

Are goblet cell carcinoids a group of heterogeneous tumors?

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Background. Goblet cell carcinoids belong to neuroendocrine tumors, according to the WHO classification. The tumors are diagnosed based on a typical histological pattern and using neuroendocrine markers. However, some tumors do not react with these markers and yet expression of proliferative markers is high. Do these tumors belong to G1 and G2 neuroendocrine tumors?

Methods. The sample comprised nine cases of tumors of the appendix identified by immunohistological methods as goblet cell carcinoids or adenocarcinoma ex goblet cell carcinoid.

Results. In six cases, hematoxylin and eosin staining revealed tumors completely or 90% made of characteristic large tumor cells observed in typical goblet cell carcinoids. The remaining three cases were identified as adenocarcinomas arising ex goblet cell carcinoids. Immunohistological examination revealed that in four cases of typical goblet cell carcinoids, expression of neuroendocrine markers was low or completely negative. Yet in two cases, the Ki-67 proliferative index exceeded the 20% cut-off for inclusion in the G1 and G2 category.

Conclusions. Goblet cell carcinoids are a heterogeneous group of tumors that may express neuroendocrine markers in a small number of tumor cells or are negative to these markers. However, high expression of the proliferative marker Ki-67 exceeds the criteria for G1 and G2 neuroendocrine tumors. It is our opinion that these tumors may be classified as a specific type of carcinoma.

Key words: goblet cell carcinoid, appendix, immunohistology, negative neuroendocrine markers

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INTRODUCTION

Tumors referred to as goblet cell carcinoids (GCCs) are uncommon¹⁻³. They are made of large cells. The cytoplasm is filled with mucin, the nuclei are pushed to the periphery under the cell membrane. Mucin production has always been thought to be related to adenocarcinomas but mucin has also been demonstrated in carcinoids^{4,6}. Gagné et al.⁷ described three tumors of the appendix considered to be a transitional type between carcinoid and adenocarcinoma. Höfler et al.⁸ reported production of mucins and 5-hydroxytryptamine in a single tumor cell.

These tumors are not difficult to recognize. The tumor cells have an appearance resembling goblet cells or signet ring cells with mild cytological atypia. The cells form irregular, mostly small nonlumenized aggregates without the palisade arrangement of tumor cells near the stroma. There are no signs of desmoplasia around the tumor. Unlike “classical” carcinoids, they do not form solid tumor masses that are macroscopically visible. A striking feature is invasion of the tumor into all layers of the appendix. The entire thickness of the appendix wall is involved. The way the tumor spreads makes a pathologist suspect a primary lesion in another location such as in the cecum or elsewhere in the gastrointestinal tract. According to some authors⁹, tumors with a similar appearance may occur outside the appendix. However, some more recent studies show that tumors with an appearance

similar to GCCs are rarely found in other locations. The prognosis is less favorable compared to “classical” G1 and G2 neuroendocrine tumors. In different cases, immunohistological examination results vary considerably. Frequently, neuroendocrine markers are positive in only small numbers of tumor cells or completely negative. To a large extent, pathological diagnosis relies on the special appearance and arrangement of tumor cells. Classification of these tumors remains a matter of discussion. The study aimed at comparing expression of neuroendocrine markers with expression of a marker of proliferation (Ki-67 proliferative index).

MATERIALS AND METHODS

In archives of the Department of Pathology, First Faculty of Medicine, Charles University and General University Hospital in Prague and the Department of Pathology, Faculty of Medicine, University of Ostrava and University Hospital in Ostrava, a total of nine cases were found that met the diagnostic criteria for GCC.

Immunohistological examination was carried out using the avidin-biotin complex (ABC) method. The following antibodies were used (working dilutions are given in parentheses): AE1-AE3 clone AE1-AE3 (1:50), Ck20 clone Ks 20.8 (prediluted), Ki-67 clone MIB-1 (1:50), Ck7 clone OU-TL 12/13 (1:50), NSE clone 2F11 (1:50), rabbit

Table 1. Immunoprofile of GCC and adenocarcinoma ex GCC (Nos. 1, 4, 7).

Case	1	2	3	4	5	6	7	8	9
Synapto	30 %	Neg.	80 %	60 %	80 %	10 %	40 %	5 %	Neg.
Chromo	Neg.	Neg.	80 %	30-40 %	40-50 %	Neg.	Neg.	Neg.	Neg.
CD56	50 %	5-10 %	Neg.	30-40 %	90 %	20 %	40 %	Neg.	Neg.
NSE	80 %	10 %	30 %	1 %	50 %	20 %	40 %	Neg.	Neg.
Ck7	50 %	20 %	40 %	80 %	50 %	50 %	90 %	80 %	Neg.
Ck20	80 %	80 %	80 %	80 %	90 %	90 %	80 %	90 %	90 %
Ki-67	20 %	10-20 %	40 %	30 %	60-70 %	5 %	5-20 %	20-40 %	80 %
CEA	90 %	100 %	100 %	90 %	100 %	100 %	90 %	100 %	90 %
AE1-AE3	100 %	100 %	90 %	90 %	100 %	100 %	100 %	90 %	100 %
Somato	0	0	Neg.	Neg.	Neg.	Neg.	Neg.	0	Neg.
Sero	0	0	Neg.	20 %	30 %	Neg.	Neg.	0	Neg.
Gluca	0	0	0	0	Neg.	0	0	0	Neg.
E-cad	100 %	80 %	80 %	Neg.	100 %	0	0	100 %	100 %
P53	Neg.	10 % C	Neg.	Neg.	80 % C	0	0	0	0
Beta-cat	100 %	100 % +/-	100 %	0	100 %	0	0	100 %	100 %

Legend: Synapto – synaptophysin; Chromo – chromogranin; Somato – somatostatin; Sero – serotonin; Gluca – glucagon; E-cad – E-cadherin; 0 – not performed; Neg. – negative; C – cytoplasmic positivity; beta-cat – beta-catenin

Table 2. Metastasis to lymph nodes and distant metastasis

Metastasis to lymph nodes (the numbers refer to cases in Table 1)	Tumor spreading to the surrounding tissues and distant metastasis (the ovary, uterus and omentum)
3, 5, 8	1, 3, 8

anti-human somatostatin polyclonal antibody (1:1000), E-cadherin clone NCH-38 (1:50), beta-catenin clone beta-catenin-1 (1:400) – all antibodies produced by DAKO, Glostrup, Denmark; synaptophysin clone 27G12 (1:100), chromogranin A clone 5H7 (1:100), CD56 clone 1B6 (1:50), p53 clone DO-7 (1:400) – all antibodies produced by Novocastra, Newcastle-upon-Tyne, UK; and CEA rabbit polyclonal antibody (1:50) – produced by Biogenex.

RESULTS

The unselected sample of nine patients comprised six females and three males; their ages ranged from 53 to 73 years. All patients were admitted and operated on for appendicitis. In six cases, hematoxylin and eosin staining revealed tumors completely or 90% made of characteristic GCC structures (Fig. 1). The remaining three cases (Table 1, Nos. 1,4,7) had a different histological pattern. This was characterized by medium-sized cells with morphology different from that of goblet or signet ring cells. These formed trabecules, bands, tubules; alternatively, isolated tumor cells infiltrated the appendix wall. The nucleoplasmic ratio was in favor of the volume of the nucleus. The nuclei were of irregular shape in some areas, hyperchromatic and, sometimes, with distinct nucleoli. Large cells with mucin, characteristic for GCC, were found in 10–20% of tumor cells in these cases. Such tumors were identified as adenocarcinomas arising ex GCC (Fig. 2, 3, 4).

Immunohistological examination revealed that in four cases, GCC-like tumor cells did not react with neuroendo-

crine markers (in particular synaptophysin), either at all or in only a small proportion (5% and 10% of the cells) (Fig. 5a, 5b). Yet the Ki-67 proliferative index was positive in 10–20% of cells of one tumor (No. 2) and in 5% of cells of another tumor (No. 6). In the third (No. 8) and fourth (No. 9) cases, the rates were 20–40% and 80%, respectively (Fig. 6). In the remaining cases, neuroendocrine markers and, especially, positivity against synaptophysin were demonstrated in as much as 80% of the cell population, with a high Ki-67 proliferative index, ranging from 30% to 60% for individual patients. Positive results for an antibody against chromogranin were noted in only three cases. All tumors showed positive results with antibodies against cytokeratins AE1-AE3, CEA and cytokeratin 20. In eight cases, positivity with an antibody against Ck7 was observed. With an antibody against the p53 protein, the finding was negative. In two cases, cytoplasmic positivity was documented. Positive findings with an antibody against E-cadherin were seen in six out of seven cases. With an antibody against beta-catenin, positive findings were found in six out of six cases (Table 1).

Table 2 summarizes cases of metastasis to lymph nodes or local spread and distant metastasis to the ovary, uterus and omentum (the numbers refer to cases shown in Table 1).

DISCUSSION

GCC was first described in 1974 (ref.¹⁰). According to some authors³, it accounts for approximately 14% of all appendix tumors. In the WHO classification of carci-

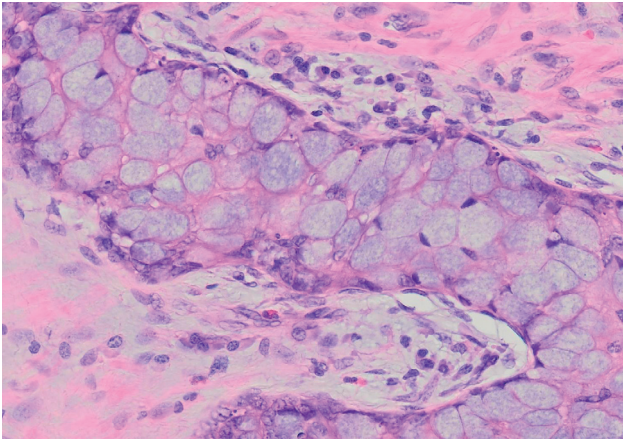


Fig. 1. Characteristic histological pattern of tumor cells with an appearance consistent with goblet cells or signet ring cells (HE, 200x).

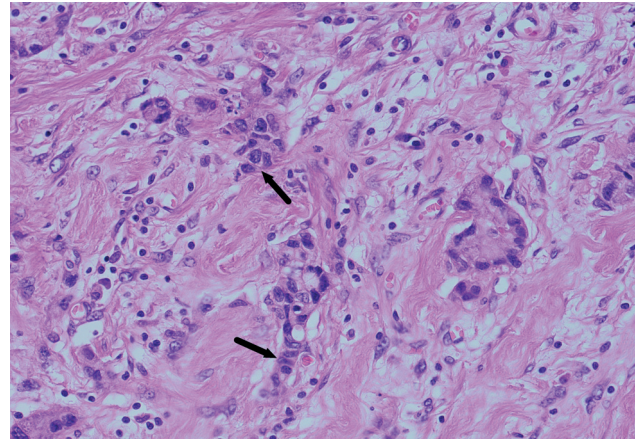


Fig. 2. Tumor GCC transforms into adenocarcinoma ex-goblet cell carcinoid (arrows point to adenocarcinoma cells) (HE, 200x).

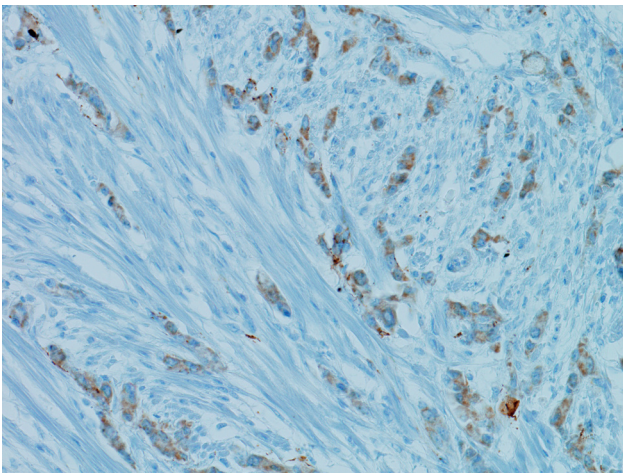


Fig. 3. Adenocarcinoma ex-goblet cell carcinoid cells are positive with a marker against synaptophysin (synaptophysin, 200x).

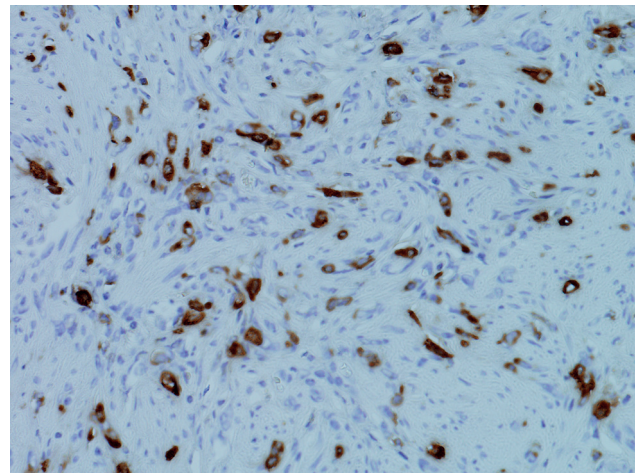


Fig. 4. Isolated cells of adenocarcinoma ex-goblet cell carcinoid positively react with an antibody against chromogranin (chromogranin, 200x).

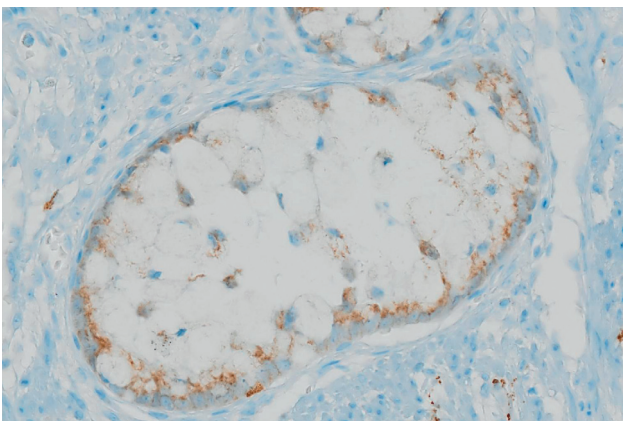


Fig. 5a. Comparison with a synaptophysin-positive focus of GCC (case No. 7) (synaptophysin, 200x).

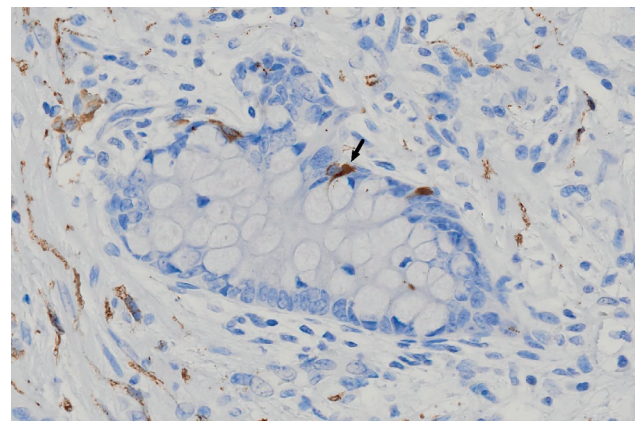


Fig. 5b. Immunohistochemical reaction with an antibody against synaptophysin. The tumor cells are negative. There are sparse non-tumor positive neuroendocrine cells (arrow) (synaptophysin, 200x).

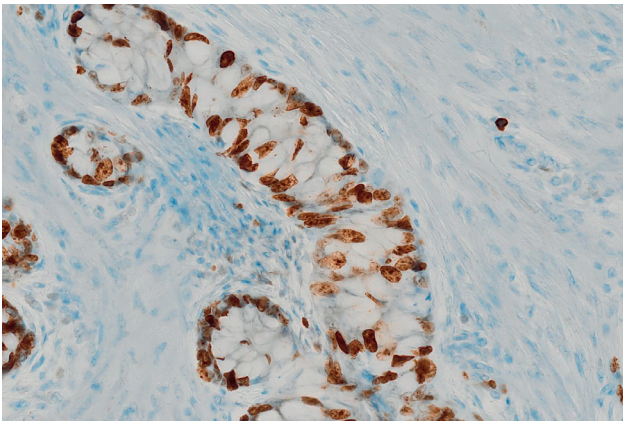


Fig. 6. Immunohistological reaction with the Ki-67 antibody. Significant positivity of most tumor cells (Ki-67, 200x).

noids, it is regarded as a separate entity and not as a variant of “classical” carcinoids of the appendix. Relatively frequently, GCC transforms into adenocarcinoma. This, together with other phenotypic and immunohistological characteristics distinguishing GCCs from “classical” carcinoids made some authors conclude that it is carcinoma from the very beginning. They referred to it as crypt cell carcinoma, microglandular goblet cell carcinoma or composite goblet cell carcinoid-adenocarcinoma^{1,11-14}. GCC is histogenetically derived from pluripotent epithelial cells at the crypt base whereas “classical” carcinoids arise from subepithelial neuroendocrine cells originating from the neural crest^{12,15-18}. The differences between the two types (both morphological and clinical) allowed for publication of a novel variant of neuroendocrine tumors of the appendix, namely combined classical carcinoid and GCC tumor¹⁵. GCC is characterized by production of neutral and acid glycosaminoglycans in the cytoplasm of tumor cells, demonstrated by the PAS method and alcian blue staining. At the same time, positive results are obtained by immunohistological examination with neuroendocrine markers such as synaptophysin, chromogranin and CD56. In some cases, however, positivity rates using these markers is relatively low or tumors are completely negative. According to some authors¹², the diagnosis is more dependent on the morphological pattern than immunohistology results which, in rare cases, may be negative. The question arises as to which criteria should be applied to classify such tumors as neuroendocrine. In the present study, positivity rates with the Ki-67 proliferative index was considerably higher in two cases (Nos 8 and 9) than in G1 and G2 neuroendocrine tumors, ranging from 20% to 40% (No. 8) and reaching as much as 80% (No. 9). Even though the Ki-67 index is much higher than in “classical” carcinoids, an association between prognosis of the disease and the Ki-67 proliferative index has not been demonstrated as yet^{19,20}. Tang et al.¹ studied a sample of 63 GCC patients divided into three groups based on tumor cell differentiation. Only in the third group, including cases with poorly differentiated adenocarcinomas ex GCC, the Ki-67 positivity rates reached 80%, as was the case in adenocarcinomas of the appendix. It means that

there is a relationship between grading and positivity of the marker Ki-67. Higher Ki-67 positivity rates (> 10–20%) are also observed in tumors that metastasize. Tumors with the Ki-67 below 5% usually grow locally²¹. In the present study, two cases of typical GCC showed positivity rates well above 20%, thus exceeding the range for G1 and G2 neuroendocrine tumors as defined by the current WHO criteria.

The results of immunohistological examination using the Ki-67 proliferative index are not always reliable for assessment of the aggressive behavior of tumors. Pericleous et al.²² reported a case of GCC with lung metastasis and a Ki-67 proliferative index of less than 5%. In four cases of metastasis to lymph nodes and distant metastasis identified in the present study, the Ki-67 proliferative index ranged from 20% to 70%.

A considerable number of cases of adenocarcinoma of the colon or appendix are associated with mutations in the genes for beta-catenin and p53. In the present study, p53 protein overexpression was not detected; intracytoplasmic positivity was observed in only two cases. Using an antibody against beta-catenin, positive findings were seen in six out of six cases. In one case, the intensity of staining was low. With an antibody against E-cadherin, positive findings were documented in six out of seven cases. Positive findings were seen in 80–100% of cells. In one case, the finding was negative. O'Dowd and Gosney²³ failed to detect p53 protein overexpression in “classical” carcinoids in the gastrointestinal tract. Similar results were observed in GCC (ref.¹).

So far, molecular genetic pathology results have shown genetic alterations compatible with GCC rather than with appendix tumors^{1,24-26}. On the other side, van Eeden et al.²⁷ used 21 immunohistological markers in their study and found expression of the transcription factors Math1 and HD5 in GCC and adenocarcinomas but not in “classical” carcinoids. The authors inferred that GCC and adenocarcinomas of the appendix have more common features than “classical” carcinoids. The marker CEA, positive in colon adenocarcinoma, was significantly positive in all cases in the present study. It tends to be negative in “classical” carcinoids. We assume that cases with negative or very little positive (< 10% positivity) neuroendocrine markers and highly positive findings with Ki-67 should be identified as a special group referred to as a specific type of carcinoma by van Eeden et al.²⁷.

CONCLUSION

GCC of the appendix continue to be included among neuroendocrine tumors in the WHO classification, even though, in some cases, expression of neuroendocrine markers is low or completely absent. Yet the Ki-67 proliferative index exceeds values set for G1 and G2 neuroendocrine tumors. Patients with these tumors have a poorer prognosis than those with “classical” G1 and G2 neuroendocrine tumors. GCCs seem to be a heterogeneous group of tumors with an identical phenotype but different immunohistological profiles. It is our opinion

that such tumors should be classified as a specific type of carcinoma, as suggested by van Eeden et al.²⁷.

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Conflict of interest statement: The authors state that there are no conflicts of interest regarding the publication of this article.

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