

## Molecular basis of gastrointestinal disease T133-T140

T133

## MODULATION OF ERB B3 GENE EXPRESSION IN HEPATOCYTES FOLLOWING GROWTH FACTOR STIMULATION IN VITRO.

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ERB-B3 is a gene encoding a transmembrane tyrosine kinase, and is part of the EGF-receptor family. Its ligand is unknown. In view of the known involvement of the EGF-receptor (c-erb-b1 product) in hepatic resection, and the identification of another tyrosine kinase (the c-met oncogene product) as the receptor for HGF, we investigated the hypothesis that growth factor-initiated hepatocyte DNA synthesis would modulate c-erb-b3 expression.

Rat hepatocytes in primary culture were stimulated with Hepatocyte growth factor (HGF), EGF, insulin or basal medium control. Gene expression was determined by Northern analysis: membranes were hybridised at high stringency with a full length 4.8 kb cDNA to erb b3.

Expression was compared with constitutively expressed glyceraldehyde-3-phosphate dehydrogenase and total 28S ribosomal RNA. Unstimulated hepatocytes in culture expressed the 6.3 kb transcript of erb b3. There was a striking reduction in mRNA expression, resulting transiently in undetectable levels of the 6.3kb transcript after 8h, when DNA synthesis was stimulated by EGF or HGF with or without insulin. Thus, erb b3 gene expression is strikingly modulated by growth factor stimulation in hepatocytes, but not up-regulated. This contrasts with the changes in the EGF-receptor and HGF-receptor mRNA after receptor-ligand interaction, where receptor mRNA is up-regulated following receptor occupancy.

These findings suggest that none of these growth factor combinations interacts directly with erb b3 protein as a receptor. One explanation for the modulation observed is that constitutive erb b3 expression plays a role in maintaining hepatocytes in their normal non-proliferating state.

T135

## PROGNOSTIC VALUE OF p53 AND c-Ki-ras GENE MUTATIONS IN COLORECTAL CANCER

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Mutations in Ki-ras codon 12 and the p53 gene are common abnormalities in colorectal cancer. To determine if the occurrence of p53 and/or Ki-ras codon 12 mutations are related to patient survival we have analysed 100 colorectal adenocarcinomas for the presence of these oncogenic abnormalities. p53 staining was detected by immunohistochemistry in 45% of tumours. A higher frequency of p53 over-expression was identified in DNA aneuploid and left-sided tumours when compared to DNA diploid and right-sided tumours. Mutations in Ki-ras codon 12 were identified in 24% of carcinomas using the polymerase chain reaction and a restriction enzyme digestion method. Ki-ras mutations did not correlate with any clinicopathological feature.

In this series of colorectal cancers, mutations in Ki-ras codon 12 or p53 were not prognostic indicators of survival when considered individually. However, a statistically significant difference in survival was identified when these two oncogenic abnormalities were analysed together. The median survival of patients whose tumours contained both oncogenic mutations was a third of that of patients with either mutation alone or without either abnormality ( $p < 0.005$ ). Thus the screening for multiple genetic abnormalities in colorectal cancers excised at surgery may prove to be a useful tool in determining prognosis.

T134

## MECHANISMS OF INDUCTION AND INHIBITION OF A SIALYLTRANSFERASE IN HEP G2 CELLS.

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Many cell recognition phenomena are mediated by surface oligosaccharides, whose composition is determined by the glycosyltransferases (GT) expressed by a particular cell. We reported previously that *n*-butyrate (*n*B) inhibits expression of a GT,  $\beta$ -galactoside  $\alpha$ 2,6-sialyltransferase (Gal  $\alpha$ 2,6-ST), by >80% in Hep G2 cells but does not inhibit transcription of Gal  $\alpha$ 2,6-ST mRNA. We report here the effect of *n*B on mRNA turnover and the effects of several cytokines on Gal  $\alpha$ 2,6-ST expression in Hep G2 cells.

Northern blots of Hep G2 RNA were probed with a human cDNA for Gal  $\alpha$ 2,6-ST. Gal  $\alpha$ 2,6-ST enzyme activity of crude Hep G2 homogenates was measured by transfer of CMP-[<sup>14</sup>C]sialic acid to asialo- $\alpha$ -acid glycoprotein. Transcriptional effects were determined in nuclear run-on assays. Turnover of mature mRNA was assessed by use of inhibitors of transcription and protein synthesis.

In the presence of 5 mM *n*B,  $t_{1/2}$  of Gal  $\alpha$ 2,6-ST mRNA fell from 30 to 5 h, an effect that was negated by concurrent inhibition of transcription (actinomycin D) or protein synthesis (cycloheximide). In contrast, incubation with 100 U/ml of human recombinant interferon alpha-2a (Roferon-A, Roche, rIFN $\alpha$ ) increased Gal  $\alpha$ 2,6-ST mRNA level and enzyme activity  $\geq$ 3-fold, and this was a direct transcriptional effect. Incubation of Hep G2 cells with recombinant human interferon  $\gamma$  or interleukin-6 did not alter Gal  $\alpha$ 2,6-ST expression.

We conclude that *n*B inhibits Gal  $\alpha$ 2,6-ST expression in Hep G2 cells by increasing mRNA turnover, whereas rIFN $\alpha$  induces expression of the same enzyme by a direct transcriptional effect. Further elucidation of the mechanisms by which the ~100 mammalian GT are regulated may provide useful insights to normal cell-cell recognition and disease processes, such as tumor invasion and metastasis.

T136

## EXPRESSION OF OESTROGEN RECEPTORS AND AN OESTROGEN INDUCED PROTEIN IN LARGE BOWEL MUCOSA AND CANCERS

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The epidemiology of colo-rectal cancer suggests a role for sex steroid hormones in its development: differences exist between the sexes in the site specific incidence of this cancer. There is also a higher than expected incidence of colo-rectal cancer amongst women who have had breast cancer. In addition, a negative correlation exists between increasing parity and the risk of developing colo-rectal cancer.

We have attempted to demonstrate the expression of oestrogen receptors and an oestrogen induced protein, pS2, in large bowel mucosa and cancers. In breast cancers pS2 expression correlates with oestrogen receptor expression, and is predictive of response to hormonal therapy.

Twelve normal mucosa/cancer pairs were examined by northern blot analysis using cDNA probes for oestrogen receptor and pS2. Messenger RNA for oestrogen receptor, 6Kb in size, was detected in all samples. There was no quantitative difference between normal mucosae or cancers. Messenger RNA for pS2, 0.7Kb in size was detected in all samples; cancers had lower expression in 9 out of the 12 cases. In situ hybridisation using <sup>35</sup>S labelled riboprobes identified expression of pS2 in epithelial cells and oestrogen receptor. Immunostaining using an anti-pS2 monoclonal antibody and an anti-oestrogen receptor related protein antibody (ERD5) also demonstrated expression of pS2 and oestrogen receptor in normal tissues and cancers.

We have demonstrated the presence of oestrogen receptors in the large bowel mucosa and cancers; the co-expression of pS2 is evidence of receptor function.

T137

## EXPRESSION OF LACTASE mRNA IN RABBIT SMALL INTESTINE

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Lactose intolerance and the regulation of lactase activity after weaning is poorly understood. In addition, lactase expression is modified during certain intestinal disease states. We have therefore developed molecular probes to examine the cellular expression of lactase mRNA. Samples of duodenum (10 cm from the pylorus), jejunum (50% of length) and distal ileum (10 cm from the ileal-caecal junction) were taken from post-weaned rabbits (10 week old, n=6) and rapidly frozen. Cryostat sections were cut, fixed in 4% paraformaldehyde and stored in 95% ethanol at 4°C. 'Sense' and 'antisense' oligonucleotide probes (45mers) were synthesised from the rabbit lactase-phlorizin hydrolase cDNA sequence (nucleotides 1420-1464) and labelled with [<sup>32</sup>S]ATP. After hybridisation and autoradiography, sections were examined using a microdensitometer and consecutive density readings were taken over the enterocytes from the crypt base to the villus tip.

The 'antisense' probe specifically hybridised to the enterocyte population in all samples tested, no hybridisation was observed to any other intestinal cell type. No specific hybridisation of the control 'sense' probe to the tissue was observed. Expression of lactase commenced at the crypt/villus junction and after an initial rapid increase, reached a maximum at approximately 300 µm above this point. The signal then declined towards the villus tip. Maximum expression in the duodenum tended to be lower than in the jejunum (5.17 vs 8.15 AU). However, lactase mRNA was undetectable in the distal ileum of these animals.

This is the first study to examine the transcription of the lactase gene using *in situ* hybridisation histochemistry. These results demonstrate that there is regional variation in the abundance of lactase mRNA both along the length of the small intestine and the crypt/villus axis of post-weaned rabbit. This technique has proven useful for the study of lactase expression; future work will examine the molecular control of transcription for the lactase gene during post-natal development and intestinal disease.

T139

## ANALYSIS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS OF T-CELL RECEPTOR GENES IN FAMILIES MULTIPLY-AFFECTED WITH COELIAC DISEASE.

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Susceptibility to coeliac disease is at least in part genetically determined and a primary association has been demonstrated with the MHC Class II alleles DQA1\*0501 and DQB1\*0201. In Western European populations, 25% of normal individuals possess these alleles. Several epidemiological studies have suggested that a second genetic susceptibility gene exists and that this gene is not MHC-linked. The T-cell receptor (TcR) is responsible for recognising antigenic peptides when presented by MHC molecules. Germ-line polymorphisms of the T-cell receptor genes have been shown to be associated with other autoimmune disease states.

To investigate whether the T-cell receptor genes exert an additional genetic influence on susceptibility to coeliac disease, we have studied the germ-line polymorphisms of the TcR β gene complex located on chromosome 7 in 114 individuals from 13 families multiply-affected by coeliac disease. A Cβ DNA probe was used to examine the restriction fragment length polymorphisms (RFLP) following digestion of whole genomic DNA with the restriction enzymes Kpn I and Bgl II. These RFLP patterns are associated with polymorphisms within the TcR Cβ complex. Whilst these two restriction enzyme sites are not linked, the Bgl II restriction site is in linkage disequilibrium with the Jβ and Dβ genes as well as the telometric region of the Vβ gene complex.

No significant association was found between either RFLP pattern and coeliac disease, irrespective of MHC typing. These results suggest that susceptibility to coeliac disease is not related to the TcR β gene complex on chromosome 7.

T138

## DETECTION OF HERPESVIRUS DNA IN THE LARGE INTESTINE OF PATIENTS WITH ULCERATIVE COLITIS AND CROHN'S DISEASE USING THE NESTED POLYMERASE CHAIN REACTION

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Herpesviruses have been implicated in the pathogenesis of ulcerative colitis. This study examined the prevalence of herpesvirus DNA in inflammatory bowel disease tissue.

**METHODS:** DNA was extracted from resection and biopsy specimens of large intestine from patients with ulcerative colitis (n = 21), Crohn's disease (n = 29), and patients with non-inflammatory bowel disease (controls) (n = 21). The nested polymerase chain reaction was used to detect viral DNA using primer pairs specific for either cytomegalovirus (CMV), herpes simplex virus 1 (HSV1), human herpesvirus 6 (HHV6), varicella zoster virus (VZV) or Epstein Barr virus (EBV).

**RESULTS:** HSV1 and VZV DNA were not detected in any of the tissue samples. There was a high prevalence of CMV (81%), HHV6 (76%) and EBV (76%) DNA in ulcerative colitis tissue compared to Crohn's disease tissues (CMV 66%, HHV6 45%, EBV 55%). Control tissue had a relatively low frequency of CMV (29%) and EBV (19%) DNA, but a prevalence of HHV6 DNA similar to that of ulcerative colitis (86%). However, the simultaneous presence of HHV6 and CMV and/or EBV DNA in ulcerative colitis tissue (76%) was much greater than in either Crohn's disease tissues (38%) or control tissue (29%) (p < 0.05).

**CONCLUSIONS:** Infection of colonic tissues with HHV6 has not been described previously. CMV and EBV are capable of reactivating HHV6: the high prevalence of coexistent HHV6 infection with either or both of these two viruses in ulcerative colitis tissue suggests that these herpes viruses may play a synergistic role in the aetiology of this disease.

T140

## VITAMIN C REDUCES GASTRIC MUCOSAL DNA DAMAGE

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The persistence of covalently bound carcinogen-DNA adducts in a tissue has been associated with an increase in the risk of developing cancer in that tissue. We have examined DNA extracted from gastric mucosa for the presence of DNA adducts both before and after a course of vitamin C in order to clarify the role vitamin C plays in gastric carcinogenesis.

49 volunteers were entered into the study. At a first attendance for upper gastrointestinal endoscopy, biopsies of gastric mucosa were taken for DNA analysis and samples of both serum and gastric juice were taken for vitamin C estimation. Each patient then received a course of vitamin C (1g daily for 28 days) prior to undergoing a further endoscopy at which time the sampling procedures were repeated. DNA adducts were assayed by <sup>32</sup>P-postlabelling and vitamin C levels estimated by the method of Lowry.

Serum vitamin C levels were elevated in all patients following vitamin C supplementation as were gastric juice vitamin C levels in those patients with normal histology. In the presence of chronic atrophic gastritis or intestinal metaplasia the alteration in gastric juice vitamin C level was variable.

Gastric mucosal DNA adduct levels were significantly lower following supplementation. The mean level before vitamin C was 7.9 adducts/10<sup>8</sup> nucleotides and after supplementation was 5.4 adducts/10<sup>8</sup> nucleotides (p=0.01; Wilcoxon sign rank test).

This suggests that vitamin C may protect against the development of gastric cancer by reducing gastric mucosal DNA damage.