Molecular Characterization of Appendiceal Goblet Cell Carcinoid

Hiroyuki Arai[1], Yasmine Baca[2], Francesca Battaglin[1], Natsuko Kawanishi[1], Jingyuan Wang[1], Shivani Soni[1], Wu Zhang[1], Joshua Millstein[3], Curtis Johnston[2], Richard M. Goldberg[4], Philip A. Philip[5], Andreas Seeber[6], Joanne Xiu[2], Jimmy J. Hwang[7], Anthony F. Shields[5], John L. Marshall[8], W. Michael Korn[2], and Heinz-Josef Lenz[1]

ABSTRACT

Goblet cell carcinoid (GCC) is a distinct subtype of appendiceal neoplasm that exhibits unique clinical and pathologic features. We aimed to reveal the molecular profiles of GCC compared with other appendiceal tumors, such as adenocarcinomas and neuroendocrine tumors. A total of 495 appendiceal tumor samples (53 GCCs, 428 adenocarcinomas, and 14 neuroendocrine tumors) were tested with next-generation sequencing (NGS) on a 592-gene panel and IHC. Microsatellite instability (MSI)/mismatch repair (MMR) status was tested with a combination of NGS, IHC, and fragment analyses. Tumor mutational burden (TMB) was evaluated by NGS, and PD-L1 expression was tested by IHC (SP142). The most prevalent mutated genes within GCCs were *TP53* (24.0%), *ARID1A* (15.4%), *SMAD4* (9.4%), and *KRAS* (7.5%). Pathway-specific alterations were dominantly observed in cell cycle, MAPK, epigenetic, and TGFβ signaling pathways. GCCs as compared with adenocarcinomas exhibited significantly lower mutation rates in *KRAS, GNAS*, and *APC*, and significantly higher mutation rates in *CDH1, CHEK2, CDC73, ERCC2*, and *FGFR2*. GCCs as compared with neuroendocrine tumors showed significantly lower mutation rates in *KRAS, APC, BRCA2*, and *FANCA*. In GCCs, MSI high/MMR deficient, TMB high (≥17 mutations/Mb), and PD-L1 expression were seen in 0.0%, 0.0%, and 2.0% of tumors, respectively. No significant differences were observed in any immunotherapy-related markers examined when compared with adenocarcinomas and neuroendocrine tumors. In conclusion, GCCs had considerably distinct mutational profiles compared with appendiceal adenocarcinomas and neuroendocrine tumors. Understanding these molecular characteristics may be critical for the development of novel and more effective treatment strategies for GCC.

Introduction

Goblet cell carcinoid (GCC) is a very rare tumor, almost exclusively found in the appendix, with an incidence of approximately 0.01–0.05/100,000 per year (1). GCC clinically behaves as a malignant disease with a tendency to spread to the surrounding bowel, lymph nodes, peritoneum, and ovaries thus, resulting in poor prognosis (2). According to a population-based analysis of appendiceal tumors, the reported 3-year overall survival rates of patients with GCC were 96.6%, 91.7%, 65.3%, and 32.9% for stage I, II, III, and IV diseases, respectively, highlighting the aggressive character of GCC, particularly in the advanced stage, with similar survival rates of colorectal adenocarcinoma (3).

GCC arises from pluripotent, intestinal crypt base stem cells that are able to differentiate into both mucinous and neuroendocrine cells. The histologic patterns of GCC vary and consist of a mixture of glandular and neuroendocrine components (4). Their classical pathologic features include a composition of predominant goblet cells, which include intracytoplasmic mucin, with a few neuroendocrine cells (5). Recent data show the coexistence of poorly differentiated or signet-ring cell adenocarcinoma in at least half of GCC cases (i.e., "adenocarcinoma ex-GCC"), as well as rare cases with greater amounts of neuroendocrine components (6, 7). A poorly defined exocrine–endocrine hybrid appearance can confuse pathologists, surgeons, and oncologists attempting to diagnose and treat patients with GCC (8).

Whether GCC should be considered as a special form of adenocarcinoma or a neuroendocrine tumor variant remains a matter of debate (9). In fact, there are some disparities between the classification system currently used and clinical guidelines. The 2010 World Health Organization classification for appendiceal tumors classifies GCC under the category of neuroendocrine tumors (10). On the other hand, both consensus guidelines from the European Neuroendocrine Tumor Society (ENETS) and the North American Neuroendocrine Tumor Society (NANETS) recommend regarding GCC as a colorectal adenocarcinoma when managing patients, given its aggressive clinical course (1, 11). Concerning treatment, both statement guidelines are based only on expert opinions following retrospective review due to a lack of any evidences from prospective clinical trials. The current situation of a lack of consensus between the classification system and treatment strategy is partly due to the unknown molecular mechanisms of GCC. There are very few studies focusing on the genetic

[1]Division of Medical Oncology, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, California. [2]Caris Life Sciences, Phoenix, Arizona. [3]Department of Preventive Medicine, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, California. [4]West Virginia University Cancer Institute, Morgantown, West Virginia. [5]Department of Oncology, Karmanos Cancer Institute, Wayne State University, Detroit, Michigan. [6]Department of Hematology and Oncology, Comprehensive Cancer Center Innsbruck, Innsbruck Medical University, Innsbruck, Austria. [7]Levine Cancer Institute, Carolinas HealthCare System, Charlotte, North Carolina. [8]Ruesch Center for The Cure of Gastrointestinal Cancers, Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, D.C.

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Corresponding Author: Heinz-Josef Lenz, Norris Comprehensive Cancer Center, University of Southern California, 1441 Eastlake Avenue, Los Angeles, CA 90033. Phone: 323-865-3967; Fax: 323-865-0061; E-mail: lenz@usc.edu

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differences between GCC and other types of appendiceal tumors (12). However, a better understanding of the molecular background of this disease could facilitate not only differential diagnoses, but also could facilitate better consideration of an optimal treatment strategy for GCC. To address this issue, we performed genetic and molecular profiling of GCC compared with appendiceal adenocarcinoma and neuroendocrine tumor using a comprehensive tumor profiling platform.

Materials and Methods

Samples submitted to a commercial Clinical Laboratory Improvement Amendments–certified laboratory (Caris Life Sciences) from April 2015 to September 2019 were analyzed for molecular profiles. Formalin-fixed, paraffin-embedded (FFPE) samples submitted from clinical physicians around the world were sent for analysis. The tissue diagnoses were made on the basis of pathologic assessments from physicians who requested the assays, and were further verified by a board-certified oncological pathologist at the Caris laboratory. A total of 495 appendiceal tumor samples (53 GCCs, 428 adenocarcinomas, and 14 neuroendocrine tumors) were analyzed. This study was conducted in accordance with guidelines of the Declaration of Helsinki, Belmont Report, and U.S. Common Rule. In keeping compliance with policy 45 CFR 46.101(b) (4), this study was performed using retrospective, deidentified clinical data. Therefore, this study is considered institutional review board exempt and no patient consent was necessary.

Mutation analyses

Next-generation sequencing (NGS) was performed on genomic DNA isolated from FFPE samples using an NGS Platform (Illumina, Inc.). A custom-designed SureSelectXT assay was used to enrich 592 cancer-related whole-gene targets (Agilent Technologies). All variants were detected with >99% confidence based on allele frequency and amplicon coverage, with an average sequencing coverage depth of 750 and an analytic sensitivity of 5%. Identified genetic variants were analyzed by board-certified molecular geneticists and categorized as follows according to the American College of Medical Genetics and Genomics standards: "pathogenic," "presumed pathogenic," "variant of unknown significance," "presumed benign," or "benign." When assessing mutation frequencies of individual genes, "pathogenic" and "presumed pathogenic" were counted as mutations, whereas "variant of unknown significance," "presumed benign," and "benign" were excluded.

Immunotherapy-related biomarkers

Microsatellite instability (MSI) and mismatch repair (MMR) status was tested with a combination method employing IHC, fragment analysis, and NGS, with resulting status defined as either MSI-high (MSI-H)/MMR-deficient (dMMR) or microsatellite stable/MMR-proficient. Detailed methods for assessment of MSI/MMR status are documented in the Supplementary Data.

Tumor mutational burden (TMB) was measured by counting all nonsynonymous missense mutations found per tumor (592 genes and 1.4 MB sequenced/tumor). The threshold for a TMB-high (TMB-H) definition was ≥17 mutations/MB. This threshold was established by comparing TMB with MSI via fragment analysis in colorectal cancer cases based on reports of TMB exhibiting high concordance with MSI-H in colorectal cancer.

PD-L1 expression was tested by IHC using SP142 Antibody (Spring Biosciences). The staining intensity on the tumor cells membrane was assessed on a semiquantitative scale: 0 for no staining, 1+ for weak staining, 2+ for moderate staining, and 3+ for strong staining. Tumors exhibiting ≥5% of tumor cells stained as 2+ or 3+ were regarded as being PD-L1 positive.

From February 2019 to September 2019, mRNA expression data were obtained from isolated FFPE tumor samples using Illumina NovaSeq Platform (Illumina, Inc.) and Agilent SureSelect Human All Exon V7 Bait Panel (Agilent Technologies). Microenvironment cell population-counter (MCP-counter) was used for quantification of the abundance of immune and stromal cell populations using transcriptomic data as described previously (13).

Statistical analyses

Patient and molecular characteristics of GCCs were compared with those of adenocarcinomas and neuroendocrine tumors. Student *t* test and nonparametric Kruskal–Wallis test were used to analyze age and TMB distribution, respectively. Other categorical data were analyzed using Fisher exact test. Cases with any missing data information were not included in the analysis. All statistical analyses were performed with SPSS v23 (IBM SPSS Statistics), and all tests were two-sided at a significant level set to 0.05.

Results

Patient characteristics

Baseline characteristics of the 495 enrolled patients are shown in **Table 1**. Average age at diagnosis of GCC was significantly higher than that of neuroendocrine tumor (57.6 vs. 44.4 years, respectively; $P < 0.01$) and equivalent to that of adenocarcinoma (57.6 vs. 58.2 years; $P = 0.75$). A gender preference was not observed for GCC (47% male vs. 53% female), and the gender proportions did not differ between GCC and adenocarcinoma/neuroendocrine tumor. The information of tumor–node–metastasis (TNM) staging was available only in limited patients ($N = 142$). In any type of tumor, stage IV was the most common (75% or more; Supplementary Fig. S1).

Table 1. Baseline characteristics.

Characteristics		GCC ($n = 53$)	AC ($n = 428$)	NET ($n = 14$)	P	
Age	Average	57.6	58.2	44.4	GCC vs. AC	0.75
					GCC vs. NET	<0.01
Sex	Male (%)	25 (47)	193 (45)	7 (50)	GCC vs. AC	0.77
	Female (%)	28 (53)	235 (55)	7 (50)	GCC vs. NET	0.85

Abbreviations: AC, adenocarcinoma; GCC, goblet cell carcinoid; NET, neuroendocrine tumor.

Analyses of genetic alterations

In total, 50 "pathogenic" or "presumed pathogenic" mutations were detected within 25 genes in patients with GCC (Supplementary Fig. S2). Among them, pathway-specific mutations were dominantly observed within the following: cell cycle (13 mutations in *TP53*), MAPK (seven in *KRAS, BRAF*, and *NF1*), epigenetic (six in *ARID1A, CDC73, KDM6A, KMT2D*, and *SMARCA4*), and TGFβ signaling (six in *SMAD2* and *SMAD4*) pathways. Whereas the mutations present in the WNT (two in *APC* and *RNF43*) and PIK3 signaling (one in *PIK3CA*) pathways were less frequent (Supplementary Fig. S1). Genes

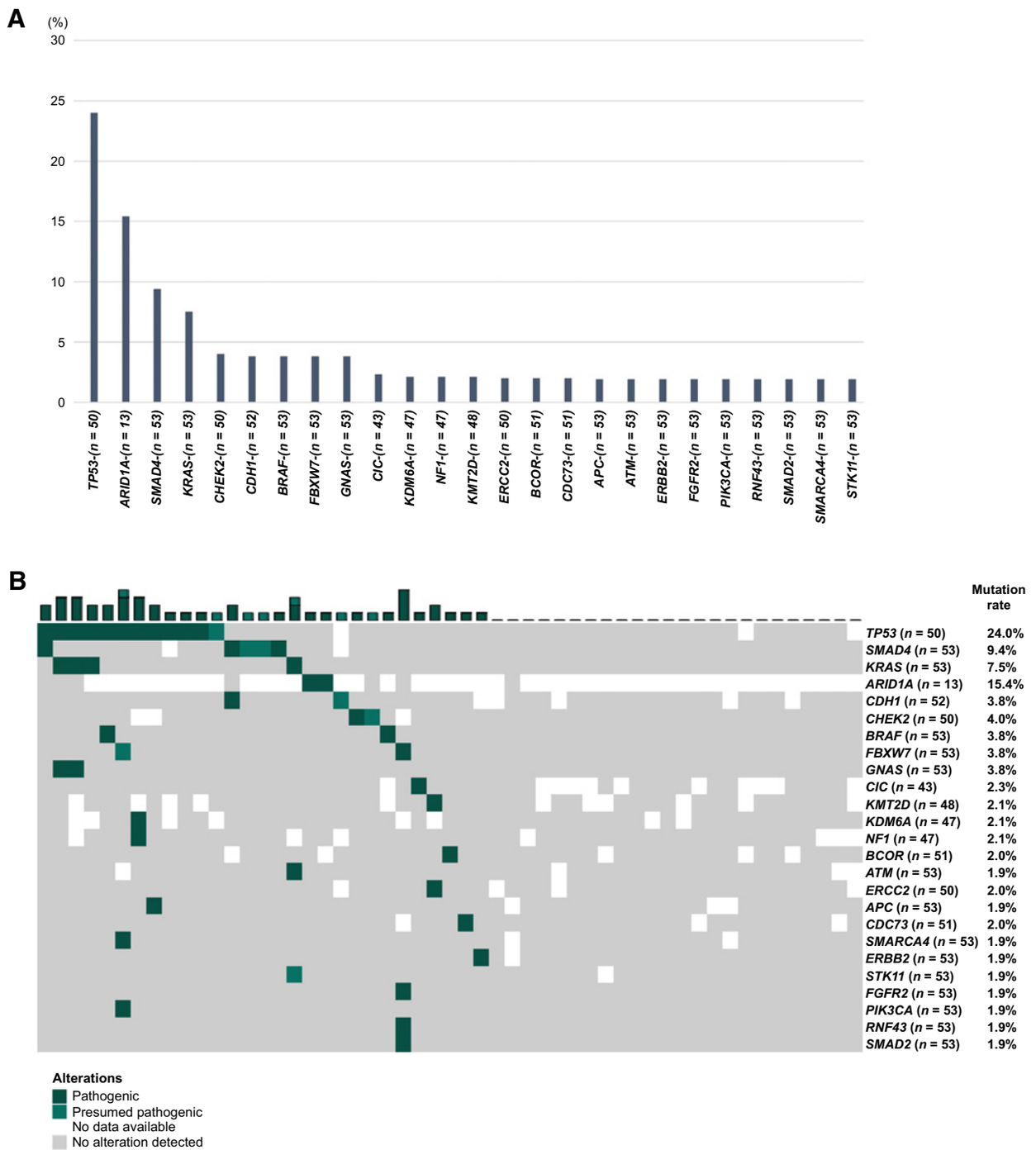

Figure 1.

Mutation profile of GCC. **A,** Most prevalent mutations within GCC. **B,** "Pathogenic" or "presumed pathogenic" mutations identified within GCC. The "*n*" in parentheses indicates the total number of tumors tested for the biomarker.

showing the highest mutation rate in patients with GCC were *TP53* (24.0%), *ARID1A* (15.4%), *SMAD4* (9.4%), and *KRAS* (7.5%). The other 21 genes were mutated in less than 5% of patients (**Fig. 1**). When comparing 26 ex-GCCs and 27 pure GCCs, no differences in genetic alterations were observed (Supplementary Table S1).

In this study, a total of 71 mutated genes were identified in appendiceal adenocarcinoma (**Table 2**). Among them, the most frequent mutations were observed in *KRAS, TP53, GNAS, ARID1A, SMAD4*, and *APC* (mutation rate > 10%). Compared with these mutation profiles of adenocarcinoma, GCC exhibited significantly lower mutation rates in *KRAS* (7.5% vs. 60.4% for GCC and adenocarcinoma, respectively), *GNAS* (3.8% vs. 34.4%), and *APC* (1.9% vs. 11.7%), and significantly higher mutation rates in *CDH1* (3.8% vs. 0.7%), *CHEK2* (4.0% vs. 0.3%), *CDC73* (2.0% vs. 0.0%), *ERCC2* (2.0% vs. 0.0%), and *FGFR2* (1.9% vs. 0.0%; **Fig. 2**; **Table 2**). As for *TP53*, which was the second most frequently mutated gene in adenocarcinoma, GCC showed a marginally lower mutation rate as compared with adenocarcinoma (24.0% vs. 37.0%, respectively; $P = 0.070$).

Within appendiceal neuroendocrine tumor, only nine mutated genes were observed: *KRAS, APC, TP53, CDH1, BRAF, BCOR, BRCA2, FANCA*, and *ERBB2* (**Table 3**). GCC showed significantly lower mutation rates when compared with appendiceal neuroendocrine tumor in *KRAS* (7.5% vs. 28.6%, respectively), *APC* (1.9% vs. 28.6%), *BRCA2* (0.0% vs. 7.1%), and *FANCA* (0.0% vs. 7.1%; **Fig. 2**; **Table 3**). GCC showed a numerically higher mutation rate in *TP53* (24.0% vs. 14.3%), but the difference was not statistically significant ($P = 0.437$).

Gene amplifications in GCC were observed in *MDM2* (3.8%), *FUS* (2.0%), *SF3B1* (2.0%), and *FGF23* (2.0%), while amplified *MYC* (2.4%), *CCND1* (2.2%), *FGF19* (1.7%), and *FGF4* (1.5%) represented the most frequent copy-number alterations observed in adenocarcinoma, and no copy-number alterations were observed within neuroendocrine tumor (Supplementary Table S2). No notable gene rearrangements were detected in GCC.

Immunotherapy-related biomarkers

Mean TMB was 5.8/Mb in GCC, which was lower than that of adenocarcinoma (7.6/Mb) and higher than that of neuroendocrine tumor (4.1/Mb). The frequency of TMB-H patients was virtually equivalent among all tumor types (GCC, 0%; adenocarcinoma,

Table 2. Comparison of mutation frequency between appendiceal adenocarcinoma and GCC.

	AC			GCC					AC			GCC			
Gene	MT	Total	Mutation rate (%)	MT	Total	Mutation rate (%)	*P* (AC vs. GCC)	Gene	MT	Total	Mutation rate (%)	MT	Total	Mutation rate (%)	*P* (AC vs. GCC)
KRAS	256	424	60.4	4	53	7.5	**<0.01**	*RAD50*	3	351	0.9	0	44	0.0	0.54
TP53	152	411	37.0	12	50	24.0	0.07	*PRKDC*	3	390	0.8	0	49	0.0	0.54
GNAS	146	424	34.4	2	53	3.8	**<0.01**	*CDH1*	3	423	0.7	2	52	3.8	**0.04**
ARID1A	18	90	20.0	2	13	15.4	0.69	*MRE11*	3	410	0.7	0	52	0.0	0.54
SMAD4	78	426	18.3	5	53	9.4	0.11	*PIK3R1*	3	417	0.7	0	52	0.0	0.54
APC	50	427	11.7	1	53	1.9	**0.03**	*KMT2A*	3	423	0.7	0	53	0.0	0.54
PIK3CA	30	427	7.0	1	53	1.9	0.15	*BLM*	3	425	0.7	0	53	0.0	0.54
RNF43	25	427	5.9	1	53	1.9	0.23	*STK11*	3	426	0.7	1	53	1.9	0.37
ATM	22	427	5.2	1	53	1.9	0.29	*U2AF1*	3	426	0.7	0	53	0.0	0.54
BRAF	17	427	4.0	2	53	3.8	0.94	*BRCA1*	3	427	0.7	0	53	0.0	0.54
FBXW7	15	415	3.6	2	53	3.8	0.95	*CDK12*	3	427	0.7	0	53	0.0	0.54
ASXL1	10	291	3.4	0	32	0.0	0.29	*EP300*	3	427	0.7	0	52	0.0	0.54
MED12	4	132	3.0	0	16	0.0	0.48	*MTOR*	3	427	0.7	0	53	0.0	0.54
KDM6A	10	374	2.7	1	47	2.1	0.82	*FANCE*	2	328	0.6	0	41	0.0	0.62
BRCA2	11	427	2.6	0	53	0.0	0.24	*WRN*	2	408	0.5	0	53	0.0	0.61
KDM5C	4	158	2.5	0	21	0.0	0.46	*FANCC*	2	422	0.5	0	52	0.0	0.62
SMAD2	10	425	2.4	1	53	1.9	0.83	*FH*	2	422	0.5	0	53	0.0	0.62
KMT2C	7	366	1.9	0	45	0.0	0.35	*POT1*	2	422	0.5	0	52	0.0	0.62
CDKN1B	8	425	1.9	0	52	0.0	0.32	*NBN*	2	423	0.5	0	53	0.0	0.62
KMT2D	7	379	1.8	1	48	2.1	0.91	*CCND3*	2	426	0.5	0	53	0.0	0.62
BCOR	7	415	1.7	1	51	2.0	0.89	*CREBBP*	2	426	0.5	0	53	0.0	0.62
AMER1	7	423	1.7	0	53	0.0	0.35	*MAX*	2	426	0.5	0	53	0.0	0.62
ATRX	3	203	1.5	0	22	0.0	0.57	*MITF*	2	426	0.5	0	52	0.0	0.62
SMARCA4	6	420	1.4	1	53	1.9	0.79	*SETD2*	2	426	0.5	0	52	0.0	0.62
AKT1	6	424	1.4	0	53	0.0	0.38	*FLCN*	2	427	0.5	0	53	0.0	0.62
MUTYH	6	425	1.4	0	53	0.0	0.38	*HNF1A*	2	427	0.5	0	53	0.0	0.62
ERBB2	6	427	1.4	1	53	1.9	0.78	*IDH1*	2	427	0.5	0	53	0.0	0.62
PTCH1	4	317	1.3	0	41	0.0	0.47	*MLH1*	2	427	0.5	0	53	0.0	0.62
PTEN	5	419	1.2	0	53	0.0	0.42	*PALB2*	2	427	0.5	0	53	0.0	0.62
NRAS	5	426	1.2	0	53	0.0	0.43	*CHEK2*	1	399	0.3	2	50	4.0	**<0.01**
NF1	4	341	1.2	1	47	2.1	0.59	*CIC*	1	380	0.3	1	43	2.3	0.06
BCL9	5	427	1.2	0	53	0.0	0.43	*CDC73*	0	402	0.0	1	51	2.0	**<0.01**
MSH6	4	421	1.0	0	53	0.0	0.48	*ERCC2*	0	418	0.0	1	50	2.0	**<0.01**
ARID2	4	424	0.9	0	53	0.0	0.48	*FGFR2*	0	426	0.0	1	53	1.9	**<0.01**
PMS2	2	230	0.9	0	27	0.0	0.63								

Note: The bold *P* values indicate significant difference ($P < 0.05$).
Abbreviations: AC, adenocarcinoma; GCC, goblet cell carcinoid; MT, mutant.

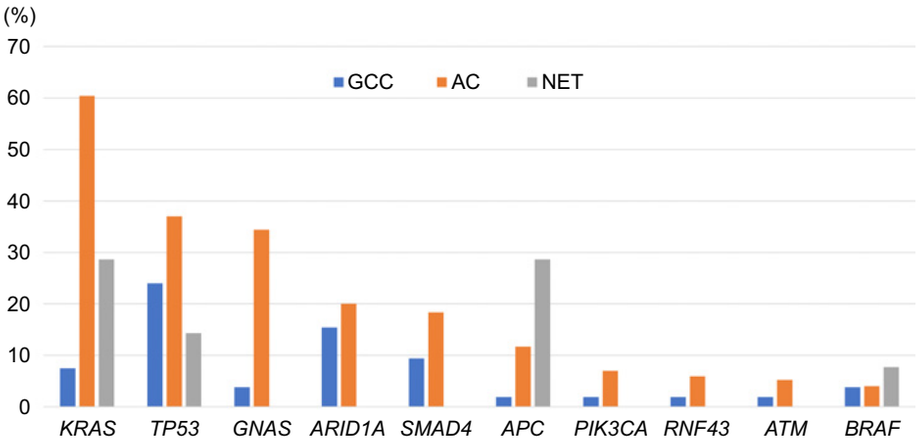

Figure 2.
Comparison of major gene mutation rates among different appendiceal tumors. The top 10 major genes in which mutations were identified in appendiceal adenocarcinoma. Details for all data in the comparative analysis are shown in **Tables 2** and **3**. AC, adenocarcinoma; GCC, goblet cell carcinoid; NET, neuroendocrine tumor.

1.7%; and neuroendocrine tumor, 0%). The frequency of MSI-H/dMMR patients was 0% for GCC, 1.9% for adenocarcinoma, and 0% for neuroendocrine tumor. PD-L1 positivity was 2.0% in GCC, 2.9% in adenocarcinoma, and 0% in neuroendocrine tumor. No significant difference was observed in these immune profiles when GCC was compared with adenocarcinoma/neuroendocrine tumor (**Table 4**). The results of MCP-counter were obtained for 86 samples (GCC, nine; adenocarcinoma, 76; and neuroendocrine tumor, one). Neuroendocrine tumors only had one case with mRNA data thus, the comparative analysis was only done between GCC and adenocarcinoma. While natural killer cells were the only cell population showing a trending difference, other nine cell populations did not show any difference between GCC and adenocarcinoma (Supplementary Fig. S3).

Discussion

To the best of our knowledge, this is the largest study investigating the molecular profiles of appendiceal GCC, in which 53 patient samples were compared with other appendiceal tumors (428 adenocarcinomas and 14 neuroendocrine tumors). We demonstrated that GCC consists of considerably different genetic alterations as compared with appendiceal adenocarcinoma and neuroendocrine tumor. Our data further increase the understanding of GCC biology, emphasizing that GCC is a molecularly distinct entity from other appendiceal tumors.

The epidemiology of GCC has been well-documented, with an average age of diagnosis about 10 years higher than appendiceal neuroendocrine tumor, and no gender preference (1). In our study we confirm that the average age of diagnosis in patients with GCC was 13.2 years higher compared with neuroendocrine tumor, and no differences of distribution exist between genders.

We report here the largest studied cohort of GCC to date with comprehensive molecular profiling using a 592-gene target panel. The most prevalent mutations observed were present within *TP53* (24.0%), *ARID1A* (15.4%), *SMAD4* (9.4%), and *KRAS* (7.5%), and 21 minor mutant genes accounted for a small subset of patients with GCC. In addition, the mutational spectrum reflected dominant alterations in cell cycle, MAPK, epigenetic, and TGFβ signaling pathways, indicating that these pathways are critical for GCC pathogenesis. Of note, the WNT and PIK3 signaling pathways were infrequently altered, although the well-known function of these pathways is as a key driver for tumorigenesis and progression of

Table 3. Comparison of mutation frequency between appendiceal NET and GCC.

	NET			GCC					NET			GCC			
Gene	MT	Total	Mutation rate (%)	MT	Total	Mutation rate (%)	*P* (NET vs. GCC)	Gene	MT	Total	Mutation rate (%)	MT	Total	Mutation rate (%)	*P* (NET vs. GCC)
KRAS	4	14	28.6	4	53	7.5	**0.03**	*ATM*	0	14	0.0	1	53	1.9	0.60
APC	4	14	28.6	1	53	1.9	**<0.01**	*FBXW7*	0	14	0.0	2	53	3.8	0.46
TP53	2	14	14.3	12	50	24.0	0.44	*KDM6A*	0	11	0.0	1	47	2.1	0.63
CDH1	1	13	7.7	2	52	3.8	0.55	*SMAD2*	0	14	0.0	1	53	1.9	0.60
BRAF	1	13	7.7	2	53	3.8	0.54	*KMT2D*	0	14	0.0	1	48	2.2	0.59
BCOR	1	13	7.7	1	51	2.0	0.29	*SMARCA4*	0	14	0.0	1	53	1.9	0.60
BRCA2	1	14	7.1	0	53	0.0	**0.05 (0.049)**	*NF1*	0	14	0.0	1	47	2.1	0.58
FANCA	1	14	7.1	0	53	0.0	**0.05 (0.049)**	*STK11*	0	14	0.0	1	53	1.9	0.60
ERBB2	1	14	7.1	1	53	1.9	0.30	*CIC*	0	13	0.0	1	43	2.3	0.58
GNAS	0	14	0.0	2	53	3.8	0.46	*CHEK2*	0	12	0.0	2	50	4.0	0.48
ARID1A	0	4	0.0	2	13	15.4	0.40	*CDC73*	0	14	0.0	1	51	2.0	0.60
SMAD4	0	14	0.0	5	53	9.4	0.23	*ERCC2*	0	14	0.0	1	50	2.0	0.59
PIK3CA	0	14	0.0	1	53	1.9	0.60	*FGFR2*	0	14	0.0	1	53	1.9	0.60
RNF43	0	14	0.0	1	53	1.9	0.60								

Note: The bold *P* values indicate significant difference ($P < 0.05$).
Abbreviations: GCC, goblet cell carcinoid; MT, mutant; NET, neuroendocrine tumor.

Table 4. Comparison of immunotherapy-related markers between GCC and appendiceal adenocarcinoma/NET.

Biomarker		GCC	AC	NET	*P*	
Mean TMB	(/Mb)	5.8	7.6	4.1	GCC vs. AC	<0.01
					GCC vs. NET	0.02
TMB-H	(%)	0.0	1.7	0.0	GCC vs. AC	0.34
					GCC vs. NET	NA
MSI-H/dMMR	(%)	0.0	1.9	0.0	GCC vs. AC	0.31
					GCC vs. NET	NA
PD-L1 positive	(%)	2.0	2.9	0.0	GCC vs. AC	0.70
					GCC vs. NET	0.60

Note: TMB/MSI status/PD-L1 positivity were tested in 52/53/51 patients with GCC, 409/427/412 patients with AC, and 14/14/14 patients with NET, respectively. TMB-H was defined as 17 or more mutations/Mb.
Abbreviations: AC, adenocarcinoma; GCC, goblet cell carcinoid; NA, not assessed; NET, neuroendocrine tumor.

colorectal adenocarcinoma (14). Our findings are consistent with a previous smaller study, which showed a unique distribution of altered pathways with frequent alterations in the epigenetics pathway and rare alterations of the WNT pathway within GCC (15). Our results also suggest that there is a significant overlap of molecular alterations found in pure GCC and ex-GCC, which is consistent with a previous report suggesting that both represent a single tumor type with varying differentiation grades (15).

As previously reported, the mutational profiles of appendiceal adenocarcinoma are distinct from those of colon adenocarcinoma. Specifically, appendiceal adenocarcinoma shows lower mutation rates compared with colon adenocarcinoma in *TP53, APC, PIK3CA*, and *FBXW7*, and higher mutation rates in *GNAS* and *SMAD4* (16). In this study, the molecular profiles between 53 GCCs and 428 appendiceal adenocarcinomas were compared; we observed less frequent mutation rates in *KRAS, GNAS*, and *APC* within GCC. On the other hand, some less common mutations were more frequently detected within GCC (*CDH1, CHEK2, CDC73, ERCC2*, and *FGFR2*). In addition, the copy-number alteration profiles did not overlap between GCC and appendiceal adenocarcinoma, showing more frequent amplification in *MDM2, FUS, SF3B1*, and *FGF23* for GCC. These results suggest a variable pathogenesis of GCC with potentially different key driver alterations compared with appendiceal as well as colorectal adenocarcinoma, as observed in the previously described "adenoma-carcinoma sequence" (14).

A previous study showed that LOH within 11q, 16q, and 18q might play a role in the pathogenesis of ileal carcinoid as well as GCC (17). The most frequently reported mutated gene in gastrointestinal neuroendocrine tumor (GI-NET) is *CTNNB1* (18, 19). However, information concerning the genetic profiles of appendiceal neuroendocrine tumor has not yet been reported. Our data are the first to show appendiceal neuroendocrine tumors exhibit mutations in nine different genes (*KRAS, APC, TP53, CDH1, BRAF, BCOR, BRCA2, FANCA*, and *ERBB2*) and a lack of mutations in *CTNNB1* (**Table 3**). These findings suggest that appendiceal neuroendocrine tumor may be molecularly distinct from other GI-NET. Importantly, the findings in this study indicate that GCC contains significantly different mutation profiles compared with appendiceal neuroendocrine tumor, as well as other described GI-NET.

Certain biomarkers may become critical for patient selection for immunotherapies, including immune checkpoint inhibitors (ICI). Patients with MSI-H colorectal cancer have been shown to significantly benefit from ICI therapies (20–23). In addition to MSI status, PD-L1 expression and TMB are related to efficacy of ICIs within other cancer treatments (24). In patients with GCC analyzed in this study, we did not detect any cases exhibiting MSI-H and/or TMB-H, but we found 2.0% of PD-L1–positive cases. Thus, GCC is considered to be an immunologically cold tumor. The nonactivated immune profiles described herein were similar to those of appendiceal adenocarcinoma and neuroendocrine tumor. Of note, MCP-counter results showed almost similar abundance of immune and stromal cell populations in tumor microenvironment between GCC and adenocarcinoma. These results indicate that ICIs may not be a promising treatment for GCC nor for the other types of appendiceal tumors.

Current clinical guidelines established by the ENETS and NANETS recommend that patients with GCC are treated in accordance with colorectal cancer treatment given the aggressive clinical course (1, 11). Specifically, right hemicolectomy for resectable GCC and palliative 5-fluorouracil–based chemotherapy for metastatic GCC are the recommended standard treatments. However, based on the findings of this study, the question arises as to whether the same treatment strategy for colorectal cancer should be used for GCC, based on the significant differences observed in molecular profiling of GCC compared with adenocarcinoma. Finding more effective and rationally based treatment strategies for patients with GCC is needed. There is no data suggesting that GCC should be treated as a neuroendocrine tumor, as significant molecular differences between these tumor types were demonstrated in this study. Our findings suggest that GCC treatment strategies should be reconsidered and instead focus on therapies targeting cell cycle, MAPK, epigenetic, and TGFβ signaling pathways. Studies of preclinical models are critical to transition new therapies into the clinic for this rare tumor.

There are some limitations within our study. First, the retrospective design could not completely exclude a selection bias. Second, we did not have certain important clinical data for the patients enrolled in this study. We just had limited information of TNM stage, but the details of treatment regimens and survival time were not available at all. Further investigations including this information would allow us to better understand the association between the genetic alterations of GCC and clinical stage, prognosis, and treatment outcome.

In conclusion, GCC has distinct genetic backgrounds compared with appendiceal adenocarcinoma and neuroendocrine tumor. These findings raise a question about reconsidering the currently used classification system and treatment strategies for this rare disease.

Disclosure of Potential Conflicts of Interest

R.M. Goldberg reports personal fees from Amgen (lecture honorarium and travel expenses), Genentech (expert testimony in a legal matter), Novartis (drug development advice), Bayer (drug development advice), Taiho (drug development advice), and BMS (drug development advice) outside the submitted work. J. Xiu reports personal fees from Caris Life Sciences (employment) during the conduct of the study. J.J. Hwang reports grants and personal fees from Boehringer Ingelheim and personal fees from Bristol Myers Squibb, Ipsen, Bayer, Eisai, Genentech/Roche, Taiho, Amgen, and Celgene outside the submitted work. A.F. Shields reports other from Caris Life Sciences (research support) during the conduct of the study and Caris Life Sciences (travel, speakers bureau) outside the submitted work. J.L. Marshall reports personal fees from Caris Life Sciences (SAB member) outside the submitted work. W.M. Korn reports other from Caris Life Sciences (employment, ownership interest) and personal fees from Merck, Sharp and Dohme, outside the submitted work. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

H. Arai: Conceptualization, investigation, methodology, writing-original draft, project administration. **Y. Baca:** Resources, data curation, formal analysis, methodology, writing-review and editing. **F. Battaglin:** Writing-review and editing. **N. Kawanishi:** Writing-review and editing. **J. Wang:** Writing-review and editing. **S. Soni:** Writing-review and editing. **W. Zhang:** Writing-review and editing. **J. Millstein:** Writing-review and editing. **C. Johnston:** Writing-review and editing. **R.M. Goldberg:** Writing-review and editing. **P.A. Philip:** Writing-review and editing. **A. Seeber:** Writing-review and editing. **J. Xiu:** Resources, data curation, writing-review and editing. **J.J. Hwang:** Writing-review and editing. **A.F. Shields:** Writing-review and editing. **J.L. Marshall:** Writing-review and editing. **W.M. Korn:** Supervision, writing-review and editing. **H.-J. Lenz:** Conceptualization, supervision, funding acquisition, project administration, writing-review and editing.

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