacambra et al. have introduced "*PRRX1-NCOAx*-rearranged fibroblastic tumor," expanding this subclassification.(1) In 2023, Warmke et al. described additional cases of this new discovered subtype, one of which harbored a *PRRX1::KMT2D* gene rearrangement. Furthermore, they observed a variable degree of S100 and SOX10 expression in a subset of these tumors and therefore proposed the term “*PRRX1*-rearranged mesenchymal tumor” to encompass fusion partners beyond *NCOA1/2* and to acknowledge the potential for partial neural or neuroectodermal differentiation within these tumors.(4)

The two presented cases share both clinical and morphological characteristics consistent with previous literature descriptions. Specifically, our cases are mainly subcutaneous lesions located on the shoulder of middle-aged individuals. The literature records a range of subcutaneous locations, including the neck (n=4), abdominal wall (n=3), groin (n=2), shoulder (n=2), axilla (n=1), scalp (n=1), back (n=1), flank (n=1), knee (n=1), thigh (n=1) and hip (n=1). (1, 4-7) Our additions contribute to a total of four cases specifically situated in the shoulder region, underscoring a slight preference for the occurrence of these tumors in the head and neck region. Only two cases were observed intramuscular: one in the chest wall and another on the forehead.

Microscopically, they are composed of bland spindle to stellate cells within a myxo-collagenous stroma. Minimal to mild cytologic atypia is observed. Notably, hemangiopericytoma-like vessels are observed at the lesion periphery. Furthermore, certain areas within the lesion show the presence of ropey-type collagen and mast cells, resembling spindle cell lipoma. Cloutier et al. reported a solitary case of *PRRX1*-rearranged mesenchymal tumor accompanied by the presence of pigment-laden epithelioid and dendritic cells.(7) This unique feature has not been noted in any other published case, including our own case. While the entrapment of mature adipocytes, as observed in one of our cases, has been previously documented by Warmke et al. in one case (4), the entrapment of pre-existing adnexal structures has not been reported in the literature.(4)

On immunohistochemistry, one of our cases showed strong S100 positivity, while both cases lacked SOX10 expression. These findings align with the observations of Warmke et al., who showed co-expression of S100 and SOX10 in a subset of *PRRX1*-rearranged mesenchymal tumors.(4) Furthermore, expression of desmin, SMA, cytokeratin, STAT6 and MUC4 has not been observed. This is helpful in the differential diagnosis since the presence of staghorn-like vessels or hyaline rosettes requires the exclusion of solitary fibrous tumor and low-grade fibromyxoid sarcoma, respectively.

The identification of a *PRRX1::NCOA1* gene fusion, through RNA NGS in both our cases, led to the diagnosis of *PRRX1*-rearranged mesenchymal tumor. This gene fusion is identical to others documented in the literature, characterized by the fusion of exon 1 of *PRRX1* with exon 13 of *NCOA1*.

Interestingly, both of the presented cases exhibited loss of *RB1* expression, a finding that has not been documented in existing literature. However, it’s worth nothing that Dermawan et al. reported retained immunohistochemical RB1 expression in two cases, while Chen et al. reported similar findings in one case.(5, 6) Importantly, these cases did not undergo further molecular investigation using FISH analysis. It's noteworthy that none of the other cases mentioned in the literature were subjected to these investigations. The identification of *RB1* loss in both our cases is particularly intriguing considering the morphological similarities they share with spindle cell/pleomorphic lipoma, cellular angiofibroma and mammary-type myofibroblastoma, which all belong to the *RB1*-deficient family of tumors. These tumors are characterized by bland spindled cells embedded in a myxoid stroma with the presence of ropey collagen.(8-13) However, unlike the aforementioned PRRX1-rearranged mesenchymal tumors in the literature, these entities exhibit positive staining for CD34. Interestingly, one of our cases did show focal CD34 positivity, further indicating a potential overlap with *RB1*-deficient tumors. Further investigation is required to elucidate the exact role of *RB1* loss in *PRRX1*-rearranged mesenchymal tumors and its potential relationship with *RB1*-deficient tumors.

Furthermore, in one of our cases positive staining for pan-TRK was observed, a finding that was also reported in a case by Dermawan et al.(4) However, it's important to emphasize that no NTRK gene rearrangements were detected in any of the documented *PRRX1*-rearranged mesenchymal tumors, including the ones presented here. Additionally, one of our cases also displayed strong ALK immunoreactivity. This finding was also observed in three cases of Dermawan et al. (with one case stained using ALK D5F3 and two using ALK1 clones).(5) Notably, no *ALK* gene rearrangements were detected in any of the *PRRX1*-rearranged mesenchymal tumors. The identification of pan-TRK and ALK immunopositivity in mesenchymal tumors can raise diagnostic questions and should prompt additional molecular investigations. Preferably, RNA NGS should be performed to identify potential gene rearrangements. The proportion of *PRRX1*-rearranged mesenchymal tumors exhibiting immunohistochemical positivity for pan-TRK and ALK needs further investigation.

**Conclusion**

Based on this case series, we tried to further elucidate the immunohistochemical as well as the molecular profile of the emerging entity of ‘*PRRX1*-rearranged mesenchymal tumors’. Of particular significance is the loss of *RB1* seenin both cases and the focal CD34 expression in one case, which could suggest the diagnosis of an *RB1-*deficient tumor. Furthermore, the presence of ALK and pan-TRK positivity in one case should prompt further molecular investigations, in order to find underlying gene alterations aiding in the differential diagnosis. The integration of molecular assays into the diagnostic evaluation of fibroblastic neoplasms can enhance our comprehension of their molecular mechanisms, refine their (sub)classification, and provide valuable guidance for more precise therapeutic decisions.

**Figures**

**Figure 1: Case 1**

The tumor is composed of bland spindle to stellate cells, with minimal to moderate atypia. The cells are arranged as individual cells, in ill-defined fascicular bundles or solid nests (A, HE original magnification 200x) The cells are embedded in a myxo-collagenous background with presence of ropey collagen (B, HE original magnification 200x). The lesion is well-circumscribed with staghorn-like vessels at the periphery (C, HE original magnification 40x). Adnexal structures are entrapped in the lesion, as well as adipocytes. (D, HE original magnification 200x)

**Figure 2: Case 1 IHC**

The tumor cells show strong diffuse expression of S100 (A and B, original magnification 100x and 400X) and p16 (B, original magnification 100x).

**Figure 3: Case 1 IHC**

Tumoral areas exhibit loss of expression of RB1, with retained expression in endothelial cells (A, original magnification 200x). Positivity for CD34 (B, original magnification 100x), pan-TRK (C, original magnification 100x) and ALK (D, original magnification 100x) was observed in the more nested areas.

**Figure 4: Case 1 FISH RB1**

FISH RB1/13q12 revealed monosomy of chromosome 13q (1 red/1 green signal) in the tumor cells.

**Figure 5: Case 2**

Well-circumscribed mesenchymal tumor (A, HE original magnification 12x) composed of bland spindle to stellate cells embedded in a collagen-rich stroma (B, HE original magnification 100x). Large, slit-like and branching vessels are present (C, HE original magnification 40x). At the periphery a more myxoid stroma with presence of ropey collagen and mast cells was observed (D, HE original magnification 200x).

**Figure 6: Case 2 IHC**

The tumor cells were negative for CD34 (A, original magnification 50x) and S100 (B, original magnification 200x). Tumoral areas showed loss of RB1 expression (C, original magnification 400x). There was diffuse positivity of p16 in the tumor cells (D, original magnification 200x).

**Figure 7**

Both cases revealed a *PRRX1::NCOA1* gene rearrangement, characterized by the fusion of exon 1 of *PRRX1* with exon 13 of *NCOA1*. Notably, this fusion preserves the transactivator domains of NCOA1 while losing the basic helix-loop-helix-Per-ARNT-Sim (bHLH-PAS) domain, which is integral to the DNA-binding function of *NCOA1*. This alteration may result in subsequent transcriptional dysregulation.

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