CASE REPORT

A tumour with a neuroendocrine and papillary serous component: two or a pair?

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Aims: To examine the clonal origin of a tumour, 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/Results: A 70 year old woman, known to have a metastasised 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, and both components showed 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 tumour and the immunohistochemical profile of both components are consistent with a different clonal origin. The tumour is probably a collision tumour, in which the papillary serous carcinoma must have been of peritoneal origin because necropsy revealed a normal uterus and normal ovaries.

A composite tumour is defined by the mixture or coexistence of distinct components made up of different histological features. Composite tumours are relatively rare and they can be encountered at various locations. Composite tumours made up of a neuroendocrine component and an adenocarcinoma component have been described in the gastrointestinal tract, breast, bladder, ovary, and prostate.^{1,2}

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An interesting issue concerning these composite tumours is whether the histologically distinctive parts of the tumour reflect the ability of one tumour cell to proliferate and differentiate in two different directions, or whether the tumour 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 tumour can be considered as a collision tumour.³

Using immunohistochemistry, similarities and differences in protein expression between the various tumour components can be demonstrated, but this mostly provides

information on the differentiation of the tumour cells and does not necessarily provide information on their developmental route. However, the study of genetic changes, which are 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 tumour can provide a powerful means of answering this question of origin.

Here, we report a case of a metastasised 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 tumour resembling a papillary serous carcinoma, it was thought possible that the papillary serous component had arisen from the neuroendocrine tumour 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 (fig 1A). At the age of 67 she had 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 tumour mass in the right upper abdomen. A second primary colonic tumour was excluded by endoscopy. A liver biopsy showed metastases of a low grade neuroendocrine carcinoma (carcinoid tumour; fig 1B). Urinary 5-hydroxyindole acetic acid excretion was not raised. To choose the optimal palliative treatment nuclide imaging was performed.^{4,5} Both the somatostatin receptor imaging with ¹¹¹In pentreotide and the ¹³¹I 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 tumour mass was present near the pancreas and the ileotransversostomy. This process had induced ischaemia of the distal ileum. The distal part of the ileum and the obstructing mesenterial tumour mass were resected and the vascularisation improved. However, three days later signs of ischaemia

Abbreviations: CEA, carcinoembryonic antigen; LOH, loss of heterozygosity; PCR, polymerase chain reaction

Figure 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.

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 several similar looking, white tumour 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 tumour 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 1C,D). Both components had a different immunohistochemical pattern of protein expression (table 1). The neuroendocrine component was positive for the common neuroendocrine markers such as chromogranin, synaptophysin, and neurone specific enolase, but negative for cytokeratin 7, cytokeratin 20, carcinoembryonic antigen (CEA), CA125, and the protein product of the tumour suppressor gene p53. The papillary serous component did not express neuroendocrine markers, cytokeratin 20, CEA, or p53, but was CA125 positive. Because the patient apparently had a CA125 positive papillary serous tumour, 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 tumour was found.

Table 1 Immunohistochemical results

Marker	Neuroendocrine carcinoma	Papillary serous adenocarcinoma	Colonic adenocarcinoma
Chromogranin	Positive	Negative	Negative
Synaptophysin	Positive	Negative	Negative
NSE	Positive	Negative	Negative
Cytokeratin 7	Negative	Positive	Negative
Cytokeratin 20	Negative	Negative	Negative
CA125	Negative	Positive	Negative
CEA	Negative	Negative	Positive
p53	Negative	Negative	Positive

CEA, carcinoembryonic antigen; NSE, neurone specific enolase.

Table 2 Pattern of loss of heterozygosity (LOH) in the neuroendocrine carcinoma (T1) and the papillary serous adenocarcinoma (T2)

Marker	Chromosome arm	T1 conclusion	T1 LOH allele	T2 conclusion	T2 LOH allele
D1S228	1p36	NI	–	NI	–
D1S2766	1p22	ROH	–	LOH	Allele 1
D4S430	4q28	LOH	Allele 1+F30	LOH	Allele 2
D5S346	5q22–5q23	NI	–	NI	–
D5S644	5q15	ROH	–	LOH	Allele 1
D8S258	8p22	NI	–	NI	–
D8S552	8p22	NI	–	NI	–
D9S171	9p21	ROH	–	ROH	–
D9S288	9p23–9p21	NI	–	NI	–
D9S286	9p21	ROH	–	ROH	–
D10S192	10q23.3–10q24.1	ROH	–	ROH	–
D10S217	10q25–10q26	ROH	–	ROH	–
D14S68	14q24.3–14q31	LOH	Allele 2	ROH	–
D17S588	17	LOH	Allele 1	LOH	Allele 2
D18S58	18q22–18q23	LOH	Allele 2	ROH	–

NI, not informative; ROH, retention of heterozygosity.

We then reviewed the slides of the adenocarcinoma of the colon, for which the patient underwent surgery 18 years earlier. The tumour was a mucinous colonic adenocarcinoma and this tumour did not show histological similarities to either the neuroendocrine carcinoma or the papillary serous adenocarcinoma. Immunohistochemical staining for CEA and p53 was positive, whereas the tumour 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.

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 histopathological evaluation of the tumour. Sections were dewaxed by standard procedures. The unstained sections were incubated for 16 hours in 1M 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 tumour percentage was scraped with a scalpel from the glass slide, and transferred to a tube containing digestion buffer: 2 mg/ml proteinase K (Roche, Basel, Switzerland) in 10mM Tris/HCl (pH 8.0), 50mM KCl, 2.5mM MgCl₂, 0.5% Tween 80, and 0.1 mg/ml gelatin. The estimated tumour 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 tumour and normal tissue obtained from a separate tissue block containing a lymph node were analysed using 15 polymorphic microsatellite repeat markers selected through the Genomic Data Base (table 2). The fluorescent labelling of the primer allows the semiquantitative evaluation of the polymerase chain reaction (PCR) products using the ABI377 automatic sequencer. The peak intensity of the PCR products was determined and used to calculate the intensity ratio between the two allele peaks of the heterozygotes. This ratio is subsequently normalised using the peak intensity of the normal DNA (divided by the ratio of the normal alleles), resulting in an LOH index. An LOH index below 0.75 was interpreted as evidence of LOH, whereas an

Figure 2 The allelic imbalance of the various microsatellite markers used for the loss of heterozygosity (LOH) analysis of the two tumours. LOH is seen at markers D4S430 (chromosomal arm 4q) and D17S588 (chromosome 17) in both tumours, but involving different alleles (indicative of a distinct origin of the two tumours). Diamond shapes, tumour 1 (neuroendocrine carcinoma); squares, tumour 2 (papillary serous adenocarcinoma). NR, normal ratio; TR, tumour ratio.

index above this value was considered to be retention of heterozygosity (ROH).

Table 2 and fig 2 show the results of the LOH analysis. The most important finding is that the neuroendocrine and papillary serous components both have LOH 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.

DISCUSSION

Here, we describe a patient with a widely disseminated neuroendocrine carcinoma of unknown origin, who underwent resection of an obstructing mesenteric tumour 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, Duke's stage B, which had been resected completely.

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

Immunohistochemical studies of composite carcinoma-carcinoid tumours compared with pure carcinoid tumours and adenocarcinomas of the gastrointestinal tract have shown that sometimes one component of a composite tumour expresses a protein that normally would only be expected in its counterpart.¹ This was used as an argument in favour of the hypothesis that these tumours come 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 cannot 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 tumour because this methodology focuses directly on the developmental route of the tumour by studying 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 tumours.^{6,7}

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 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 2; fig 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 tumour. It is very unlikely that two components of the same tumour 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.⁸ Nevertheless, loss of a different allele at the same locus within one tumour 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.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

Take home messages

- Our patient had a composite tumour with both a neuroendocrine component and areas of papillary serous adenocarcinoma
- Loss of heterozygosity (LOH) analysis and immunohistochemistry suggested that the two tumour components had a different clonal origin, so that this was a collision tumour
- Because the ovaries and uterus did not contain tumour, the papillary serous adenocarcinoma must have been of peritoneal origin
- LOH techniques are useful for investigating the origins of composite tumours

tumour.^{8,10} Within a genetically less stable tumour, such loss could occur twice and affect different alleles.

"In view of the negative findings in the ovaries and uterus at necropsy, the papillary serous adenocarcinoma must be of peritoneal origin"

Although these mechanisms can account for genetic heterogeneity within one tumour, we feel that in this case the total pattern of LOH and the pattern of protein expression suggest that a collision tumour has arisen from a metastasised 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 tumours, CA125 positivity has been described.¹¹ Moreover, the site of the tumour and the normal aspect of the other visceral organs at necropsy are in accordance with this diagnosis.

The absence of microscopic similarities between the colonic adenocarcinoma and either the neuroendocrine carcinoma or the papillary serous tumour, the differences in protein expression between the tumours found by immunohistochemistry, and the interval of 18 years between the diagnosis of colonic adenocarcinoma and metastasised neuroendocrine carcinoma make it very unlikely that there is a relation between the colonic adenocarcinoma and the other two tumours. Unfortunately, because of 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 tumour consisting of a neuroendocrine component and areas of papillary serous adenocarcinoma. By means of LOH analysis and immunohistochemistry, a different clonal origin of both tumour components is suggested. Because the ovaries and uterus did not contain tumour, the papillary serous adenocarcinoma must have been of peritoneal origin.

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