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ORIGINAL ARTICLE

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CDX-2, cytokeratin 7 and cytokeratin 20 immunohistochemical expression in the differential diagnosis of primary adenocarcinomas of the sinonasal tract

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Abstract Because the histopathological features of some primary adenocarcinomas of the sinonasal tract may show considerable overlap, we assessed the diagnostic value of a panel of immunohistochemical markers in the distinction between these malignancies. Paraffin-embedded tumour tissue sections from a series of 39 primary adenocarcinomas of the sinonasal tract, including 25 cases of intestinal-type adenocarcinoma (ITAC), 10 cases of salivary gland-type carcinoma and 4 cases of tubulopapillary low-grade adenocarcinoma were immunostained for CDX-2, cytokeratin 7 and cytokeratin 20. Diffuse nuclear staining for CDX-2 was identified in 80% of ITACs, while all non-ITACs were negative. Staining for cytokeratin 20 was positive in 84% of ITACs, including all cases negative for CDX-2, but negative in all other adenocarcinomas. Cytokeratin 7 was consistently positive in 88% of ITACs and in 100% of non-ITACs. Normal sinonasal epithelia expressed cytokeratin 7, but not CDX-2 and cytokeratin 20. Staining for CDX-2 and cytokeratin 20 has potential use in separating ITACs from other primary malignant glandular neoplasms of the nasal cavities and paranasal sinuses.

Keywords Nasal cavity · Paranasal sinuses · Intestinal-type adenocarcinoma · Salivary gland-type carcinoma · Low-grade adenocarcinoma

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Introduction

Sinonasal adenocarcinomas account for 10–40% of sinonasal epithelial malignancies [5, 13, 17, 20, 22]. This is a heterogeneous group of neoplasms, with different epidemiological features and variable clinical behaviour, which is rather poorly characterised from the histopathological point of view, also due to the rarity of these malignancies. A significant proportion of sinonasal adenocarcinomas show histological features reminiscent of colonic adenomas and adenocarcinomas and have therefore been termed intestinal-type adenocarcinomas (ITACs) [3]. Another major group of malignant glandular tumours of the nasal cavities and paranasal sinuses are histologically identical to major and minor salivary gland carcinomas and can be further classified according to salivary gland tumour classification schemes. Finally, there are reports concerning a rather ill-defined group of low-grade adenocarcinomas, most of which show papillary–tubular structures formed by bland cuboidal and columnar neoplastic cells [14, 19].

The differential diagnosis between these entities is clinically relevant, because ITACs are characteristically associated with occupational exposure to wood and leather dusts, and have an aggressive behaviour characterised by repeated local recurrences and ominous outcome [3, 9, 12]. Conversely, low-grade adenocarcinomas and salivary gland-type carcinomas tend to have a more indolent clinical course [19, 20]. However, due to their rarity and, in some instances, to the considerable overlap of their histological features, the distinction between sinonasal ITACs and non-ITACs may be difficult, especially in small biopsy material. In addition, there are only a few systematic immunohistochemical studies of sinonasal adenocarcinomas aimed at identifying potentially useful diagnostic markers to distinguish between these entities.

In a preliminary report, we analysed the expression of a marker of intestinal differentiation, the CDX-2 homeobox gene, and of cytokeratin 7 and 20 in a group of sinonasal ITACs [11]. The aim of the present study was to

Table 1 Antibodies used in this study

Antibody	Dilution	Pretreatment	Source
CDX-2	1:100	Citrate buffer pH 6, microwave (30 min)	Bio Genex, San Ramon, CA, USA
Cytokeratin 7	1:800	0.5% Protease XIV 20°C (15 min)	Bio Genex, San Ramon, CA, USA
Cytokeratin 20	1:60	0.5% Protease XIV 20°C (15 min)	Bio Genex, San Ramon, CA, USA

verify the possible use of these markers in the differential diagnosis of primary adenocarcinomas of the sinonasal tract.

Materials and methods

Sinonasal adenocarcinoma samples were obtained from tissue archives of the Department of Human Pathology and Oncology of the University of Florence, and from the Unit of Anatomic Pathology, Department of Laboratory Medicine, Empoli Hospital (Italy). The series included 25 cases of ITAC, 10 cases of salivary gland-type carcinoma and 4 cases of tubulopapillary low-grade adenocarcinoma. Paraffin-embedded tumour tissue sections were immunostained for CDX-2, cytokeratin 7 and cytokeratin 20 using an automated labelled streptavidin-biotin-peroxidase method on the GenoMX i6000 immunostainer (Biogenex). Table 1 reports the antibody source, dilution and antigen retrieval protocols used. Fragments of colorectal mucosa and colorectal adenocarcinomas were used as positive controls for CDX-2 and cytokeratin 20. Non-neoplastic sinonasal mucosa served as internal positive control for cytokeratin 7. Negative controls were performed by substituting primary antibody with non-immune mouse serum.

Cases were considered positive if more than 10% of neoplastic cells showed nuclear (CDX-2) or cytoplasmic (cytokeratin 7 and 20) immunoreactivity.

Results

The results of the immunohistochemical studies are summarised in Table 2. The group of ITACs included 6 well-differentiated, 9 moderately differentiated, 5 poorly differentiated, and 5 mucinous adenocarcinomas [9]. Nuclear immunostaining of neoplastic cells for CDX-2 was observed in all cases. In 5 adenocarcinomas (20%; 1 well differentiated, 2 moderately differentiated, 1 poorly differentiated and 1 mucinous) the immunoreactivity was focal (<10% of neoplastic cells); while, in the other cases, there was strong and diffuse (>50%) nuclear staining (Fig. 1D and G). Surface and glandular epithelia of the sinonasal mucosa were always negative for CDX-2 (Fig. 1A). Twenty-two ITACs (88%) showed cytoplasmic staining for cytokeratin 7 in the majority of neoplastic cells (Fig. 1E and H). Two mucinous adenocarcinomas and one moderately differentiated adenocarcinoma were negative or showed only focal immunoreactivity. Strong immunostaining for cytokeratin 7 was also observed in surface and glandular epithelia of normal sinonasal mucosa (Fig. 1B). Cytokeratin 20 expression was detected in 21 ITACs (84%), including 6 of 6 well differentiated, 7 of 9 moderately differentiated, 4 of 5 poorly differentiated and 4 of 5 mucinous adenocarcinomas (Fig. 1F and I). The 5 adenocarcinomas showing focal staining for CDX-2 were positive for cytokeratin 20. No cytokeratin 20 immunostaining was identified in normal sinonasal epithelia (Fig. 1C).

The group of non-intestinal-type adenocarcinomas was characterised by a uniform immunoprofile. All cases were strongly and diffusely positive for cytokeratin 7 (Fig. 1M and P), while no immunoreactivity for cytokeratin 20 and CDX-2 was observed (Fig. 1L, N, O and Q).

Table 2 Results of the immunohistochemical analysis of 39 primary adenocarcinomas of the sinonasal tract (cases showing positive staining, >10% of neoplastic cells)

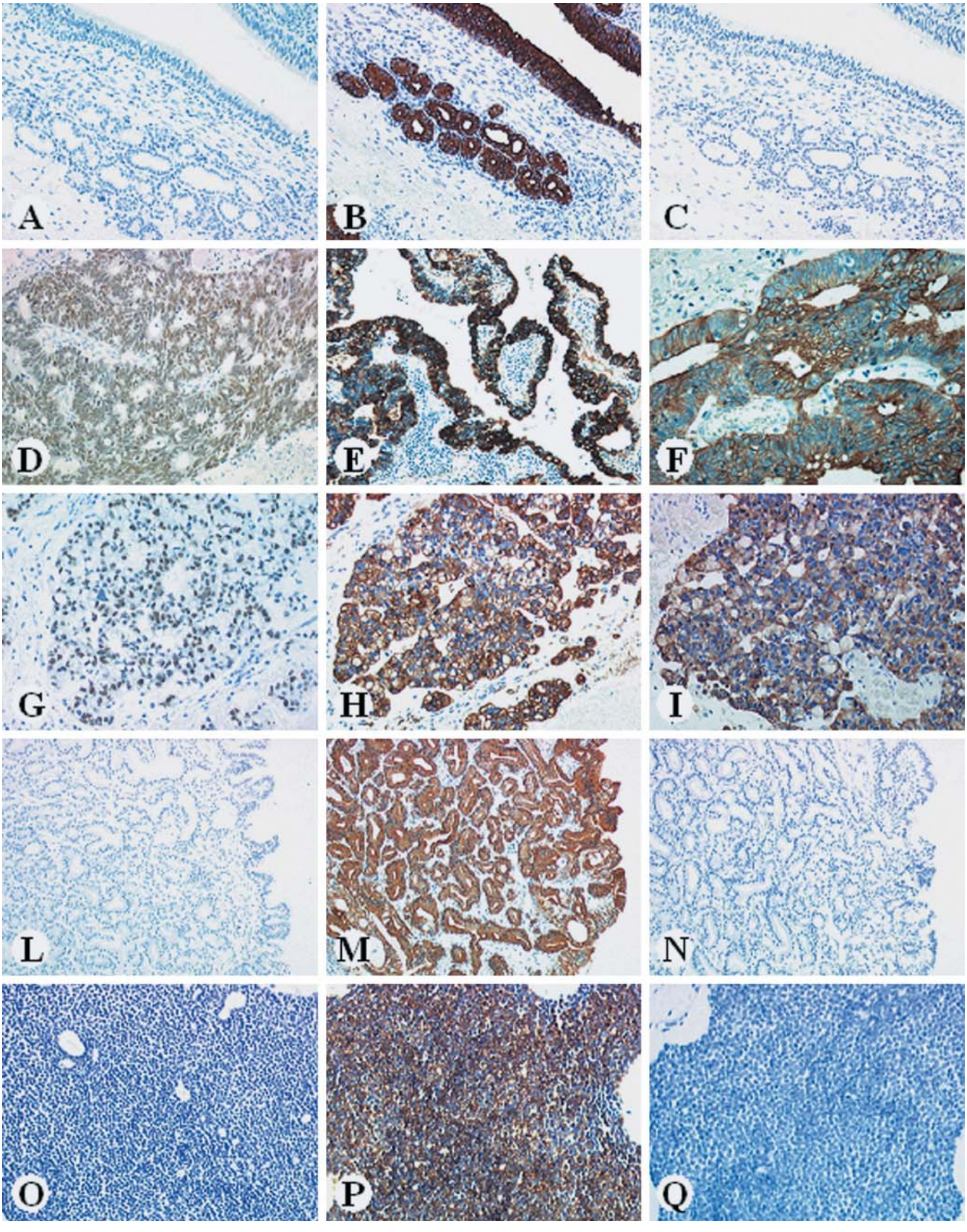
Tumor type	Cytokeratin 7	Cytokeratin 20	CDX-2
ITAC			
Well differentiated	6/6	6/6	5/6
Moderately differentiated	8/9	7/9	7/9
Poorly differentiated	5/5	4/5	4/5
Mucinous	3/5	4/5	4/5
Non-ITAC			
Salivary gland-type	10/10	0/10	0/10
Tubulopapillary	4/4	0/4	0/4

Discussion

Our analysis indicates that primary glandular epithelial malignancies of the sinonasal tract show a different immunoprofile. On the one hand ITAC is characterised by an immunophenotype resembling that of colonic adenocarcinoma, with the expression of CDX-2 and cytokeratin 20; on the other non-ITACs are characterised by the expression of cytokeratin 7 only, with consistent negative staining for CDX-2 and cytokeratin 20.

CDX-2 is a caudal-related homeobox transcription factor involved in the regulation of proliferation and differentiation of intestinal cells. In normal adult tissues its expression is restricted to the intestinal epithelium and to portions of the pancreatic duct system [16]. Immuno-

Fig. 1 Representative images of the immunohistochemical characterisation of sinonasal adenocarcinomas. *First column* CDX-2, *second column* cytokeratin 7, *third column* cytokeratin 20. *First row* normal sinonasal mucosa, *second row* moderately differentiated intestinal-type adenocarcinoma, *third row* mucinous intestinal-type adenocarcinoma, *fourth row* low-grade tubulopapillary adenocarcinoma, *fifth row* adenoid cystic carcinoma. Normal sinonasal mucosa and non-intestinal-type adenocarcinomas (salivary gland type and low-grade tubulopapillary adenocarcinomas) were characterised by expression of cytokeratin 7 only, whereas intestinal-type adenocarcinomas consistently expressed CDX-2 and both cytokeratins tested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histochemical surveys in tumour tissues have demonstrated that this marker is significantly expressed by colonic adenocarcinomas and by a subset of gastric, oesophageal, bilio-pancreatic and ovarian carcinomas [1, 2, 16, 23]. Overall, CDX-2 appears to be a marker of intestinal differentiation in tumours, and its presence in ITACs of the sinonasal tract reinforces the notion that these neoplasms exhibit a high degree of overlap with normal intestinal epithelial cells and intestinal neoplasms. Moreover, it is conceivable that the acquisition of CDX-2 expression is a key event in the pathogenesis of ITAC, considering that the CDX-2 gene may regulate several genes involved in the acquisition of the mature intestinal phenotype.

The acquisition of cytokeratin 20 expression appears to be another aspect of the intestinal differentiation that characterizes ITAC. Indeed, this marker is absent in normal sinonasal epithelia, whereas, according to our study and to other reports [7, 15, 18], it is expressed by the majority of ITACs of the nasal cavities and paranasal sinuses, regardless the histologic subtype. Other immunohistochemical markers shared by ITAC and colorectal adenocarcinoma are CEA, MUC2 and sialosyl-Tn-antigen [8, 10, 15, 18, 21]. Interestingly, CK20 expression has also been evidenced in areas of intestinal metaplasia present in nasal respiratory type mucosa adjacent to ITAC, which may represent a precursor of these tumours [8].

The data presented in this study indicate that strong and diffuse CDX-2 and cytokeratin 20 immunostaining is very common in sinonasal ITACs, while no expression is detectable in non-ITACs. Such differential expression of CDX-2 protein and cytokeratin 20 in primary sinonasal adenocarcinomas may be of diagnostic aid in selected cases. Well-differentiated ITAC showing a predominance of tubulopapillary architecture and little or no cellular atypia should be differentiated from tubulopapillary low-grade adenocarcinoma, which is also characterised by formation of tubular structures and papillae [19]. This distinction is clinically relevant, because ITAC tends to pursue a more aggressive clinical course than tubulopapillary low-grade adenocarcinoma. Therefore, positivity for CDX-2 and/or cytokeratin 20 in a papillary–tubular adenocarcinoma of the sinonasal tract supports a diagnosis of ITAC.

Another setting in which immunostaining for CDX-2 and cytokeratin 20 may be of diagnostic aid is in the distinction of poorly differentiated ITAC, which shows minimal gland formation, from other primary epithelial neoplasms of the sinonasal tract, including poorly differentiated carcinomas of salivary gland origin, such as adenoid cystic carcinoma with predominantly solid pattern. Again, positivity for CDX-2 and/or cytokeratin 20 is in favour of a diagnosis of ITAC.

Both ITAC and non-ITAC groups showed expression of cytokeratin 7, a marker of normal sinonasal epithelia, which is also found in other sinonasal epithelial malignancies [10]. In our series, cytokeratin 7 was identified in 88% of ITAC, a level of expression which is comparable to the 100% expression detected in a previous report [15]. At variance, Sandison et al. found a positive rate of only 59% in a series of 22 primary sinonasal ITACs arising in woodworkers [18], similar to the 50% rate observed by Choi et al. in a study of 6 cases [7]. Despite these variable results, cytokeratin 7 immunostaining may be useful in the differential diagnosis between sinonasal ITAC and metastasis from a colorectal adenocarcinoma. Metastatic adenocarcinoma to the nasal cavity and paranasal sinuses from a primary adenocarcinoma of the gastrointestinal tract is a very uncommon occurrence [4, 7]. However, cases of gastrointestinal adenocarcinoma presenting with metastasis to the head and neck region have been reported [6], and it seems prudent to exclude this possibility before making a diagnosis of ITAC. Considering the overlap in the histological appearance between ITACs and metastatic lesions, immunohistochemistry may be of help in the differential diagnosis. In this setting, although sensitivity may not be high, positive staining for cytokeratin 7 supports the diagnosis of sinonasal ITAC.

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