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# Genomic characterization of HPV-related and gastric-type endocervical adenocarcinoma: Correlation with subtype and clinical behavior

**Anjelica Hodgson, MD**[1], **Brooke E. Howitt, MD**[2], **Kay J. Park, MD**[3], **Neal Lindeman, MD**[4], **Marisa R. Nucci, MD**[4], **Carlos Parra-Herran, MD**[1]

[1]Sunnybrook Health Sciences Centre and University of Toronto, Toronto, ON, Canada

[2]Stanford University Medical Centre, Stanford, CA, USA

[3]Memorial Sloan Kettering Cancer Center, New York, NY, USA

[4]Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

## Abstract

The majority of endocervical adenocarcinomas (EAs) are caused by human papillomavirus (HPV). Gastric-type EA, the second most common EA and unrelated to HPV, is biologically different with a more aggressive clinical course. Our knowledge of the molecular fingerprint of these important EA types and its role in diagnosis, prognosis and management is still evolving. Thus, we sought to evaluate the genomic profile of HPV-related and gastric EA. Clinical information including patient outcome was gathered for fifty-six tumors (45 HPV-associated and 11 gastric-type) which were evaluated by a targeted massively parallel sequencing assay (OncoPanel platform) which surveys exonic DNA sequences of 447 cancer genes and 191 regions across 60 genes for rearrangement detection. *KRAS, TP53*, and *PIK3CA* were the most commonly mutated genes (10, 10, and 9 cases, respectively). Alterations in *TP53, STK11, CDKN2A, ATM*, and *NTRK3* were significantly more common in gastric-type EA ($p < 0.05$, Fisher's exact test). Disease recurrence and/or death occurred in 14/49 (29%) cases with clinical information available (7 HPV-related (18% of HPV-related cases with clinical information available) and 7 gastric-type (64% of gastric-type cases with clinical information available). Tumors associated with adverse outcome, regardless of histotype, more commonly had alterations in *KRAS (2 HPV-related, 4 gastric-type), GNAS (3 HPV-related, 1 gastric-type)*, and *CDKN2A (0 HPV-related, 3 gastric type)* compared to indolent-behaving cases ($p < 0.05$, Fisher's exact test). A total of 8/56 (14%) tumors harbored at least one actionable mutation; of these, 6 (75%) were associated with recurrence and/or cancer-related death. Copy number variations were detected in 45/56 cases (80%). The most frequent were chromosome 20 gain and 16q loss, identified in 7 cases each (all HPV-associated EA). The mutational profile of EA is diverse and correlates with clinical behavior and EA subtype. Thus, targeted sequencing assays can potentially serve as a diagnostic and prognostic tool. It can also identify targetable alterations, which may benefit patients with recurrent/metastatic disease.

**Corresponding author:** Carlos Parra-Herran, Associate Professor, Dept. of Laboratory Medicine and Pathobiology, University of Toronto; Sunnybrook Health Sciences Centre, 2075 Bayview Ave Room E4-27a, Toronto, ON, Canada M4N 3M5, Phone: 416-480-6100 ext. 4987; Fax: 416-480-4271; carlos.parraherran@utoronto.ca.

### Keywords

Cervix; endocervical adenocarcinoma; histotype; next generation sequencing; outcome

## INTRODUCTION

Endocervical adenocarcinoma (EA) is the 2nd most common malignancy of the uterine cervix[1]. Like its squamous counterpart, a large proportion of EAs are associated with oncogenic human papilloma virus (HPV) infection. However, it is well recognized that a significant proportion of EAs are not associated with HPV infection[2–8]. Because of this dichotomy in pathogenesis, an international group of pathologists recently proposed an updated classification scheme entitled the International Endocervical Criteria and Classification (IECC) which categorizes EAs based on the presence or absence of HPV infection-related features[9] (Figure 1). The IECC is further supported by the original descriptions of gastric-type EA (the most common HPV-unrelated EA) which described aggressive clinical behavior in this tumor type (Figure 2)[10–12]. Recent clinicopathologic studies based on the IECC system have confirmed the differences in presentation and prognosis between gastric-type EA and HPV-associated EA[13,14].

Genomic evaluation of EA thus far has shown diverse results. Recurrently altered genes include *PIK3CA*, *KRAS* and *PTEN* (PI3K-Akt-mTOR pathway), mostly found in HPV-related adenocarcinomas[15–21]. Interestingly, mutations in both *PIK3CA* and *KRAS* have been shown to be prognostically significant[22,23] and additionally, targeted therapies exist against alterations in these genes[24,25]. In contrast to HPV-associated EAs, gastric-type tumors tend to be enriched for *TP53*, *STK11*, *GNAS, SMAD4*, and *CDKN2A* mutations[26,27]; in addition, there appears to be some molecular overlap between this subtype and HPV-associated EAs as *KRAS* and *PIK3CA* alterations have also been described. Importantly, previous studies have not separated cases into subtypes as per the IECC and were based mostly on targeted sequencing panels.

With this information in mind, we sought to evaluate the genomic profile of an institutional cohort of HPV-associated EAs and gastric-type EAs using a comprehensive massively parallel sequencing platform, with the aim of identifying prevalent and specific alterations for IECC subtypes and correlating these molecular alterations with patient outcome.

## MATERIALS AND METHODS

### Case selection and review

Consecutive cases of invasive EA from Sunnybrook Health Sciences Centre (Toronto, Ontario, Canada) diagnosed between 2002 and 2018 were retrieved for initial review (see Figure 3). The search included resection (cone biopsy, loop electrosurgical excision procedure, trachelectomy, or hysterectomy) and biopsy specimens. Those with histologic material available for examination were further reviewed and complete slide sets for each case were examined by one gynecologic pathologist (CPH) in order to confirm the diagnosis of EA. Tumors of uncertain origin, adenosquamous carcinomas and metastases to the cervix

were excluded. After the diagnosis of EA was confirmed, subtyping as per the IECC[9] was completed by three gynecologic pathologists (BH, MN, CPH) who reviewed full slide sets (hematoxylin and eosin stained slides only) for each case. Fully concordant diagnoses (agreement by all three pathologists) required no further review. Discrepant cases were assigned a majority diagnosis (when two reviewers concurred). The reviewers were blinded to all clinicopathologic characteristics as well as immunohistochemistry and HPV in situ hybridization and/or polymerase chain reaction results (if available).

For each case, age at diagnosis, stage at diagnosis and follow up information was collected (if available) including documentation of tumor recurrence, time to recurrence, time to last follow up (in months) and clinical status at last follow up (alive with no evident disease, alive with ongoing disease, died of disease, or died of other causes). Detailed clinical and pathologic information of the cohort, including patient outcome, was previously documented by our group[13].

## HPV in situ hybridization and polymerase chain reaction

In situ hybridization with chromogen for HPV types was performed on tissue microarrays, which were constructed using two 2-mm cores of representative paraffin-embedded tissue blocks per case. Normal tissue controls were included in each TMA block. In situ hybridization was completed using the Advanced Cell Diagnostics (Hayward, CA) RNAscope® system (catalogue no. 312598). The RNAscope® Probe “HPV HR18” contains probes targeting E6 and E7 mRNA for the following high risk types: HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82. Historic tumors known to contain high risk-HPV were used as positive controls, while those known to be high risk HPV-negative were used as negative controls during assay optimization. Subsequently, a negative control slide lacking application of the probes was prepared and examined, particularly when adjudicating tumors with rare or equivocal signals. A full range of cytoplasmic and nuclear signals were encountered, as has been previously described[28].

Tumor tissue for polymerase chain reaction testing was attempted in tumors not represented in tissue microarrays. The Roche cobas 4800 system (Pleasanton, California) was utilized for human papillomavirus detection which evaluates for the presence of 14 types of the following HPV DNA: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

## Genomic DNA extraction and sequencing

Tumor samples from selected paraffin-embedded blocks were processed at the Center for Advanced Molecular Diagnostics at Brigham and Women’s Hospital (Boston, MA, USA) for all stages of the next generation sequencing assay. Tumor cellularity was estimated by one pathologist (CPH) as a percentage of the overall cellularity in each selected tumor block (all cases > 20% cellularity). Unstained 4-micron formalin-fixed paraffin-embedded tissue sections of tumor were manually scraped for all cases and DNA was isolated using a commercially available kit (Qiagen, Valencia, California) following the manufacturer’s instructions. Of note, paired normal tissue samples were not analyzed. DNA was quantified (PicoGreen) and samples with at least 50 ng/ul of DNA proceeded to library preparation. Hybrid capture libraries were prepared following previously published

protocols[29,30]. Sheared DNA was hybridized to a set of custom-designed capture probes (Agilent SureSelect) targeting the complete exonic regions of 447 cancer genes and 191 intronic regions across 60 genes for the evaluation of structural rearrangements (OncoPanel). Sequencing was performed using Illumina HiSeq 2500.

## Sequencing data analysis

Data were analyzed by an internally developed bioinformatics pipeline composed of reconfigured publicly available tools and internally developed algorithms (VisCap Cancer, Phaser, BreaKmer)[29]. Pooled sample reads were demultiplexed using Picard (http://picard.sourceforge.net/command-line-overview.shtml), aligned to Human Genome Reference Consortium reference sequence GRCh37p13[31] and duplicate reads were removed. GATK[32] was used to refine alignments around insertion/deletion (indel) sites. Single nucleotide variants were called using MuTect[33] and indels using Indelocator (http://www.broadinstitute.org/cancer/cga/indelocator). Annotation was performed using Oncotator[34]. Because tumor tissues were tested without a paired normal from individual patients, additional informatics steps were taken to identify common single nucleotide polymorphisms: any single nucleotide polymorphism present at >0.1% in Exome Variant Server, NHLBI GO Exome Sequencing Project, Seattle, WA (URL: http://evs.gs.washington.edu.myaccess.library.utoronto.ca/EVS/) was filtered, however, variants also present in the Catalogue of Somatic Mutations in Cancer (COSMIC; cancer.sanger.ac.uk) were rescued for manual review. VisCap Cancer calls copy number changes based on log2 ratios that are calculated using a normalized depth of coverage against a median from a panel of normal (non-cancer) samples. Circular binary segmentation was used to segment the data; segments were called via strict thresholding. Unique, aligned (hg19) sequence reads with PHRED>30 were reviewed, annotated, and interpreted using Integrated Genome Viewer (Broad Institute, Cambridge, MA) and a suite of internally developed Web-based tools. Samples with a mean target coverage of <50 × were failed and excluded from further analysis. Individual variants present at <10% allele fraction or in regions with <50 × coverage were flagged for manual review and interpreted by the reviewing laboratory scientists and molecular pathologists (NL, BH, CPH) based on overall tumor percentage, read depth, complexity of alteration, and evidence for associated copy number alterations. Manual inspection also included cross-reference with the Catalogue of Somatic Mutations in Cancer (COSMIC) database (https://cancer.sanger.ac.uk/cosmic). After filtering and manual inspection, the number of single nucleotide variations per case was obtained (mutational burden).

Prevalent mutations (seen in two cases or more) and copy number variations were correlated with clinicopathologic variables including tumor subtype and patient outcome (in terms of disease-free survival, disease-specific survival and overall survival). “Actionable” pathogenic mutations were defined as an alteration which applies to at least one of the following contexts: predicts response to treatment with an FDA-approved therapy or an investigational therapy in clinical trials for the same cancer type, proven association of response to treatment with an FDA-approved therapy in a different type of cancer, or is a similar alteration with a proven association with response with an FDA-approved therapy in this cancer type.

### Statistical analysis

Inferential analysis was performed to compare clinicopathologic variables and genomic data using GraphPad QuickCalcs (GraphPad Software Inc, La Jolla, CA, United States). Categorical variables were compared using the Fisher's exact test, and numerical variables with the unpaired t-test. A p value of less than 0.05 was considered statistically significant.

## RESULTS

Case selection and final inclusion is depicted in Figure 3. A total of 184 cases of EA were identified in our database. Of these, 86 cases were excluded for a number of reasons: external cases seen in consultation, incomplete slide set or no blocks available, or revised diagnosis (non-EA or mixed carcinoma). Of the remaining 98 tumors, 35 were further excluded due to insufficient tissue for tissue coring or after failed sequencing and 63 cases were successfully sequenced. After exclusion of other tumor histotypes (with very low frequency for further analysis), the final cohort consisted of 56 EAs: 45 (80%) HPV-associated EAs and 11 (20%) gastric-type EAs. The 45 HPV-associated tumors were further subclassified into subtypes according to the IECC as follows: 31 usual type, 8 mucinous not otherwise specified, 1 mucinous intestinal type, and 5 invasive stratified mucin producing carcinoma. The distribution amongst tumor categories was the result of full concordance (3/3 reviewers) in 36 (64%) cases and majority consensus (2/3 reviewers) in 20 (36%) cases. Instances of complete disagreement among the three reviewers were not encountered. Classification of the included cases is shown in Table 1.

### Clinical information

The mean and median age at diagnosis for HPV-associated EAs was 43 and 40 years, respectively. Gastric-type EA patients were older, with mean and median age of 56 and 58 years, respectively ($p = 0.005$, unpaired t test). Staging information was available for 53/56 cases (95%), of which 42 were HPV-associated EAs and 11 were gastric-type EAs. 37/42 (88%) HPV-associated EAs were stage 1 while the remaining 5 (12%) were stage 2; there were no stage 3 or stage 4 HPV-associated EAs. In contrast, 3/11 (27%) of gastric-type EAs were stage 1, 1 (9%) was stage 2, 2 (18%) were stage 3, and the remaining 5 (46%) were stage 4. The difference in stage distribution between HPV-associated EAs and gastric type EAs was statistically significant ($p = 0.0002$, Fisher's exact test).

A total of 49/56 cases (88%) had clinical outcome information available. The mean time of follow up was 33 months (range 1–133 months). Tumor recurrence and/or cancer-related death was documented in 14/49 (29%) patients. Of these, 7 were HPV-associated (18% of total HPV-associated EAs with outcome information available) and 7 were gastric-type (64% of total gastric-type tumors with outcome information available). Importantly, of the HPV-associated EAs that recurred, 4 were invasive stratified mucin producing carcinomas, 2 were usual type and 1 was an intestinal type mucinous carcinoma.

In order to mitigate bias, we compared the clinico-pathologic profile of the 56 included cases with the 36 excluded due to insufficient tissue or failed sequencing. No statistically significant clinicopathologic differences were observed between these groups in terms

of patient age, histologic type distribution, stage at presentation or clinical outcome (Supplemental File 1).

## HPV detection analysis

49/56 tumors (88%) were successfully tested for the presence of HPV (42/45 HPV-associated tumors and 7/11 gastric-type tumors). Of these, 45 were tested by in-situ hybridization (cases represented in tissue microarrays) and 4 by PCR. The remaining 7 lesions had no remaining material for HPV testing. All 42 cases (100%) classified as HPV-associated EA per methodology were positive for HPV. Similarly, all 7 (100%) of the tumors classified as gastric-type EA that were tested for HPV resulted negative (Table 1).

## Mutation profile of HPV-associated and gastric-type endocervical adenocarcinomas

Prevalent altered genes across the entire cohort are shown in Table 2 and Figure 4. Specific mutations are listed in Supplemental File 2. All single nucleotide variants identified after filtration and manual inspection are listed as pathogenic or likely pathogenic in the COSMIC database. Overall, *KRAS* and *TP53* were most commonly altered (10 cases each), followed by *PIK3CA* (9 cases), and *GNAS* (7 cases). *TP53, CDKN2A, STK11, ATM and NTRK3* alterations were more frequently seen in gastric-type compared to HPV-associated tumors ($p < 0.05$ for all, Fisher's exact test). Of note, we found 2 HPV-associated EA which had *STK11* alterations; interestingly, both of these cases were classified as invasive stratified mucin producing carcinomas, a recently described entity with specific architectural and cytomorphology[35]. A higher mutation burden was noted in gastric-type EAs (average 3.2 per case) compared to HPV-associated EAs (average 2.0 per case; $p = 0.04$, unpaired t test).

Table 3 depicts the number of cases in the cohort with actionable mutations. Overall, 8 cases were found to have actionable mutations, of which 4 (9%) were HPV-associated EAs and 4 (40%) were gastric-type EAs ($p = 0.027$, Fisher's exact test).

## Correlation of mutation profile and clinical outcome

Overall, EAs with adverse outcome had a higher mutational burden compared to those cases which did not have an adverse outcome (3.2 vs. 1.7 gene alterations / case, $p = 0.004$, unpaired t test) and more commonly had mutations in *KRAS, GNAS*, and *CDKN2A* ($p < 0.05$ for all, Fisher's exact test), compared to cases without an adverse outcome. Worse outcome in *STK11*-altered cases approached significance. Of note, 6/8 (75%) of the patients who had tumors with actionable mutations suffered a recurrence and/or died of their disease; 4 of those 6 were gastric-type tumors. Indeed, 100% of gastric type tumors which had actionable alterations suffered some form of adverse outcome. To the best of our knowledge, no tumors in the cohort were treated with targeted therapy.

## Copy number variations in HPV-associated and gastric-type endocervical adenocarcinomas

Copy number variations were detected in 45/56 cases (80%) of which 36 were HPV-associated EAs and 9 were gastric-type EAs. Gastric-type EAs did not show any significant recurring copy number variations, although whole chromosome 4 and arm level chromosome 13q losses were each seen in 2 cases. The most common recurring copy

number alterations were identified exclusively in HPV-associated EAs: arm level (20p) or whole chromosome 20 gain, arm level chromosome 16q loss (7 cases each), arm level chromosome 5p gain (5 cases each), and arm level chromosome 3p and 1q losses (3 cases each). Some copy number alterations were identified in both HPV-associated and gastric-type EA: arm level chromosome 18q loss in 5 cases (4 HPV-associated EA, 1 gastric-type EA) and arm level 17p loss in 4 cases (3 HPV-associated EA, 1 gastric-type EA). Gene specific amplifications were identified in a minority of cases. *MYC* amplifications (chromosome 8q) were identified in two HPV-associated EAs (4%) and *ERBB2* (HER2) amplification was noted in one gastric-type EA (9%). None of the identified copy number variations correlated significantly with patient outcome.

## DISCUSSION

Previous studies have evaluated the genomic landscape of EAs although only a fraction have separated lesions based on subtype (i.e. HPV-related versus unrelated). In HPV-associated tumors, *PIK3CA* and *KRAS* alterations have been reported in several studies[15–21], and we again show that these genes are prevalently altered in our cohort of EAs. We also identified copy number variations (ex. involving chromosome 18 and 20) in our study which have also been previously reported[16] and we confirm that the vast majority of these are found in HPV-associated EAs.

Gastric-type EA, including minimal deviation adenocarcinoma, is associated with Peutz-Jehger's syndrome, a hereditary tumor disorder characterized by alterations in *STK11*. The STK11 protein is a serine/threonine kinase which functions, in part, to regulate the AMP-activated protein kinase pathway and therefore, has an important role in the regulation of cell metabolism. Previous studies have demonstrated alterations in *STK11* in gastric type EA[27,36,37], a finding also observed in our series. Nonetheless, the specificity of *STK11* mutations for gastric-type EA does not appear to be as good as predicted in earlier studies as we identified *STK11* alterations in two HPV-associated EAs, both of which were positive for HPV by in situ hybridization and were classified as invasive stratified mucin producing carcinomas. It is important to note that the mutations in those cases were different than those in the gastric-type EA group and that no case from either group shared an identical *STK11* mutation. All of the detected *STK11* mutations appear to involve the protein kinase domain of the STK11 protein and although the exact biochemical significance of all of the mutations is not entirely clear, the majority have been reported to negatively impact protein function[38–41]. Overall, 2/5 (40%) invasive stratified mucin producing carcinomas in the cohort had *STK11* mutations. Recently, our group studied the clinicopathologic characteristics of EAs including invasive stratified mucin producing carcinoma: these neoplasms behave more aggressively than other HPV-associated EAs[13]. Of note, none of the patients with *STK11*-mutated tumors (either HPV-related or gastric-type) had clinical findings compatible with Peutz-Jehgers Syndrome.

Murali *et al*[26] noted the morphologic and genomic similarities between gastric-type EA and pancreatic ductal adenocarcinoma. Modern molecular analysis of pancreatic ductal adenocarcinomas has shown a number of recurrently altered genes including *KRAS, TP53, GNAS, CDKN2A*, and *SMAD4*[42] and we show, like Murali *et al*, that gastric-type EA tends

to harbor alterations in the these genes[26]. Interestingly, alterations in all of these genes can be also seen in HPV-associated tumors, albeit in different proportions.

Of interest, we identified both *NTRK1* and *NTRK3* mutations in our cohort. *NTRK* rearrangements have been described in cervical sarcomas as well as some carcinomas and Larotrectinib, a tropomyosin receptor kinase (TRK) inhibitor, has been approved for use in the treatment of solid tumors which harbor an *NTRK* gene fusion. However, little data exists regarding the use of Larotrectinib in the setting of *NTRK* mutations. As such, the therapeutic significance of these alterations in not only EAs, but other tumor types with NTRK mutations, remains to be established.

Importantly, *KRAS, GNAS*, and *CDKN2A*-altered tumors, irrespective of histotype, behaved more aggressively than tumors without alterations in these genes. This finding suggests that information from next generation sequencing may be used in a prognostic sense. Potentially actionable mutations were seen in 14% of the total cohort (in addition to one additional case with *ERBB2* amplification). This is a relatively small but clinically important finding: a) all patients with gastric-type EA and adverse outcome (4/10, 40%) harbored an actionable mutation, and b) 2 of the 4 patients with HPV-associated tumors with actionable mutations also recurred and/or died of their disease. This observation further underscores the potential utility of next generation sequencing in the assessment of EAs, particularly of those tumors from patients who are suffering from recurrent and/or refractory disease. Of note, efforts to successfully target *KRAS* mutations pharmacologically are ongoing[43] while *PI3K* inhibitors such as Alpelisib and Idelalisib are FDA-approved and are already being used in the clinic in the treatment of solid tumors such as carcinomas arising from the breast[44] and different hematological malignancies[45].

Our study is limited by the relative paucity of gastric-type EAs compared to HPV-associated EAs. However, the distribution of cases in our cohort generally reflects the expected proportion of EAs[9]. We also purposely excluded other HPV-unrelated variants of EA (clear cell, mesonephric etc.) as we had too few patients in these categories. Larger studies with proper representation of these variants are certainly warranted. In addition, a number of cases were excluded which, theoretically, could introduce bias into the analysis. However, we found no clinicopathologic differences between these cases and those which were included and thus, we can be reassured that our results are applicable to a larger and general population.

In summary, in this comprehensive analysis of EA we demonstrate a correlation between the mutational profile and the etiologic subtype, namely HPV-related versus gastric-type EA. Nonetheless, no single gene alteration is entirely sensitive or specific for either type. Furthermore, we show an association between certain genomic alterations and clinical behavior. Lastly, next generation sequencing serves to identify actionable mutations. Thus, sequencing of EAs can serve as a prognostic and predictive clinical tool, particularly in patients with recurrent or refractory disease.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Conflicts of Interest and sources of funding:

The authors have no conflicts of interest to disclose.

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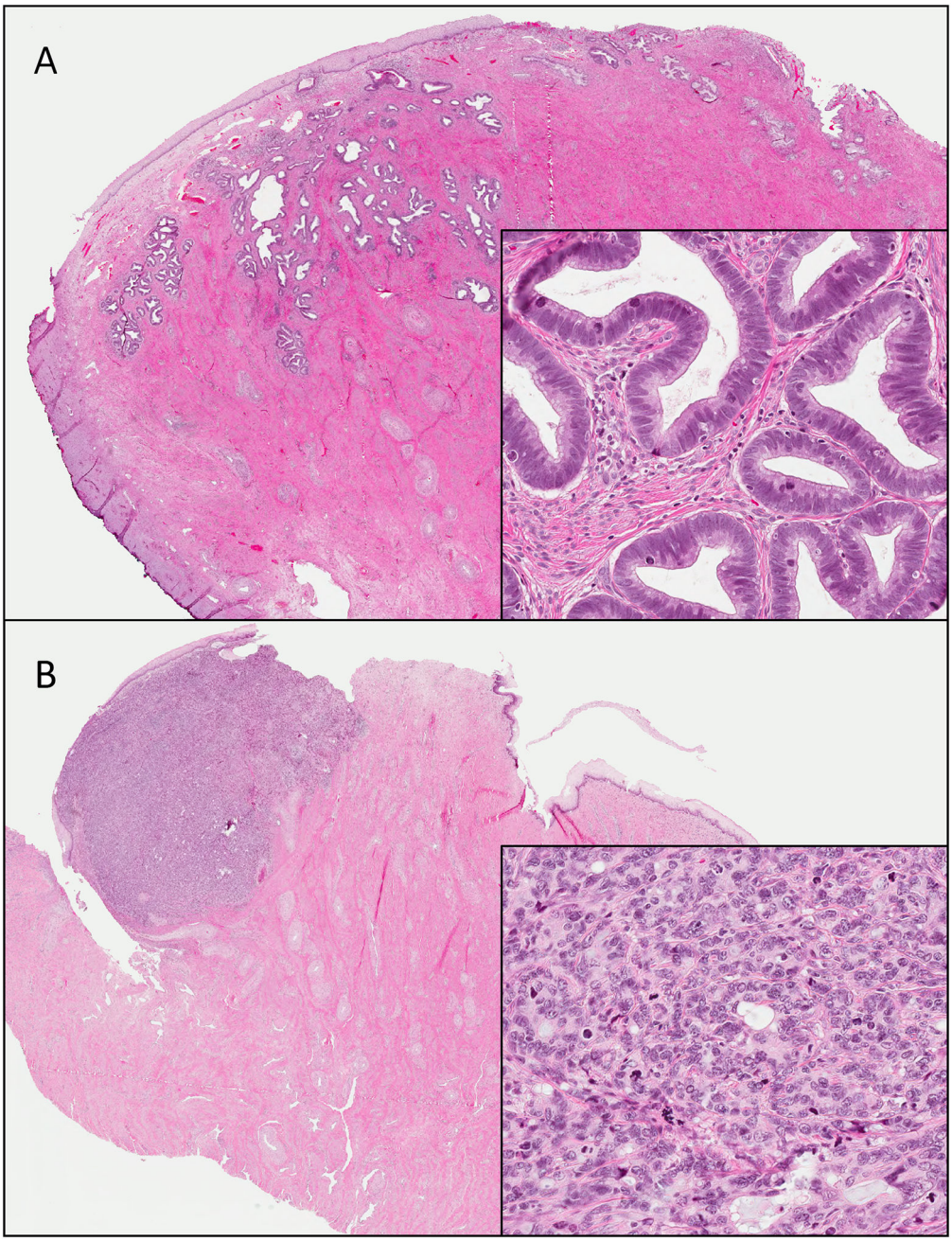


**Figure 1.**

Human papillomavirus-associated endocervical adenocarcinoma. These lesions are centered in the squamous-columnar junction. Tumors ranged from gland-forming / well-differentiated (A) to solid and poorly differentiated (B). Note in both the presence of conspicuous mitoses and apoptoses.

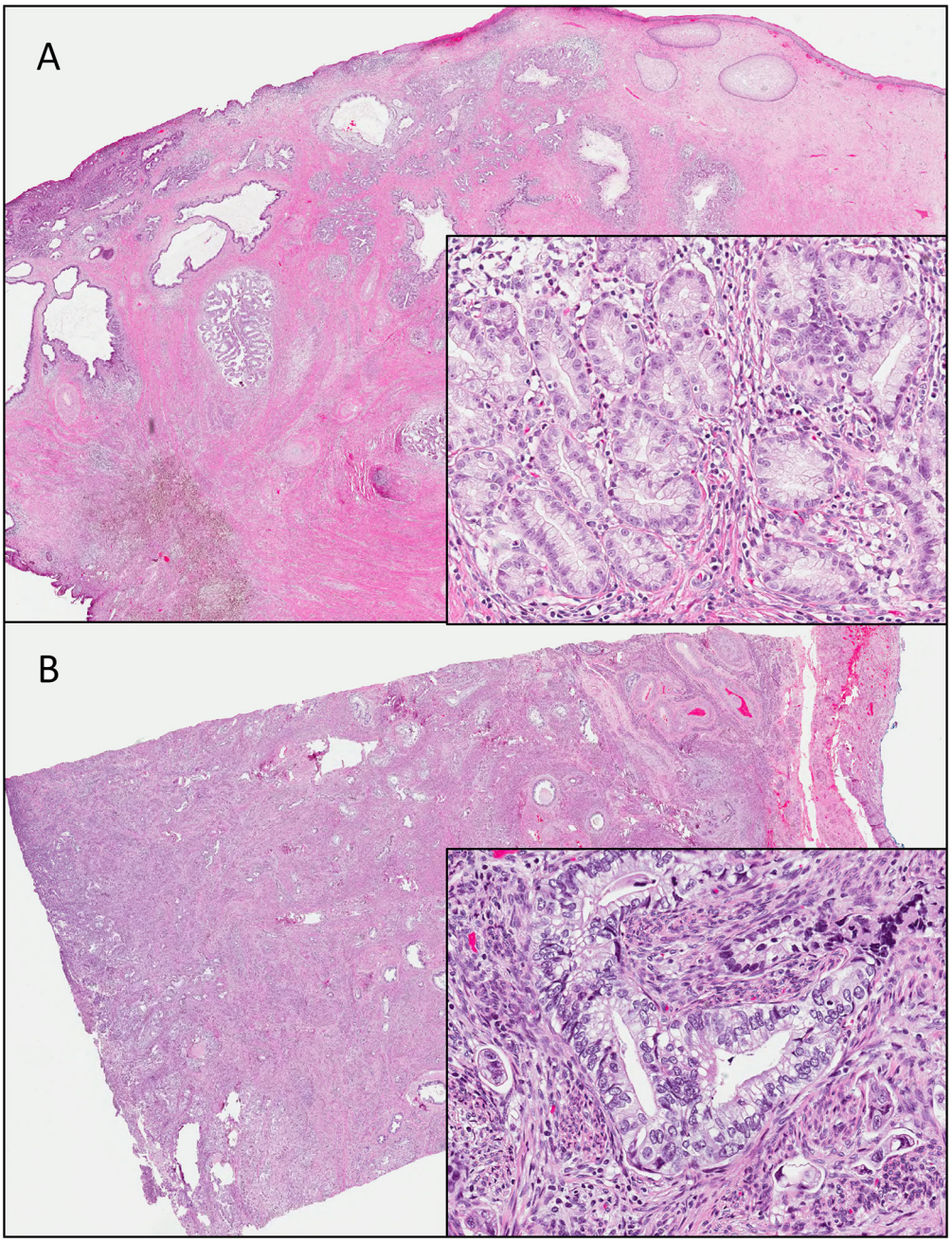


**Figure 2.**

Gastric-type endocervical adenocarcinoma. The tumor is composed of mucinous cells with clear to foamy cytoplasm, distinct cell borders and atypical round nuclei. Tumors range from well-differentiated (A) to poorly differentiated (B).

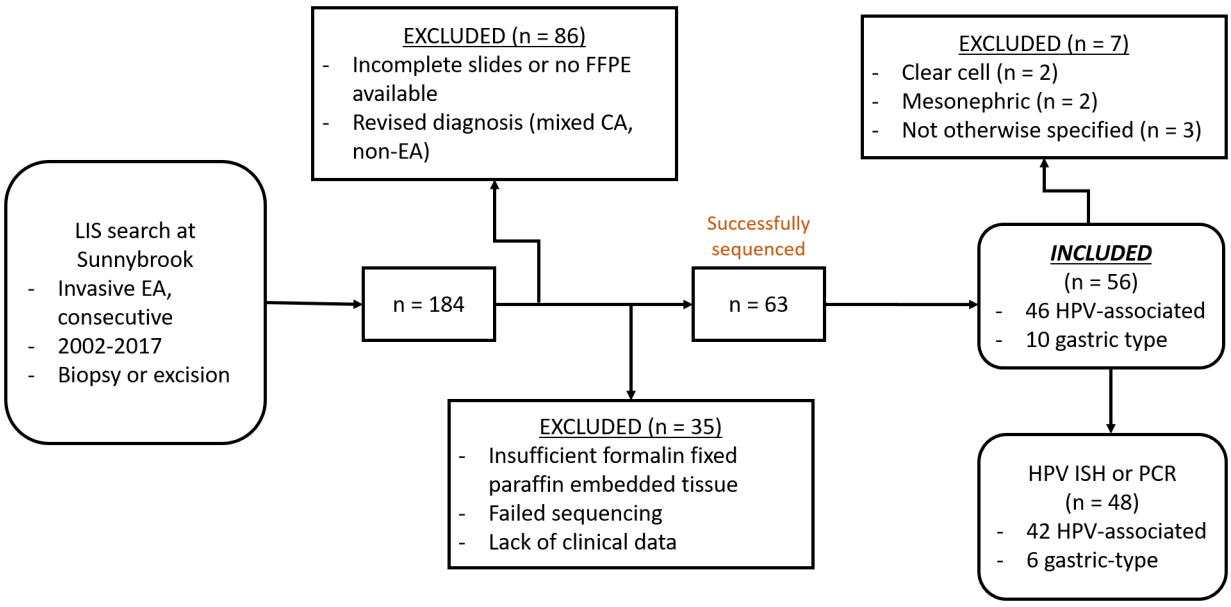


**Figure 3.**

Flowchart depicting case selection and inclusion. LIS, laboratory information system; EA, endocervical adenocarcinoma; FFPE, formalin fixed paraffin embedded; HPV, human papillomavirus; PCR, polymerase chain reaction; ISH, in situ hybridization.

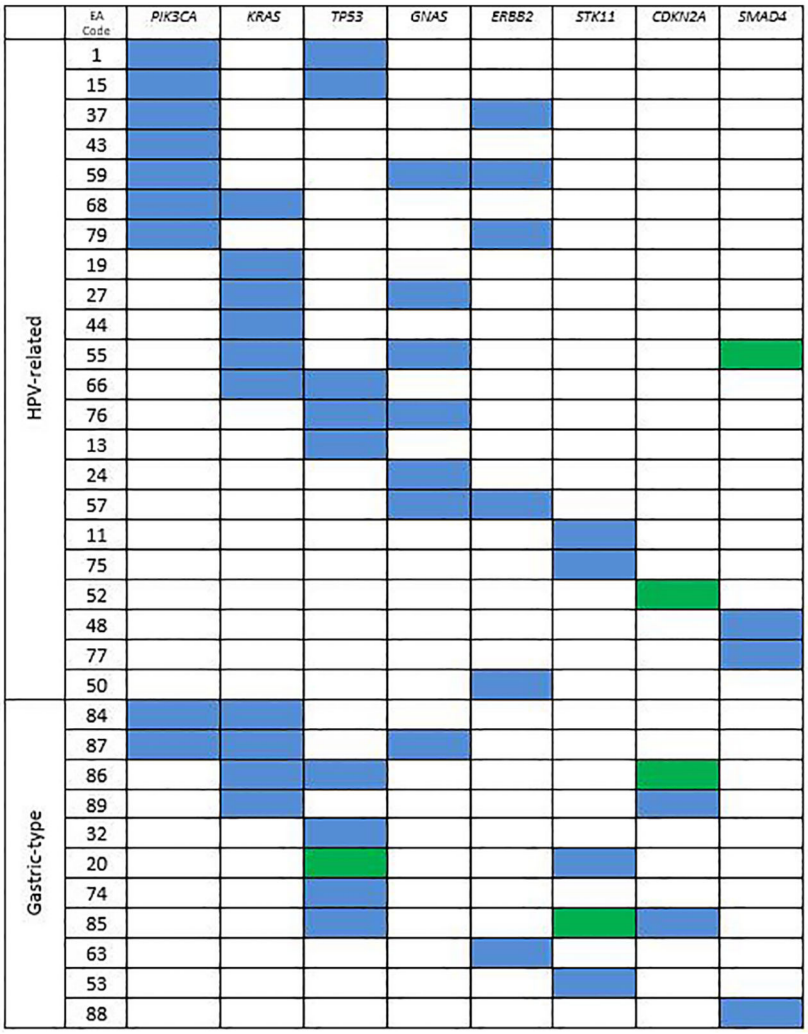


**Figure 4.**

Most commonly altered genes in endocervical adenocarcinoma. Blue = missense mutation, green = truncating/frameshift mutation.

**Table 1.**

Case distribution and HPV status for 56 endocervical adenocarcinomas morphologically classified according to the International Endocervical Adenocarcinoma Criteria and Classification (IECC)

| | Number of cases | Number of cases tested for HPV by ISH or PCR | Number of cases positive for HPV by PCR or ISH |
|---|---|---|---|
| **All endocervical adenocarcinoma cases** | 56 | 49 | 42 |
| | | | |
| **HPV-associated endocervical adenocarcinoma subtypes** | 45 | 42 | 42 |
| Usual | 31 | 29 | 29 |
| Mucinous, not otherwise specified | 8 | 7 | 7 |
| Mucinous, intestinal type | 1 | 1 | 1 |
| Invasive stratified mucin producing carcinoma | 5 | 5 | 5 |
| **Gastric-type endocervical adenocarcinoma** | 11 | 7 | 0 |

HPV: human papillomavirus; ISH: in situ hybridization; PCR: polymerase chain reaction.

**Table 2.**

Altered genes seen in at least 2 cases of endocervical adenocarcinoma cohort

| Gene | Number of total affected cases | Human papillomavirus-associated endocervical adenocarcinoma (n = 45) | Gastric-type endocervical adenocarcinoma (n = 11) | p value (Fisher's exact test) |
|---|---|---|---|---|
| | n (%) | | | |
| *KRAS* | 10 (18%) | 6 (13%) | 4 (36%) | 0.0934 |
| *TP53* | 10 (18%) | 5 (11%) | 5 (46%) | **0.0179** |
| *PIK3CA* | 9 (16%) | 7 (16%) | 2 (18%) | 1.00 |
| *GNAS* | 7 (12%) | 6 (13%) | 1 (9%) | 1.00 |
| *ERBB2* | 6 (11%) | 5 (11%) | 1 (9%) | 1.00 |
| *STK11* | 5 (9%) | 2 (4%) | 3 (27%) | **0.0468** |
| *CDKN2A* | 4 (7%) | 1 (2%) | 3 (27%) | **0.0211** |
| *SMAD4* | 4 (7%) | 3 (7%) | 1 (9%) | 1.00 |
| *ATRX* | 3 (5%) | 3 (7%) | 0 | 1.00 |
| *TET2* | 3 (5%) | 3 (7%) | 0 | 1.00 |
| *ATM* | 2 (4%) | 0 | 2 (18%) | **0.0357** |
| *NTRK3* | 2 (4%) | 0 | 2 (18%) | **0.0357** |
| *ERBB3* | 2 (4%) | 2 (4%) | 0 | 1.00 |
| *SF3B1* | 2 (4%) | 2 (4%) | 0 | 1.00 |
| *NTRK1* | 2 (4%) | 2 (4%) | 0 | 1.00 |
| *CARD11* | 2 (4%) | 2 (4%) | 0 | 1.00 |
| *CREBBP* | 2 (4%) | 2 (4%) | 0 | 1.00 |
| *MED12* | 2 (4%) | 2 (4%) | 0 | 1.00 |
| *HNF1A* | 2 (4%) | 2 (4%) | 0 | 1.00 |
| *MAP2K2* | 2 (4%) | 2 (4%) | 0 | 1.00 |
| *TSC2* | 2 (4%) | 2 (4%) | 0 | 1.00 |
| *CHEK2* | 2 (4%) | 2 (4%) | 0 | 1.00 |
| *ETV6* | 2 (4%) | 2 (4%) | 0 | 1.00 |

**Table 3.**

Genes with actionable alterations in endocervical adenocarcinoma

| Gene | Human papillomavirus-associated endocervical adenocarcinoma (n = 45) | Gastric-type endocervical adenocarcinoma (n = 11) |
|---|---|---|
| *KRAS* | 1 | 2 |
| *PIK3CA* | 2 | 0 |
| *KRAS and PIK3CA* | 0 | 2 |
| *ERBB2* | 1 | 0 |
| | | |
| Total number of cases with actionable alterations | 4 (9%) | 4 (36%) |