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Expression of endothelial adhesion molecules in colorectal neoplasia

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Intercellular adhesion molecule-1 (ICAM-1), intercellular adhesion molecule-3 (ICAM-3), vascular cell adhesion molecule-1 (VCAM-1) and endothelial cell adhesion molecule-1 (ELAM-1) or E-selectin are inducible endothelial surface molecules involved in leucocyte adhesion. These molecules may also have an important role in the process of tumour metastasis, through their interaction with tumour cells. We examined the expression of these adhesion molecules in the endothelium of 46 colorectal carcinomas, 13 adenomas and in normal colorectal mucosa in frozen tissue sections. ICAM-1 was strongly expressed in the endothelial cells of both small arteries and veins in all normal and neoplastic cases. VCAM-1 showed a variable expression, with focal strong positivity in arteries and mild to moderate staining in most of the small veins in all carcinoma cases, and only mild staining in adenomas and in the endothelium of normal mucosa. E-selectin and ICAM-3 were not expressed by normal mucosa vessels, but while all carcinomas and 11/13 adenomas showed staining in the small venules with E-selectin, ICAM-3 was positive in the endothelium of only 3/46 carcinomas. Our findings suggest that colonic neoplasia induces a variable upregulation of the expression of endothelial adhesion molecules. This upregulation is probably a result of cytokine stimulation and may affect the host inflammatory response to the tumour, as well as the metastatic process.

W62

THE CORRELATION BETWEEN ANAL SPHINCTER DEFECTS AND PRESSURE PROFILES.

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To study the relationship between ultrasonographic sphincter defects and pressure profiles we investigated 115 patients with faecal incontinence. Endoanal ultrasound was performed using a rotating 7.5 MHz probe and the anal pressure profile was obtained using a water-filled microballoon system. 63 of the 115 (55%) patients had a demonstrable sphincter defect. There was no significant difference in the mean ages of these patients [55.2 (2.6) vs 55.5 (2.4)]. 8 patients had an internal anal sphincter (IAS) defect, 30 patients had an external sphincter (EAS) defect and 28 patients had a defect in both the IAS as well as the EAS. There was no significant difference in the mean basal pressures (cm H_20) in the sphincter defect and no sphincter defect groups [63.6 (4.9) vs 61.2 (3.5), $p>0.05$]. Similarly there was no significant difference in the squeeze pressures between the two groups [126.7 (9.5) vs 107.8 (5.9), $p>0.05$]. When the site of the defect was taken into account, there was no significant difference in the basal pressures regardless of whether the defect was in the IAS, EAS or both. However, the squeeze pressure was significantly lower pressure in the group with an EAS defect ($p<0.05$). Squeeze pressures in the group with IAS defects and combined defects were not significantly different from those with no defect.

These data show that sphincter pressures are a poor indicator of the morphological integrity of the anal sphincter complex.

W63

GENETIC ALTERATIONS IN THE PLASMA OF PATIENTS WITH COLORECTAL CANCER.

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Malignant cells are shed into the sputum, urine, pancreatic juice, faeces and blood of some patients with cancer. In addition, DNA can be found circulating in the plasma of both healthy individuals and cancer patients, with increased quantities present in those with cancer. Codon 12 K-*ras* gene mutations are found in approximately 50% of colorectal cancers and we hypothesised that identical mutations might also be detected in DNA extracted from the plasma of patients with this disease.

Paired tumour and plasma samples were collected from 14 patients with colorectal cancer (stage A to D; mean age 63.4 years, range 41-78; males 10). DNA was isolated from blood plasma using previously described extraction procedures. Codon 12 K-*ras* gene alterations were detected by a sensitive polymerase chain reaction (PCR) assay which uses sequence specific primers to amplify mutant K-*ras* copies (PASA-PCR). Results were confirmed by restriction fragment length polymorphism (RFLP) PCR with product sequencing. In addition, the first exon of the K-*ras* gene was cloned and sequenced from DNA extracted from the plasma of 4 patients displaying different mutations.

Seven patients (50%) harboured a K-*ras* mutation within their primary tumour. Identical mutations were found in DNA extracted from the plasma of 6 (86%) of these, including one patient with stage A disease. Mutations were not detected in negative control tissues, in the plasma DNA of healthy controls or in the plasma DNA of 7 colorectal cancer patients whose tumours were negative for K-*ras* gene mutations. Similar results were obtained using all three molecular biological techniques.

We conclude that genetic abnormalities can be detected in circulating DNA extracted from the plasma of colorectal cancer patients. This may have applications for cancer diagnosis, screening and follow-up in the future.

Neoplasia W64–W71

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THE CCK-B/GASTRIN RECEPTOR IN HEPATOCELLULAR CARCINOMA

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Background: Gastrin is known to be a trophic hormone binding to the CCK-B/gastrin receptor and perhaps other receptor sites in the gastrointestinal tract. There is little knowledge of the effect of gastrin on the liver although some studies have suggested a trophic action on hepatocytes in cell culture and a trophic role in the regenerating liver. It is known that in chronic liver disease there are high circulating serum gastrin levels. The gastrin receptor has not previously been identified in the liver.

Hypothesis: That in man gastrin is trophic to hepatocytes in conditions such as cirrhosis and hepatocellular carcinoma.

Aim: To identify the CCK-B/gastrin receptor in hepatocellular carcinoma.

Methods: Paraffin sections from 20 consecutive patients with hepatocellular carcinoma and 10 consecutive "control" patients who had histologically normal biopsies (but abnormal liver function tests) were assessed for CCK-B/gastrin receptor. Immunocytochemistry was performed with a polyclonal CCK-B/gastrin receptor antibody using an avidin-biotin method. The specificity of this new antibody was demonstrated by pre-absorbence with the CCK-B/gastrin receptor epitope and control staining using pre-immune serum and substitution of specific antibody by buffer.

Results: 16 of the 20 patients with hepatocellular carcinoma had marked nuclear staining involving at least 50% of tumour cells; of these 3 patients additionally had marked cytoplasmic staining also involving at least 50% of the tumour cells. 4 patients had no nuclear staining and 17 had no cytoplasmic staining. In the control group only 2 of 10 patients had nuclear staining and another 2 patients had cytoplasmic staining.

Conclusion: The CCK-B/gastrin receptor may have a role in liver regeneration and hepatocellular carcinoma; its physiological significance needs to be studied further.

W65

INTRA-ARTERIAL RADIOTHERAPY WITH ^{131}IODINE-LIPIODOL FOR IRRESECTABLE HEPATOCELLULAR CARCINOMA (H.C.C.)

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The results of Lipiodol targeted chemotherapy for irresectable H.C.C. suggest a modest survival benefit compared to historical controls. The effect of intra-arterial Lipiodol-I131 was assessed in 26 patients with irresectable H.C.C. confined to the Liver [M = 22, F = 4; median age = 64 years (range 25-75)] (Okuda stage I = 6 , II = 19 , III = 1). Mean tumour volume was 446 (15-1200) cm^3 . Bolus Lipiodol-I131 was administered via the hepatic artery. Mean total activity was 953 MBq (247-1315). Further courses of Lipiodol-I131 were given at 2-3 months if clinically indicated.

Selective localisation was seen in all patients with mean tumour:liver ratio 10.4 (0.4-30.0). The mean radiation dose was 34 Gy (0.4-76.7) to tumour, 3.5 Gy (0.9-4.4) to liver, and 4.7 Gy (0.7-7.5) to the lungs. Partial response (reduction in tumour size > 50%) was seen in 8 patients (40%) with no change in 7 and disease progression in 7 , related to radiation dose (p=0.04). Cumulative survival rates were 61% at 6 months and 31% at 1 year. Median survival = 4 (1.1-19.1) months. (Stage I = 16.2 months ; stage II = 5.0 months). Four patients died from hepatic failure associated with cirrhosis (Child-Pugh grade B). Complications of treatment included transient pyrexia (38%) and abnormal liver function (27%). There was no marrow suppression.

Lipiodol-Iodine131 can deliver targeted internal radiation to H.C.C. , with some evidence of objective response and with relatively few adverse effects. Comparison to historical controls indicated a potential survival benefit.

W66

MUTATIONS IN CODONS 12 & 13 OF THE *KIRSTEN - RAS* GENE ARE NOT RELATED TO RELAPSE IN PATIENTS WITH EARLY COLORECTAL CANCERS

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Background: Molecular biological techniques may complement existing clinical and histological parameters in determining which patients with colorectal cancer are at higher risk of relapse. Using information such techniques provide, it may become possible to select those patients most likely to benefit from adjuvant therapy.

Methods: Patients were identified who had undergone resection for colorectal adenocarcinoma and were at low risk of recurrence by conventional criteria. Their histology was reviewed. The presence or absence of a *Ki-ras* exon-1 mutation was determined using PCR amplification and direct sequencing. To determine whether tumour had recurred, hospital records were checked and general practitioners and the Thames Cancer Registry were contacted. *Ki-ras* status was correlated with tumour recurrence and with clinical and histopathological features.

Results: Ninety eight consecutive patients with early colorectal cancer were identified. The Astler-Coller modification of Dukes staging was stage A in 22, B1 in 55, B2 in 20 patients and uncertain (≥B1) in one. Forty five were men and 53 women. Median age was 70 years (range 33 - 90). Median follow up was 37 months (range 6.5 months - 8 years).

Kirsten-ras mutations were present in 26 patients (26.5%). A mutation was present in 4 of 14 patients (28.5%) who developed tumour recurrence compared to 22 of 79 patients (28%) who have remained well with no evidence of relapse (p=0.86 log rank test). There were 5 peri-operative deaths and 2 other deaths unrelated to the cancer. The spectrum of mutations detected was similar to that reported by other groups.

There was a trend for cancer to recur in patients who were older at the time of surgery even when stratified for stage (p=0.05) and for those with a higher stage according to Astler-Coller's modification (p=0.06). However, the presence of a *Ki-ras* mutation was not associated with other histological features or clinical parameters.

Conclusions: This report clarifies previous findings and suggests that direct sequencing of the *Kirsten-ras* gene in whole tissue sections from primary tumours is not prognostic for Dukes A and early Dukes B colorectal cancer.

W67

bcl2 ONCOPROTEIN EXPRESSION IN RECTOSIGMOID ADENOMAS AS A PREDICTIVE MARKER FOR PROXIMAL ADVANCED NEOPLASMS.

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Histology and size of rectosigmoid adenomas (RSA) have been considered as predictors of proximal advanced neoplasms (PAN) The bcl2 proto-oncogene is a known inhibitor of apoptosis that allows accumulation and propagation of cells containing genetic alterations. The aim of this study was to determine whether bcl2 oncoprotein expression in RSA can be used for further identification of patients at risk for PAN. Fifty consecutive symptomatic average-risk patients who underwent total colonoscopy and had RSA were included. An adenoma was considered as advanced if villous histology and/or severe dysplasia and/or diameter >1cm were present. bcl2 Oncoprotein expression was immunohistochemically examined on paraffin embedded microwaved tissue sections using the monoclonal mouse anti-human bcl2 antibody, clone 124 (DAKO). The bcl2 score was calculated by multiplying the percentage of bcl2 positive cells (0-4) and the staining intensity (1-3). In total, bcl2 expression was observed in 46 (92%) of the 50 RSA tested; the mean bcl2 score was relatively higher in the 34 advanced (9.4±3.3) than in the 16 non-advanced RSA (7.3±4.1, P=0.08). PAN were detected in 6 (12%) of the 50 patients [3 had advanced adenoma(s) and 3 colon cancer]. PAN were present only in patients with advanced RSA(6/34 or 17.6%) and in none of the 16 patients with non-advanced RSA (R.R.:7.5, P=0.08). The mean bcl2 score in RSA was significantly higher in the 6 patients with PAN (11.3±1.6) than in the 44 patients without PAN (8.3±3.7, P=0.04). Moreover, PAN were present in 5 (25%) of the 20 patients with RSA of the highest (12) bcl2 score and in only one (3.3%) of the 30 patients with RSA of bcl2 score ≤9 (R.R.:9.7, P=0.03). Our preliminary data in patients with RSA suggest that 1. bcl2 oncoprotein expression in RSA is higher in patients with advanced PAN and 2. the highest bcl2 score in RSA may be used as a predictive marker for synchronous PAN.

W68

BCL-2 EXPRESSION DOES NOT INFLUENCE RESPONSE TO CHEMOTHERAPY IN COLORECTAL CANCER

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BACKGROUND: The Bcl-2 gene product blocks the induction of programmed cell death (apoptosis) and inhibits apoptosis caused by a number of agents, including drugs that are used in the treatment of cancer. A number of studies have determined that bcl-2 is expressed in colorectal tumours, but have not examined its influence on response to chemotherapy. In this study we have investigated the effect of bcl-2 expression on the response of colorectal tumours to chemotherapeutic regimens that include 5-fluorouracil, which is known to induce apoptosis.

PATIENTS AND METHODS: We identified archival tumour tissue from 262 patients, 87 of which were Dukes' stage A, 45 stage B, 60 stage C and 70 stage D. One hundred and sixty six patients had advanced or metastatic disease. They had received no prior chemotherapy or radiotherapy and were enrolled in three clinical trials at the Royal Marsden Hospital. Ninety six patients had disease that was considered to have good prognosis and were treated by surgical resection alone. Expression of bcl-2 was determined by immunohistochemistry and staining was assessed by an independent observer.

RESULTS: Sixty percent (159/262) of the tumours stained with a heterogeneous pattern for bcl-2. There was no association between bcl-2 expression and stage, primary tumour site, age of paraffin block or age of slide. There was a weak association of bcl-2 with low plasma CEA (p=0.029) and an inverse association between bcl-2 expression and tumours that were poorly differentiated (p=0.026). There was no difference in bcl-2 expression between the surgery alone or chemotherapy groups. Nor was there any association between bcl-2 expression and response or failure to respond to chemotherapy. In both groups of patients the bcl-2 positive patients had marginally increased event free and overall survival that failed to reach significance (p>0.1).

CONCLUSION: There was a weak association between bcl-2 expression and those histological parameters which are considered to convey a better prognosis. However, bcl-2 expression did not influence response to chemotherapy.

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W69

CD44v6 Expression Does Not Predict Outcome In Early Colorectal Cancer

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BACKGROUND:
Metastatic cancers share several common properties with lymphocytes, in particular their ability to colonise lymph nodes and permeate tissues. CD44 is a cell surface protein expressed on lymphocytes which facilitates both these functions. The discovery that variant forms of CD44 are expressed on certain tumour cells may lead to further understanding of carcinogenesis. This study was performed to examine the relationship between the expression of v6 variant (CD44v6) and clinical outcome in early colorectal cancer.
METHODS:
The records at 2 district general hospitals were reviewed to identify patients who had undergone potentially curative bowel resection for early colorectal cancer between 1987 and 1994. The pathology was reviewed. Expression of the CD44v6 variant protein was determined immunohistochemically using tissue blocks from the original tumour. Staining was assessed by an independent observer. Details of clinical outcome were obtained from hospital records and by contacting the patients' General Practitioners. Outcome measures used were evidence of disease relapse and death from recurrence.
RESULTS:
97 patients were identified who had early primary colorectal cancers (85 Dukes A, 12 Dukes B) treated by surgical resection between 1987-94. 45 were men and 52 women. Median age was 70 years (range 33-90). Median follow up was 37 months (range 0.5-8 years). 14 patients had disease relapse. 11 died and 3 patients underwent further surgery for local recurrence. 36 tumours were positive for CD44v6. Staining was heterogeneous and generally of low intensity when compared with a positive control of normal keratinocytes. 3 of 11 cancer deaths and 1 of 3 local recurrences were CD44v6 positive. Of the 14 patients with disease relapse, 9 (60%) did not express CD44v6.
CONCLUSION:
CD44v6 expression did not correlate with recurrence, survival or any histological marker examined.

W71

BILE FROM TRANSGENIC MICE CARRYING A MUTATION IN THE *Apc* GENE INDUCES MORE DNA DAMAGE THAN DOES BILE FROM WILD-TYPE MICE FROM THE SAME GENETIC BACKGROUND.

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Patients with familial adenomatous polyposis (FAP) carry germline mutations in the *APC* gene and are at high risk of developing duodenal neoplasms. The periampullary clustering of these duodenal tumours suggests that biliary carcinogens contribute to their formation. This hypothesis is supported by studies showing that levels of DNA adducts (promutagenic markers of carcinogen exposure) are higher in FAP duodenum than in normal duodenum, and that FAP bile produces more adducts than does normal bile in rat small-bowel *in vivo*, and in human cell lines and naked DNA *in vitro*. We now show that bile from transgenic mice that are heterozygous for a chain-termination mutation (*Apc*1638N) in the 15th exon of the mouse *Apc* gene, and which develop intestinal tumours similar in type and progression to human FAP, produces significantly more adducts when incubated with DNA *in vitro* than does bile from wild-type mice.

	DNA adducts per 10^9 nucleotides, median (range) of 4 replicate ^{32}P-postlabelling assays		*p* (Mann-Whitney)
Expt	Wild type	*Apc*1638N heterozygotes	
1	36.5 (12.4-54.4)	168.1 (80.4-235.5)	0.0143
2	59.0 (32.9-69.7)	89.4 (79.4-146.8)	0.0286

These results show that the increased capacity of human FAP bile to produce DNA damage can be replicated in a mouse model, opening the way for experimental studies of the factors that may influence the development of foregut neoplasia in FAP.

BSG/BBSRC W72–W88

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PHASE I/II TRIAL OF A MATRIX METALLOPROTEINASE INHIBITOR IN PATIENTS WITH MALIGNANT ASCITES.

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Malignant ascites is associated with a poor prognosis, measured in weeks. Intraperitoneal administration of Batimastat, a synthetic matrix metalloproteinase inhibitor, has been shown to resolve ascites in animal models. We, therefore, studied whether a single intraperitoneal dose of Batimastat could resolve ascites in symptomatic patients.

Nine patients with proven malignant ascites were recruited, with ethical approval and underwent intraperitoneal administration of a 500ml suspension of Batimastat after removal of an equal volume of ascites. Response to treatment was assessed by weight, abdominal girth and drainage.

Rapid systemic drug absorption was achieved with drug levels remaining elevated for 6 weeks and were higher than in a corresponding study where the ascites was drained to dryness prior to drug administration. Side effects consisted of abdominal pain of mild to moderate intensity (6 pts), pyrexia (2 pts), nausea (3 pts) and vomiting (2 pts). Only abdominal pain (3 pts) and scrotal oedema continued beyond 72 hours. A treatment response was seen in 5 out of 9 patients.

Intraperitoneal Batimastat is well absorbed and the large volume of dissolution(ascites not drained) improved absorption.Our results suggest that this agent may be useful in controlling ascites though further studies are required to confirm this.

W72

THE EFFECT OF BOLUS ENTERAL FEEDING ON HUMAN COLONIC MOTOR ACTIVITY.

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Introduction: Up to 25% of patients receiving continuous enteral feeding (CEF) develop diarrhoea, the pathogenesis of which remains unclear. Our previous studies show that CEF related diarrhoea is likely to be due to an abnormal colonic response, occurring earlier and more commonly with continuous intragastric than with intraduodenal feeding. The aim of the present study was to determine whether the abnormal colonic response to CEF could be overcome by administering intragastric enteral feed using a bolus technique to simulate more closely normal feeding patterns.
Methods: Intraluminal pressure recordings in the unprepared descending and sigmoid colon were studied in 6 healthy subjects using an established technique on 3 separate occasions in random order. Continuous recordings were made for 8.5 hours; 3 hours before and 5.5 hours after the start of a 15 minute intragastric tube fed bolus infusion of a polymeric enteral diet. Group 1 were fed 83mls over 15 minutes hourly for 4 instillations (5.53kcal/min, 34.9mgN/min); group 2 were fed 250mls over 15 minutes 2 hourly for 2 instillations (16.7kcal/min, 105mgN/min) and group 3 were fed 500mls over 15 minutes once (50kcal/min, 313mgN/min) feed. The pressure records were analysed in 30 minute epochs for the study segment (sum of 4 channels) activity index (AI = area under the curve: mmHg.min) by fully automated computer analysis.
Results:1 of group 1, all 6 of group 2 and 5 of 6 subjects in group 3 developed diarrhoea. There were no significant differences in AI between the 3 groups prior to feeding: group 1 - 2670 (132); group 2 - 2957 (227); group 3 - 3021 (214) mmHg.min, mean (SE). In group 1 there were no significant differences in AI before and after feeding (2694 (28)). In group 2 the AI fell to 2505 (72), $p<0.05$ and in group 3 the AI fell to 2455 (86), $p<0.04$. **Conclusion:** Low load bolus infusion of polymeric diet in Group 1 subjects was without effect on the colonic motility response. However bolus infusion of 16.7kcal/min or greater resulted in significant suppression of colonic motor activity, associated with a high incidence of diarrhoea.