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Original contribution

Genomic profile of appendiceal goblet cell carcinoid is distinct compared to appendiceal neuroendocrine tumor and conventional adenocarcinoma☆,☆☆

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Summary Goblet cell carcinoid (GCC) is a rare appendiceal tumor with unique morphologic features that shows glandular and neuroendocrine differentiation on immunohistochemistry. An additional component of adenocarcinoma (AC) can be present (GCC-AC). Both GCC and GCC-AC are staged and treated like AC. The histogenesis and genetic alterations underlying GCC and GCC-AC are unclear. Capture-based next-generation DNA sequencing targeting 479 cancer genes was performed on 19 appendiceal tumors: 4 GCC, 9 GCC-AC, 3 neuroendocrine tumors (NET), and 3 AC (2 conventional, 1 mucinous). Somatic coding mutations were not seen in any NET. Pathogenic (P)/likely pathogenic (LP) mutations were present in 1 GCC, 8 GCC-AC and all 3 AC cases. P/LP mutations in chromatin remodeling genes were seen in 4 (44.4%) GCC-AC cases, but not in NET, GCC or AC. In GCC-AC, P/LP mutations in *ARID1A* and *RHOA* were each present in 3 cases, and *KDM6A* and *SOX9* mutations were each seen in 2 cases. *APC* and *KRAS* mutations were present in 1 conventional AC case, but were not observed in any GCC or GCC-AC. This limited series reveals mutations in *SOX9*, *RHOA*, and chromatin-modifier genes in goblet cell tumors, and shows that the mutational profile of GCC/GCC-AC is distinct from NET and conventional appendiceal AC.

1. Introduction

Goblet cell carcinoid (GCC) is a malignant appendiceal tumor with a unique morphology that appears to recreate the intestinal crypt. The tumor cells resemble goblet cells and often show neuroendocrine differentiation evidenced by chromogranin and synaptophysin expression [1-3]. GCC typically has

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low-grade nuclear atypia and lacks destructive invasion and desmoplasia; however, aggressive pathologic features like transmural spread, perineural invasion, and vascular invasion can be present, and there is a high propensity for lymph node, peritoneal, and ovarian involvement [4-7]. In some cases, there are areas with more pronounced cytologic atypia, architectural abnormalities, frequent mitoses, higher Ki-67 proliferation index, and/or a distinct adenocarcinoma component. The latter can have conventional, mucinous, or signet ring cell histologic types. These tumors have been termed as mixed goblet cell carcinoid-adenocarcinoma (GCC-AC), and as "mixed adenoneuroendocrine carcinoma" in the WHO 2010 classification [1]. A proposed 3-tier (groups A-C) scheme by Tang et al [8] designates pure GCC as group A, while tumors with an adenocarcinoma component are designated as "adenocarcinoma ex goblet cell carcinoid". These can be type B (signet ring cell type) showing an infiltrative architecture with a partial or complete loss of clustering of goblet cells (but no diffuse sheets) or type C (poorly differentiated type) with a component of poorly differentiated adenocarcinoma or sheets of signet ring cells [8].

Both GCC and GCC-AC are staged as adenocarcinomas (AC) as per American Joint Committee on Cancer (AJCC) recommendations [9-11]. The consensus guidelines by North American Neuroendocrine Tumor Society (NANETS) and European Neuroendocrine Tumor Society (ENETS) recommend right hemicolectomy after initial appendectomy for GCC [10,11].

There are limited studies examining molecular aspects of GCC. The lack of mutations in *KRAS* has been noted in GCC [12,13], while *KRAS* mutation was reported in one case in another study [2]. Immunohistochemistry for p53 was negative in some studies [2,12], while *TP53* mutation was reported in 25% of GCC in another series [13]. Positive results with p53 immunohistochemistry have also been observed in GCC associated with poorly differentiated AC [8]. *CTNNB1* mutations are not present in GCC [12], and nuclear pattern of staining with beta-catenin was noted in only 12% of GCC [2]. *DPC4* mutation has not been reported [12] and DPC4 is intact by immunohistochemistry [2]. Allelic loss of chromosomes 11q, 16q and 18q has been reported in GCC [12]. This study examines the genomic profile in a small series of GCC and GCC-AC and compares it with appendiceal neuroendocrine tumor (NET) and conventional AC.

2. Materials and methods

2.1. Case selection

The study group comprises 3 NET, 4 GCC, 9 mixed GCC-AC, and 3 appendiceal adenocarcinomas (2 conventional and 1 mucinous). All cases were reviewed to confirm the diagnosis. The study was approved by the institutional review boards of participating institutions.

2.2. Capture-based next-generation DNA sequencing

Capture-based next-generation sequencing (NGS) was performed in all 19 cases at the UCSF Clinical Cancer Genomics Laboratory, using an assay (UCSF500 panel) that targets the coding regions of 479 cancer-related genes, select introns from approximately 47 genes, and the *TERT* promoter with a total sequencing footprint of 2.8 Mb. Matched normal tissue was sequenced in 11 cases. Sequencing libraries were prepared from genomic DNA extracted from punch biopsies or macrodissected unstained sections from formalin-fixed paraffin-embedded tissue. Target enrichment was performed by hybrid capture using a custom oligonucleotide library. Sequencing was performed on a HiSeq 2500 (Illumina, San Diego, CA). Duplicate sequencing reads were removed computationally to allow for accurate allele frequency determination and copy number calling. The analysis was based on the human reference sequence UCSC build hg19 (NCBI build 37), using the following software packages: BWA: 0.7.10-r789, Samtools: 1.1 (using htslib 1.1), Picard tools: 1.97 (1504), GATK: 2014.4–3.3.0–0-ga3711, CNVkit: 0.3.3, Pindel: 0.2.5a7, SATK: 2013.1–10- gd6fa6c3, Annovar: v2015Mar22, Freebayes: 0.9.20 and Delly: 0.5.9.20, 21, 22, 23, 24, 25, 26, 27, 28, 29. Only insertions/deletions (indels) up to 100 bp in length were included in the mutational analysis. Somatic single-nucleotide variants and indels were visualized and verified using Integrated Genome Viewer. Genome-wide copy number analysis based on on-target and off-target reads was performed by CNVkit and Nexus Copy Number (Biodiscovery, Hawthorne, CA).

The genetic variants were classified as pathogenic/likely pathogenic (P/LP) or variant of uncertain significance (VUS). P/LP variants include recurring activation ("hotspot") mutations in established oncogenes and truncating, splicing or recurrent mutations in known tumor suppressors. Alternatively, they may represent point mutations with loss of heterozygosity (LOH) in genes known to be mutated in the cancer type being evaluated. P/LP variants tend to be reported in multiple cancer types and/or multiple times in each cancer type. VUS are those not known to be mutated in the cancer type of interest, do not inactivate a tumor suppressor, and/or are not seen in cancer databases such as COSMIC or BioPortal.

3. Results

3.1. Clinicopathologic features

The study group comprised 10 (53%) women (Table 1). The average age at diagnosis was 22.3 years (range 20–25) for NET, 64.3 years (range 58–73) for GCC, 59.8 years (range 34–91) for GCC-AC, and 54.0 years (range 39–74) for AC (Fig. 1).

All 4 GCCs were characterized by low-grade nuclear atypia, predominant arrangement as nests or crypt-like