Highly proliferative anal neuroendocrine carcinoma: molecular and clinical features of a rare, recurrent case in complete remission

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Abstract

Background: Poorly differentiated anal neuroendocrine carcinomas (ANECs) are rare lesions with poor prognosis, and the molecular etiology is only partially understood.

Case presentation: At our institution, we have treated and followed a patient with such a rare ANEC. He had primarily surgery followed by three rounds of repeated surgery for loco-regional recurrences. He also received three different combinations of chemotherapy and external beam radiation. At last follow-up 13 years since the primary diagnosis, the patient had been in complete remission for nine years.

The patient’s medical files were re-examined, including laboratory, radiology and clinical examinations. Histopathology was re-assessed, and expanded immunohistochemistry was performed from tissue specimens from the four surgical procedures. In addition, the molecular genetic status was evaluated through next-generation sequencing.

The initial tumor was consistent with a 59 mm small cell neuroendocrine cancer with a Ki-67 index of 80%. Regional lymph node metastases were evident, and immunohistochemistry supported a neuroendocrine origin. A PCR screening detected human papilloma virus type 45 DNA (high-risk subtype), and focused next-generation sequencing found a missense mutation in the Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha (PIK3CA) gene. In tissues representing subsequent recurrences, the Chromogranin A expression was lost, and the Ki-67 index increased to 90%.

Conclusions: For the first time, we report the detection of HPV45 in a case of ANEC. To our belief, PIK3CA mutations have also not been previously demonstrated in this tumor entity. In highly malignant ANECs, cure can in rare cases be achieved. Although speculative, expression of HPV45 and/or the PIK3CA mutation may have contributed to the favorable outcome.

Keywords: Anal neuroendocrine carcinoma, Remission, HPV, PIK3CA, Mutation, Case report
Background
Carcinomas of the anal tract are mainly squamous cell carcinoma (SCC) or adenocarcinomas, with anal neuroendocrine carcinomas (ANECs) representing only 1% of anal malignancies [1]. Small, localized and well-differentiated neuroendocrine tumors are much more common, are usually <10 mm in size and have rarely invaded or metastasized at diagnosis [2]. Neuroendocrine carcinomas with neuroendocrine features are graded by a Ki-67 proliferation index, ranging from G1 (Ki-67 index <3%), G2 (3–20%) and G3 (>20%). G3 tumors are further subdivided into well-differentiated NET G3 and NECs [3]. The pathogenesis is unclear, but ANECs can occur synchronously with squamous cell cancer [4] or adenocarcinoma [5], but does not seem to develop as part of a dedifferentiation from well-differentiated neuroendocrine tumors [6].

Treatment with surgery and/or chemotherapy is normally preferred, initially often platinum-etoposide or based on 5-fluorouracil [7], but the outcome is generally poor, and the five-year survival rate has been estimated to 3–27% when distant metastases are present [8, 9]. Due to the rarity of ANECs, prospective studies are lacking and no standard treatment regimen has been developed. Moreover, guidelines as to if surgery should be performed unless the tumor is localized, or if chemotherapy should be the therapy of choice, are also lacking.

At our institution, we have now treated and followed a patient diagnosed with ANEC during 13 years. This male patient described herein has had surgery four times, and has been treated with chemotherapy and external radiation, and is now recurrence-free. Our aim is to describe the clinical and histopathological features of this highly malignant tumor presenting with a successful long-term outcome. The patient's medical files were re-examined, including laboratory, radiology and clinical examinations. Histopathology was re-assessed, and expanded immunohistochemistry was performed from tissue specimens from the four surgical procedures.

All immunohistochemical stainings were carried out in our pathology laboratory using the Ventana Benchmark Ultra system (Ventana Medical Systems, Tucson, AZ, USA). All stainings were analyzed by an endocrine pathologist (CCJ).

DNA extraction from formalin-fixed paraffin-embedded (FFPE) tissues was performed using the Maxwell® DNA FFPE Tissue kit from Promega, and the Oncomine Solid Tumor Panel (Ion Torrent S5, Hi-Q Chef; Thermo Scientific) was used to screen for >1800 cancer related mutations within the following genes: EGER, KRAS, NRAS, PIK3CA, BRAF, ALK, ERBB2, ERBB4, FGFR1, FGFR2, FGFR3, MET, DDR2, AKT1, PTEN, MAP 2 K1, STK11, NOTCH1, CTNNB1, SMAD4, FBXW7 and TPS3. The input DNA was 10 ng.

For HPV detection, real-time PCR was performed in duplicate with probes detecting HPV high-risk types 16, 18, 31, 33, 35 and 45 using tumor DNA and an established protocol used in clinical routine. Specific PCR cycling conditions are available upon request.

Case presentation
A 37-year-old male without any previous medical history was examined for a painful anal fissure in 2006, and a biopsy was consistent with small cell ANEC with a Ki-67 proliferation index of 80%. Laboratory with blood count, liver and kidney parameters were normal, including CEA 4.5 μg/L (reference: <4.7). An abdominoperineal resection (APR) was performed, and the patient was subsequently treated with adjuvant cisplatin/etoposide (six rounds) as well as external radiation therapy (46.8 Gy), followed by further chemotherapy for 1 year. Two ensuing recurrences with subcutaneous lymph node metastases in the right inguinal area were noted shortly afterward, and the metastatic tissue was removed surgically in two subsequent operations 2 years after initial presentation (Fig. 1). The first metastasis measured 3.7 cm and was excised with positive margins, and the second subcutaneous relapse was 4 cm with extensive perinodal extension, almost penetrating the overlying skin. Post-operatively, the patient was administered three rounds of doxorubicin and docetaxel, with additional radiation 12.6 Gy. Soon after this, an additional subcutaneous recurrence within the abdominal wall was noted, which expanded from two to 6 cm within 2 months. Extended surgery was performed, excising a 7 cm large metastasis with negative resection margins. The resection included the spermatic cord and right testicle, but these structures were seen without tumoral engagement. This procedure was completed with reconstructive surgery using a flap technique and a skin transplant. After this, adjuvant treatment with eight rounds of irinotecan/fluorouracil and folic acid (leucovorin) were administered for 2 years. Since the last relapse 2 years after initial presentation and to our last follow-up 13 years after initial presentation, there have been no recurrences during clinical investigations and imaging performed with repeated computed tomography (CT), magnetic resonance imaging (MRI) and fluorodeoxyglucose–positron emission tomography (FDG-PET) (Fig. 1). The patient is still followed clinically by our Department of Breast and Endocrine Surgery, and a follow-up MRI of the abdomen and pelvic area is planned in the autumn of 2020. If this future radiological examination is negative for recurrences, the patient will be discharged as an outpatient. The clinical course of our patient is schematically presented in Table 1.

Histopathology of the primarily surgically resected anal lesion as well as three subsequent local recurrences...
was reviewed by an experienced endocrine pathologist (CCJ) to verify the original diagnoses. In this process, additional immunohistochemical and molecular analyses were ordered, and are detailed below.

The primary anal lesion resected was found to be a 59 mm undifferentiated tumor growing in solid formations, with tumor nuclei displaying a dense chromatin and focal nuclear molding (Fig. 2a-b). Plenty of apoptotic bodies were also noted, in addition to widespread tumor necrosis and > 20 mitoses per ten high-power fields. The tumor infiltrated through the muscularis propria into the pericolic fat, but displayed negative margins. Metastases were found in 2 of 19 regional lymph nodes (Fig. 2c).

By immunohistochemistry, the tumor cells were seen positive for Chromogranin A, Synaptophysin, CD56, INSM1, OSCAR, somatostatin receptor type 2 (SSR2), P16 and focally for ISLET1 (Fig. 2d-f). Negative immunoreactivity was noted for P53, CK20, MCV-LT, CDX2, AFP, PSA, HCG, Uroplakin, GATA3, OCT3-4, PLAP, GLP-1, SOX10R and CD45. The diagnosis was consistent with a poorly differentiated small cell ANEC with a Ki-67 index of 80% (Fig. 2g). The TNM staging was pT3N1a R0.

![Fig. 1 Imaging of the lower abdominal cavity and pelvic region visualizing one of the local recurrences as well as the radiological evidence of complete remission after the final round of surgery. Left columns depict conventional computerized tomography (CT) scans and right columns visualize the positron emission tomography with fluorodeoxyglucose (PET F18 FDG) findings. Top row: White arrowhead highlights one of the local recurrences, a right-sided subcutaneous inguinal lymph node metastasis. Bottom row: No remaining tumor tissue is evident after the fourth and final round of surgery. Left side is marked by the letter “L.”](image)

| Year | 2006 | 2008 | 2008 | 2008 |
|------|------|------|------|------|
| **Surgery** | Primary surgery (abdomino-perineal resection) | Resection of first recurrence | Resection of second recurrence | Resection of third recurrence |
| **Diagnosis** | Primary ANEC with regional lymph node metastases | Inguinal lymph node metastasis | Inguinal lymph node metastasis | Abdominal wall metastasis |
| **Tumor size (cm)** | 5.9 | 3.7 | 4.0 | 7.0 |
| **Chromogranin A IHC** | Positive | Negative | Negative | Negative |
| **Synaptophysin IHC** | Positive | Positive | Positive | Positive |
| **Ki-67 index** | 80% | 90% | 90% | 90% |
| **Adjuvant chemotherapy** | Cisplatin, Etoposide | Doxorubicin, Docetaxel | Irinotecan, Fluorouracil |
| **Adjuvant radiotherapy** | 46.8 Gy | – | 12.6 Gy | – |

IHC immunohistochemistry, cm centimeter, ANEC anal neuroendocrine carcinoma, Gy Grey, –; not administered
Given the strong P16 stain, a real time PCR screening of high-risk HPV (HR-HPV) was ordered using FFPE material from the primary tumor, and HPV type 45 DNA was detected – thereby strongly suggesting an active HR-HPV infection in the excised lesion. Indeed, the combination of P16 positivity and the detection of HR-HPV DNA is a strong indicator of HPV early-gene expression [10]. Interestingly, our patient had no other known HPV associated manifestations of the genital or extra-genital regions.

When analyzing tumor DNA extracted from the primary tumor, a focused next-generation sequencing approach detected a PIK3CA mutation in exon 10 (c.1624G > A p.Glu542Lys; E542K. This specific mutation is recurrently found in carcinomas of the breast and endometrium and is therefore believed to be pathogenic. We performed an in silico analysis using PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/), in which the mutation scored 0.995, indicating a high probability of this missense variant of having impact on the overall PIK3CA function.

Subsequent characterization of the three separate subcutaneous metastases displayed a small cell tumor with a similar histological appearance as the primary small cell ANEC diagnosed earlier. The tumor cells were now negative for Chromogranin A but positive for synaptophysin and pan-cytokeratins, and the Ki-67 index was consistently 90% in all three metastatic lesions (Fig. 2h-i). The absence of Chromogranin A expression in the subsequent relapses do not argue against an ANEC diagnosis, as reduced or absent expression of this marker is not uncommonly observed in metachronously biopsied NECs from the same individual. This reduction in sensitivity is well established and possibly reflects the underlying tumor de-differentiation process [11].
**Discussion and conclusions**

We describe a patient with a highly proliferative ANEC who had surgery four times, received six different cytotoxic drugs in three combinations and has no residual disease at last follow-up 13 years after the primary diagnosis. From the first round of surgery, the tumor differentiated further, losing Chromogranin A expression and increasing in Ki-67 index. Intriguingly, the tumor exhibited HPV 45 DNA and strong P16 staining - suggesting the intra-tumoral presence of active HR-HPV. Moreover, sequencing analyses revealed a cancer-associated PIK3CA mutation. These two molecular aberrancies have never before been synchronously reported in ANECs.

ANECs are rare tumors, not least reflected in our own material. After probing our departmental pathology database using a SNOMED based search criteria with morphology codes “malignant neuroendocrine neoplasm” (M82463) and “neuroendocrine cancer” (M82493) with topography codes of “rectum” (T68000) and “anus” (T69000), we found no additional cases of ANECs over a time period of almost 30 years. However, when specifically searching through our consultation cases (second opinion reviews of tumors initially resected outside of our department), we did identify one additional patient, an 80-year-old female, with a biopsy suggesting small cell ANEC. Given the scarce availability of tissue after exhausting the FFPE block for immunohistochemical purposes, no molecular analyses had been performed. However, this tumor was also strongly P16 positive, possibly arguing for an active HPV infection also in this case (data not shown). This assumption is also in line with previous results from more comprehensive case series, in which HR-HPV DNA is recurrently detected in ANECs [5].

Complete remission of poorly differentiated NECs is indeed rare. In metastatic NECs from various sites, the median survival is 7.6 months for lung NEC, 7.5 months for gastrointestinal NEC, and 2.5 months for NEC of unknown primary [12]. However, some factors have been in favor for this patient. His age at presentation (37 years) was one of them. The median age at presentation of NECs in general is often much older [13], and sometimes concomitant diseases such as HIV or inflammatory bowel disease contributes to the poor prognosis. Our patient did not exhibit distant metastases at diagnosis, and the observed recurrences were only local with inguinal and lymph node metastases. Also, the initial surgery was performed with negative margins.

In ANEC, the molecular drivers have been poorly understood. HPV is an etiological agent of malignant diseases of the cervix, vulva, penis, anal canal, larynx as well as the head and neck region [14]. HPV is also a pathogenic factor in anal neoplasms, with up to 90% in anal SCC [15]. In individuals with HPV, viral DNA can be integrated in the genome of the host cell and this is an important route for progression of pre-neoplastic lesions to invasive carcinoma. The Rb protein is a critical negative regulator of the cell cycle that binds the transcription factor E2F and thus blocks entry into S-phase [16]. The oncoprotein E7 from HR-HPV can cause inactivation and increase degradation of Rb, by which Rb loses its ability to halt cell cycle progression and can bring about oncogenesis in infected individuals [17].

High risk papilloma virus is associated with anal adenocarcinoma and anal squamous skin cancer [5], and HPV subtypes 16 and 18 have also been previously reported in ANEC [5, 18]. Presence of HR-HPV may also be associated to a better outcome, as in stage III anal squamous cell carcinoma in which a more favorable response to chemotherapy has been seen with a better 5-year disease free survival (DFS) [19]. If this is true also for ANECs is not known.

The dimeric enzyme kinase lipid family, phosphoinositide 3-kinases (PI3Ks), acts in regulating cellular functions as proliferation, differentiation and survival [20]. In SCC of the anal canal, PIK3CA mutations have been previously described [21]. Patients with genomic alterations in PIK3CA have also been demonstrated in seven patients with colorectal NECs and HR-HPV type 18 [5], but if the PIK3CA mutations were present in the two ANEC patients included in this study is not evident. To the best of our knowledge this is therefore the first publication where such a gene alteration has been found in anal NECs in association with HR-HPV type 45.

How to treat ANECs has been debated. Similar progression-free survival (PFS) or overall survival (OS) numbers have been found in cases treated with surgery vs. chemotherapy [8, 22]. In a publication by Brieau and colleagues, there was no difference in PFS or OS in 24 patients treated with surgery vs. chemotherapy [23]. Radiation is also a treatment possibility that should not be overlooked [24]. Debulking or resection of metastases has generally not been recommended due to the poor prognosis in metastatic disease, but remains today the only possible cure [25, 26].

Chemotherapy options as a treatment algorithm for ANECs has primarily been based on platinum-derived agents (i.e. cisplatin), which is the recommended drug of choice according to the Swedish national guidelines and the European Society for Medical Oncology (ESMO) – and was also the case for our patient [27]. This compound inhibits cell division by interference with DNA transcription and/or replication [12, 28]. In our case, cisplatin was combined with etoposide, a compound that also induces DNA damage. Even so, our patient developed metastatic recurrences despite this treatment. The
second round of chemotherapy was doxorubicin (preventing DNA replication) and docetaxel (promoting tubulin assembly in microtubules and inhibits their depolymerization). By this, docetaxel acts as a mitotic poison and induces a mitotic block in proliferating cells [29]. Also, the second round of chemotherapy was largely unsuccessful. After the fourth surgical procedure, irinotecan was administered - an enzyme inhibitor blocking the enzyme topoisomerase causing double strand DNA breakage and cell death. This drug was given together with 5-fluoruracil, another enzyme inhibitor acting on thymidylate blocking formation of thymidine required for DNA synthesis [30]. During and after this treatment, following the fourth surgery, the patient has remained healthy. However, from this experience it cannot be decided if irinotecan/5-fluoruracil had an additive cytotoxic effect – but it is tempting to speculate that the combination of these drugs in association to the rare occurrence of HPV45 and/or the PIK3CA mutation could have influenced the disease course. Even so, we are not in a position to advocate changes to current treatment algorithms based on a single case.

Mutations in PI3K is frequent in most solid cancers [31], and investigations are ongoing if PI3K/mTOR inhibitors, PI3K pan-inhibitors or isoform-selective PI3K inhibitors could have an effect on various PI3K mutated tumors [32]. Of recent note, approval has been granted for the isoform-selective PI3K inhibitor alpelisib in the treatment of refractory breast cancer. Although speculative, such therapy may also be a potential adjuvant treatment modality for PI3K mutated ANECs. Ongoing studies will also clarify whether peptide receptor radionuclide therapy and/or immunotherapy with pembrolizumab or nivolumab will add to the therapeutic possibilities, not only in anal SCC but also in ANECs [33]. Moreover, vaccination against HR-HPV types 6, 11, 16, and 18 may also decrease the development of papilloma virus associated malignancies. Trials are also aiming at delivering HPV oncprotein E6 and E7 antigens to elicit cytotoxic T-cell activation [34].

We conclude that ANEC is a rare disease. Repetitive surgery and chemotherapy may in occasional instances induce cure. The manifestation of HR-HPV type 45 and a PIK3CA mutation may possibly have contributed to treatment response and an overall favorable prognosis despite disseminated disease, but this has to be confirmed in future investigations.

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Authors’ contributions
J.C. and H.F. conceived the study. C.C.J. performed the histological and molecular work-up. M.K. and J.A. gave input from a surgical perspective, and S.W. from an oncological angle. All authors discussed the results and contributed to the final manuscript. The author(s) read and approved the final manuscript.

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All data generated or analysed during this study are included in this published article.

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Ethical approval was granted by the Swedish Ethical Review Authority. Written consent to participate in this study was obtained from the patient.

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Written consent to write and publish this manuscript was obtained from the patient.

Competing interests
The authors declare that they have no competing interests.

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