Mixed Adenoneuroendocrine Carcinoma of the Colon: Molecular Pathogenesis and Treatment

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Abstract. Background/Aim: We report a case of a mixed adenoneuroendocrine carcinoma developed in a colorectal adenocarcinoma with lymph node and liver metastases exclusively emanating from the neuroendocrine carcinoma component. The patient underwent right hemicolectomy and postoperatively received chemotherapy with cisplatin and etoposide and subsequent high-dose induction chemotherapy, followed by autologous stem cell transplantation. Following this treatment, there was a complete remission. Currently, thirty months after treatment, the patient is in unmaintained complete remission. Comparative exome sequencing of germline DNA and DNA from the two separate malignant components revealed six somatic changes in cancer consensus genes. Both components shared somatic mutations in Adenomatous polyposis coli (APC), Kirsten rat sarcoma viral oncogene homolog (KRAS), B-cell CLL/lymphoma 9 (BCL9) and Forkhead Box P1 (FOXP1) genes. Mutation in SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (SMARCA4) was only found in the neuroendocrine carcinoma component. The finding of several identical somatic mutations in both components supports a clonal relationship between the neuroendocrine carcinoma and the adenocarcinoma. We suggest that a mutation in SMARCA4 could be responsible for the transformation of the adenocarcinoma component into the neuroendocrine phenotype.

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High-grade neuroendocrine tumours of the colon are rare (1, 2). Herein, we report a case of an aggressive mixed adenoneuroendocrine carcinoma (MANEC) that developed in a low-grade colorectal adenocarcinoma. There are only a few descriptive reports on large-cell colorectal neuroendocrine carcinomas arising in adenomas or adenocarcinomas of the colon (2).

Case Report

A 30-year-old male patient presented with abdominal discomfort and unexplained weight loss. His past medical history was unremarkable. His father was diagnosed at the age of 60 years with multiple adenomatous polyps of the colon and his paternal grandmother underwent hemicolectomy because of malignancy. Further familial history was unremarkable. Physical examination showed hepatomegaly. Laboratory findings revealed inflammation and elevated liver enzymes.

A colonoscopy showed a large polyp at the flexura hepatica coli. A biopsy was performed. The lumen of the bowel appeared to be violated and the patient underwent emergency laparotomy. Intraoperative inspection revealed a polypoid tumour of 7×4.5 cm in the colon ascendens, with several palpable lymph nodes and multiple liver metastases, without peritoneal carcinomatosis. The patient underwent right hemicolectomy and a liver biopsy.

The resection margin of the colectomy specimen was tumour-free. Five out of the 37 dissected regional lymph nodes were found to be involved. No other distant metastases were found. Ultrasound of the liver showed a dilated bile duct due to the massive liver metastases.

Pathological examination of the colonic tumour revealed an invasive carcinoma that had developed from a tubulovillous adenoma. Two different, closely-intermingled, invasive components were present. One part was consistent...
with a low-grade gland-forming adenocarcinoma (Figure 1A). The other part of the tumour consisted of solid nests of tumour cells and large cells with large vesicular nuclei with prominent nucleoli (Figure 1B). There were numerous mitotic figures. The Ki67 index was 75% in the latter component as opposed to 25% in the low-grade adenocarcinoma. These cells were found to be positive for synaptophysin and CD56, and negative for chromogranin. These histological and immunohistochemical findings are consistent with a high-grade large cell neuroendocrine carcinoma. The liver biopsy and the involved lymph nodes were compatible with a metastasis of the large cell neuroendocrine carcinoma only (Figure 1C).

The same Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation (G12D) was found in the large cell neuroendocrine carcinoma component, in the adenocarcinoma component in the colon and in the liver metastasis, showing a clonal relationship between the two malignant phenotypes. Immunohistochemistry for DNA mismatch repair (MMR) proteins mutL homolog 1 (MLH1), post-meiotic segregation increased 2 (PMS2), mutS homolog 2 (MSH2) and mutS homolog 6 (MSH6) showed preserved nuclear expression.

There was an elevation of neuron-specific enolase (NSE; 276.7 μg/l) and chromogranin (251 μg/l). Beta-human chorionic gonadotropin was within normal limits. Thus, we concluded that the patient had a mixed low-grade adenocarcinoma (pT1N0M0) and a high-grade large cell neuroendocrine tumour (pT1N2aM1a) in a tubulovillous adenoma of the colon.

The patient received postoperative chemotherapy with cisplatinum and etoposide. After four cycles of chemotherapy, a partial remission was obtained. Because of the high chemosensitivity of the tumour, he received subsequent high-dose induction chemotherapy with carboplatin, mitoxantrone and cyclophosphamide, followed by autologous stem cell transplantation. A total of 3.6×10^6 stem cells/kg bodyweight was administered. The neutropenic phase post-transplantation was complicated by grade 2 mucositis. Haematological recovery was prompt with neutrophil engraftment day 13 post-transplantation.

Following this treatment, there was a clinical and radiological complete remission with normalisation of the imagining (Figure 2) and tumour markers (NSE and chromogranin). Thirty months after treatment, the patient is alive and in unmaintained complete remission.

The occurrence of an aggressive high-grade neuroendocrine carcinoma in a low-grade colonic adenocarcinoma without any intermediate phenotypes was intriguing. Therefore we wanted to investigate the genomic events that might have caused this abrupt transformation. Subsequently, DNA was extracted from formalin-fixed blocks containing each of the malignant components, followed by comparative whole-exome sequencing of both components using the Illumina TruSeq DNA library preparation and Exome Capture kits and an Illumina HiSeq2000 sequencer. Somatic changes were indentified as described previously (3). On average, we obtained exome data with an average coverage (to refer to the average number of times both exomes were sequenced as a quality control measure) of 31.9 and 37.7x, respectively, allowing us to identify 6 somatic changes in cancer consensus genes.

Somatic stop-gain and frameshift mutations were detected in Adenomatous polyposis coli (APC) (R1096X and L1382fs, respectively), and Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation (G12D) in both tumour components was confirmed. Additionally, we detected two other mutations in B-cell CLL/lymphoma (BCL9) and Forkhead Box P1 (FOXP1) in both tumours, as well as a missense mutation in SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (SMARCA4) (R849W) in the neuroendocrine carcinoma component only. All mutations were confirmed using Sequenom Mass Array and were absent in germ-line DNA. There were no gross differences in the amplification/deletion patterns detected in both tumours.

**Discussion**

High-grade neuroendocrine carcinoma of the colon is very rare. Almost all are metastasized at the time of diagnosis and carries a poor prognosis (1, 2). Once metastasized, high-grade digestive neuroendocrine carcinoma has a very poor prognosis, with no curative treatment available.

Only one other case of a 60-year-old man with a synchronous high-grade large cell neuroendocrine carcinoma and a low-grade adenocarcinoma of the colon has been described as far as we were aware of, however, without metastases. He received postoperative chemotherapy (oxaliplatin, 5-fluouracil and leucovorin) and had no sign of tumour progression or distant metastasis six months after treatment (2).

Most patients with high-grade metastatic digestive neuroendocrine tumours are not candidates for curative resection (4). Patients with a high-grade neuroendocrine tumours of the colon, as well as of other digestive primary sites, have a high response rate to cisplatin and etoposide, which in most patients is short-lived, and the survival is limited to less than one year (4). There are no long-term stage IV survivors reported. A retrospective study assessing the efficacy of cisplatin-plus-etoposide in metastatic high-grade neuroendocrine tumours of the colon reported a radiographic complete response in one out of eight patients, and four with a partial response. The median progression-free survival was 4.5 months and the median overall survival was 9.5 months.
Figure 1. Histological features of tumours in this case. A: Low-grade gland-forming adenocarcinoma, H&E staining (magnification ×500). B: Area of high-grade large cell neuroendocrine carcinoma closely-intermingled with the low-grade gland-forming adenocarcinoma component. Left: H&E staining (magnification ×1000); right: immunohistochemistry for synaptophysin (magnification ×500). C: Area of high-grade large cell neuroendocrine carcinoma in the liver. Left: H&E staining (magnification ×1,000); right: immunohistochemistry for synaptophysin (magnification ×1,000).
(4). No patient survived beyond 17 months. This is in line with the treatment and overall outcome of stage IV extrapulmonary high-grade neuroendocrine carcinomas in general (5).

Because of the reported poor outcome perspectives with conventional chemotherapy alone, we applied a high-dose chemotherapy followed by autologous stem cell transplantation. We utilized a high-dose chemotherapy regimen that had been successful in a patient with a metastatic stage primitive neuroectodermal tumor, an incurable disease in adults (6), who is now alive and has been in unmaintained complete remission for 20 years.

Previous literature has not been able to establish a clonal relationship between occurrences of high-grade neuroendocrine carcinoma and synchronous adenocarcinoma. If a clonal relationship exists, the second question that arises is what event is responsible for the transformation from an adenocarcinoma phenotype to a neuroendocrine phenotype?

Therefore, we investigated whether a clonal relationship exists. The identical KRAS mutation in both components of the tumour already supported a clonal relationship between the MANEC and the adenocarcinoma and is further supported by the finding of several shared somatic mutations in both components in the subsequent exome sequencing.

The histological examination suggested that the MANEC arose abruptly in the low-grade adenocarcinoma polyp. Such an abrupt transition could have been caused by a discrete genomic event. Another possibility is that a genomic catastrophe (chromothripsis) occurred in an adeno(carcinoma) cell, leading to widespread mutations in the cancer genome responsible for the aggressive neuroendocrine clone (8). We, therefore, used comparative exome sequencing of the adenocarcinoma component and the neuroendocrine carcinoma. Remarkably, the only unique event detectable in the aggressive neuroendocrine clone affected the helicase domain of SMARCA4. Heterozygous missense mutations in this domain are the most frequent somatic event in the Wingless-type MMTV integration site family member (WNT) subgroup of medulloblastoma tumours, where they disrupt chromatin remodelling of WNT-responsive genes (9). There are indeed multiple similarities between neurons and neuroendocrine cells, not only morphologically and functionally but also in terms of gene expression profiles (10). Our observations therefore suggest that SMARC4A inactivation was responsible for the transdifferentiation of an adenocarcinoma into an aggressive neuroendocrine phenotype and could possibly represent a common driver mutation between such neuroendocrine tumours and other tumours of neuroectodermal origin, including medulloblastoma (11).

**Conclusion**

We highlight a clonal relationship between a low-grade adenocarcinoma of the colon and an aggressive neuroendocrine carcinoma that abruptly arose in the adenocarcinoma. A discrete mutation in the SMARC4A gene is implicated in the sudden transformation.

**Conflicts of Interest**

No conflicts of interest were declared.
References


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