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A Study of Appendiceal Crypt Cell Adenocarcinoma (So-Called Goblet Cell Carcinoid and Its Related Adenocarcinoma)

Daisuke Nonaka, MD ^{1,4}, George Papaxoinis, MD, PhD ², Angela Lamarca, MD, PhD ², Paul Fulford, MBBS, FRCS ³, Juan Valle, MBChB, MSc, FRCP ^{2,5}, Bipasha Chakrabarty, MBBS, MD, FRCPath ¹

1. Department of Histopathology, The Christie NHS Foundation Trust, Manchester, UK

2. Department of Medical Oncology, The Christie NHS Foundation Trust, Manchester, UK

3. Department of Surgery, The Christie NHS Foundation Trust, Manchester, UK

4. Institute of Cancer Sciences, The University of Manchester, Manchester, UK

5. Institute of Cancer Studies, The University of Manchester, Manchester Academic Health Science Centre, Manchester, UK

Corresponding author: Daisuke Nonaka, M.D.

Address: St Thomas' Hospital, Department of Cellular Pathology
2nd Floor, North Wing, Westminster Bridge Road
London SE1 7EH, United Kingdom

Telephone number: +44 (0)207 188 2945, Fax number: +44 (0)207 188 2948

E-mail address: DNonaka@msn.com

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Abstract

Goblet cell carcinoids (GCCs) of the appendix are rare tumors, characterized by a carcinoid-like organoid growth pattern. Despite the term carcinoid, neuroendocrine features are inconspicuous, and its behavior is distinct from carcinoid. Its high grade counterpart is designated as adenocarcinoma ex GCC. We conducted a retrospective study of 105 tumors to find prognostic values of a variety of clinico-pathologic features.

The tumors were subclassified as low grade, equivalent to classic type, and high grade, defined as loss of organoid pattern, and a proportion (%) of low and high grades were documented in each tumor. Correlations between survival and various clinico-pathologic parameters were investigated. One-third were pure low grade while the remainder contained variable high grade component ranging 5-95%. Neuroendocrine cell component ranged 0-90% (median 5) while mucus cell component ranged 5-100% (median 70). By univariate analysis, size, stage, high grade component, nuclear grade, surgery and chemotherapy correlated with cancer-related survival (CSS), and by multivariate analysis, stage ($p=0.001$), high grade component ($p=0.008$) and tumor size ($p=0.005$) correlated with CSS. There was significant difference in CSS when the cases were grouped in high grade component <40%, 40-90 and $\leq 90\%$ ($p<0.001$).

Our results indicate that staging and proportion of high grade histology may provide important prognostic information. Neuroendocrine component was insignificant in both low and high grade areas. In light of our findings, this tumor type is best regarded as a variant of adenocarcinoma, and the term crypt cell adenocarcinoma more appropriately reflects the nature and origin of this tumor group.

INTRODUCTION

Goblet cell carcinoid (GCC) and adenocarcinoma ex GCC represent the low grade and high grade spectrum of a group of rare appendiceal tumors with distinct clinical and pathologic characteristics. A few studies have demonstrated a correlation between high grade transformation (so-called adenocarcinomatous transformation) and clinical outcome [1-4]. In this study, we performed a comprehensive analysis of 105 tumors to describe various clinical and pathologic features, and investigate potential prognostic factors, with an aim to identify an informative and objective grading system. Issues regarding whether GCC is a variant of adenocarcinoma or carcinoid tumor will also be discussed.

MATERIAL AND METHODS

GCC of appendix and its related adenocarcinoma (adenocarcinoma ex GCC) were retrieved from the histopathology archives of The Christie NHS Foundation Trust during the period from 1993 to 2016. Inclusion criteria for the study were a primary appendiceal tumor treated by resection, availability of histologic slides, and follow-up information. Tumors arising in association with mucosal dysplastic lesion such as tubular adenoma were not included in the study. A total of one hundred five tumors (46 goblet cell carcinoids and 59 adenocarcinomas ex goblet cell carcinoid) were investigated.

Hematoxylin and eosin (H&E) stained sections from only primary appendiceal tumor were evaluated for a variety of morphological features described below. When the tumor directly extended to the neighboring organs, the whole tumor involving the

appendix as well as invading the other organs was evaluated for histologic features. The number of slides examined for the primary tumor per case ranged from 2 to 51 (median, 16).

Tumors were assessed for low grade and high grade components, and proportions (%) of both components in 5% increments were documented. Low grade tumor component was defined as organoid nests of cells constituting an admixture of four cell types: mucus cells, eosinophilic cuboidal to columnar cells, neuroendocrine cells, and Paneth cells. The nests were generally rounded with smooth contour, but could display compressed linear configuration, when they were seen in the muscularis propria [1,5]. Some nests also contained lumens, which were mostly small, but could have dilatation due to accumulated intraluminal mucus secretion or necro-inflammatory debris [5]. Low grade component encompassed, on one end of the spectrum, classic GCC, where the tumor was composed of nests of predominantly goblet cells, and on the other end of the spectrum, tubular adenocarcinoid by Warkel or microglandular adenocarcinoma by Wolff, where the tumor was composed of small discrete acini or tubules lined by a single layer of cuboidal or columnar cells with eosinophilic cytoplasm [1,6,7]. Of note, the latter type (tubular adenocarcinoid) is different from so-called tubular carcinoid, a variant of classic carcinoid tumor, which comprises exclusively neuroendocrine cells.

High grade component was defined by any signs of loss of organoid pattern and acquired irregularity and complexity in nests, including complex branching cords, enlarged or confluent nests or irregular nests with jagged contours, and fused or cribriform glands. Furthermore, patterns generally indicating poorly differentiated

tumor, such as large lobules and solid sheets, and individual discohesive (poorly cohesive) single cells or single files were also included in high grade category.

Nuclear grade was divided to low and high grade. Low grade was defined by small size, round and smooth nuclear contour, uniform chromatin pattern, and small pinpoint-sized nucleoli. Prominent nucleoli were often seen in eosinophilic cuboidal to columnar cells [6]. If other nuclear features were those of low grade, presence of enlarged nucleoli per se was not regarded as high nuclear grade feature. High nuclear grade was defined by larger size, chromatin irregularity, vesicular chromatin, and prominent nucleoli, often accompanied by increased mitotic figures and apoptotic bodies.

Cellular component was subdivided to mucinous cells (goblet cells, signet ring cells) and non-mucinous cells, and a proportion (%) of each cell type in 5% increments was documented. The presence or absence of Paneth cells was also documented. Additionally, in 77 tumors, immunohistochemistry for chromogranin A (cocktail of clones LK2H10 and PHE5, 1:200, MenaPath, Berkshire, UK) and synaptophysin (clone 27G12, 1:50, Novocastra, Newcastle, UK) were performed on a Ventana Benchmark Ultra automated staining instrument (Tucson, AZ, USA) according to the manufacturer's instructions. Neuroendocrine cell component was evaluated based on the synaptophysin and chromogranin A stained sections. Proportion (%) of positive staining for each neuroendocrine marker in 5% increments was documented, and the greater percentage in the two stains was regarded as the representative neuroendocrine component in the given tumors.

The following pathological and clinical parameters were recorded: age at the time of initial diagnosis, gender, tumor size, perineural invasion, lymphovascular

invasion, vascular invasion, resection margin, stage (appendiceal carcinoma, TNM 7th edition), type of initial surgery, chemotherapy, and recurrence. Follow-up information was obtained from the hospital medical records, and follow-up period and length of survival were documented.

The statistical analysis was performed using Statistical Package for the Social Sciences for Windows version 22 (SPSS, Inc., Chicago, IL, USA). Parametric and non-parametric tests were used in the analysis. Cancer-specific survival (CSS) was defined as the time from surgery to death from GCC. Patients who died from other causes were censored at the date of death. Median CSS was calculated by Kaplan-Meier curves and log-rank test was used for median CSS comparisons. Cox proportional hazard models were used for univariate and multivarilte analysis. Multivariable Cox-regression model analysed baseline variables for possible prognostic significance, with a forward selection procedure and a removal criterion of $p > 0.10$. All analyses were two-tailed and p values of ≤ 0.05 were considered significant.

RESULTS

Clinical Features

Clinical and pathologic features are listed in Table 1. The most common clinical presentation was symptoms suggestive of acute appendicitis (48/105, 46%), followed by abdominal pain (31/105, 30%). Symptoms due to abdominal or pelvic mass of presumed ovarian origin were documented in 17% (18/105), and constituted 33% of female patients. Two tumors were an incidental finding during surgery for unrelated disease. An appendiceal mass was found on CT in a patient with

ulcerative colitis. One patient presented with mucocoele. Information was not available in three patients.

Nineteen patients underwent only appendectomy (18%) while the remainder received more extensive surgeries. Ten patients were treated by appendectomy and concurrent salpingo-oophrectomy with or without hysterectomy for metastatic tumors. 42 patients received right hemicolectomy, twelve of which followed initial appendectomy. Among the 42 patients, nine also underwent salpingo-oophrectomy with or without hysterectomy for metastatic tumors. One patient with ulcerative colitis received a total colectomy. 30 patients received appendectomy, followed by cytoreduction and hyperthermic intraperitoneal chemotherapy (HIPEC) while 3 patients received right hemicolectomy, followed by cytoreduction and HIPEC. One patient with peritoneal disease received cytoreduction and HIPEC as an initial surgical treatment. Overall 34 patients received cytoreduction and HIPEC. 40 patients subsequently received systemic chemotherapy.

Pathologic Features

Macroscopically, the appendix had features of acute appendicitis in 44 cases (42%). Mass was observed in 29 cases (28%). In 19 cases, the appendix was indurated, thickened or strictured, without mass formation. In one case, the appendix was partially replaced by mucoid material. Other findings included luminal dilatation in three cases, edematous change in two, adhesions in one, congestion in one, and pale discoloration in one. The appendix was unremarkable in two cases. No gross findings were documented in two cases.

Microscopically, the appendiceal wall was circumferentially infiltrated by neoplastic cells often without a mass formation, and with relative preservation of the

pre-existing anatomic structure. 35 tumors (33%) showed exclusively low grade histology while the remaining 70 tumors (67%) contained variable proportion of low and high grade histology, with high grade component ranging from 5 to 95%. Among 35 tumors with pure low grade morphology, 24 tumors showed exclusively conventional GCC morphology (Figure 1A) while the remaining 11 tumors contained variable proportion of microglandular pattern, ranging from 5 to 95% (Figures 1B-1D). The latter type often composed of eosinophilic cells with scattered goblet cells, remarkably resembling crypt architecture (Figure 1B).

In the majority of tumors (81%), more than one growth pattern was seen and various patterns were often intermixed. Notably high grade histology comprised a wide range of histologic features as recently described [8]. As a definition of our system, there was loss of organoid morphology, including irregular, angulated, enlarged, or confluent nests (Figures 2A-2D), complex branching cords, diffuse sheets, large lobules, discohesive cells, and single cell files (Figures 3A-3D). Foci with morphological features similar to enteric type adenocarcinoma were also seen. Neuroendocrine components ranged from 0 to 90% (median 5) while mucus cell components ranged in amount from 5 to 100% (median 70) (Figures 4A-4D). There was no correlation between the proportion of neuroendocrine cell component and high grade histology component ($p=0.366$), or between the proportion of mucinous cell component and high grade histology component ($p=0.355$). Mucin extravasation was seen in 58 tumors, ranging from 0 to 95% (median 30%). The tumors with copious extracellular mucin resembled mucinous (colloid) carcinoma. Paneth cells were seen in 34 tumors (32%), and generally only a few cells were seen in the tumor by close inspection at high power magnification, but in three tumors, clusters of Paneth cells were noted. While each pattern of growth in high grade components

could resemble other tumor types, e.g., gastric signet ring cell carcinoma, mammary lobular carcinoma, colorectal adenocarcinoma, mucinous (colloid) carcinoma and so forth, it did not present as a pure histologic pattern, and the proportion of these patterns within a given tumor was highly variable and different patterns were intermingled. Low grade organoid architecture was also identifiable even in tumors with predominantly high grade histology.

Stage was divided to stage I/II, III and IV. There were 57 patients with stage I/II (54.3%), 13 with stage III (12.4%), and 35 with stage IV (33.3%) (Table 1). Distribution of high grade component values was stratified by stage (Figure 5). Median value of high grade component was significantly higher in stage III/IV group compared to stage I/II group ($p < 0.001$).

Survival Outcomes and Prognostic Factors

Follow-up information was available in all patients, with a follow-up period ranging from 4 to 277 months (median 56). 45 patients (42.9%) were alive with no evidence of disease, 9 (8.6%) were alive with disease, and 6 (5.7%) were alive with disease status unknown while 43 patients (40.9%) died of tumor, and 2 (1.9%) died of other causes. Median CSS was 67 months (95%CI, 38.2-95.8). All the patients who died of disease had progressive peritoneal disease. In three patients, extra-peritoneal distant metastasis occurred after uncontrolled peritoneal disease; one developed liver metastasis, another had brain and bone metastases, and the third had bone metastasis. No patients developed extra-peritoneal distant metastasis without progressive peritoneal disease.

Univariate Cox regression analysis for CSS is shown in Table 2. Primary tumor size, stage (Figure 6A), nuclear grade, high grade component (%), type of

surgery and administration of chemotherapy were found to be unfavorable prognostic factors. Multivariate Cox-regression analysis confirmed that stage, high grade component (%) and primary tumor size were independently associated with CSS.

Cases were subdivided according to the high grade component after taking into consideration the median value (40%) and the high number of cases sequestered in two extremes, 0% and 90-100%. According to this concept, cases were initially allocated in 4 groups, those without high grade component, those with 1-39% high grade component, those with 40-89% high grade and finally those with 90-100% high grade. Because of low number of cancer-related deaths, the first 2 groups were examined as a single group (0-39%). CSS was significantly different between the 3 groups (0-39% vs. 40-89%: HR=3.91, 95%CI=1.42-10.81, $p=0.009$; 0-39% vs. 90-100%: HR=13.17, 95%CI=5.71-30.38, $p<0.001$; 40-89% vs. 90-100%: HR=2.86, 95%CI=1.20-6.81, $p=0.018$) (Figure 6B). We also investigated our cases with different cut-off points, 25 and 50%, the similar system used in the study by Taggart MW et al [2]. <25% vs. 25-50% did not differ in CSS (chi-square 0.646, $p=0.422$). >50% had poorer CSS compared to <25% (chi-square 53.010, $p<0.001$) and 25-50% (chi-square 12.114, $p=0.001$).

Interestingly, although increasing size of the primary tumor was an adverse prognostic factor in univariate analysis, this was associated with favorable outcome in multivariate analysis. Subgroup analysis showed that increasing tumor size was not associated with outcome in patients with stages I-II (HR 0.99, 95%CI 0.93-1.06, $p=0.820$), while in patients with stages III-IV, increasing tumor size had a trend for longer CSS (HR 0.99, 95%CI 0.97-1.00, $p=0.084$). A statistically significant interaction was found between tumor size and stage with regard to their effect on

CSS ($p=0.008$). Median tumor size in patients with stages III-IV was 45mm. Patients with stages III-IV and size ≥ 45 mm (above or equal to median) had median CSS 2.5 years (95%CI 1.4-3.6) which was statistically longer than those with smaller tumors (median CSS 1.5 years, 95%CI 0.6-2.6, $p=0.048$).

DISCUSSION

GCC is a rare tumor with distinct morphologic features, and it almost exclusively occurs in the appendix. The prototypical GCC is composed of small uniform nests of tumor cells. The tumor is characterized by a submucosal growth, with a concentration of neoplastic elements around the basiglandular portion of the mucosa, the findings suggesting that the tumor arises from the crypts, while the mucosa is characteristically spared and lacks dysplastic change [9]. In 1969, Gagne F et al reported, in French literature, three cases of this tumor type as an intermediate form between carcinoid and adenocarcinoma of the GI tract [10]. However, this appendiceal tumor type had been recognized as adenocarcinoma prior to this publication [11]. For example, figures 114 and 115 in the section of adenocarcinoma of the appendix vermiformis in the AFIP fascicle published in 1967 illustrate typical examples of GCC [11]. Since Gagne's report, a number of case reports and series of this tumor type have been published under a variety of terms: adenocarcinoid [6], mucinous carcinoid tumor [12], microglandular goblet cell carcinoma [7], and crypt cell carcinoma [13]. Its high grade progression / transformation has been called mixed carcinoid-adenocarcinoma, mixed adenoneuroendocrine carcinoma, and adenocarcinoma ex-GCC [1,3,14].

It has been believed that the justification for including this tumor within the carcinoid group rests on rather superficial histological resemblances to classical carcinoid tumors [6,13], and there have been questions over placing GCC into a category of neuroendocrine tumors [9] given that the presence of neuroendocrine cells have also been reported in adenocarcinoma of GI tract [15-18]. The term GCC was originally coined by Subbuswamy SG, et al [19]. It is of interest that their article states, "The number of argentaffin cells observed was considerable, but no greater than is seen in some adenocarcinomas of the stomach and colon", "It might be argued that this tumor is a type of very well-differentiated mucinous adenocarcinoma. However, comparison with such tumors in other parts of the gastrointestinal tract reveals important differences.", "we suggest the term goblet cell carcinoid, if only because the principal cell type closely resembles, both morphologically and functionally, the goblet cell of the intestinal tract." It is evident from their description that the emphasis was on goblet cells but not on neuroendocrine cells. Indeed, the neuroendocrine cells are variably present and often inconspicuous, or completely absent in some of the tumors as our study also confirmed [2,7,19-25].

Ultrastructurally, some have claimed that neurosecretory granules were demonstrated within mucin-containing goblet cells [5,26], indicating amphicrine (endo-exocrine) nature, which is regarded as a special form of neuroendocrine cells, hence, supporting the notion that GCC is an example of carcinoid tumor. However, others could not identify cells containing both structures in GCC [25]. In our study, strong expression of synaptophysin and/or chromogranin to a variable extent was seen in mucinous cells in a subset of tumors, which may indicate that some of mucinous cells are indeed amphicrine. Of note, neuroendocrine differentiation has been well-documented in mucinous adenocarcinoma. The high frequency of

expression of neuroendocrine markers has been found in gastric adenocarcinomas, with reported rate ranging from 18.7 to 75% of cases [27,28], and neuroendocrine differentiation is more common in the signet ring cell type [28]. Another notable example is so-called type B mucinous carcinoma of the breast, which often contains cells with signet ring morphology [29]. Currently, the significance of neuroendocrine differentiation or amphicrine nature in those mucinous adenocarcinomas is unknown, but such tumors are regarded as an adenocarcinoma. Furthermore, demonstration of neuroendocrine expression is not required in routine diagnostic work-up for these mucinous carcinomas. Taken all together, GCC is best regarded as a distinct variant of adenocarcinoma.

GCC is much more aggressive than carcinoid tumors, and metastases have been documented in 8-20% of the cases [6,7,19,30]. Some patients with GCC present with stage IV disease [1,2,31]. Even localized disease can result in fatal course [32,33]. In our series, 6 patients with pure low grade histology died of disease within a period from 34 to 98 months after the initial surgery, and all of them initially presented as localized disease. Our findings support the notion that GCC, even as a pure form and at low stage, should be regarded as a malignant neoplasm.

Tang LH et al attempted to classify this group of tumor into type A, B and C, and they demonstrated the subtyping correlated with survival, with type A and C being at low and high grade end, respectively [1]. Their results were reproduced by one study [31] but were not confirmed by two other studies [2,8]. Our group also published analysis regarding the prognostic value of this system in a cohort similar to the current study [6]. The univariate analysis demonstrated prognostic significance but the multivariable analysis did not. This discrepancy might be explained by the fact that each tumor can contain a variety of growth patterns, cellular components,

and nuclear grade, which makes the assignment of a given tumor to a single category of A, B, and C considerably challenging. Additionally, subgrouping to type B and C, under the terms adenocarcinoma ex GCC signet ring cell type and poorly differentiated carcinoma type are not ideal because the presence of signet ring cells generally signifies a poorly differentiated histology, associated with an aggressive clinical course [34].

Taggart MW, et al, classified their 74 tumors into 3 groups: group 1, GCC or GCC with less than 25% of adenocarcinoma; group 2, GCC with 25-50% of adenocarcinoma; group 3, GCC with more than 50% of adenocarcinoma, and compared the tumors among each group as well as with group 4, which was adenocarcinoma without GCC [2]. By multivariate analysis, only stage and tumor category were independent predictors of overall survival.

Most recently, Lee LH, et al, subdivided their 78 tumors to low and high grade histology using a scoring system, based on a combination of cytologic atypia, stromal desmoplasia and solid growth pattern, and found their two-tier system was predictive of overall survival when controlled for TNM stage [4]. However, the study did not investigate other common high grade morphologic pattern; i.e., individual discohesive (poorly cohesive) single cells or single files.

In our study, the increasing percentage of high grade component correlated with unfavorable outcome by both univariate and multivariate analysis. The results were expected as high grade histology as an unfavorable prognostic factor is commonly used in adenocarcinomas of a variety of organs. The tumors were also subdivided to three groups; 0-39% of high grade component, 40-89%, and more than 90%, and there was significant difference in cancer specific survival among the three

groups. We also investigated our cases with different cut-off points, 25 and 50%, used in the study by Taggart MW et al [2]. <25% vs. 25-50% did not differ in CSS while >50% had poorer CSS compared to <25% and 25-50%. Although our results were not too different from theirs, we could not reproduce the exact system, probably due to differences in distribution of high grade component, patient cohort, and treatment modalities. Further studies are needed to determine the cut-off value between the subgroup.

Interestingly, although increasing size of the primary tumor was an adverse prognostic factor in univariate analysis, this was associated with favorable outcome in multivariate analysis. Subgroup analysis showed that increasing size was not associated with outcome in patients with stages I-II while increasing tumor size had a trend for longer CSS in stages III-IV. The reason for this discrepancy is not certain, but one could speculate that a small size of a primary tumor in high stage disease may reflect a biologically, more aggressive phenotype. Similar associations between smaller tumor size and poorer CSS in high stage disease have been reported in colon cancers [35].

In summary, our study demonstrated tumor staging and proportion of high grade component to be important prognostic factors. This tumor group is characterized by the presence of hallmark GCC morphology and a wide variety of other morphologic patterns, which intermingle with each other. In spite of the morphologic diversity, it can be simply subdivided to low and high grade morphology for prognostification. Our findings are in agreement with others in that this tumor group represents a continuum of low grade (GCC) and high grade (adenocarcinoma ex GCC), and that varying proportions of low and high grades are seen in given tumors [1,2,8]. Neuroendocrine component was generally insignificant, and

furthermore there was no significant difference in amounts of neuroendocrine cells and mucinous cells between low grade and high grade areas. The findings in our study indicate that this tumor type, regardless of low or high grade, is best regarded as a variant of adenocarcinoma. Given that the low grade tumor recapitulates crypt structure, the term crypt cell adenocarcinoma, originally coined by P Isaacson [13], more appropriately reflects the nature and origin of this tumor group.

FIGURE LEGENDS

FIGURE 1: (A) Classic morphology of GCC, that is, organoid nests lined by goblet cells with minimal stromal reaction (Hematoxylin and eosin / H&E stain, x100), (B) infiltrating tubules, which show remarkable resemblance to non-neoplastic crypts (H&E stain, x40), (C) dilated tubules due to secretion and necrotic debris (H&E stain, x40), (D) tubules markedly dilated due to necroinflammatory debris, the morphology merging to that of well differentiated adenocarcinoma (H&E stain, x40)

FIGURE 2: (A) Markedly expanded nests, (B) irregular nests, (C) complex branching nests, (D) angulated, irregular nests within extracellular mucin pools (H&E stain, x100, respectively)

FIGURE 3: (A) Solid sheets (upper half) and confluent and irregular nests of goblet cells (lower half) (H&E stain, x40), (B) area of signet ring cell carcinoma (H&E stain, x100), (C) area resembling lobular carcinoma of breast (H&E stain, x100), (D) single cell file (upper half) and intestinal-like adenocarcinoma (lower half) (H&E stain, x100)

FIGURE 4: (A) Typical GCC (H&E stain), (B) only a few tumor cells positive for synaptophysin, while there are several neural fibers positive for synaptophysin, (C)

irregular, large clusters of signet ring cells (H&E stain), **(D)** diffusely positive for synaptophysin (x100, respectively)

FIGURE 5. Distribution of high grade component values stratified by stage. Median high grade value was significantly higher in stages III/IV compared to stages I/II (95% vs. 0%, $p<0.001$).

FIGURE 6. (A) Cancer-specific survival (CSS) curves in patients with stage I/II, III and stage IV disease, **(B)** Cancer-specific survival (CSS) curves in patients with tumors with high grade component of 0-39%, 40-89% and 90-100%.

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Highlights:

1. Goblet cell carcinoid tumor group can be simply subdivided to low and high grade morphology.
2. Staging and proportion of high grade component were shown to be important prognostic factors.
3. Term crypt cell adenocarcinoma more appropriately reflects nature and origin of this tumor group.