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Immunohistochemical profiling of mucins in sinonasal adenocarcinomas

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ABSTRACT

In this study we investigated the expression of mucins (MUC1, MUC2, MUC4, MUC5AC and MUC6) in a series of 66 sinonasal adenocarcinomas, in order to establish their distribution and the possible correlation with clinicopathological and prognostic parameters. The series included 51 intestinal type adenocarcinomas, 4 non-intestinal type adenocarcinomas, and 11 salivary gland type carcinomas. The immunohistochemical analysis was conducted on a tissue microarray obtained from formalin fixed-paraffin embedded tumor tissue samples. Thirty-nine adenocarcinomas (59.1%) resulted positive for MUC1, 21 (41.2%) for MUC2, 47 (71.2%) for MUC4, and 16 (24.2%) for MUC5AC, while MUC6 was negative in all cases tested. MUC1 was significantly more expressed in ITACs than in non-ITACs (70% vs 20%, $p = 0.0007$) while MUC2 was expressed only in ITACs ($p = 0.0015$) with a clear prevalence in the mucinous subtype ($p < 0.0001$). Conversely, MUC4 and MUC5AC were similarly expressed in the sinonasal adenocarcinoma subtypes tested. High expression of MUC 1 was related to a significantly shorter overall survival, both in the whole series ($p = 0.04$), while adenocarcinomas positive for MUC 2 tended to have a worse overall survival ($p = 0.07$). In addition, MUC2 expression was higher in ITACs with distant metastasis, being expressed in 4 out of 5 cases ($p = 0.015$). We conclude that sinonasal adenocarcinomas have a characteristic expression of different mucin types, with significant clinicopathologic correlations. In view of the extensive involvement of mucins in different aspects of tumor growth and their emerging role as possible therapeutic targets, our study suggests that these factors could be considered clinically relevant biomarkers and attractive targets for new treatments in sinonasal adenocarcinomas.

1. Introduction

Sinonasal adenocarcinomas comprise a wide spectrum of epithelial tumours with glandular differentiation, representing the second malignancy after conventional squamous cell carcinoma in this anatomic site (20–25% of sinonasal tumors) [1]. According to the 4th edition of the WHO classification, these tumors can be distinguished into salivary and non salivary type, the latter further divided into intestinal (ITAC) and non-intestinal type (non-ITAC) [2].

Because of the lack of specific symptoms, the diagnosis is often made when the neoplasm is extensive and invades surrounding tissues (T3 and T4 disease) [3]. Treatment of choice is radical surgical resection when possible, with open or endoscopic techniques, associated with adjuvant radiotherapy when the disease is in advanced stage, to increase local control. Systemic treatment is less standardized[3] and elective neck dissection is not routinely performed because of the limited risk of regional metastases (7%) [3,4]. Overall, the prognosis of most sinonasal adenocarcinomas still remains poor, manly because the high risk of local recurrences which are often unresectable and re-irradiation is not possible because the procedure is often performed as part of the treatment at the first presentation of the tumor [3,4]. Therefore, there is strong need to investigate new possible therapies based on detection of specific tumoral targets.

Mucins are high-molecular weight glycoproteins, containing a polymorphic central domain that comprises a variable number of tandem repeats, a hallmark of the mucin family [5,6]. A total of 21 different mucins have been found in human tissues, functionally classified into two major groups: secreted (MUC2, MUC5AC, MUC5B, MUC6-MUC8 and MUC19) and membrane-bound (MUC1, MUC3, MUC4, MUC12, MUC13, MUC15-MUC17, MUC20 and MUC21) [5]. In normal tissues, mucins are expressed in a relatively organ- and cell-specific manner [6–9]. For example, MUC1 is found in most epithelial

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Table 1
Antibodies employed in this study.

Antibody	Clone and provider	Species and diluition	Antigen retrieval
MUC1	Monoclonal, clone H23, Roche Tissue Diagnostics, Monza, Italy	Mouse, prediluted,	CC1
MUC2	Monoclonal, clone MRQ-18, Roche Tissue Diagnostics, Monza, Italy	Mouse, prediluted	CC1
MUC4	Monoclonal, clone 8G7, Roche Tissue Diagnostics, Monza, Italy	Mouse, prediluted	CC1
MUC5AC	Monoclonal, clone MRQ-19, Roche Tissue Diagnostics, Monza, Italy	Mouse, prediluted	CC1
MUC6	Monoclonal, clone MRQ-20, Roche Tissue Diagnostics, Monza, Italy	Mouse, prediluted	CC1

cells, most of all in breast and the digestive, respiratory, and genitourinary tracts [7,8,10]. In contrast, the distribution of MUC2 and MUC5AC seems to be more restricted, with MUC2 specifically expressed in goblet cells of the small intestine and colon [10,11] and MUC5AC preferentially expressed in the stomach and respiratory tract [8–12].

Mucins are historically thought to represent the main constituent of mucus in human body [5,9]. Several studies have confirmed that they also have important role in cellular differentiation, modulation of cell adhesion, immune response and cell signalling [13,14]. For these reasons, mucins have been studied in relation to cancerogenesis and it is now clear that they are associated with tumor progression in different ways. Indeed, they are target for several growth factors and cytokines, and they promote proliferation and metastasis of cancerous cells, being potential targets for new therapies [14–18]. In some neoplasms, the aberrant expression of mucins is also related to the clinical outcome [19–21].

In the head and neck region, expression of some of these mucins, in particular MUC1, MUC2, MUC4, MUC5AC and MUC6 was studied both in normal and in pathological conditions in various sub-sites, including the upper respiratory tract [8,11], oral cavity [18–20], larynx [22,23] and salivary glands [21,24], but no study has been undertaken so far on sinonasal neoplasms. MUC1, MUC2, MUC4, and MUC5AC were found mainly in the epithelial goblet cells, whereas MUC5B and MUC7 were expressed mostly by mucous glands [11]. In head and neck carcinomas and in some salivary gland neoplasms, mucins expression is often dysregulated and a strong relation to the outcome of patients has been documented in some studies [18,19,21,22,24].

The aim of this study is to investigate the expression of mucins and their correlation with clinical behavior in a series of sinonasal adenocarcinomas.

2. Patients and methods

2.1. Patients

A total of 66 consecutive patients treated at our hospital for sinonasal adenocarcinoma between January 1995 and December 2016 were selected for this study. Clinical and pathologic data were retrospectively collected, and a complete follow-up was obtained (range 5–194 months; mean 56, median 42). The series included 51 intestinal type adenocarcinomas, 4 non-intestinal type adenocarcinomas (3 low-grade and 1 high grade), and 11 salivary gland type carcinomas (9 adenoid cystic carcinomas, 1 polymorphous adenocarcinoma, 1 myoepithelial carcinoma).

There were 55 males and 7 female subjects, ranging in age between 42 and 83 years (mean 63.7 years). Occupational exposure was known for 55 patients: 26 had been employed in leather industry, 16 in wood industry, and 13 had no significant work exposure. According to AJCC TNM staging system (Seventh Edition, 2009), which could be determined for 54 subjects, the majority of tumors was in locally advanced stage, 14 being T3 and 19 T4 lesions. Neck lymph node metastases were present in 2 patients, while 7 had distant metastasis. Complete follow-up was available for 57 patients, ranging between 3 and 110 months, (median 38 months): 31 patients (54.4%) experienced local recurrence and 29 (50.9%) died of disease.

Patients were treated by different modalities: exclusive surgery, surgery (open or transnasal endoscopic assisted, with or without skull base reconstruction) followed by radiotherapy or radio and chemotherapy, and radio-chemotherapy alone. Selected cases were treated by neo-adjuvant radiotherapy followed by chemoterapy. In each case, all available histological slides were reviewed and the tumors were classified according to the WHO Classification scheme in intestinal (n = 51), non intestinal (n = 2), and salivary gland subtypes (n = 13).

The Ethical Committee of the Azienda Ospedaliera Universitaria "Careggi" approved the study.

2.2. Immunohistochemistry

Formalin fixed, paraffin embedded tissue samples were retrieved for all cases. For tissue microarray construction, areas of interest rich in non-necrotic tissue were selected from corresponding haematoxylin and eosin stained sections and mark on the source paraffin block. For each case, two cores of different tumor areas were arrayed. The source block was cored and a 1-mm core was transferred to the recipient master block using the Beecher Tissue Microarray (Beecher Instruments, Silver Spring, MD). In addition, 8 cores of non-neoplastic sinonasal mucosa were included. Sections (5-μm thick) were obtained from the block, which were stained with haematoxylin and eosin, or utilized for the immunohistochemical analysis

For immunohistochemical staining, tissue sections (5 μm) were deparaffinised, hydrated, and after endogenous peroxidase inactivation immunostained with BenchMark Ultra stainer (Ventana, Tucson, AZ), and revealed with i VIEW DAB detection kit, providing a brown reaction product. Table 1 shows the antibody source, dilution, and antigen retrieval protocol. After completing the staining process, the slides were removed from the autostainer, counterstained with haematoxylin, dehydrated, and mounted with a permanent medium. As a negative control, we substituted the primary antibody with a Ventana dispenser filled with nonimmune serum at the same concentration for each immunohistochemical reaction.

The semi-quantitative evaluation of the results of the immunohistochemical studies was conducted considering both the staining intensity and the percentage of positive cells. The staining intensity was evaluated on a 4-point scale (0–3), and the proportion of positive cells was evaluated according to the scale 0 (< 5%), 1 (5–20%), 2 (21–50%), 3 (> 50%). The two values were summed to obtain a total score ranging between 0 and 8. For statistical analysis, cases with score ≥4 were considered positive.

2.3. Statistical analysis

All statistical tests were performed using SPSS software (release 21.0). Associations between categorical variables were assessed by means of the chi square test and Fisher exact test. For analysis of survival, the endpoints considered in this study were disease free interval and overall survival. Survival functions were modelled using the Kaplan-Meier method. The difference of survival between groups was assessed with Log Rank (Mantel-Cox) chi square test. Two-tailed $P < 0.05$ were considered significant.

Table 2
Correlation between MUC expression and clinico-pathologic variables.

	MUC1+ n (%)	P	MUC2+ n (%)	P	MUC4+ n (%)	P	MUC5+ n (%)	P
Age (median 65) (n = 66)								
Age ≥ 65 (36)								
Age < 65 (30)	23 (63.9) 16 (53.3)	0.4551	11 (30.5) 9 (30)	1.0	26 (72.2) 21 (70)	1.0	13 (36.1) 3 (10)	0.0202
Gender (n = 66)								
M (56)	36 (64.3)	0.174	20 (35.7)	0.150	42 (75%)	0.136	14 (25)	1.0
F (10)	4 (40)	6	1 (10)	5	5 (50)	3	2 (20)	
Occupational								
Exposure (n = 56)								
Yes (36)	25 (69.4)	0.091	15 (41.7)	0.143	27 (75)	1.0	10 (27.8)	0.178
No (20)	9 (45)	9	4 (20)	2	15 (75)		2 (10)	2
Histologic type (n = 66)								
ITAC (51)								
Non-ITAC (15)	36 (70.6)	0.000	21 (41.2)	0.001	40 (78.4)	0.024	15 (29.4)	0.092
	3 (20)	7	0 (0)	5	7 (46.7)	8	1 (66.7)	7
Lymph node metastases								
(n = 43)								
No (39)	20 (51.3)	1.0	10 (25.6)	1.0	27 (69.2)	1.0	7 (17.9)	1.0
Yes (4)	2 (50)		1 (25)		3 (75)		1 (25)	
Distant metastases								
(n = 40)								
No (35)	18 (51.4)	0.355	7 (20)	0.015	25 (71.4)	0.626	6 (17.1)	0.256
Yes (5)	4 (80)	5	4 (80)	2	3 (60)	6	2 (40)	8
Local recurrence (n = 59)								
No (35)								
Yes (24)	17 (48.6)	0.192	8 (22.9)	0.253	21 (60)	0.160	9 (25.7)	0.529
	16 (66.7)	9	9 (37.5)	7	19 (79.2)	6	4 (16.7)	2

3. Results

The results of the immunohistochemical studies are summarized in Table 2 and illustrated in Fig. 1.

3.1. MUC 1

Normal sinonasal mucosa showed partial positivity for MUC1 in the apical aspect of the membrane of ductal and acinar cells of seromucous glands, whereas the surface epithelium was negative. Among 66 sinonasal adenocarcinomas, 39 (59.1%) resulted positive for MUC1, both in cytoplasm and in cellular membrane. Regarding carcinoma subtypes, 70% of ITAC vs 20% of non-ITAC were positive (p = 0.0007). No significant difference in MUC1 expression was seen in different subtypes of ITAC (p = 0.244) (Table 3). High expression of MUC 1 was related to a significantly shorter overall survival in the whole series (p = 0.04) (Fig. 2), while no significant differences were noted for disease-free interval (p = 0.137). Considering the group of ITACs, still high MUC1 expression was associated with shorter overall survival (p = 0.05).

3.2. MUC2

In sinonasal healthy mucosa, MUC2 positivity was seen only in goblet cells interspersed in the surface epithelium, while the mucosal glands were negative. MUC2 was positive in 21 (41.2%) of tumors, mainly with a cytoplasmic distribution, but in several mucinous ITACs the staining was limited to the apical membrane of the cells. Interestingly, only ITACs expressed this marker (p = 0.0015), and among ITACs, the mucinous subtype showed a higher expression than the other variants (p < 0.001) (Table 3). Adenocarcinomas positive for MUC 2 tended to have a worse overall survival (p = 0.07) (Fig. 3), while no significant differences were noted for disease-free interval (p = 0.382). In addition, MUC2 expression was higher in ITACs with distant metastasis, being expressed in 4 out of 5 cases (p = 0.015). However, the overall survival was not related to MUC2 expression in the group of ITACs (p = 0.4).

3.3. MUC 4

In normal sinonasal mucosa, MUC4 was diffusely expressed in surface epithelium, while seromucous glands were negative. MUC4 was the most frequently expressed mucin in adenocarcinomas of the sinonasal tract. Indeed, 47 adenocarcinomas stained for this marker (71.2%), with a membrane and cytoplasmic distribution. Both ITAC and non-ITAC showed expression of MUC4, with a higher frequency in ITAC (78.4% versus 46.7%; p = 0.0248). Among ITACs, all the 15 mucinous adenocarcinomas were positive versus 62.7% of non mucinous ITACs (p = 0.0034) (Table 3). No correlation with disease free and overall survival was found (p = 0.617 and 0.988, respectively).

3.4. MUC 5AC

MUC5AC was focally expressed in ductal and acinar cells of the seromucous glands of sinonasal mucosa, and in rare surface epithelial cells. MUC5AC was positive in 16 adenocarcinomas (24.2%), including 29.4% of ITACs and 66.7% of non-ITAC, even though the difference was not significant (p = 0.927). Among ITACs, the mucinous subtype was more frequently positive than other subtypes (p = 0.0055) (Table 3). No correlation with disease free and overall survival was found (p = 0.476 and 0.275, respectively).

3.5. MUC6

MUC6 was not expressed in any of the adenocarcinomas as well as in normal sinonasal epithelium.

4. Discussion

To the best of our knowledge, this study shows for the first time the immunohistochemical profile of mucins in sinonasal adenocarcinoma and correlates findings with clinicopathological data and patients overall survival. Mucins are aberrantly expressed in many carcinoma types, including pancreatic [25–28], gastroenteric [29–37], breast [38–40], reproductive female system [41–44], kidney [45–47], urogenital [48–51] and lung [51–54]. Their expression is also often

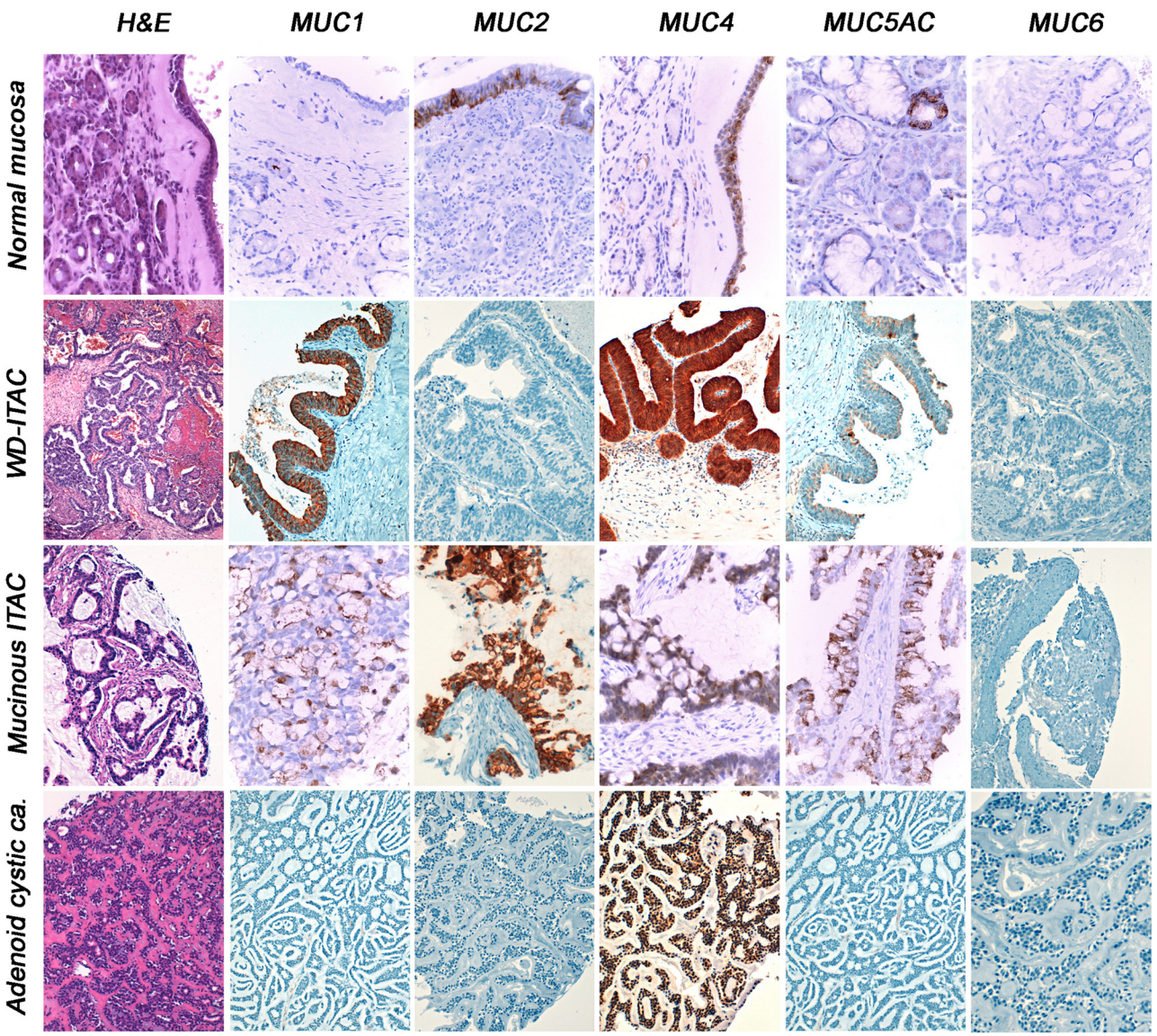

Fig. 1. Differential expression of mucins in non-neoplastic sinonasal mucosa (first row), in a case of well-differentiated intestinal type adenocarcinoma (second row), in a case of mucinous intestinal type adenocarcinoma (third row) and in a case of adenoid cystic carcinoma (fourth row). The mucin type is indicated at the top of the column. H&E: hematoxylin and eosin staining.

related to adverse prognosis and resistance to chemotherapy, in particular in pancreatic cancer [14,15]. According to our study, sinonasal adenocarcinomas also show aberrant expression of several mucin types in comparison with normal sinonasal mucosa.

MUC1 is frequently overexpressed in sinonasal adenocarcinomas, particularly in ITACs, and high expression is associated with worse prognosis. The expression of MUC1 in other head and neck subsites has been well documented, especially in oral squamous epithelium and in salivary gland tissue in normal and pathological conditions [18–24]. Indeed, in oral squamous cell carcinoma, several studies show overexpression of MUC1, with a frequency ranging between 18% and 96% [18]. In addition, some studies document a correlation between the overexpression of this mucin and prognosis, but results of other studies are conflicting [18,19]. In salivary gland neoplasms, up-regulation of MUC1 has been documented in the progression from pleomorphic adenoma to carcinoma ex pleomorphic adenoma [18]. Moreover, mucoepidermoid carcinomas with overexpression of MUC1 shows poor prognosis [21,24]. MUC1 is also expressed in normal and cancerous tissues of several organs. In the gastro enteric tract, MUC1 is expressed in most normal epithelia and in the malignant counterpart in pancreatic [15,25,26], stomach [29,30] and colon carcinomas [35]. In gastric carcinomas, few studies show a deregulation of MUC 1 expression [29–31], mostly with overexpression and association to poor prognosis [30], but other studies suggest a downregulation of this protein [29]. In colorectal adenocarcinomas, the expression of this protein is often present, while data on prognosis are conflicting [35]. On the other

Table 3
Distribution of positivity for mucins in sinonasal ITAC subtypes.

	MUC1 + n (%)	p-value	MUC2 + n (%)	p-value	MUC4 + n (%)	p-value	MUC5AC + n (%)	p-value
Non-mucinous (n = 51, 77.3%)	28 (54.9%)	0.244	7 (13.7%)	< .001	32 (62.7%)	0.0034	8 (15.7%)	0.0055
Mucinous (n = 15, 22.7%)	11 (73.3%)		14 (27.4%)		15 (100%)		8 (53.3%	

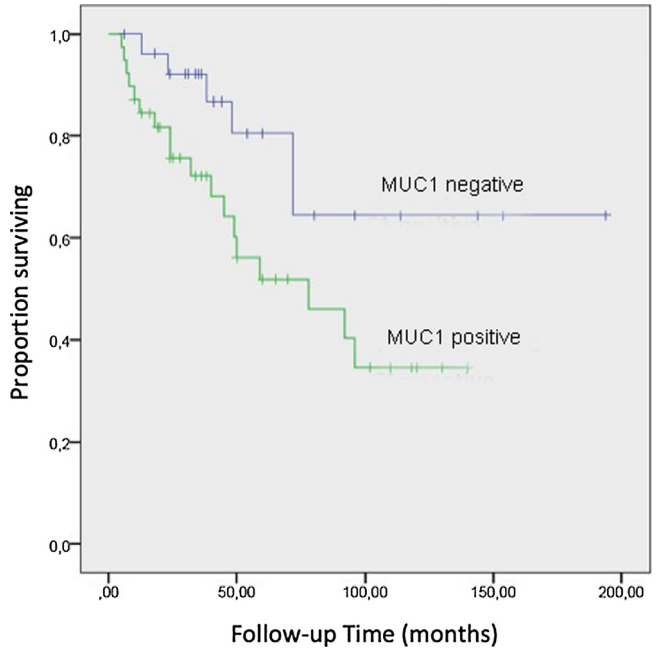

Fig. 2. Overall survival according to MUC 1 expression. High expression of MUC 1 was related to a significantly shorter overall survival (p = 0.04).

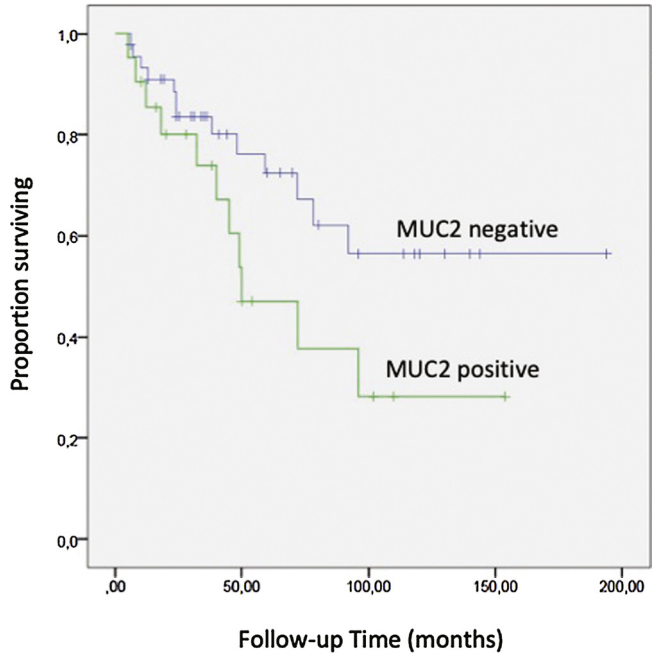

Fig. 3. Overall survival according to MUC 2 expression. Adenocarcinomas positive for MUC 2 tended to have a worse overall survival (p = 0.07).

hand, in pancreatic carcinoma both the expression and the correlation to poor prognosis are well documented [26]. MUC1 is expressed in normal breast epithelium [38] and overexpressed in carcinomas, both in situ and invasive lesions [38–40]. MUC1 expression is related to higher tumor grade in "in situ" carcinoma [39], while in invasive cancer marked heterogeneity is found regarding subcellular localization and prognosis. Indeed, the presence of MUC1 in the apical part of the cell (an indicator of intact MUC1 pathway) is associated with well differentiated tumours and good prognosis, while aberrant patterns of expression of MUC1 in cytoplasm or non-apical membrane are associated with a high tumor grade and shorter survival time [38]. In the female reproductive system, MUC1 is expressed in normal tissue of the ovary and in most benign and malignant serous lesions, as well as in a minor proportion of mucinous and endometrioid carciomas [41]. Regarding the uterus, MUC1 is expressed both in normal and in neoplastic endometrium [42,43] and cervix [44]. Interestingly, MUC1 expression is related to worse prognosis in mucinous adenocarcinoma of the cervix [43]. In the urinary tract, normal kidney [45], prostate [49,50] and bladder [48,51] epithelia express MUC1 and carcinomas of all these subsites are also positive. In renal cell carcinoma, one study suggests the correlation between the expression of MUC1 and adverse clinicopathological features [46], as in prostate carcinoma [50] and in bladder cancer [51]. In the lung, the expression of MUC1 has been widely studied [52,53]. In normal epithelium MUC1 expression is weak in tracheobronchial and collecting ductal epithelium, and it is negative in submucosal glands and bronchioles [52,53]. In malignant lesions, both squamous cell carcinomas and adenocarcinomas express MUC1, with relation to poor outcomes [52,53]. Thus, MUC1 overexpression in sinonasal adenocarcinomas and its relationship with poor prognosis is in line with the results of previous studies on different organs.

Regarding MUC2, our study shows that it is overexpressed only in ITAC, which is in line with the literature [2], with a significant predilection for the mucinous variant. In normal sinonasal mucosa, MUC2 is well known to be expressed in goblet cells of the respiratory epithelium [11]. In head and neck tumours MUC2 is often overexpressed, including in laryngeal squamous cell carcinoma [22,23], in some salivary gland neoplasm such as Warthin tumor, acinic cell carcinoma (in combination with MUC3) and in salivary duct carcinoma (with the association with the overexpression of MUC6) [18]. In carcinomas of other organs, such as stomach [29,31,32] colon [35,36], pancreatic [25,26], breast [38–40] and ovary [41], MUC2 is usually overexpressed. In some organs [10,13,25,40–42], MUC2 expression is related to mucinous variants of carcinomas, and sometimes, especially in colorectal carcinomas, in non-mucinous variant the loss or decreased expression of this protein is associated with poor prognosis [35,36]. In breast in situ carcinoma, MUC2 expression is associated with an higher grade of DCIS [39], while in invasive carcinomas there is a difference between mucinous and non-mucinous histotypes. Indeed, in mucinous variant MUC2 expression is related to better clinicopathological parameters, while in non-mucinous variant MUC2 is associated to an aggressive tumor behaviour [38,40]. In the ovary, MUC2 is strictly associated to mucinous lesions, both benign and malignant, while it is not expressed in normal ovarian tissue [41]. In the uterus, MUC2 is detected in normal endometrium exclusively in the secretory phase [44], but not in cervical mucosa [42]; in neoplastic tissue of the endometrium it is not found [44], whereas it is noted in 25%, 40%, and 22% of AIS, endocervical adenocarcinomas, and endometrial adenocarcinomas of the uterine cervix respectively [42]. In urothelial bladder carcinoma, expression of MUC2 relates with less aggressive behaviour [51], while this mucin is not expressed in prostate carcinoma [49]. MUC2 is generally observed in normal respiratory epithelium, except in the bronchioles and alveolar epithelial cells [52,53]. MUC2 is also expressed in adenocarcinomas, both mucinous and non-mucinous variants, while no expression is seen in squamous cell carcinomas [52,53].

MUC4 is the most frequently expressed mucin in sinonasal adenocarcinomas in our study, with significantly greater expression in ITAC, in particular in the mucinous subtype. In normal upper airways mucosa, MUC4 expression is seen mostly in globet cells [11]. In other head and neck sub-sites, MUC4 is frequently overexpressed, with an association with aggressive behaviour and poor outcome in oral squamous cell carcinoma [20] and a trend towards better prognosis in laryngeal squamous cell carcinoma [22,23]. MUC 4 is also expressed in normal salivary gland tissue as well as in salivary gland tumors, including secretory carcinoma and mucoepidermoid carcinoma, the latter with a relation with better prognosis [18,21,24]. In the gastroenteric tract, MUC 4 is frequently overexpressed and associated with poor prognosis in gastric carcinomas [33,34]. In colorectal adenocarcinoma, increased MUC4 expression was a predictor of poor survival, but only in patients

with early stage tumours [37]. Overexpression of MUC4 is frequently detected in pancreatic cancer [15,27,28], with a significant correlation with worst prognosis [28]. In breast carcinoma, MUC4 expression is strongly associated with higher tumor grade, but not with patient outcome [38]. In the gynaecologic tract, MUC4 is not expressed in normal ovarian tissue but it is found in borderline and malignant tumors, frequently associated to poor prognosis [52]. In the uterus, MUC4 is expressed both in normal endometrial and cervical mucosa and in endometrial and endocervical adenocarcinomas [42,43]. In the urological tract, low expression of MUC4 predicted poor prognosis in patients with clear-cell renal cell carcinoma [47], as in bladder cancer, where it is related to specific cancer death [51], while it is downregulated in prostatic cancer [48]. In lung normal epithelium MUC4 is expressed in almost every cells, including basal, ciliated and goblet cells [52,53] and it is also widely expressed in malignant lesions, both in adenocarcinomas and in squamous and in large cell carcinomas. Moreover overexpression is related with poorer survival in patients with squamous cell carcinoma and adenocarcinoma [52,53].

MUC5AC stains positive in approximately 25% of sinonasal adenocarcinomas, mostly in ITAC, with higher expression in the mucinous subtype, as seen for MUC2 and MUC4. In normal sinonasal mucosa MUC5AC is expressed in rare cells of surface epithelium and in glands [11]. In carcinomas of other head and neck subsites, MUC5AC is found in salivary gland carcinomas, mostly in mucoepidermoid carcinoma, and it could be an important tool in the differential diagnosis with squamous cell carcinoma which is MUC5AC negative [18,21,28]. MUC5AC is also present in carcinomas of the gastro enteric tract (gastric, pancreatic-biliary and colon carcinomas) and in some cases, as in gastric cancer, data on prognostic value of MUC5AC are discordant [29,32], since in early gasric cancer it seems to be related to a better prognosis [32], while in advanced stage of gastric carcinomas it is related to a worse prognosis [29]. In colorectal cancer the expression of MUC5AC is present [35,36] and it is related to better prognosis [35]. Overexpression of MUC5AC is also well documented in pancreatic neoplasms [15,25,26]. Overexpression of MUC5AC is variably noted in breast carcinomas, with frequency ranging between 6% and 37% [38,40], but without correlation with clinicopathological variables. Among tumours of the gynaecological tract, MUC5AC is expressed in borderline ovarian tumors, especially in the mucinous type and in invasive neoplasms ovary [41]. Considering uterine tumours, MUC5AC expression in endometrial adenocarcinoma shows marked variability in frequency, which is generally low, without association with clinicopathological variables [43,44] and in most of endocervical adenocarcinomas [42]. Conversely, most of the infiltrating endocervical adenocarcinomas, including adenosquamous carcinomas, express MUC5AC, a difference that could be of diagnostic relevance to separate endometrial from endocervical adenocarcinomas [43]. In normal lung tissue MUC5AC is expressed in trachea, main bronchi but not in the smaller airways. It is also expressed by goblet cells and glandular ducts [52,53]. Interestingly, MUC5AC expression is downregulated in squamous cell carcinoma while it is expressed in almost every subtypes of adenocarcinoma [52,53]. Patients with tumors expressing MUC5AC also have poorer overall survival [54].

Given the significant involvement of mucins in the development and progression of cancers at different anatomic sites, in the last few years these proteins have been widely studied as possible targets for new therapies. Interestingly, it has been demonstrated that MUC1 has a very close relation with the immune system during the cancerous processes. Indeed, MUC1 has an inhibitory effect on antitumor immune response, because of the interference with T cell proliferation and function [55]. Moreover, MUC1 secreted from cancer cells suppresses the lysis of cancer cells by natural killer cell killing [55,56]. Other studies document that there is a relation between the high level of MUC1 antibodies in cancer patient blood and MUC1-primed lymphocytes in the lymph node of the same patients [57]. Moreover, the presence of MUC1 antibodies in serum of breast cancer patients is related to better prognosis and lower frequency of metastasis [58]. In addition, patients who have anti-MUC1 antibodies in their sera probably in response to chronic inflammation or infections are at lower risk of developing cancer [59]. Based on these findings, it would be important to induce or stimulate MUC1-associated immunity with cellular or humoral response. MUC1 target therapy is based on vaccines, antibodies, drug inhibitors, cell-based therapy and miRNA based therapy. Phase 2 and phase 3 clinical trials employing vaccines are currently in progress in patients with breast, lung, colorectal pancreatic, prostate, kidney and ovarian cancer. Antibody based treatments for MUC1 are currently used in phase II studies for colorectal, gastric and ovarian cancer. MUC1 inhibitors and cell based therapy have started to be included in clinical trials, with phase II studies respectively against cutaneous T cell lymphomas/acute myeloid leukemia and ovarian cancer [13,15,60].

MUC4 is another mucin that has been widely studied as therapeutic target, especially in pancreatic cancer [13,15], where MUC4 is deregulated in several ways, including altered glycosylation, existence of multiple splice variants and multiplicity of epitopes. MUC4 has also a strong interaction with the immune system, by modulating the heterogeneity of immune cell population. Moreover, MUC4 seems to mediate drug resistance to gemcitabine, one of the most common drugs used in pancreatic adenocarcinoma. Thus, MUC4 could be a significant therapeutic target, both through the down-regulation of the expression, directly by silencing MUC4 gene targeting the transcription with miRNA, or indirectly with MUC4 pharmacological agent that inhibit the upregulation. Other therapeutic approaches include the stimulation MUC4 antitumor immune response by the use of vaccines or the induction of MUC4 overexpression for targeting cytotoxic or chemotherapeutic agents with antibodies.

A recent study on pseudomyxoma peritonei shows that even MUC2 could be a target for new treatments, especially to reduce tumor growth in mucinous adenocarcinomas, by using hypoxia (hypoxia-inducible factor-1α; HIF-1α) inhibitors [17]. Indeed, hypoxia seems to regulate the expression of MUC2 and MUC5AC, and in vitro models show that when MUC2 secreting cell are exposed to hypoxia, there is an increase of both MUC2 mRNA and protein. Accordingly, HIF-1α inhibition decreases MUC2 production and mucinous adenocarcinoma growth.

In conclusion, our data show that both normal sinonasal mucosa and sinonasal adenocarcinomas have a characteristic expression of different mucin types, with significant clinicopathologic correlations. In view of the extensive involvement of mucins in different aspects of tumor growth and their emerging role as possible therapeutic targets, our study suggests that these factors could be considered clinically relevant biomarkers and attractive targets for new treatments in sinonasal adenocarcinomas, especially in patients presenting with high stage disease or with disease recurrence. Further studies on larger series of patients are needed to explore these attractive hypotheses.

Declaration of interest

The Authors declare they have no conflict of interest.

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