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CASE REPORT

INI1 (SMARCB1)-Deficient Sinonasal Carcinoma: A Clinicopathologic Report of 2 Cases

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Abstract Poorly differentiated sinonasal malignancies are amongst the hardest differential diagnoses in pathology, owing to the large number of rare entities that arise there. Complicating the matter is that most pathologists, including those with experience in head and neck pathology, have little experience in any one of these rare entities. Most patients with sinonasal carcinoma present with locally advanced disease and in the past a combination of chemotherapy, radiotherapy, and surgery would usually be recommended without the specific disease subtype playing a large part of the decision making. However, in the era of "precision medicine" and targeted therapies, the specific tumour subtype and an accurate diagnosis will become increasingly important even for the so-called "undifferentiated carcinoma". Specific entities that tend to enter into the differential diagnosis include olfactory neuroblastoma, sinonasal undifferentiated carcinoma (SNUC), and non-keratinizing squamous cell carcinoma (viral and non-viral). However, recent new entities, such as NUT-midline carcinoma also have to be considered. Recently it was found that a subset of tumours originally diagnosed as one of the aforementioned entities all demonstrated loss of the ubiquitously expressed protein Integrase Interactor 1 (INI1; SMARCB1). These tumours were often basaloid with at least partial rhabdoid differentiation and most were considered a part of the SNUC spectrum. In this report, we describe two additional cases of INI1-deficient sinonasal carcinoma prospectively identified, both of which appeared to have a marked response to neo-adjuvant chemoradiation, a finding not previously described.

Keywords SNUC · INI1 · SMARCB-1 · Sinonasal · Carcinoma

Introduction

Sinonasal carcinoma is an uncommon malignancy, accounting for only 3 % of all neoplasms arising in the head and neck [1]. Although survival rates for sinonasal carcinoma have improved dramatically in recent decades, morbidity remains high, owing to the complicated anatomy of the head and neck. Moreover, as most patients with sinonasal carcinoma present with locally advanced disease, a combination of chemotherapy, radiotherapy, and surgery is usually recommended. The choice of therapy, however, depends on the histological subtype and the sinonasal tract is unique in that it is home to a wide variety of tumours that present similarly as poorly differentiated/undifferentiated carcinomas [2]. Specific entities that enter into the differential diagnosis include sinonasal undifferentiated carcinoma (SNUC), non-keratinizing squamous cell carcinoma, NUT-midline carcinoma, myoepithelial carcinoma, small cell undifferentiated neuroendocrine carcinoma, and olfactory neuroblastoma.

Recently it was found that a subset of tumours originally diagnosed as one of the aforementioned entities all

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demonstrated loss of the ubiquitously expressed protein Integrase Interactor 1 (INI1; SMARCB1) [3, 4]. Moreover, all of these tumours were composed as least partially by 'rhabdoid' cells, a feature with INI1-deficient tumours arising in other body sites [5]. Because the expression of INI1 can be readily detected by immunohistochemistry, these INI1-deficient sinonasal carcinomas can be distinguished from their histologically similar counterparts.

In this report we describe two additional cases of INI1-deficient sinonasal carcinoma and provide insight into the early management of these highly aggressive malignancies.

Methods

H&E and immunohistochemical stains were performed on 4 μm thick unstained sections cut from representative formalin fixed paraffin embedded blocks. The immunohistochemistry was performed by the avidin–biotin-peroxidase complex technique. All stains were performed using commercially available antibodies in a Ventana® XT instrument (Ventana Systems, Tucson AZ). The stains, sources and dilutions included CAM5.2 (CAM5.2; BD BIOSCIENCE), CK5 (XM26; LEICA (NOVOCASTRA)), S100 (Polyclonal; DAKO), BRST-2 (23A3; LEICA (NOVOCASTRA)), ER (SP1; ROCHE), PR (16; LEICA (NOVOCASTRA)), GATA-3 (L50-823; INTERMEDICO), p63 (DAK-p63; DAKO), synaptophysin (27G12; LEICA (NOVOCASTRA)), chromogranin (Polyclonal; DAKO), desmin (D33; DAKO), CD34 (Q-BEND-10; DAKO), INI-1 (MRQ-27; Cell Marque).

Results

Case #1

A 34 years old male patient presented with facial swelling and loose dentition. A CT scan demonstrated a right maxillary sinus mass with invasion of the maxillary wall and involvement of the right maxillary alveolus. There was also invasion of the orbital floor but no orbital involvement. A biopsy was performed and the tumour diagnosed as sinonasal undifferentiated carcinoma (SNUC). The patient received neoadjuvant chemo-radiotherapy (Cisplatin) followed by planned surgical resection. The patient underwent a right maxillectomy, neck dissection, and scapular free flap reconstruction. The maxillectomy specimen demonstrated a 3.7 cm tumor; however, the majority of the mass was composed of fibrosis, foamy histiocytes, and reactive changes. Two small foci of viable tumor remained, measuring 1–2 mm and these were seen at the surface of the bone but without obvious invasion of the cortex. The residual tumour showed pleomorphic cells in sheets and a nested pattern. Tumor necrosis was present with a "comedo" pattern (Fig. 1a). The tumour cells had large eccentric nuclei with prominent nucleoli and an eosinophilic cytoplasm imparting a "rhabdoid" appearance (Fig. 1b). Empty vacuoles were common, which mimicked mucinous cells, however mucin was not present. All surgical margins were free of tumour and the neck nodes were all negative. By immunohistochemistry, the tumour was diffusely positive for CAM5.2 (Fig. 1c) and focally positive for synaptophysin. The tumor was negative for CD34, S100, p63, CK5, and desmin. Immunohistochemistry for INI1 showed loss of the protein in the tumor nuclei with retention in the normal tissues (Fig. 1d). The patient remained loco-regionally controlled, but developed distant metastatic disease in the lung, pleura, bone, and liver at 10 months post surgery, and died of disease 26 months after surgery.

Case #2

A 56 years old female with a prior history of breast carcinoma presented with anosmia for 5 months with more recent epistaxis, facial paresthesia, and diplopia. The patient presented to the emergency department during an episode of epistaxis and was noted to have proptosis. A MRI scan showed a large right sinonasal mass with intracranial extension and cavernous sinus invasion (image not shown). Large intraoperative biopsies were performed and showed a poorly differentiated malignancy forming islands with comedo-necrosis. The tumor cells were arranged in a nested pattern, had large nuclei with open chromatin, conspicuous nucleoli, and prominent "rhabdoid" morphology, similar to case 1. Empty vacuoles were frequent (Fig. 2a). There were also areas of a more infiltrative growth showing artifactual clefting (Fig. 2b) and focal clear cell change was noted (Fig. 2c). No definitive gland formation or keratinization was identified. Mitoses were frequent. No surface mucosal involvement was identified.

Immunohistochemistry showed diffuse positivity for low molecular weight keratin (CAM5.2) and chromogranin A (Fig. 2d). The tumor was negative for p63, CK5, S100, synaptophysin, GATA-3, BRST-2, ER, and PR. The latter markers were performed due to the prior history of breast carcinoma. Immunohistochemistry for INI1 showed loss of the protein in the tumor nuclei with retention in the normal tissues similar to case #1 (not shown). The patient was treated with chemotherapy (Cisplatin) followed by radiation which led to an apparent complete response radiologically. As a consequence, no resection was performed. She is in remission 12 months post initial presentation and 5 months post completion of radiation therapy.

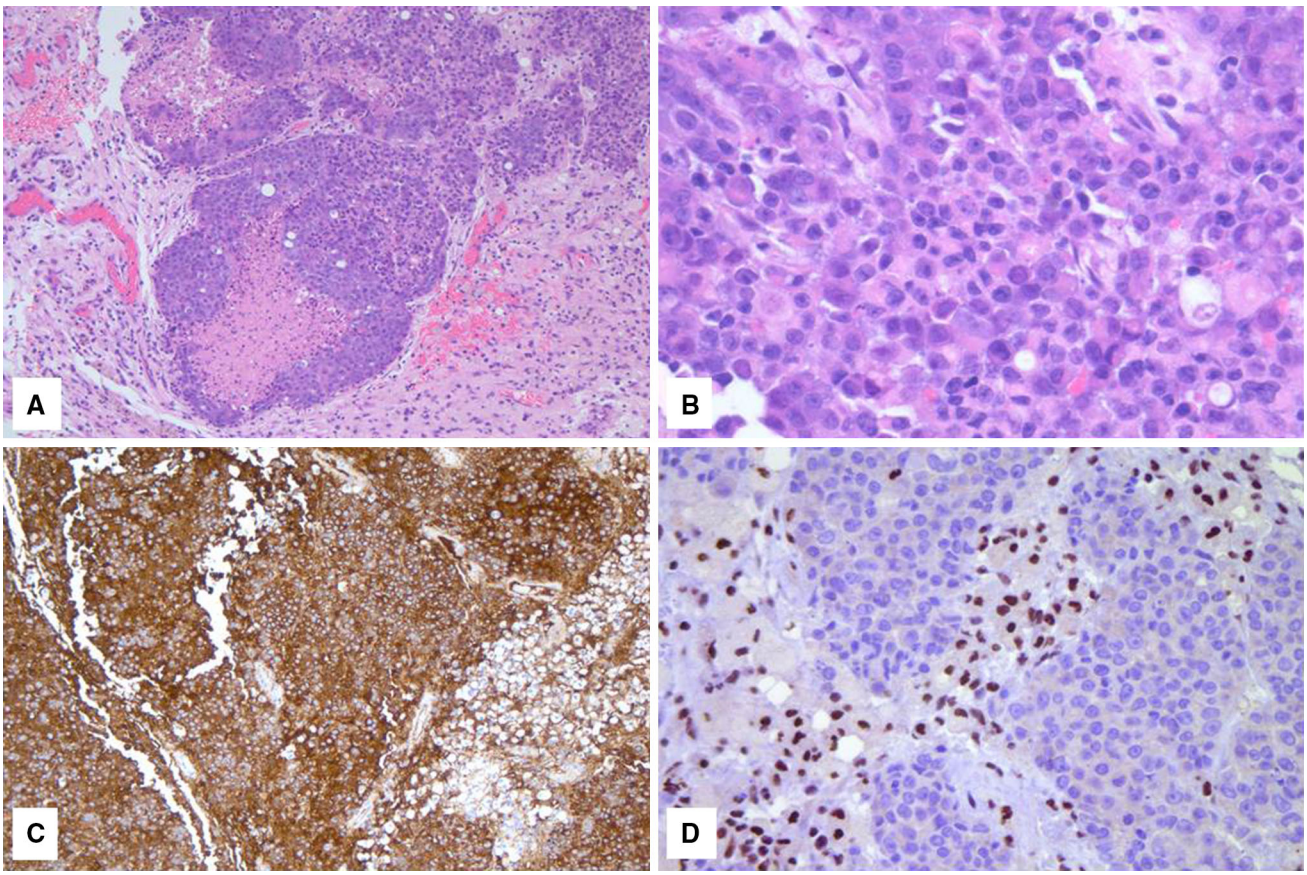

Fig. 1 **a** Low-power view of the tumor with comedonecrosis and a foamy histiocyte rich background reaction. **b** High-power view showing cells with a rhabdoid phenotype. **c** Diffuse CAM5.2 was seen in both cases. **d** Loss of INI1 in the tumor with retained staining in the normal background tissue was also a finding in both cases

Discussion

INI1-deficient sinonasal carcinoma is a relatively new entity with only 16 cases described to date [3, 4, 6]. In this report we present the clinicopathological features of two cases received by our consultation service and managed at our institution. While the previously reported cases were derived from the retrospective analysis of archived tumours, our cases were prospectively diagnosed as INI1-deficient sinonasal carcinoma and as a consequence, this report provides a unique insight into the early management of this highly aggressive neoplasm.

INI1 is a core subunit of the SWI/SNF chromatin modelling complex, an evolutionarily conserved ATP-dependent complex that is normally expressed in all eukaryotic cells. INI1 acts as a tumour suppressor as loss of the gene leads to the rapid development of cancer in experimental models [7]. The relationship between the loss of INI1 expression and the development of sinonasal carcinoma appears to involve multiple molecular pathways. Firstly, loss of INI1 activity leads to increased cellular proliferation through the over-expression of cyclin D1 which leads to the phosphorylation and inactivation of RB and progression of the cell cycle [8, 9]. Secondly, the over-expression of cyclin D1 has been shown to be a sufficient stimulus to induce a rhabdoid morphology in normal cells. Conversely, genetic ablation of cyclin D1 blocks the development of rhabdoid tumours [10]. Finally, the SWI/SNF complex plays a critical role in the maintenance of chromatin structure and the loss of functional INI1 leads to widespread epigenetic alterations, which include the silencing of genes required for normal lineage specific differentiation and maturation [11]. Interestingly, INI1-deficient tumours have been found to harbor very few somatic mutations other than the recurrent loss of INI1, which suggests that these tumours are driven largely by aberrant transcriptional regulation [12, 13]. Indeed, the loss of INI1 expression in all tumour cells without evidence of an INI1 competent precursor lesion (in our cases and in previously published cases) seems to support the theory that this molecular alteration is an initiating event in this highly aggressive malignancy. Why some people develop inactivating mutations in INI1 however has yet to be elucidated.

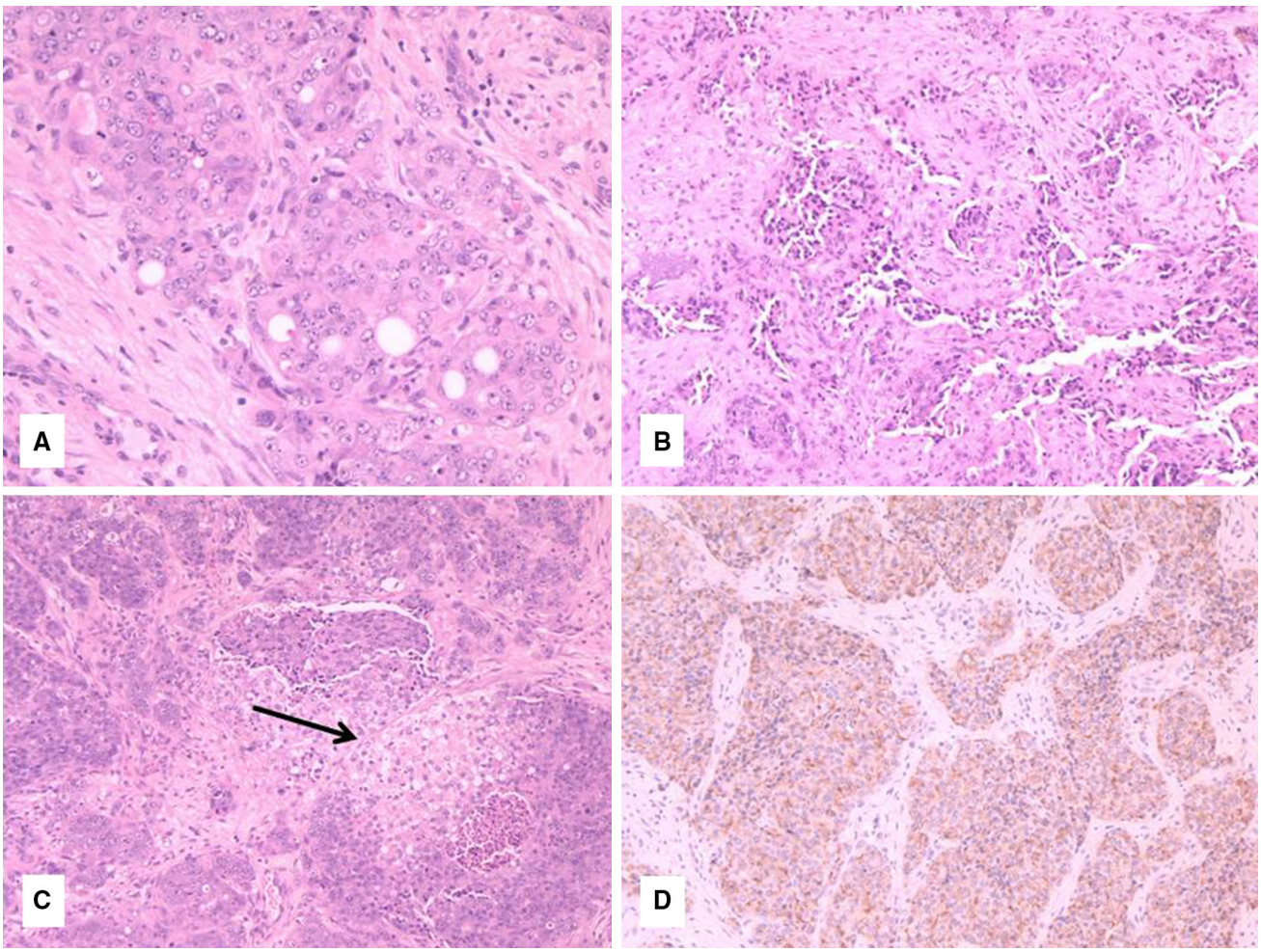

Fig. 2 **a** Prominent empty vacuoles were noted in both tumours. **b** Case 2 showed areas with a more infiltrative growth with small nests demonstrating artifactual clefting. **c** Areas of clear cell change were also seen (*arrow*). **d** Diffuse positivity for chromogranin was present in case 2

Patients with INI1-deficient sinonasal carcinoma tend to present with large and locally advanced tumours; indeed, based on the previously reported series, most INI1-deficient sinonasal carcinomas are staged as T4 at the time of diagnosis [4, 6]. Despite this, our experience suggests that these tumours respond well to neoadjuvant chemo-radiation. In both of our cases, the patient received Cisplatin, a platinum based alkylating-like agent followed by radiation therapy. This regiment resulted in a significant reduction in viable tumour in the first patient and a complete radiological response in the second patient. Because of the morbidity associated with surgical resection of large sinonasal tumours, these cases suggest a possible use of neoadjuvant chemo-radiation to reduce tumour volume prior to surgery. In contrast, patients in the previously reported series underwent surgical resection prior to adjuvant chemoradiation with variable results. In most cases, the tumours recurred locally and the majority of patients ultimately developed metastatic disease [3, 4, 6]. Future treatments with agents that target the epigenetic machinery such as inhibitors against Enhancer of Zeste homologue 2 (EZH2) or histone deactylase may prove even more effective [14, 15].

Loss of INI1 expression has been detected in a wide variety of tumours including epithelioid sarcoma, renal medullary carcinoma, myoepithelial carcinoma of soft tissue, epithelioid malignant peripheral nerve sheath tumour, extraskeletal myxoid chondrosarcoma, and gastrointestinal rhabdoid carcinoma [5]. Although INI1 gene deletions can be detected by FISH, immunohistochemistry is preferred as it documents loss of normal nuclear protein expression. Also, not all cases documented have had loss of the INI-gene. The common histological feature linking all of these unique entities is the presence of “rhabdoid” cells—large cells with eccentric nuclei, open chromatin, prominent nucleoli, and eosinophilic cytoplasmic inclusions; although the number of such cells can vary from a few discreet cells to sheets of rhabdoid cells that constitute the majority of the

tumour. In both of our cases, rhabdoid cells were readily apparent which led us to perform immunohistochemistry for INI1. However, in many cases of INI1-deficient sinonasal carcinoma, the rhabdoid cells tend to be scattered among more basaloid cells and the correct diagnosis requires a high degree of suspicion when assessing an apparently undifferentiated tumour [3, 4]. Other consistent histological features include necrosis, increased mitotic activity, infiltrative borders, and despite the aggressive nature of the tumour, minimal pleomorphism. Consistent with previous reports, we also observed numerous empty spaces containing necrotic tumour cells which tended to impart a pseudoglandular appearance at low power. We did not observe any true glandular or squamous differentiation nor have they been described in any of the cases reported to date [3, 4, 6].

By immunohistochemistry, INI1-deficient sinonasal carcinomas consistently express keratins and some, including our two cases, have been reported to express p63, CK5, chromogranin, and synaptophysin [3, 4, 6]. The proliferative index as measured by Ki-67 has been reported to exceed 50 % [3]. Although p16 has been shown to be over-expressed in some tumours, high-risk HPV has not been detected [4, 6]. These results reinforce the notion that despite the presence of rhabdoid cells, INI1-deficient sinonasal carcinoma is indeed an epithelial neoplasm that can variably express a variety of other markers but none with enough consistency to suggest a definitive line of differentiation.

The differential diagnosis for INI1-deficient sinonasal carcinoma includes other poorly differentiated/undifferentiated malignancies such as non-keratinizing squamous cell carcinoma, myoepithelial carcinoma, NUT-midline carcinoma, small cell undifferentiated neuroendocrine carcinoma, olfactory neuroblastoma, and sinonasal undifferentiated carcinoma (SNUC). Besides the presence of rhabdoid cells and the loss of INI1 expression, specific histological and immunophenotypic markers may help distinguish between these similar looking entities. For example, although INI1-deficient sinonasal carcinoma can closely resemble non-keratinizing squamous cell carcinoma histologically, the latter tumour tends to exhibit greater nuclear pleomorphism, more diffuse p63 expression, and may be associated with an in situ lesion. In contrast to INI1-deficient sinonasal carcinoma, the tumour cells in myoepithelial carcinoma may demonstrate a spindled morphology and almost always strongly express S100. Like INI1-deficient sinonasal carcinoma, NUT midline carcinoma can be a deceptively monotonous tumour, however foci of abrupt squamous differentiation can often be identified and the tumour cells can be shown to harbor the *BRD-NUT* fusions by NUT immunohistochemistry. Small cell carcinoma and olfactory neuroblastoma differ from INI1-deficient sinonasal carcinoma in that they diffusely express the neuroendocrine markers chromogranin, synaptophysin, and CD56 whereas INI1-deficient sinonasal carcinoma rarely express all three markers; olfactory neuroblastoma is also typically negative for keratins. INI1-deficient sinonasal carcinoma and SNUC are both histologically undifferentiated tumours that may only express cytokeratins. Although SNUC tend to demonstrate a greater degree of cellular atypia, immunohistochemical staining for INI1 may be the only way to definitely distinguish between these entities in many instances.

In summary, this report describes two additional cases of INI1-deficient sinonasal carcinoma, prospectively diagnosed at our institution and treated with neoadjuvant chemo-radiation. The significant reduction in tumour volume with this therapy may facilitate subsequent surgical resection and improve the chance of long-term local control, although early data and our own experience suggests mortality due to distant failure is very common.

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