LETTERS

Epithelial cells disseminate into the bone marrow of colorectal adenoma patients

Although the skeleton is not a preferred site of overt metastasis in colorectal cancer, demonstration of tumour cells in bone marrow has to be seen as evidence of the general disseminative capability of an individual tumour. Other observations such as involuntary transmission of tumour by organ grafts directly supports the notion that very few quiescent cells lodging at improbable sites, such as the kidney or heart, suffice to generate de novo metastatic disease in the organ recipient. The TNM classification recommends mention of the presence of disseminated tumour cells as a facultative factor for metastasisation (M0 (−) or M0 (mol+)) according to the immunological or molecular detection technique.

However, the results of the one and only meta-analysis available to date show that the prognostic impact of epithelial cells in the bone marrow of colorectal cancer patients has to be substantiated by further studies under standardised conditions. To further investigate this question, bilateral crest aspiration is performed routinely in our institution for neoplastic diseases. From September 1997 until July 2000, we investigated 233 patients using this method: approximately 2 million mononuclear cells were analysed from each sample and divided into 10 cytopins. One half was stained with the A45-B/B3 antibody (supplied by U. Karstens, PhD, Berlin, Germany) and the other half with Ber-EP4 (Dako, Hamburg, Germany). Staining was performed using the alkaline phosphatase anti-alkaline phosphatase technique. Histopathological staging showed that 15 of these patients suffered from an early adenocarcinoma (T1 category), and in seven patients no malignancy could be documented, in spite of complete analysis of the specimen.

Patients without cancer were of particular interest to us, for addressing the question of the early dissemination of epithelial cells in colorectal neoplasms. To our surprise, we observed the presence of disseminated epithelial cells in the bone marrow of three of these patients (table 1, fig 1).

In a previous study, we examined the clonality of disseminated tumour cells in the bone marrow of 51 colorectal cancer patients by determining the mutational pattern in codons 12 and 13 of the K-ras gene. Our results demonstrated that, at least for K-ras mutations, disseminated epithelial cells are not always clonal with the primary tumour. The type of mutations suggested also that cell dissemination might be an early event in the development of colorectal neoplasms as most bone marrow K-ras mutations were found in codon 13, a codon barely mutated in invasive colorectal cancer but frequently mutated in aberrant crypt foci. Obviously, epithelial cells can already disseminate in the polyp stage, in particular when so-called intraepithelial neoplasia is diagnosed. Indeed, dissemination of epithelial cells into the bone marrow in a stage defined as non-cancerous questions the carcinomatus nature of these cells, and in particular their micrometastatic nature. In contrast, should these cells be cancer cells—which we cannot exclude on the basis of our previous and present observations—then the benign nature of intraepithelial neoplasia should in turn be challenged.

We would be delighted to receive feedback from other researchers that would help us to interpret the present observation.

R Steinert, M Vieth, M Hantschick, M A Reymond
University of Magdeburg, Magdeburg, Germany

Correspondence to: Professor M A Reymond, University of Magdeburg, Leipziger Str 44, Magdeburg 39120, Germany; marc.reymond@medizin.uni-magdeburg.de
doi: 10.1136/gut.2004.062216

Conflicts of interest: None declared.

References


Table 1 Patients, tumours, and results of bone marrow immunohistochemistry

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (y)</th>
<th>Localisation</th>
<th>Histopathology</th>
<th>A45-B/B3</th>
<th>Ber-EP4</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>63</td>
<td>Rectum</td>
<td>Tubular adenoma with high grade intraepithelial neoplasia</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>F</td>
<td>41</td>
<td>Colon sigmoideum</td>
<td>Tubulo-adenoma with high grade intraepithelial neoplasia</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>F</td>
<td>56</td>
<td>Colon ascendens</td>
<td>Tubular adenoma with low grade intraepithelial neoplasia</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>M</td>
<td>57</td>
<td>Colon sigmoideum</td>
<td>3 tubulo-adenoma with high grade intraepithelial neoplasia</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>F</td>
<td>67</td>
<td>Rectum</td>
<td>Tubulo-adenoma with high grade intraepithelial neoplasia</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>M</td>
<td>79</td>
<td>Rectum</td>
<td>Tubulo-adenoma with high grade intraepithelial neoplasia</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>M</td>
<td>74</td>
<td>Colon sigmoideum</td>
<td>Tubulo-adenoma with high grade intraepithelial neoplasia</td>
<td>Negative</td>
<td>Positive</td>
</tr>
</tbody>
</table>

2/7 positive 3/7 positive
were analysed for DNA from blood and from frozen JNA tissue affected sibling of an FAP family. Matched carrier who underwent restorative proctoco-

dNA from a 24 year old JNA affected FAP

mutations in matched blood and tumour

System (Promega, Madison, Wisconsin, USA)

www.gutjnl.com

1046 PostScript

mones.

growth is stimulated by male sex hor-

to erectile tissue, the almost exclusive occur-

Figure 1

Histopathological appearance of the nasopharyngeal angiofibroma described in this study. The tumour is composed of dilated vascular channels set in an abundant myxoid stroma containing fusiform fibroblasts and focal mononuclear cell infiltrates (A, ×125; B, ×400).

References


8  Cama A, Palmintera R, Curia MC, et al. Multiplex PCR analysis and genotype-phenotype

Genetic evidence that juvenile

nasopharyngeal angiofibroma is an integral FAP tumour

Juvenile nasopharyngeal angiofibroma (JNA) is a rare locally invasive neoplasm composed of cavernous vascular channels set in an abundant myxoid stroma composed of cavernous vascular channels set in an abundant myxoid stroma of fibroblasts and

The histological similarity to erectile tissue, the almost exclusive occurrence in pubescent males, and expression of multiple steroid receptors suggest that JNA growth is stimulated by male sex hormones. 1, 2 The frequency of JNA is significantly increased in male familial adenomatous polyposis (FAP) patients, suggesting that it may arise through alterations of the adenomatous polyposis coli (APC) β-catenin gene pathay. 3 This was supported by the high frequency of recurrent β-catenin gene mutations detected in sporadic JNA, but no APC mutations have thus far been found. 4, 5

We analysed the sequence of the APC gene and the presence of recurrent β-catenin mutations in matched blood and tumour DNA from a 24 year old JNA affected FAP carrier who underwent restorative proctocolectomy and resection of an abdominal wall desmoid. The patient was the only JNA affected sibling of an FAP family. Matched DNA from blood and from frozen JNA tissue were analysed for APC mutations using the TNT Quick Coupled Transcription/Translation System (Promega, Madison, Wisconsin, USA) and heteroduplex analysis on agarose mini-

gel, 6 followed by sequencing. Using these techniques we detected a frameshift APC mutation, c.3927-3931delAAAGA, in both blood and JNA tissue. This mutation introduces a stop codon (pGlu1309HisX1312) in the APC gene region between the first and second 20 amino acid β-catenin binding repeats. Another frameshift APC mutation, consisting in a 5 bp deletion, c.3183-3187delACAGAA, that introduces a stop codon (p.Lys1061fsX1062) in the region encoding the first 20 amino acid β-catenin binding repeat, was detected only in JNA DNA. Using restriction enzyme analysis, 7 we ruled out the presence of the JNA associated activating mutations at codons 32 and 34 in exon 3 of the β-catenin gene. These results were confirmed in duplicate experiments. Due to lack of tumour sections, we were unable to perform laser capture microdissection to separate the vascular and stromal components of the tumour. However, the somatic mutation is expected to have been present in fibroblasts because of the clear stromal predominance in the JNA tissue analysed. In the study by Abraham et al, activating β-catenin mutations were found in 12 of 16 sporadic JNAs analysed. 7 The APC sequence corresponding to the mutation cluster region (MCR) of sporadic colorectal cancer was investigated in the four JNAs without β-catenin mutations but no mutations were detected. 8 Guerri et al analysed 11 sporadic JNAs from nine patients for mutations in the MCR of the APC gene and for loss of heterozygosity (LOH) at the APC locus. 7 No APC mutations were detected and none of the informative cases were LOH positive. 9 Ferrouz et al found no germline APC mutations in a series of nine JNA patients. 7 Thus there was no direct evidence involving the APC gene in JNA, although this rare tumour is reported to occur 25 times more frequently in FAP affected adolescents than in an age matched population. 7

This study documents for the first time the association between a somatic and a germline APC mutation in an FAP related JNA. Because of the stromal predominance in the tumour analysed (fig 1), the somatic mutation must have been present in the fibroblasts (that is, in the same cell type where nuclear accumulation of β-catenin, indicative of activation of the Wnt pathway, was previously demonstrated). 10 We cannot exclude the presence or absence of the mutation in the vascular component. Our findings agree with the well known evidence of double hit APC inactivation in FAP associated fibroblastic tumours. 7 Thus FAP associated JNA should be considered a sex dependent extraintestinal FAP manifestation.

Acknowledgements

Supported by grants from “Associazione Italiana per la Ricerca sul Cancro” (AIRC) and the Italian Ministry of University and Research.

R Valanzano

Department of Clinical Physiopathology, University of Florence, Florence, Italy

M C Curia, G Aceto, S Veschi, L De Lellis

Department of Oncology and Neurosciences, University “Gabriele D’Annunzio” Chieti-Pescara, Chieti, Italy, and Centre of Excellence on Aging, “G D’Annunzio” Foundation, Chieti, Italy

T Catalano

Department of Oncology and Neurosciences, University “Gabriele D’Annunzio” Chieti-Pescara, via dei Vestini 31, 66013 Chieti, Italy ml.curia@unic.it

doi: 10.1136/gut.2005.065508

Conflict of interest: None declared.

www.gutjnl.com

1136 PostScript

on September 14, 2023 by guest. Protected by copyright.

Evaluation of vascular signal in pancreatic ductal carcinoma using contrast enhanced ultrasonography: effect of systemic chemotherapy

Evaluation of the effect of chemotherapy for pancreatic ductal cancer (PC) is generally conducted based on changes in tumour diameter using imaging modalities; however, exact measurement is often difficult because of local inflammation, fibrotic change, and desmoplastic reaction to treatment, leading to an unreliable evaluation. PC is considered a hypovascular tumour. However, recently developed highly sensitive ultrasonic equipment has enabled the detection of vascular signals in PC; vascular signals were detected in 20–67% of cases.1 2 We focused on changes in tumour vascularity of PC associated with chemotherapy, and attempted to apply it to evaluation of the effect of treatment and usefulness in relation to prognosis. In this study, we assessed vascular images of the tumour based on the Doppler signal using contrast enhanced ultrasonography (CEUS). Thirty one histopathologically confirmed consecutive patients with PC who had distant metastases were included in the study. Informed consent was obtained from all patients and the study was approved by the ethics committee. The tumour was located in the head of the pancreas in 16 patients and in the body or tail in 15. All patients were treated with a combination of S-1, an oral fluorinated pyrimidine derivative, and gemcitabine. Chemotherapy was performed every three weeks as one cycle. CEUS was performed before and after one and two cycles of treatment using a SSA-770A (Toshiba Co. Ltd, Tokyo, Japan) and a 3.75 MHz convex probe. CEUS images were obtained by intravenous injection of 8 ml at 1 ml/s. After injection, v signals in the tumour of the pancreas were continuously observed for 120 seconds. CEUS images showing the highest intensity of the vascular signal were selected and classified into five categories according to intensity: no signal (grade 0), spotty signals (grade 1), linear signals between grades 1 and 3 (grade 2), mosaic pattern signals (grade 3), and diffuse pattern signals (grade 4). Dynamic computed tomography (CT) was performed with a helical CT scanner (Soft Imaging Ultra, GE Medical Systems) which was performed every two cycles. In this study, treatment effect after two cycles of chemotherapy was examined. The response to treatment, as determined by dynamic CT after two cycles of treatment, was as follows: partial response (PR) in five patients (16%), stable disease (SD) in 17

Figure 1 Cumulative survival rate according to changes in the v signal score after two cycles of treatment. Median survival time (MST) of patients in the non-increased v signal group (n = 22) was 382 days (range 71–484) and for those in the increased group (n = 9), 176 days (range 68–257). MST in the increased group was significantly shorter compared with the non-increased group (log rank test, p = 0.0094).

Smoking status in therapeutic trials in Crohn’s disease

We were interested to hear the results of a number of trials of novel therapies for Crohn’s disease (CD) that were presented at the 12th UEGW and reported in abstract form in Gastroenterology. Many of the studies were randomised controlled trials in which the active and control groups were reported to have identical baseline characteristics. However, in all of the studies that were reported there was no mention of the smoking status of the participants, consistent with recent therapeutic trials in CD published in high profile journals.3 4 Smoking is a well documented confounding factor in therapeutic trials in Crohn’s disease. We urge investigators to include smoking status in the abstract, text, and analyses of all therapeutic trials of CD. Furthermore, we believe that stratification for smoking should be included at the planning stage for all randomised controlled trials in CD. Investigators may wish to reanalyse published data to ensure that results have not been confounded by smoking.
Ferroportin disease due to the A77D mutation in Australia

Ferroportin disease or type 4 haemochromatosis is an autosomal dominant iron overload disorder caused by mutations in the iron exporter ferroportin. Numerous mutations in ferroportin (SLC11A3) have been identified (see review by Pietrangelo). The A77D mutation of ferroportin has thus far only been reported in Italy. We report the first A77D mutation of ferroportin which resulted in haemochromatosis in an Australian family. The study was approved by and performed in accordance with the ethical standards of the Queensland Institute of Medical Research Human Ethics Committee and the Helsinki Declaration of 1975, as revised in 1983.

The subject, a 45 year old Caucasian male, presented with complaints of lethargy and fatigue, myalgias, and arthralgia. On examination he was found to have chronic iron overload in an Australian family. The subject was referred for further evaluation after finding a high serum ferritin concentration (12.2 g/dl, white blood count of 4–8 × 10^9/l). There is no known ancestral link between the family reported here and that in Italy. Thus it is likely that the A77D mutation has occurred in the two populations separately, as appears to be the case with the V626del mutation which has so far been reported in five geographic locations.

As knowledge about ferroportin disease is uncommon in the community, unlike HFE associated haemochromatosis, it is possible that some cases of this disorder are not recognised and thus remain undiagnosed. This particular case was not diagnosed until liver biopsy was performed. The raised serum ferritin level was initially attributed to viral illness. Because transferrin saturation and HFE genotype were normal, a diagnosis of iron overload was not initially considered.

In conclusion, we report the first identification of ferroportin disease caused by the A77D mutation in a region outside of Italy. This suggests that the A77D mutation may be more widespread than initially thought. This report also suggests that some cases of ferroportin disease may go undiagnosed. Ferroportin disease should thus be considered when a patient presents with a high serum ferritin, even when transferrin saturation and HFE genotype are normal.

Acknowledgements
The authors gratefully acknowledge the immense support and encouragement of the patient and his family. This work was supported by grants from the Haemochromatosis Society of Australia and the National Health and Medical Research Council of Australia (953219) to VNS.

V N Subramaniam, D F Wallace
Membrane Transport Laboratory, Queensland Institute of Medical Research, Queensland, Australia

J L Dixon
Iron Metabolism Laboratory, Queensland Institute of Medical Research, Queensland, Australia

L M Fletcher, D H Crawford
Department of Gastroenterology and Hepatology, Princess Alexandra Hospital, and the Department of Medicine, University of Queensland, Brisbane, Queensland, Australia

Correspondence to: Dr V N Subramaniam, Membrane Transport Laboratory, Queensland Institute of Medical Research, 300 Herston Rd, Herston, Brisbane, QLD 4006, Australia; nathan58@qimr.edu.au
doi: 10.1136/gut.2005.069021
Conflict of interest: None declared.

References
There are four clinical variants of KS: classic, endemic, acquired immunodeficiency syndrome (AIDS)-associated, and iatrogenic. Excessive use of immunosuppressive drugs in the second part of the 20th century has been associated with a higher prevalence of iatrogenic KS. Start of the disease, after administration of the triggering drug in previously reported studies, ranged from less than one month to more than 20 years. The dose of steroid ranged from 5 to 125 mg/day. There was no evident correlation between the development of KS and dose or duration of steroid therapy. Our patient had been treated with 12–125 mg methylprednisolone daily for about four months when his skin lesions appeared. Reduction or discontinuation of immunosuppressive drugs often leads to considerable improvement in KS lesions. In accordance with these data, after withdrawal of steroid therapy the skin symptoms of our patient regressed spontaneously. Visceral KS is quite frequent in AIDS patients and can affect virtually all viscera, but colonic KS is rare. These patients are often asymptomatic or have aspecific symptoms. As KS affects the submucosa more often, superficial bowel biopsies frequently miss it, as happened in our case. A link between HHV-8, a gamma herpesvirus, and KS was first reported more than 10 years ago. The virus was found in more than 90% of KS samples from HIV seropositive patients but it has low prevalence in healthy controls. HHV-8 DNA persists in endothelial cells and spindle cells of KS. According to the literature, the HHV-8 virus alone is not sufficient to form KS but it may be an important cofactor in the development of the disease. In our case, we detected HHV-8 genome in native samples from skin lesions but failed to do so in paraffin embedded colonic samples. The occurrence of colonic KS and UC together is rare. We found eight similar cases in the English literature (table 1).

Our patient was the fourth who was HIV negative and developed KS in association with UC. To our knowledge he was the first proven HHV-8 positive case who developed disseminated KS during immunosuppressive treatment for UC. Our treatment policy was successful. The patient, in spite of his poor condition, tolerated the surgical therapy well. After cessation of his steroid therapy KS regressed spontaneously. He remains well 35 months after surgery.

Acknowledgements
We would like to express our thank to Professor Péter Kupcsulik for revision of the manuscript.

A Bursics
Semmelweis University Budapest, 1st Department of Surgery, Budapest, Hungary

K Morvay
Semmelweis University Budapest, 1st Department of Surgery, Budapest, Hungary

K Abraham, M Marschalkó
Semmelweis University Budapest, Department of Dermatology and Venerology, Budapest, Hungary

M Kardos, B Járó
Semmelweis University Budapest, 2nd Department of Pathology, Budapest, Hungary

Table 1: Main data from previously published articles on the coexistence of colonic Kaposi’s sarcoma (KS) and ulcerative colitis (UC)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year of publication</th>
<th>HIV status</th>
<th>Pathology of the colon</th>
<th>Skin lesion</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gordon</td>
<td>1966</td>
<td>No information</td>
<td>UC</td>
<td>No</td>
<td>Colectomy</td>
</tr>
<tr>
<td>Adlersberg</td>
<td>1970</td>
<td>No information</td>
<td>Non-specific colitis</td>
<td>No</td>
<td>Colectomy</td>
</tr>
<tr>
<td>Roth</td>
<td>1978</td>
<td>No information</td>
<td>Segmental non-specific colitis</td>
<td>No</td>
<td>Subtotal colectomy</td>
</tr>
<tr>
<td>Weber</td>
<td>1985</td>
<td>Positive</td>
<td>Non-specific colitis of the rectosigmoid colon, separate lesion in the caecum</td>
<td>Yes</td>
<td>Alpha interferon-radiotherapy of rectal KS</td>
</tr>
<tr>
<td>Biggi</td>
<td>1987</td>
<td>Positive</td>
<td>UC</td>
<td>Yes</td>
<td>Urgent colectomy for toxic megacolon and later abdomino-perineal excision for rectal KS</td>
</tr>
<tr>
<td>Melznerib</td>
<td>1987</td>
<td>Negative</td>
<td>UC distal to the descendent colon</td>
<td>Yes</td>
<td>Proctocolectomy with ileostomy</td>
</tr>
<tr>
<td>Thompson</td>
<td>1989</td>
<td>Negative</td>
<td>UC</td>
<td>No</td>
<td>Restorative proctocolectomy</td>
</tr>
<tr>
<td>Tedesco</td>
<td>1999</td>
<td>Negative</td>
<td>UC</td>
<td>No</td>
<td>Restorative proctocolectomy</td>
</tr>
</tbody>
</table>
A 100 mm segment of the proximal jejunum had an irregular outline, with areas of constriction due to scarring. Histology (fig 2) showed fibrosis of the subserosa, and interruption of the muscularis propria by fibrosis. The submucosa and epithelium were normal. The diagnosis was seat belt injury.

Two proposed mechanisms explain the occurrence of small bowel obstruction after blunt abdominal injury: direct and indirect. The direct theory postulates that viscera get compressed between the abdominal wall and spinal column under the shearing force of the fastened seat belt. In the healing process, fibrosis causes constrictions that may result in partial or complete obstruction.

In the indirect mechanism, viscera suffer from ischaemia secondary to mesenteric injury, with involvement of the superior and inferior mesenteric arteries. As Miss M’s mesenteric structures were normal on laparotomy, the scarring she sustained seems to have been the result of direct trauma to the gut. The duodenum and jejunum are particularly vulnerable in the seat belt syndrome because of their proximity to the vertebral column, as well as their relation to the fastened seat belt.

The affected segment was excised and this patient was discharged, totally recovered, nine days after surgery.

References

EDITOR’S QUIZ: GI SNAPSHOT

Answer
From question on page 927
A 100 mm segment of the proximal jejunum had an irregular outline, with areas of constriction due to scarring. Histology (fig 2) showed fibrosis of the subserosa, and interruption of the muscularis propria by fibrosis. The submucosa and epithelium were normal. The diagnosis was seat belt injury.

Two proposed mechanisms explain the occurrence of small bowel obstruction after blunt abdominal injury: direct and indirect. The direct theory postulates that viscera get compressed between the abdominal wall and spinal column under the shearing force of the fastened seat belt. In the healing process, fibrosis causes constrictions that may result in partial or complete obstruction.

In the indirect mechanism, viscera suffer from ischaemia secondary to mesenteric injury, with involvement of the superior and inferior mesenteric arteries. As Miss M’s mesenteric structures were normal on laparotomy, the scarring she sustained seems to have been the result of direct trauma to the gut. The duodenum and jejunum are particularly vulnerable in the seat belt syndrome because of their proximity to the vertebral column, as well as their relation to the fastened seat belt.

The affected segment was excised and this patient was discharged, totally recovered, nine days after surgery.

doi: 10.1136/gut.2004.058032