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BRIEF REPORT

A case of nasal low-grade non-intestinal-type adenocarcinoma with aberrant CDX2 expression and a novel *SYN2-PPARG* gene fusion in a 13-year-old girl

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Abstract
We report the first patient (a 13-year-old girl) with a sinonasal low-grade non-intestinal-type adenocarcinoma showing aberrant CDX2 expression both within morular areas and in the tubular component and demonstrate for the first time a *SYN2-PPARG* gene fusion in this tumor type. The tumor arose from the nasal septum and had not spread beyond the nasal cavity.

Keywords Sinonasal · Adenocarcinoma · Intestinal · Non-intestinal · CDX2 · SYN2-PPARG gene fusion

Introduction

Non-intestinal-type adenocarcinomas (non-ITAC) are uncommon tumors of the sinonasal tract that neither display an intestinal phenotype, nor show the features of a salivary gland neoplasm [1]. They are morphologically heterogeneous mainly with regard to grade and typically regarded as a diagnosis of exclusion, although the category also includes some specific entities that are morphologically unique, e.g., renal cell-like carcinoma.

It is of utmost clinical importance to distinguish low-grade non-ITACs from low-grade intestinal-type adenocarcinoma (ITAC). To date, low-grade non-ITAC has displayed minimal, if any, metastatic clinical behavior (mainly local recurrence) whereas low-grade ITACs (and low-grade salivary gland carcinomas) are associated with a limited, but still definitive risk of metastatic behavior. In this regard, CDX2, a marker of intestinal differentiation, is frequently being used in clinical practice as a discriminatory marker to aid in the distinction between low-grade ITACs and non-ITACs, although some investigators have recently documented CDX2 expression in morular areas in low-grade non-ITAC [2–4].

ETV6-gene rearrangements with *NTRK3* or *RET* fusion partners have recently been reported in non-ITAC, with potential diagnostic and therapeutic implications [5, 6]. We herein report the first case of low-grade non-intestinal-type adenocarcinoma with aberrant CDX2 expression both within the morular areas and in the tubular component. In addition, we demonstrate for the first time a *SYN2-PPARG* gene fusion in this tumor type.

Case report

The patient is a previously healthy 13-year-old girl who presented with a left nasal cavity tumor. Intraoperatively, the tumor arose from the nasal septum, extending to the superior region of the left nasal cavity. No extension of the tumor beyond the confines of the nasal cavity was documented. The tumor was removed completely in a piecemeal fashion by the surgeon. The case is recently diagnosed. Hence, we have no meaningful follow-up data.

Histopathologic examination revealed a tumor composed of closely packed uniform tubules lined by cuboidal cells harboring monomorphic round nuclei with no or inconspicuous nucleoli. The neoplastic tubules infiltrated between native glands in the submucosa (Fig. 1a, b). Several foci of morular metaplasia were identified (Fig. 1c). There was no tumor necrosis or mitotic activity discerned. The resected specimen

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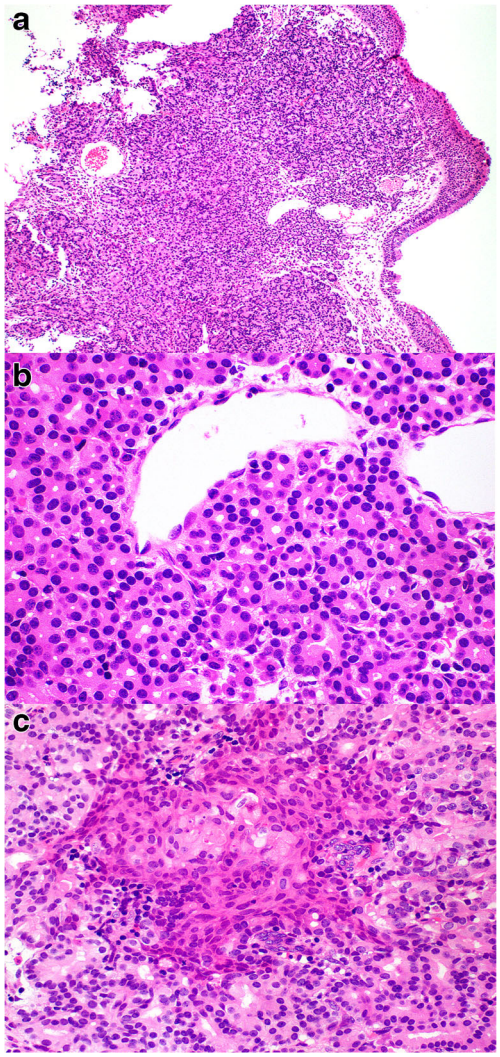

Fig. 1 Histologic findings of the sinonasal tumor showed (**a**, **b**) crowded tubules with monomorphic nuclei and (**c**) foci of morular metaplasia (hematoxylin and eosin, original magnification × 40 (**a**), × 200 (**b**, **c**))

contained osseous tissue that was not infiltrated by tumor. There was no papillary component identified.

The immunohistochemical study showed that the neoplastic cells strongly and diffusely expressed cytokeratin (CK)7 but not CK20 (Fig. 2a, b). There was variable, but significant expression of DOG1, SOX10 (Fig. 2c), and S100-protein. No p63 positive cell component in the tumor was identified. The Ki-67 proliferative index was approximately 10% (Fig. 2d). There was a patchy but significant nuclear expression of CDX2 as well as 휁-catenin, both in the tubular and in the morular areas (Fig. 2e, f).

High-throughput molecular analysis using Archer™ FusionPlex® Solid Tumor kit (AB0006, ArcherDX, Boulder, CO, USA) detected *SYN2* (*exon 10*)-*PPARG* (*intron 1-exon2*) gene fusion (Fig. 3a). A confirmatory reverse-transcriptase PCR using primers covering the breakpoint—F: CTTAAGGA TCCGGACTCAAGC; R: CTGTGTCAACCATGGTCATT TC—confirmed the breakpoint sequence (Fig. 3b).

Discussion

Most sinonasal non-ITACs are low-grade tumors occurring in patients with a wide age range from 9 to 89 years, a demographic profile that is significantly different from ITAC which are predominantly seen in older male patients and which has a well-established association with occupational wood and/or leather dust exposure. Low-grade non-ITACs are only locally destructive, but with no well-documented metastatic potential. This is in contrast to low-grade ITACs which carry some, albeit limited, metastatic potential, hence the importance of distinguishing between these two entities.

Low-grade non-ITACs display a fairly stereotypical set of histopathological features, including complex and crowded tubulopapillary architecture lined by a single layer of uniform cuboidal epithelial cells with basally located monomorphic round nuclei. In comparison, ITACs (even the well-differentiated/low-grade ones) display increased cytologic atypia compared to low-grade non-ITACs. A subset of low-grade non-ITACs (those with a tubular pattern) share similar morphology and immunohistochemical profile to sinonasal seromucinous hamartomas (SSH); these proteins associated with seromucinous differentiation (S100-protein, DOG1, and SOX10) are essentially absent in ITACs [2]. Morular metaplasia is another feature recently described in some cases of non-ITAC which appears restricted to non-ITAC and SSH and has, to the best of our knowledge, not been described in low-grade ITAC [2]. A proportion of the morular areas appear to "aberrantly" express CDX2 and also co-express nuclear 휁-catenin [2, 3]. Similar "aberrant" expression of CDX2 and 휁-catenin in morular metaplasia has also been noted in other tumors, including ovarian and endometrial endometrioid carcinoma [7]. These findings suggest that nuclear accumulation of the 휁-catenin protein in conjunction with CDX2 expression may be important for the development of the morular light microscopic phenotype [8].

The expression of CDX2 in non-ITAC (albeit within areas of morular metaplasia) may cause confusion with ITAC if not interpreted in conjunction with the tumor morphology. However, in contrast to these previously published cases, the present case exhibited CDX2 expression in the tumor cells lining the tubules, i.e., in non-morular areas. This has previously not been reported in low-grade non-ITAC and is thus a novel finding. Interestingly, as in the morular areas, there was also a similar pattern of nuclear 휁-catenin co-expression in the tubular areas with CDX2 expression. One possible explanation for this is that these areas represent early morular metaplasia before its morphologic manifestation, as suggested in endometrioid carcinoma [7]. Another possibility could be that CDX2 may not be specific for intestinal differentiation, as CDX2 expression has also been reported in other tumors without overt intestinal features,

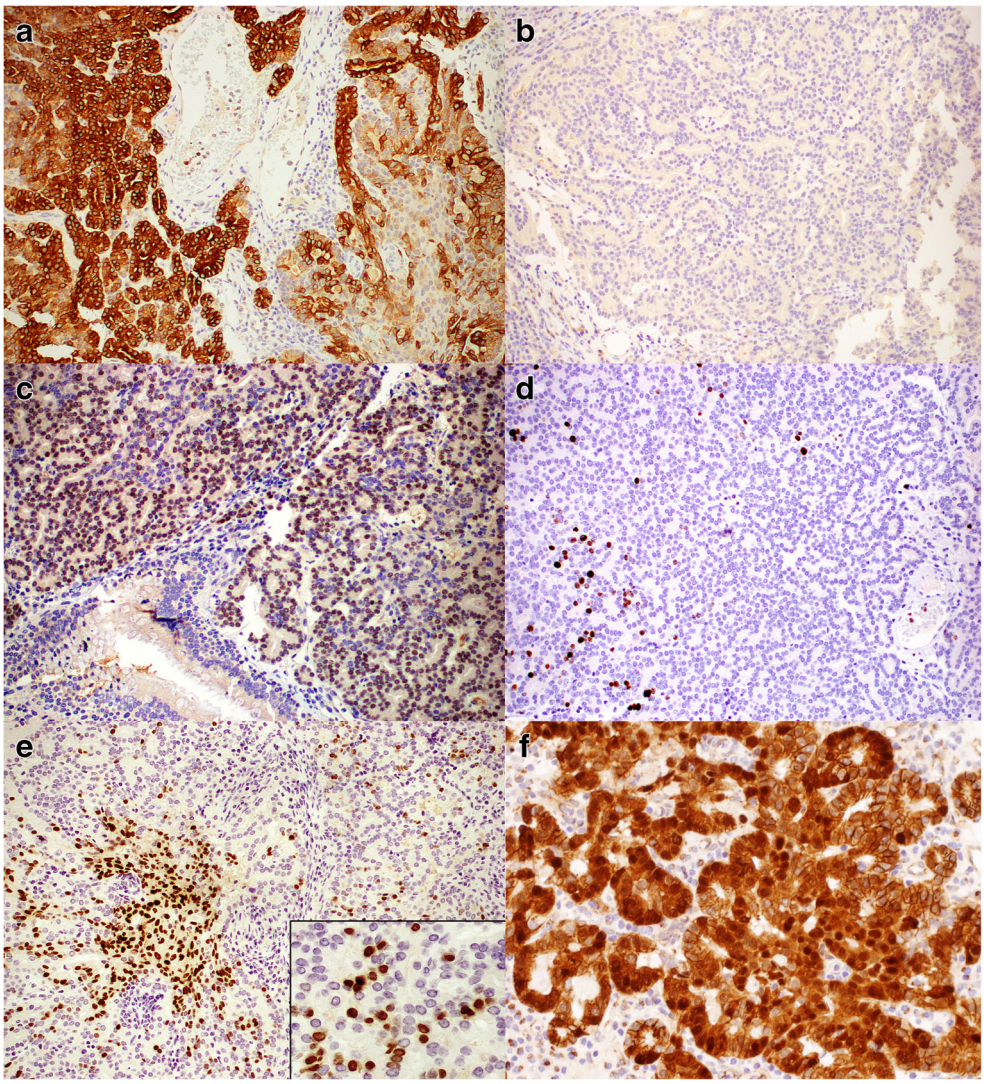

Fig. 2 Immunohistochemical findings showed strong expression of **a** CK7 but not **b** CK20. **c** There was a variable expression of SOX10. **d** Ki-67 was approximately 10%. **e** Nuclear expression of CDX2 was seen in both morular and tubular areas (inset). **f** Nuclear expression of 훽-catenin was also seen in both morular and tubular areas (original magnification × 100 (**a**–**e**), × 200 (**f**))

including high-grade sinonasal tumors, especially sinonasal undifferentiated carcinoma and non-keratinizing squamous cell carcinoma [9].

One possibility that we did not explore in our study was whether the tumor has a mutation in the Wnt/훽-catenin signaling pathway, resulting in CDX2 expression and morular metaplasia. Only rare low-grade sinonasal non-ITACs have been studied for molecular genetic abnormalities. Villatoro et al. detected *CTNNB1* mutations in 2 cases of sinonasal non-ITAC with morular metaplasia and CDX2/ -catenin nuclear expression [3]. *CTNNB1* mutations have previously also been demonstrated in other tumors with squamous differentiation/morular formation demonstrating nuclear 훽-catenin accumulation, including ovarian and endometrial endometrioid carcinoma [10]. However, Yom et al. found no genetic alterations in *KRAS*, *APC*, or *CTNNB1* in a series of 7 non-ITACs [11]. Further, Franchi et al. found two out of 12 non-ITACs with *BRAF* V600E mutation (both of which were low-grade), and absence of *EGFR* or *KRAS* mutations [12]. Recently, Andreasen et al. demonstrated *ETV6*-gene rearrangements with *NTRK3* or *RET* fusion partners in 3 cases of low-grade non-ITAC after screening 46 cases of low-grade sinonasal adenocarcinomas [5, 6]. These 3 cases had morphologic and immunophenotypic features that were distinct from *ETV6*-rearranged (mammary analogue) secretory carcinomas, which is the prototypical tumor associated with *ETV6*-rearrangement in head and neck (salivary gland) tumors, and the authors suggest that these tumors represent a discrete subset of low-grade sinonasal adenocarcinomas.

Although we did not find a *CTNNB1* mutation in our tumor, we discovered a novel *SYN2-PPARG* fusion gene which has not previously been reported in any sinonasal adenocarcinoma. The t(3;3) *SYN2-PPARG* fusion has been reported in pulmonary small cell carcinoma [13]; amplification of the region has also been well-characterized in bladder urothelial carcinoma [14]. *PPARG* (peroxisome proliferator activated receptor gamma) encodes a nuclear receptor that is implicated in adipocyte differentiation and function, while *SYN2* encodes

Fig. 3 Molecular findings. **a** A screenshot of Archer™ analysis software showing the detection of *SYN2-PPARG* fusion and the breakpoint information between the two genes. **b** RT-PCR and Sanger sequencing confirmed the fusion transcript sequence between exon 10 of *SYN2*, intron 1 of PPARG, and exon 2 of *PPARG*

a neuronal phosphoprotein. Recent research has shown that PPARgamma interacts with the canonical WNT/beta-catenin pathway in chronic inflammation and oxidative stress during carcinogenesis [15]; while it is uncertain if this has any import in our case, it would be interesting to further explore the possibility of this fusion being involved in the carcinogenesis of non-ITAC with morules. At this point, the significance of this fusion is still uncertain; future work could focus on testing for the presence of this mutation in other cases of low-grade non-ITAC or perform some functional work to determine the tumorigenic effects of this gene fusion.

In conclusion, we report the first case of sinonasal low-grade non-intestinal-type adenocarcinoma with CDX2 immunoexpression in the tubular component outside the morular areas. In addition, we document for the first time a novel *SYN2-PPARG* gene fusion in this tumor type. The implication of the novel immunohistochemical finding of CDX2 expression in the tubular component in a low-grade sinonasal adenocarcinoma is that a diagnosis of non-ITAC could still be made or suggested in the appropriate setting. Employing a panel of antibodies identifying proteins that together point towards a seromucinous phenotype, e.g., S100-protein, DOG1, and SOX10, and also, cytokeratin 7 and 20 should significantly aid the diagnostician towards establishing this diagnosis even when confronted with a limited or suboptimal biopsy or cell block specimen. Our novel molecular genetic finding of a *SYN2-PPARG* gene fusion widens the spectrum of molecular genetic alterations that low-grade non-ITAC may harbor. This molecular genetic heterogeneity may also have implications for selection of targeted therapies in patients with locally advanced low-grade non-ITACs that are deemed not amenable to surgical resection.

Authors' contributions Dr. Soon wrote the manuscript; Dr. Chang and Mr. Kuick conducted the molecular analysis; and Dr. Petersson reviewed the specimen, made the diagnosis, is responsible for the light microscopical and immunohistochemical pictures, and edited and revised the manuscript. All authors gave final their approval for publication.

Compliance with ethical standard

It is our institution's policy not to require formal ethical approval for reports on up to two patients.

Conflict of interest The authors declare that they have no conflict of interest.

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