Endocrine tumors of the pancreas and gastrointestinal tract
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CHAPTER 4

A TUMOR WITH A NEUROENDOCRINE AND PAPILLARY SEROUS COMPONENT: TWO OR A PAIR?

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A tumor with a neuroendocrine and papillary serous component

Abstract

Aims
To examine the clonal origin of a tumor, made up of a neuroendocrine component and a papillary serous component, by comparing the pattern of loss of heterozygosity (LOH) and the immunohistochemical protein expression of both components.

Methods and results
A 70 year old woman, known to have a metastasized neuroendocrine carcinoma, underwent resection of the distal part of the ileum, because of obstruction by a mesenterial mass. The macroscopically homogeneous mesenterial mass consisted histologically of an admixture of a neuroendocrine component and a papillary serous carcinoma.

Loss of heterozygosity (LOH) analysis of both components with a panel of 15 polymorphic microsatellite markers showed a distinctive pattern of LOH on chromosome 4q and 17, but involving different alleles at the same locus. Moreover, both components showed different immunohistochemical staining patterns for neuroendocrine markers, cytokeratin 7, carcinoembryonic antigen, and CA125.

Conclusion
Both LOH analysis of the neuroendocrine and papillary serous components of this tumor and the immunohistochemical profile of both components are consistent with a different clonal origin. The tumor is probably a collision tumor, in which the papillary serous carcinoma must have been of peritoneal origin, because necropsy revealed a normal uterus and normal ovaries.

Introduction
A composite tumor is defined by the mixture or coexistence of distinct components made up of different histological features. Composite tumors are relatively rare and they can be encountered at various locations. Composite tumors made up of a neuroendocrine component and an adenocarcinoma component have been described in the gastrointestinal tract, breast, bladder, ovary and prostate. An interesting issue concerning these composite tumors is, whether the histologically distinctive parts of the tumor reflect the ability of one tumor cell to proliferate and differentiate into two different directions, or whether the tumor is really composed of two neoplastic clones that have arisen from different cell types in close proximity to one another. In this last case the composite tumor can be considered as a collision tumor. Using immunohistochemistry, similarities and differences in protein expression between the various tumor components can be demonstrated, but this mostly provides information on the differentiation of the tumor cells and does not necessarily give any information on their developmental route. However, study of genetic changes, which are at the basis of malignant cell transformation, can be
very useful for determining the way in which a neoplasm has developed. Therefore, the examination and comparison of the pattern of loss of heterozygosity (LOH) in the two parts of a composite tumor can provide a powerful means of answering this question of origin.

Here, we report a case of a metastasized neuroendocrine carcinoma of the gastrointestinal tract with an admixture of areas of papillary serous carcinoma. Because the patient was not known to have a primary tumor resembling a papillary serous carcinoma, it was thought possible that the papillary serous component had arisen from the neuroendocrine tumor or vice versa. Alternatively, it could potentially represent a collision of the two neoplasms. A comparison of the pattern of LOH of a spectrum of polymorphic microsatellite markers at various chromosomal loci in both the neuroendocrine and papillary serous components, suggested that both components had originated from different cell types.

**Material and methods**

**Case report**

A 70 year old woman had a medical history of a right hemicolectomy for a Dukes's stage B mucinous adenocarcinoma of the colon at the age of 52 (figure 4.1A). At the age of 67 she suffered temporarily from abdominal crampy pain, diarrhoea, and weight loss (10 kg). Colonoscopy and other investigations were negative. Currently, the patient presented with nausea, vomiting and fatigue. At physical examination the abdomen was distended, although the liver was not enlarged. Ultrasound and an abdominal computed tomography scan revealed multiple large liver metastases, enlarged retroperitoneal lymph nodes and a tumor mass in the right upper abdomen. A second primary colonic tumor was excluded by endoscopy. A liver biopsy showed metastases of a low grade neuroendocrine carcinoma (carcinoid tumor; figure 4.1B). Urinary 5-hydroxyindole acetic acid excretion was not raised. To choose the optimal palliative treatment nuclide imaging was performed. Both the somatostatin receptor imaging with $^{111}$In pentetreotide and the $^{131}$I-meta-iodobenzyl guanidine scan showed clear retention in the liver metastases and the other abdominal localisations. Just before medical treatment was started, the symptoms of bowel obstruction at the level of the duodenum rapidly worsened. At laparotomy a tumor mass was present near the pancreas and the ileotransversostomy. This process had induced ischemia of the distal ileum. The distal part of the ileum and the obstructing mesenterial tumor mass were resected and the vascularisation improved. However, three days later signs of ischemia reappeared and at relaparotomy a resection of 40 cm of necrotic small bowel was performed. Four days after the second operation the patient's condition deteriorated acutely, as a result of massive bleeding from the superior mesenteric artery. A third laparotomy was refused and the patient died at day 9 after the first operation. A necropsy was performed. Gross examination of the distal ileum that was resected during surgery, showed
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several similar looking, white tumor nodules with a maximal diameter of 8 cm, in the fatty tissue of the mesentery, outside the small bowel wall. Microscopically, an admixture of two histologically different components was found in the tumor nodules, even though they had a homogeneous macroscopic appearance. One component consisted of islets of monotonous epithelial cells with round nuclei and granular chromatin, consistent with a localisation of the low-grade neuroendocrine carcinoma, diagnosed previously. The other component consisted of papillary formations and tubules covered with serous epithelium the cells of which contained enlarged, polymorphic nuclei with a high mitotic activity. Psammoma bodies were also present. These features were characteristic of the histological appearance of a papillary serous adenocarcinoma (figs 4.1C,D). Both components had a different immunohistochemical pattern of protein expression (table 4.1). The neuroendocrine component was positive for the common neuroendocrine markers such as chromogranin, synaptophysin and NSE, but negative for cytokeratin 7, cytokeratin 20, carcinoembryonic antigen (CEA), CA125 and the protein product of the tumor suppressor gene p53. The papillary serous component

Figure 4.1 A: Mucinous adenocarcinoma of the colon, diagnosed 18 years before the current medical history. B: Low grade neuroendocrine carcinoma. C: Papillary serous carcinoma in the mesentery of the resected ileum. D: Collision of the papillary serous and neuroendocrine component in the mesentery of the resected ileum.
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<table>
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<th>Papillary serous adenocarcinoma</th>
<th>Colonic adenocarcinoma</th>
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Table 4.1 Immunohistochemical results. CEA = carcinoembryonic antigen, NSE = neuron-specific enolase.

did not express neuroendocrine markers, cytokeratin 20, CEA or p53, but was CA125 positive. Because the patient apparently had a CA125 positive papillary serous tumor, a diagnosis of a disseminated ovarian carcinoma, in addition to the already diagnosed neuroendocrine carcinoma, was considered in this patient. Surprisingly, at necropsy both ovaries looked normal and their microscopic appearance was consistent with the age of the patient; no tumor was found. We then reviewed the slides of the adenocarcinoma of the colon, for which the patient underwent surgery 18 years earlier. The tumor was a mucinous colonic adenocarcinoma and this tumor did not show histological similarities to either the neuroendocrine carcinoma or the papillary serous adenocarcinoma. Immunohistochemical staining for CEA and p53 was positive, while the tumor was negative for CA125 and neuroendocrine markers.

To investigate whether the neuroendocrine carcinoma and papillary serous carcinoma were two unrelated neoplasms or whether they were derived from the same clonal expansion, molecular LOH analysis was performed.

Loss of heterozygosity (LOH) analysis

For molecular analysis, one area from each component was examined. From each part seven 10 μm thick serial paraffin wax embedded sections were used for DNA isolation. Adjacent thin sections were used for histopathologic evaluation of the tumor. Sections were dewaxed by standard procedures. The unstained sections were incubated for 16 hours in 1 M sodium thiocyanate at 37°C to remove crosslinks, followed by two five minute washes in phosphate buffered saline. Subsequently, guided by the thin haematoxylin and eosin section, the region with the highest tumor percentage was scraped with a scalpel from the
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**Table 4.2** Pattern of loss of heterozygosity (LOH) in the neuroendocrine carcinoma (T1) and the papillary serous adenocarcinoma (T2). Ni = not informative, ROH = retention of heterozygosity.

glass slide, and transferred to a tube containing digestion buffer: 2mg/ml proteinase K (Roche, Basel, Switzerland) in 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 2.5 mM MgCl₂, 0.5% Tween 80 and 0.1 mg/ml gelatine. The estimated tumor percentage in both samples was 90%.

Tubes were incubated for 24 hours at 55°C, proteinase K was heat inactivated at 95°C for 10 minutes, and after centrifugation the supernatant was transferred to a clean tube and stored at 4°C until use.

DNA samples isolated from the tumor and normal tissue obtained from a separate tissue block containing a lymph node were analysed using 15 polymorphic microsatellite repeat markers selected through Genomic Data Base (table 4.2). The fluorescent labelling of the primer allows the semiquantitative evaluation of
Figure 4.2 The allelic imbalance of the various microsatellite markers used for the LOH analysis of the two tumors. LOH is seen at markers D4S430 (chromosomal arm 4q) and D17S588 (chromosome 17) in both tumors, but involving different alleles (indicative of a distinct origin of the two tumors). Tumor 1 = neuroendocrine carcinoma, tumor 2 = papillary serous adenocarcinoma. NR = normal ratio, TR = tumor ratio.

The most important finding is that the neuroendocrine and papillary serous components both have loss of heterozygosity on chromosome 4q and 17, but involving different alleles at the same locus. This finding, together with the distinctive pattern of LOH in both components, suggests that they were derived from different neoplastic clones.
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Discussion

Here we describe a patient with a widely disseminated neuroendocrine carcinoma of unknown origin, who underwent resection of an obstructing mesenterial tumor mass, in which areas of papillary serous adenocarcinoma were found in close association with areas of neuroendocrine carcinoma. Furthermore, 18 years earlier, the patient underwent surgery for a mucinous adenocarcinoma of the colon, Dukes’s stage B, which had been resected completely.

When a patient presents with a tumor in which histologically distinct components are recognized, the question arises whether these components have originated from the same precursor cell followed by subsequent differentiation into different directions or whether the components represent the ‘incidental’ collision of unrelated neoplasms.

Immunohistochemical studies of composite carcinoma-carcinoid tumors compared with pure carcinoid tumors and adenocarcinomas of the gastrointestinal tract have shown, that sometimes one component of a composite tumor expresses a protein, that otherwise would be only expected in its counterpart. This was used as an argument in favor of the hypothesis that these tumors came from a common precursor cell. In the case reported here, the immunohistochemical profiles of the neuroendocrine component and the areas of papillary serous carcinoma did not correspond. Thus there is no evidence, that these components have a common progenitor cell, although this can not be ruled out completely. Molecular genetic analysis in this regard can be more distinctive and definitive than immunohistochemistry in examining the origin of two components of a composite tumor because this methodology focuses directly on the developmental route of the tumor by studying the alterations in the DNA. In this case, LOH-analysis of the areas with neuroendocrine and papillary serous differentiation was performed, in addition to immunohistochemical examination. This technique has already proved to be a powerful tool in demonstrating a common or a different clonal origin in neuroendocrine and other tumors. In our case, 10 of a panel of 15 markers were informative. Six of these 10 informative markers, showed a difference between both components (table 4.2). Importantly, two of the markers, on chromosome 4 and 17, demonstrated LOH in both components, but involving different alleles at the same locus. These results are consistent with the two components originating from a different neoplastic clone (table 4.2, fig 4.2): because several sections of each component were used for LOH analysis, the LOH pattern can be considered as relatively specific for that particular part of the tumor. It is very unlikely that two components of the same tumor would show a completely different, but specific, pattern of LOH and at the same time would have loss of a different allele at the same locus of a particular chromosome. Nevertheless, loss of a different allele at the same locus within one tumor can be encountered on rare occasions. For example, it can be caused by somatic crossing over during mitosis of a cell heterozygous for a marker. This results in two daughter cells, both of which are now homozygous.
for that marker, each having lost a different allele.\textsuperscript{9} Another possibility is that the loss of an allele on a specific chromosome, or part thereof, and the resultant haplo-insufficiency of the genes in this region, might provide a microevolutionary (growth) advantage in a specific tumor.\textsuperscript{8,10} Within a genetically less stable tumor, such loss could occur twice and affect different alleles. Although these mechanisms can account for genetic heterogeneity within one tumor, we feel that in this case the total pattern of LOH and the pattern of protein expression suggest that a collision tumor has arisen from a metastasized neuroendocrine carcinoma and a papillary serous adenocarcinoma.

In view of the negative findings in the ovaries and uterus at necropsy, the papillary serous adenocarcinoma must be of peritoneal origin. In these tumors, CA125 positivity has been described.\textsuperscript{11} Moreover, the site of the tumor and the normal aspect of the other visceral organs at necropsy are in accordance with this diagnosis. The absence of any microscopic similarities between the colonic adenocarcinoma and either the neuroendocrine carcinoma or the papillary serous tumor, the differences in protein expression between the tumors found by immunohistochemistry and the interval of 18 years between the diagnosis of colonic adenocarcinoma and metastasized neuroendocrine carcinoma, make it very unlikely that there is a relationship between the colonic adenocarcinoma and the other two tumors. Unfortunately, due to technical problems (DNA could not be isolated from the 18 year old paraffin wax embedded material) LOH-analysis of the colonic adenocarcinoma could not be performed to confirm this notion.

In summary, we report a tumor consisting of a neuroendocrine component and areas of papillary serous adenocarcinoma. By means of LOH-analysis and immunohistochemistry, a different clonal origin of both tumor components is suggested. Because the ovaries and uterus did not contain tumor, the papillary serous adenocarcinoma must have been of peritoneal origin.

References


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