In conclusion, treatment by diosmectite during 1 week delays or reduces, in vivo, two major effects of Clostridium difficile toxins in rats.

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Microvascular Endothelium from Human Intestine Is Constitutively and Functionally Different from Human Umbilical Vein Endothelial Cells


Background: Recent work has demonstrated several differences between large and small vessel endothelial cells in situ and in vitro. We therefore established a method for isolation and long-term culture of human intestinal microvascular endothelial cells (HIMEC) and compared them with human umbilical vein endothelial cells (HUVEC). Expression and regulation of the cell adhesion molecules CD36, E-Selectin, VCAM-1, and ICAM-1 were studied.

Methods: In situ phenotyping of endothelial cells was performed by double immunofluorescence staining. In vitro antigen expression was examined by a cell ELISA. Results: Only the smallest vessels in the lamina propria expressed CD36 in situ. The endothelial cells isolated from the intestine continued to express CD36 in vitro, whereas HUVEC lacked CD36 in culture. E-Selectin, VCAM-1 and ICAM-1 were upregulated in a dose-dependent fashion on HIMEC after stimulation with LPS (0–1 μg/ml), IL-1β, or TNFα (both 0–1000 U/ml) and displayed characteristic time curves comparable to HUVEC. However, IL-4 appeared to have only a negligible effect on HIMEC. Thus, both VCAM-1 and ICAM-1 were upregulated on HIMEC (IL-4: 0–1000 U/ml) but only minor responses were observed on HIMEC. E-selectin was not induced by IL-4.

Conclusion: Our data suggest that leukocyte-endothelial interactions are differently regulated on HIMEC compared with HUVEC. HIMEC are therefore a more relevant test system for studies of endothelial cell involvement in inflammatory bowel disease.

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Right-Sided and Left-Sided Colorectal Cancers Have Alternative Genetic Changes


The development of colorectal cancer (CRC) needs several genetic changes that occur in one mucosal cell. Tumor suppressor genes are frequently inactivated as an essential step in colorectal carcinogenesis, through loss and/or mutation of both gene-alleles. We have earlier reported loss of the tumor suppressor gene TP53 in 68% of CRC, and mutation of TP53 is known to occur in approximately half of the CRC.

A genetic change called "RER" (DNA replication- or repair-error) has recently been discovered, and is thought to be caused by an inherited defect in one or more DNA repair enzyme(s). We and others have reported RER to occur in colorectal CRC. Furthermore, we have also reported RER to be significantly associated with several clinicopathological variables in 252 CRC: right-sided cancer, DNA diploid tumor, poorly differentiated tumor, and a better prognosis. So far, no association has been reported between RER and other genetic changes in CRC cells.

Methods: We have analyzed 156 "sporadic" cases of CRC for RER and TP53 changes. We used PCR (for RER, 7 random loci analyzed), PCR in combination with CDGE (for TP53 mutations), and Southern technique (two loci near TP53 analyzed for loss, the losses detected are supposed to comprise TP53 in most of the cases).

Results: Loss of one TP33-allele was found in 68% (106/156), mutation of one allele in 44% (69/156). Nearly all (63/69) tumors with mutation of one allele also had loss of the remaining allele.

Rel. between loc., RER, and TP53 (loss = 1. and mutation = m.):

<table>
<thead>
<tr>
<th>RER+</th>
<th>RER-</th>
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<tbody>
<tr>
<td>TP53 I. and m.</td>
<td>l. or m.</td>
</tr>
<tr>
<td>right colon</td>
<td>left rect.</td>
</tr>
<tr>
<td>1/3</td>
<td>3/10</td>
</tr>
<tr>
<td>1/3</td>
<td>3/10</td>
</tr>
</tbody>
</table>

1. RER was demonstrated in 16% of CRC occurring in 30% of right-sided and 10% of left-sided CRC (p < 0.001).
2. Right-sided cancers with RER have no complete TP53 inactivation, whereas left-sided CRC-positive cancers have similar TP53 inactivation as RER-negative CRC (p < 0.05).

One hypothesis is that right-sided CRCs have more inherited genetic changes/defects, and thus need fewer acquired genetic alterations in order to become malignant, whereas in left-sided CRC the situation is opposite. Our results agree with this.

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Prognostic Significance of Retinoblastoma Gene Product Expression in Colorectal Cancer

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Loss or inactivation of the retinoblastoma (RB) gene has been demonstrated in several non-GI human malignancies. Recently, altered RB gene expression has been reported in colorectal cancer (CRC) but the prognostic significance of this finding has not been determined. The aim of this study therefore was to correlate RB gene product expression with longterm survival in CRC.

Patients and Methods: CRC samples from 76 patients collected over 3 years (1984–1988) were analysed for RB gene product nuclear protein expression by immunohistochemistry using the monoclonal antibody PMG3 245. Unlabelled CRC tissue and bladder cancer tissue were used as negative and positive controls, respectively. Prognostic significance was established from Kaplan-Meier curves which were generated from actuarial disease free survival, with differences in survival being compared by means of logrank analysis.

Results: Positive RB expression of >80% in tumour cells was observed in 30/76 (40%) patients. Heterogeneous RB expression (20–80%) was seen in 39/76 (51%) patients, while no RB expression occurred in the remaining 7/16 patients (5% [RB expression of 100%]) (p = 0.03).

Conclusion: These findings suggest that the RB gene is of prognostic significance in CRC and has an oncogenic-like function, in contrast to its reported tumour suppression activity in other human malignancies.

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Overexpression of p53 in Relation to Long-Term Prognosis in Gastric Carcinoma

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Overexpression of p53 protein in the cell nucleus as a result of mutation is very common in human neoplasia, but the relationship of this phenomenon to prognosis remains to be defined. We investigated the relationship between p53 expression in gastric cancer and the outcome of the disease. The expression of p53 protein was analysed immunohistochemically in 170 gastric carcinomas by use of CM 1 antibody and routinely fixed tissue. A significant association was found between p53 overexpression in cancer tissue and poor prognosis. The proportions in cancer tissue and poor prognosis. The proportions of patients with 5-year survival with or without p53 overexpression were 4% and 49% respectively (p < 0.001).

In a multivariate analysis against 10 established prognostic factors, p53 overexpression was the strongest predictor of poor prognosis followed by metastases to the regional lymph-nodes (p < 0.001), RR = 6.7 and p = 0.03, RR = 2.66 respectively.

The results suggest that in gastric cancer an immunohistochemical detection of p53 can be helpful to identify patients at high risk of tumour recurrence and death for whom the use of an aggressive surgery and adjuvant chemotherapy should be considered.

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Programmed Cell Death Throughout the Colorectal Tumor Sequence

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Homeostasis of the renewal of normal tissue, progression and regression of tumor lesions are regarded as the result of the interactions between cell proliferation and programmed cell death (PCD). There is abundant evidence that cell proliferation changes in histologically normal intestinal mucosa are early events directly and closely associated with colorectal carcinogenesis, whereas little is known about the role of PCD.

In this investigation PCD was evaluated in intestinal mucosa of control subjects (n = 10), in histologically normal mucosa with cell proliferation abnormalities from subjects with colorectal neoplasia (n = 10), in colorectal adenomas (n = 20) and adenocarcinomas (n = 10). Cells engaged in PCD were identified through immunohistochemical detection of DNA strand breaks that follow activation of endogenous endonuclease. The method is based on nick-end labeling with biotinylated polydeoxyadenyliate introduced by terminal deoxynucleotidyl transferase, and then stained with avidin-conjugated peroxidase. PCD Index was calculated as the percentage ratio between labeled and total cells.

In normal mucosa, most PCD-positive cells were found at the mouth of the crypt and in the surface epithelium, in sectors where the physiological shedding of the epithelium takes place, though sporadic activity was present at
the base of the crypt, in the stem cell compartment (mean PCD Index: 4.2%). No changes in the distribution and quantity of PCD-positive cells were noted in the high-risk mucosa of subjects with colorectal neoplasia, even when cell proliferation was already subverted. Two PCD activation levels were found in adenomas (low PCD index: from 0.5% to 1.5%; high PCD index: from 1.5% to 2.5%). They were not correlated with either polyp size or the grade of dysplasia. PCD Index was significantly higher in adenomas from patients with familial adenomatous polyposis than in sporadic adenomas.

These results indicate that PCD is not a sensitive marker of the risk of tumor transformation in the intestinal mucosa. It is suggested that PCD can play a central role in the evolution of premalignant lesions, and that may identify slowly growing or regression-prone colorectal adenomas.

145 LDL Receptors and Polyamine Levels in Human Colorectal Adenocarcinoma


Low density lipoprotein receptor (LDLR) is a cell surface protein that binds LDL, the major cholesterol-transport protein in plasma. This binding leads to cellular uptake of LDL, providing the cell with cholesterol for new membrane synthesis. Rapidly growing cells have high numbers of LDLRs. Recently, we have demonstrated the presence of LDLRs in 17% of 53 human neoplastic colorectal adenocarcinomas. Besides, polyamines – putrescine (put), spermidine (spd) and spermine (spm), are indispensable compounds in the proliferation of gastrointestinal mucosa and they are considered as a reliable marker of cellular proliferation. Our aim was to evaluate the polyamine levels in 25 colorectal adenocarcinoma samples with LDLRs and without LDLR (n = 13). LDLRs. Neoplastic surgical samples were obtained from 19 Males and 6 Females (range 40-83 yrs). LDLRs were evaluated by EIA and polyamine levels by HPLC as previously described by us. The results were analyzed by Wilcoxon rank sum test for unpaired data. Polyamine levels are expressed as nmol/mg wet tissue (median and range).

<table>
<thead>
<tr>
<th>LDLR+ (12)</th>
<th>LDLR− (13)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>1004 (902-1992)</td>
<td>675 (286-1413)</td>
</tr>
<tr>
<td>Putrescine</td>
<td>21 (3-156)</td>
<td>17 (5-75)</td>
</tr>
<tr>
<td>Spermidine</td>
<td>276 (129-622)</td>
<td>195 (60-425)</td>
</tr>
<tr>
<td>Spermine</td>
<td>657.5 (269-1484)</td>
<td>442.9 (87-2017)</td>
</tr>
<tr>
<td>Ratio</td>
<td>0.48 (0.15-2.47)</td>
<td>0.41 (0.27-0.66)</td>
</tr>
</tbody>
</table>

Our finding shows the presence of increased levels of polyamines in LDLR+ colorectal neoplastic samples compared to LDLR− ones. This evidence suggests that colorectal adenocarcinomas with LDLR+ show an increased proliferative activity.

146 Dietary Arginine Reduces Tumour Area and Volume in a Rodent Model of Colorectal Cancer


Arginine (Arg), a semi-essential amino acid, modulates host immune function resulting in variable responses against tumour induction and development in chemically-induced mammary and transplantable solid tumours.

Aim To assess the effect of supplemental Arg on tumour incidence and growth in rats exposed to 1,2-dimethylhydrazine (DMH).

Methodology Colorectal tumours were produced in male Wistar rats (n = 9 per group) by 20 weekly subcutaneous injections of DMH (20 mg/kg body weight). Treatment regimes comprised either 1% Arg (in tap water) for 22 weeks or 1% Arg during the last 12 weeks of the promotion period. DMH was used as a control. Animals were sacrificed 22 weeks after their first injection. The endpoint was established as the presence of both adenomas and adenocarcinomas (tumour area: (mm²) = 3.14 x long radius; x short radius; tumour volume: (mm³) = ½ x long diameter x square of short diameter).

Results (mean ± sem) All DMH control animals developed tumours compared to 78% of both groups of animals receiving Arg. The area and volume of adenomas in both Arg groups were not significantly different compared to controls. The area and volume of adenocarcinomas in both Arg-supplemented groups were significantly lower than controls.

<table>
<thead>
<tr>
<th>Adenoma</th>
<th>Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area</td>
<td>Volume</td>
</tr>
<tr>
<td>DMH Control</td>
<td>5.71 ± 1.73</td>
</tr>
<tr>
<td>DMH + Arg</td>
<td>5.08 ± 2.38</td>
</tr>
<tr>
<td>DMH + 12 weeks Arg</td>
<td>10.16 ± 4.19</td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.005 vs DMH control (Mann Whitney).

Conclusion Supplementation Arg significantly reduces colorectal adenocarcinoma area and volume in the DMH rat model of colorectal cancer. Treatment appears beneficial even when administered only during the latter half of the promotion stage.

147 Family Screening of Patients with Wilson Disease by Using Restriction Fragment Length Polymorphism (RFLP) of D13S31

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Wilson disease (WD) is an autosomal recessively inherited disorder of hepatic copper metabolism. The gene has been mapped to chromosome 13q14.3. Several markers from the same region, including D13S31, have been identified, which is bracketed by the two markers D13S31 (0.4 cM upstream) and D13S59 (1.2 cM downstream). By RFLPs with these markers genetic analysis in families of index cases is possible. Previous studies reported a high frequency of the smaller allele of D13S31 (4.6 kb) in patients with hepatocellular degeneration, the larger allele (6.7 kb) was associated with neurologic disease. The aim of this study was to investigate the distribution of these two alleles in a large group of patients in Austria and to correlate them with disease presentation. 25 index patients, 4 known affected siblings and their families (total number of examined persons: 155) were examined. 21 index cases were from Austrian origin, each one of Czech, Bosnian, Hungarian, and Russian-Jewish origin. DNA was extracted from peripheral blood mononuclear cells, and digested with TaqI, PvuII, BanII, BclI, EcoRI, HphI, Apal, Hind III and Ral. After Southern blotting following DNA-probes labeled with 32p-PdCTP, were used for hybridization. D13S31, D13S326, ESD and RIB. Results are only shown for the marker, D13S31 (TaqI, PvuII), which was associated with the WD locus in each family. Ten of the index patients (+2 affected siblings) were homozygous for the 4.6 kb allele, 8 for the 6.7 kb allele, and 7 (+2 affected siblings) had both alleles. Based on the RFLPs the inheritance of WD was diagnosed in 2 new cases. Besides, polyamines were not correlated with the WD locus in each family. Two index patients (+2 affected siblings) were identified that colorectal adenocarcinomas were not correlated with the WD locus in each family. Two index patients (+2 affected siblings) were identified that colorectal adenocarcinomas were not correlated with the WD locus in each family. Two index patients (+2 affected siblings) were identified that colorectal adenocarcinomas were not correlated with the WD locus in each family. Two index patients (+2 affected siblings) were identified that colorectal adenocarcinomas were not correlated with the WD locus in each family. Two index patients (+2 affected siblings) were identified that colorectal adenocarcinomas were not correlated with the WD locus in each family. Two index patients (+2 affected siblings) were identified that colorectal adenocarcinomas were not correlated with the WD locus in each family. Two index patients (+2 affected siblings) were identified that colorectal adenocarcinomas were not correlated with the WD locus in each family. Two index patients (+2 affected siblings) were identified that colorectal adenocarcinomas were not correlated with the WD locus in each family. Two index patients (+2 affected siblings) were identified that colorectal adenocarcinomas were not correlated with the WD locus in each family. Two index patients (+2 affected siblings) were identified that colorectal adenocarcinomas were not correlated with the WD locus in each family. Two index patients (+2 affected siblings) were identified that colorectal adenocarcinomas were not correlated with the WD locus in each family. Two index patients (+2 affected siblings) were identified that colorectal adenocarcinomas were not correlated with the WD locus in each family. Two index patients (+2 affected siblings) were identified that colorectal adenocarcinomas were not correlated with the WD locus in each family. Two index patients (+2 affected siblings) were identified that colorectal adenocarcinomas were not correlated with the WD locus in each family. Two index patients (+2 affected siblings) were identified that colorectal adenocar