Placenta growth factor expression is correlated with survival of patients with colorectal cancer

S-C Wei, P-N Tsao, S-C Yu, C-T Shun, J-J Tsai-Wu, C H H Wu, Y-N Su, F-J Hsieh, J-M Wong

Background: Overexpression of vascular endothelial growth factor (VEGF) correlates with vascularity, metastasis, and proliferation in colorectal cancer but the role of its homologue, placenta growth factor (PIGF), is unknown. The aim of this study was to evaluate expression and clinical implications of PIGF in colorectal cancer.

Methods: We investigated 74 tumour/non-tumour pairs of colorectal cryosections. Clinical staging was based on the UICC-TNM classification. Expression levels of mRNA for PIGF and VEGF were analysed with quantitative real-time reverse transcription-polymerase chain reaction. Proteins were analysed by immunochemical staining and enzyme linked immunosorbant assay. Analysis of the differences in PIGF and VEGF levels between tumour and non-tumour tissues in the same patient were performed by paired t-test; differences between localised and advanced disease by the Mann-Whitney, z2, and Fisher’s exact tests and survival curves by the Kaplan-Meier method.

Results: Expression levels for both growth factors were significantly higher in tumour than in non-tumour tissues (p < 0.001). The ratio of PIGF expression in tumour to non-tumour in the advanced disease group was significantly higher than for the localised disease group (p = 0.009). Patients with more tumour PIGF mRNA had shorter survival (p = 0.028). The majority of PIGF was expressed in tumour cells.

Conclusions: Our results suggest that PIGF expression correlates with disease progression and patient survival and may be used as a prognostic indicator for colorectal cancer.

Materials and Methods

Materials
Seventy four tumour and non-tumour pairs of colorectal cryosections were included from samples collected from September 2000 to June 2003, following the rules set forth by the ethics committee of the National Taiwan University Hospital. All tissues were freshly frozen or immersed in OCT, and kept at −80°C until use. Expression levels of PIGF or VEGF were analysed by quantitative reverse transcription-polymerase chain reaction (RT-PCR) and the ratio of expression levels in the tumour tissue to those in non-tumour tissue was calculated. Clinical staging of cancers was determined based on the UICC-TNM classification. All patients received identical treatment programmes in this study. No preoperative chemotherapy or radiotherapy had been given while stage III and IV patients were subjected to postoperative chemotherapy with 5-fluorouracil and...
leucovorin. Stages I and II were collectively termed as the localised disease group and stages III and IV as the advanced disease group in this study. Follow up duration was defined as the period between the operation date and day of the last visit, according to the patient’s chart.

**Quantitative RT-PCR**

Total RNA was isolated using an RNA extraction kit (Qiagen Inc., California, USA) from tissue homogenised with Trizol (Invitrogen Inc., Carlsbad, California, USA), according to the manufacturer’s instructions. For confirming expression levels of PlGF in tumour tissue, cells were collected from 15 stage III/IV patients by laser capture microdissection (LCM), as previously reported. Briefly, 5 mm sections tissue were mounted on silicon coated slides. Slides were cleared in xylene and dehydrated in graded alcohols. After staining with haematoxylin-eosin, LCM using a PixCell I system (Arcturus Engineering, Mountain View, California, USA) was used to separate tumours and normal cells. LCM parameters included a laser power of 90 mW, laser pulse duration of 1.2 ms, and laser spot size of 7.5–15 µm in diameter. A cap devised by Arcturus Engineering was used for the transfer film. RNA was extracted and isolated using the PicoPure RNA Isolation kit (Arcturus Engineering) according to the manufacturer’s instructions.

Primers and probes were designed using the Primer Express program (Perkin-Elmer Applied Biosystems, California, USA) and purchased from the same vendor. The primers used, based on the cDNA sequence of PlGF, were as follows: forward primer 5'-GGG CGG AGT AGA TGC ACT GTG T-3'; reverse primer 5'-TCC CCA GAA CGG ATC TTT AGG-3'. The sequence of the probe used to detect and quantify the RT-PCR product was 5'-AGA CGG CCA ATG TCA CCA TGC AGC-3'. The primers and probe used for VEGF mRNA were as follows: forward primer 5'-TAC CTC CAC CAT GCC AAAG T-3'; reverse primer 5'-GAT GAT TCT GCC CTC CTC TT-3'; probe 5'-TCC CAG GCT GCA CCC ATG GC-3'. The primers and probe used for the GAPDH (internal control) mRNA were as follows: forward primer 5'-AGA CGG CCA ATG TCA CCA TGC AGC-3'. The primers and probe used for the GAPDH mRNA were as follows: forward primer 5'-TCC CAG GCC ATC TTC TT-3'; reverse primer 5'-AGA GGT GAG GGT CGG AGT-3'; probe 5'-GAA GGT GAT GGT GGT TT-3'. Samples to produce the standard curve for real time RT-PCR were prepared by serial dilution of a specific RNA sample as the unknown samples were determined from this standard curve. Concentrations of PlGF in tumour and non-tumour tissues were quantified using a “Quantikine” human PlGF immunoassay (R&D Systems Inc., Minneapolis, Minnesota, USA). Diluted protein lysate was incubated in triplicates overnight at 4°C on microtitre plates coated with a murine monoclonal antibody against human PlGF. Unbound proteins were washed off, and an enzyme linked polyclonal antibody specific for PlGF was added to “sandwich” the PlGF immobilised during the first incubation. A substrate solution for horseradish peroxidase was added, and colour developed in proportion to the amount of antibody bound PlGF. Absorbance of the colour was read at 450 nm. A standard curve, consisting of known amounts of PlGF, was carried through the above procedure, and concentrations of PlGF in the unknown samples were determined from this standard curve. Concentrations of PlGF were expressed as pg/mg of protein.

**Data analysis**

Differences in PlGF and VEGF mRNA levels, and PlGF protein levels between tumour and non-tumour tissues in the same patient were analysed using a paired t test while differences between localised disease and advanced disease patients were analysed using the Mann-Whitney, χ², and Fisher's exact tests. Correlations between PlGF or VEGF expression levels and clinical stages, and PlGF expression levels between mRNA and protein, were analysed by Spearman’s correlation coefficient. A survival curve was obtained using the Kaplan-Meier method. Differences in cancer specific survival time for patients with tumours with high or low expression levels of PlGF or VEGF were analysed using the Breslow test.

**Immunohistochemical staining**

Serial sections of frozen colon tissues were mounted on poly-L-lysine coated slides and allowed to dry for 30 minutes before fixation in 100% acetone for 15 minutes. Tissue sections were washed for five minutes, three times, in phosphate buffered saline (PBS), immersed in 2% H₂O₂ in methanol for 25 minutes to inactivate endogenous peroxidase, and blocked with 1% bovine serum albumin. Blocked sections were incubated with antibodies against PlGF, VEGF, or Flt-1 (Santa Cruz Biotechnology, Inc., Heidelberg, Germany) at dilutions of 1:25, 1:50, and 1:25, respectively, for two hours at room temperature, washed with PBS, reacted with biotinylated secondary antibody with antigen antibody (dilution of 1:500; Santa Cruz Biotechnology, Inc.) for PlGF, and antirabbit antibody (dilution of 1:500; Vector Laboratories Inc, Burlingame, California, USA) for VEGF and Flt-1 for one hour, followed by incubation with streptavidin conjugated horseradish peroxidase (peroxidase substrate kit, DAB; Vector Laboratories Inc.) which was used to reveal localisation of the antibodies, and tissues were counterstained with Mayer’s haematoxylin.

**ELISA (enzyme linked immunosorbent assay)**

Protein lysate from each specimen was prepared using 10 mg tissue cut into tiny pieces, suspended in cell lysis buffer (0.15 M NaCl; 0.1 M Tris, pH 8.0; 1 mM EDTA, pH 8.0; 1 mM PMSF) and mechanically homogenised with a polytron PT 10/35 (Brinkman Instruments, Westbury, NY) for 15 minutes at full speed. Insoluble material was removed by centrifugation at 10,000 g at 4°C for 15 minutes, and the supernatant was used for the assay. VEGF was measured using the Quantikine ELISA kit (R&D Systems, Minneapolis, Minnesota, USA). The concentration of VEGF was calculated from the absorbance at 450 nm. In summary, we chose GAPDH mRNA as an internal control. The relative amount of unknown sample (PlGF mRNA), standardised against the amount of GAPDH mRNA, was expressed as –ΔCT 6 = – [CT(plgf) – CT(gapdh)]. The ratio of the number of PlGF mRNA copies to the number of GAPDH mRNA copies was then calculated as 2^[ΔCT K+K], where K is a constant.
Colon cancer tumour tissues increased gradually from stages 0.77, and 1.79, respectively. Expression levels of VEGF in higher than in non-tumour tissues ($p < 0.001$). Expression levels of PlGF and VEGF in tumour tissues were upregulated in colorectal cancer and the extent of upregulation correlated with disease progression.

- Both PlGF and VEGF mRNA expression were upregulated in colorectal cancer and the extent of upregulation correlated with disease progression.

- **Table 1** Summary of the demographic data, placenta growth factor (PlGF), vascular endothelial growth factor (VEGF) mRNA expression, and survival.

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<th>Advanced disease</th>
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†Age and follow up period are presented as means (SEM).
‡Differences in survival among different stages was statistically significant.
§Survival between PlGF ≥ median and PlGF < median was statistically significant with $p = 0.028$ by the $\chi^2$ test.
|| Comparison of localised and advanced disease groups. Sex and survival were analysed using the $\chi^2$ method and Fisher’s exact test; age, follow up period, $-\Delta C_T$ of PlGF, and $-\Delta C_T$ of VEGF were analysed using the Mann-Whitney test. |
|---|---|---|---|---|---|---|
| p | | | | | | 0.05. |

RESULTS

**Basic data**

A total of 74 colorectal cancer patients, 39 males and 35 females, were included in the study (table 1). There were 14 rectal cancers and 60 colon cancers. However, there were no significant differences in PlGF, VEGF expression level (either tumour tissue or tumour to non-tumour ratio), age, sex, or cancer specific survival between the rectal and colon cancer groups. Age at first diagnosis ranged from 26 to 94 years (mean 68.4). Follow up for these 74 patients ranged from 1 to 47 months (mean 26.0). During follow up, 14 patients died of colorectal cancer. According to the UICC-TNM classification, there were seven poorly differentiated, two well differentiated, and 65 moderately differentiated tumours and there were no statistically significant differences in PlGF or VEGF expression among these three groups.

- A positive correlation was observed between expression levels of PlGF and VEGF in tumour tissues ($r = 0.747$, $p<0.001$) as well as in non-tumour tissues ($r = 0.67$, $p<0.001$). The ratio of PlGF level in tumour tissue to PlGF level in non-tumour tissue showed a positive correlation ($r = 0.744$, $p<0.001$) with the ratio of VEGF level in tumour tissue to VEGF level in non-tumour tissue, as shown in fig 1C.

**PIGF, but not VEGF, mRNA expression is a prognostic marker for colorectal cancer**

In this study, $-\Delta C_T$ value of PlGF for the tumour samples ranged from −6.56 to 4.58, with a median value of 0.87, whereas values for VEGF ranged from −2.72 to 4.34 (median 0.79). Patients were divided into low and high expression groups, based on whether they were above or below the median value. Patients in the high PlGF mRNA expression group were more likely to have advanced disease than those in the low expression group (stage IV, $p = 0.047$) (table 1). The probability of cancer specific survival was significantly lower in the high PlGF mRNA expression group (mean survival 35.8 months (95% confidence interval [CI] 30.5–41.1) than that of the low PlGF mRNA expression group (mean survival 43.3 months (95% CI 39.8–46.7); Breslow test, $p = 0.040$) (fig 2A). In contrast, the difference between the probabilities of cancer specific survival in the high VEGF mRNA expression group and the low VEGF mRNA expression group was not statistically significant ($p = 0.41$) (fig 2B).
Placenta growth factor predicts colon cancer

For further comparison, patients with stages I and II were categorised as the localised disease group and patients with stages III and IV as the advanced disease group. Table 1 also shows that PlGF expression levels, the ratio between PlGF levels in tumour tissues and PlGF levels in non-tumour tissues, and cancer specific survival were significantly different between the localised disease and advanced disease groups (p = 0.013 and 0.001, respectively). Differences in sex, age, follow up period, VEGF expression, and ratio between VEGF levels in tumour tissues and VEGF levels in non-tumour tissues were not statistically significant. As deaths were mostly observed for the advanced disease group, we tried to determine whether there was any independent factor for predicting outcome in these advanced disease patients. We found that there was a trend for PlGF expression as a predictor of survival as the probability of survival was lower in the high PlGF mRNA expression group (mean survival 30.7 months) than in the low PlGF mRNA expression group (mean survival 36.5 months; Breslow test, p = 0.12) (fig 2C). However, the difference was not significant due to the small sample size and short follow up period.

PIGF, VEGF, and Flt-1 localisation in colorectal cancer

Immunohistochemical staining analysis (fig 3) showed that both PlGF and VEGF were expressed mainly in tumour cells. In contrast, Flt-1, the receptor for PlGF and VEGF, was expressed in both tumour cells as well as in endothelial cells.
PIGF protein levels correlated with mRNA levels
Protein levels of PIGF in tumour tissues ranged from 26.6 to 2498.4 pg/mg (mean (SEM) 281.3 (38.8) pg/mg). In corresponding non-tumour tissues, protein levels of PIGF ranged from 26.6 to 358.4 pg/mg (mean (SEM) 128.7 (8.5) pg/mg). PIGF protein levels in tumours were significantly higher than those of corresponding non-tumour tissues (p<0.001) (fig 4A). PIGF protein expression correlated well with mRNA from the same tissue (r = 0.308, p<0.001) (fig 4B).

DISCUSSION
Angiogenesis is required for tumour growth and metastasis. Recently, PIGF has been shown to be a key molecule in the angiogenic switch under pathological conditions, including cancer. In the present study, we demonstrated that PIGF expression levels were significantly higher in tumour tissues compared with non-tumour tissues. The ratio between PIGF levels in tumour tissues and PIGF levels in the non-tumour tissue correlated well with the stage of colon cancer. Furthermore, higher PIGF expression in the tumour, as well as a higher ratio of PIGF levels in tumour tissues to PIGF levels in non-tumour tissues, correlated with a poorer prognosis. It has been proposed that PIGF stimulates angiogenesis through displacement of VEGF from the Flt-1 sink, thereby increasing the fraction of VEGF available for activation of Flk-1. Alternatively, PIGF can modulate the function of VEGF by regulating intermolecular and intramolecular crosstalk between Flt-1 and Flk-1. Moreover, PIGF alone can trigger its own signalling, independent of the VEGF/Flk-1 pathway, and can trigger Flt-1 dependent functions such as proliferation, apoptosis, and angiogenesis.

In addition, loss of PIGF activity impaired angiogenesis in pathological conditions such as ischaemia, inflammation, and cancer. These results suggest that PIGF may be a key molecule in regulating the angiogenic switch under pathological conditions. These data are consistent with our results that PIGF expression levels are significantly higher in tumour tissues than in non-tumour tissues and that the ratio between PIGF levels in tumour tissues and PIGF levels in non-tumour tissues correlates with colon cancer stage.

Several studies have demonstrated that VEGF and PIGF expression correlate with tumour growth and angiogenesis. In our study, we showed that expression levels of PIGF and VEGF in tumour tissues were higher than in non-tumour tissues of the same colorectal cancer patients. These results suggest that both PIGF and VEGF may play roles in colorectal cancer formation and tumour progression, consistent with the hypothesis of Carmeliet et al that PIGF exerts a synergistic effect on VEGF driven angiogenesis under pathological conditions. VEGF stimulates growth of new vessels that are fragile, leaky, and prone to regression. PIGF can recruit smooth muscle cells/precursors, and thereby stimulate vessel maturation and stabilisation. Therefore, PIGF and VEGF should be upregulated in parallel during angiogenesis to stimulate the formation of mature non-leaky vessels. PIGF expression could be regulated by oxygen tension and cytokines. However, the mechanism of increased PIGF expression in colorectal cancer remains unclear. Elevated expression of VEGF in tumour tissues of colorectal cancer patients was observed in many studies. There was generally no correlation between elevated VEGF expression and patient survival, with only one exception, that of Lee et al who reported that VEGF correlated with survival. However, it did not emerge as an independent risk factor in a multivariate analysis. Our studies also showed that VEGF expression increased in tumour tissues but did not correlate with survival. In contrast, PIGF expression levels not only increased in colorectal cancer but also correlated with survival. This indicates that PIGF, in addition to its synergistic effect on VEGF driven angiogenesis, may have its own distinct effect on colorectal cancer. The clinical implication is that PIGF may be useful as a prognostic indicator, especially important in TNM stage III and IV patients. For stage III and IV patients with low PIGF levels, aggressive treatment is still recommended.

Figure 3 Immunohistochemical stain showed a positive placenta growth factor (PIGF) (A) and vascular endothelial growth factor (VEGF) (B) reaction, mainly in tumour cells, while Flt-1 (C) protein was expressed in both tumour cells and endothelial cells. A–C, 200 x; stage III; arrows, tumour cells; arrowhead, endothelial cell.

Figure 4 (A) Protein levels of placenta growth factor (PIGF) were significantly higher in colorectal tumour tissues than in corresponding non-tumour tissues, measured by an ELISA method. (B) PIGF protein expression levels were significantly correlated with mRNA expression levels.
Immunohistochemical localisation studies showed that PlGF, VEGF, and their receptor, Flt-1, were all localised in tumour cells. We hypothesised that colon cancer cells secrete PlGF as well as VEGF and that both factors serve paracrine and autocrine functions in stimulating angiogenesis as well as cancer growth and metastasis.

Several clinical efforts are currently underway to evaluate the therapeutic potential of inhibitors of VEGF or VEGFR-2. In recent studies, the VEGF specific antibody bevacizumab prolonged survival of colorectal cancer patients and had a direct and rapid antivascular effect in human rectal cancer. 27 28 However, VEGF and VEGFR-2 are not only involved in pathological angiogenesis but also in normal vessel growth and maintenance. 29 Furthermore, VEGF has a direct effect on motor neurone survival, 30 lung maturation, 31 liver regeneration, 32 control of blood pressure, 33 and glomerular development. 34 This raises the question of whether long term inhibition of VEGF may also affect these processes. 35 PlGF, in contrast with VEGF, affects blood vessel formation only under pathological and not under physiological conditions, 35 except during normal physiological angiogenesis in the placenta. 36 37 Our results showed that PlGF, but not VEGF, was significantly upregulated in colorectal cancer and correlated with survival. Thus PlGF may be a safer therapeutic target for colorectal cancer.

In summary, we demonstrated that both PlGF and VEGF expression levels in tumour tissues are higher than in non-tumour tissues in patients with colorectal cancer. The ratio of PlGF levels in tumour tissues to PlGF levels in non-tumour tissues, but not the analogous ratio for VEGF, correlated with stage of colorectal cancer. We conclude that PlGF expression correlates with disease progression and survival status, and may be used as a prognostic indicator for colorectal cancer.

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*S-C Wei and P-N Tsao contributed equally to this study.

Conflict of interest: None declared.

Part of the results were presented at the 95th AACR (American Association for Cancer Research) in March 2004.

REFERENCE

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Liver failure after delivery

Clinical presentation
A 32 year old, grvidra 2, para 2, was admitted at 36 weeks’ gestation with upper abdominal pain. During her second pregnancy she frequently had epistaxis. At 39 weeks’ gestation she delivered a healthy girl after an elective caesarean section which was complicated by a total blood loss of 1.5 litres. Progressive liver failure and shock led to her transport to our hospital one day post partum. She had no history of alcohol abuse, viral hepatitis, and was not receiving any medications.

Continuing abdominal bleeding was suspected and at laparotomy 5 litres of blood were found in the abdominal cavity from sites of recently lysed adhesions. On day 4 post partum, liver failure worsened with grade IV encephalopathy necessary to perform an orthotopic liver transplantation. The procedure was complicated by severe intraoperative bleeding (30 litres). A section of the explanted liver is illustrated in fig 1. Postoperative follow up was complicated by intra-abdominal infections and steroid refractory chronic rejection leading to failure of the graft. She was successfully retransplanted four months later and is currently doing well.

Question
What was the mechanism of liver failure in this patient?
See page 709 for answer
This case is submitted by:

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Figure 1

33 Li B, Ogasawara AK, Yang R, et al. KDR (VEGF receptor 2) is the major mediator for the hypotensive effect of VEGF. Hypertension 2002;39:1095–100.

EDITOR’S QUIZ: GI SNAPSHOT

Robin Spiller, Editor

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Figure 1
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 (i.e) or M0 (mi+)) 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 patients undergoing colorectal surgery 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 cytospins. One half was stained with the A45-B/B3 antibody (supplied by U Karstens, PhD, Berlin, Germany) and the other half with Ber-EP4 (supplied by U Karstens, PhD, Berlin, Germany) and the corresponding large (60×45 mm) tubulovillous adenoma of the right colon, with low grade intraepithelial neoplasia (haematoxylin-eosin staining, magnification 40×).

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 carcinomatous 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.

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Conflict of interest: None declared.

References

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

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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 of fibroblasts and myofibroblasts. 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. 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 pathway. 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.

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 desmoid. The patient was the only JNA affected FAP family. Matched desmoid. The patient was the only JNA affected FAP family. Matched desmoid.

Mutations were found in 12 of 16 sporadic JNAs analysed. 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. 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. No APC mutations were detected and none of the informative cases were LOH positive.

We performed laser capture microdissection to study the tumour tissue. The tumour is composed of dilated vascular channels set in an abundant myxoid stroma containing fusiform fibroblasts and focal mononuclear cell infiltrates. This FAP associated JNA should be considered a sex dependent extraintestinal FAP manifestation.

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References


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).
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, newly developed highly sensitive ultrasonic equipment has enabled the detection of vascular signals in PC; vascular signals were detected in 20–67% of cases. 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 metastasis 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 Advanced Dynamic Flow mode, which is wideband Doppler sonography with a high sensitivity and resolution. The contrast agent was Levovist (SHU 508 A; Schering AG, Berlin, Germany), which was administered at a concentration of 300 mg/ml by intravenous injection of 8 ml at 1 ml/s. After injection, 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 (Light Speed 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 patients (55%), and progressive disease (PD) in nine (29%). A significant decrease in grade 3 from grade 2 was observed in PR compared with SD or PD after one cycle of treatment (p = 0.0009 and p = 0.0017, respectively). After two cycles of treatment, the decrease was conspicuous in PR (p = 0.0022 and p = 0.0021, respectively) whereas in PD a significant increase in the signal intensity in the tumour was observed compared with SD (p = 0.0160). In the present study, the increase in the v signal (before the second cycle) was a significant prognostic factor (p = 0.0130). Median survival time of patients in the non-increased v signal group (n = 22) after two cycles of treatment was 382 days (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).

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 Gut. Many of the studies are summarised 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. Smoking is a well documented and universally recognised risk factor for increased CD severity as smokers are more likely to relapse and require corticosteroids, immunosuppressants, and surgery. Furthermore, smokers are more likely to have a less favourable response to infliximab. Smoking status is therefore a potential 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 re-analyse published data to ensure that results have not been conflated 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 Pietrangeli). 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. Informed and written consent was obtained from the patient and family members.

The subject, a 45 year old Caucasian male, presented with complaints of lethargy and malaise, who had no risk factors for viral hepatitis, consumed minimal alcohol (20 g/week), and was married with two children. Physical examination was normal, including a normal body mass index.

Initial investigations revealed a haemoglobin level of 12.2 g/dl, white blood cell count of 3.8×10^9, and platelet count of 135×10^9. Serum ferritin concentration was 3500 μg/l with a transferrin saturation (TS) of 29%. Molecular analysis did not reveal the presence of the C282Y, H63D, or S65C mutations.

Liver biopsy was performed and revealed significant Kupffer cell iron loading with minimal staining in hepatocytes, as detected by Perls’ Prussian blue staining. No fibrosis was detected. Hepatic iron concentration was 96 μmol/g dry weight (normal 5–35 ) with a hepatic iron index of 2.1 (normal <1.1). No other secondary cause for iron loading (for example, thalassemia, porphyria cutanea tarda, or chronic liver disease) was detected. Liver histology and biochemistry were suggestive of ferroportin disease. The entire coding region and splice sites of the ferroportin gene from the proband were polymerase chain reaction amplified and sequenced, as previously described. Other family members were subsequently evaluated.

The presence of a cytokine to adenoine change at nucleotide 230 of ferroportin, which results in mutation of an alanine to aspartic acid at amino acid 77 (A77D) was identified in the proband. Subsequently, this change was also identified in the proband’s father, sister, and daughter (fig 1). This is the same mutation which was identified in Italy by Montosi and colleagues. 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 V62del 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.

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References
HHV-8 positive, HIV negative disseminated Kaposi’s sarcoma complicating steroid dependent ulcerative colitis: a successfully treated case

We present the case of a 49 year old man who had suffered histologically confirmed ulcerative colitis (UC) since 1998. He had been asymptomatic for four years when in August 2002 an acute relapse developed. Colonoscopy and histology of a superficial bowel biopsy showed clear signs of active UC without any significant malignancy. Despite adequate therapy, he failed to improve and was referred for restorative proctocolectomy because of steroid dependency and end stage colitis. By the time of his referral, violaceous reddish-brown nodules had developed on his extremities. Skin biopsy showed spindle cells and vascular slits. Histological diagnosis was Kaposi’s sarcoma (KS) of the skin. He underwent a restorative proctocolectomy in March 2003. The pathological examination of the colon showed features of UC and surprisingly, characteristic signs of KS also. Human immunodeficiency virus (HIV) tests were negative. Human herpesvirus-8 (HHV-8) DNA was detected in native samples from affected skin but not in peripheral blood or the large intestine. The virus persisted in endothelial cells and spindle cells of KS. According to the literature, the HHV-8 genome in native 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.

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Table 1

<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>
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</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>Adlerberg</td>
<td>1970</td>
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<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>Yes</td>
<td>Subtotal colectomy</td>
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<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>
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<tr>
<td>Biggi</td>
<td>1987</td>
<td>Positive</td>
<td>UC</td>
<td>Yes</td>
<td>Urgent colectomy for toxic megacolon and severe diarrhoea, subtotal colectomy with ileostomy</td>
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<tr>
<td>Meltzer</td>
<td>1987</td>
<td>Negative</td>
<td>UC distal to the descending colon</td>
<td>Yes</td>
<td>Restorative proctocolectomy</td>
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<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 relation to the fastened seat belt. Their proximity to the vertebral column, as well as their particularly vulnerable in the seat belt syndrome because of direct trauma to the gut. The duodenum and jejunum are mesenteric structures were normal on laparotomy, the superior and inferior mesenteric arteries. As Miss M’s secondary to mesenteric injury, with involvement of the bowel obstruction after blunt abdominal injury: direct and indirect. The direct theory postulates that viscera get bowel obstruction after blunt abdominal injury: direct and indirect. The direct theory postulates that viscera get

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

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 submucosa, and interruption and replacement 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.

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

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The list of authors for the Editor’s quiz: GI snapshot on page 672 of the May issue was published in the incorrect order (CJM de Groot et al. Liver failure after delivery. 2005;54:672). The correct author list is as follows: CJM de Groot, GM van Goor, MF Stolk, G Kazemier, PE Zondervan, HJ Metselaar, IR Wanless, and HLA Janssen.

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