**Colorectal cancer**

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The analysis of proliferation state patterns in colorectal mucosa has been found to be a useful risk marker for colorectal cancer (CRC). The standard method for this is incorporation of a thymidine analogue, bromodeoxyuridine (BrdU) and its subsequent visualisation using immunohistochemistry. The use of PC10, a monoclonal antibody to proliferating cell nuclear antigen (PCNA) may have advantages because no incorporation step is necessary and fixed archival samples may be assessed.

Proliferation patterns in colorectal mucosa of 44 patients in differing risk groups for CRC were studied. Proliferation was expressed as labelling indices (LI). Immunohistochemistry using a peroxidase streptavidin-biotin complex method was performed on paraffin sections, using monoclonal antibodies to BrdU and PCNA.

Two methods of statistical analysis were used to establish whether PC10 and BrdU immunohistochemistry were interchangeable. Spearman Rank Correlation Analysis demonstrated a strong association between BrdU LI and PCNA LI (r = 0.64, p < 0.01). The second method of analysis was one specifically designed to assess agreement between two methods of measurement. This revealed unacceptable limits of agreement between the two methods of assessing proliferation.

We conclude that PC10 immunohistochemistry cannot be substituted for BrdU immunohistochemistry when assessing proliferation patterns in preneoplastic colorectal mucosa.


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**PROGRAMMED DEATH (APOPTOSIS) IN COLONIC CELLS.** S. Lifshitz, B. Schwarzt, R. Mikhailowycz, Y. Nir, S. A. Lamprecht. Gastroenterology Department, European Biotechnology Unit, Soroka Medical Center, Ben-Gurion University of the Negev, Beer-Sheba, Israel.

The orderly progression of tissue growth and differentiation requires the systematic death and removal of individual cells without disruption of the systems from which they originate. This form of altruistic programmed cell death is known as apoptosis. The colonic epithelium provides a unique model of cells at various stages of proliferation and differentiation aligned in an orderly pattern along the crypt axis. The sizes of colon proliferative and maturational compartments are maintained within precise boundaries. We propose that programmed cell death plays a central role in the maintenance of colonic cell number and the ordered renewal of colonic cells. To test the validity of this hypothesis, apoptotic-related events were studied in rat colonoctyes harvested from the crypt continuum at precise stages of their biological life span. Degradation of DNA into oligonucleosome-sized fragments, a hallmark of apoptosis, was measured in the murine colonic cells. The characteristic nucleosome ladder was detected by gel electrophoresis in rat colonoctyes harvested at all stages of proliferation/differentiation. Likewise, the extent of DNA fragmentation, calculated according to Sellsins and Cohen (J. Immunol., 139:3199, 1987) was similar in all colonic cells. No apoptotic DNA degradation was detected in HT-29, a human adenocarcinoma cell line. Ultrastructural correlates provided typical morphological evidence for the programmed demise of the colonic cell. Our findings indicate that the apoptotic program is active in normal murine colonic cells. A key question addressed pertains to the cellular signaling which controls cell survival and cell death in a precise zonal and temporal context within the colonic crypt. The present findings support the notion that the apoptotic process is part of the stringent regulatory mechanisms that keep constant the boundaries of the colonic compartments. We surmise that the time-space confined apoptotic program of the colonic cell is markedly perturbed during colon tumorgenesis.

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**a2b1 INTEGRIN CONTROLS THE MORPHOLOGICAL DIFFERENTIATION OF COLORECTAL TUMOUR CELLS IN VIVO AND IN VITRO.** A. K. Nigam, D. Li, I. J. Savage, G. WH. Stamp, PB. Boulos, M. Pignatti.

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Intercellular and cell-matrix interactions are mediated through several families of cell adhesion molecules (CAMs), of which integrins and cadherins comprise the main subgroups expressed by normal and transformed epithelial cells. Integrins are heterodimers composed of α and β subunits and are subclassified according to the associations of their subunits. Previous studies have indicated that the α1 integrins and E-cadherin have strong morphoregulatory activities in a colorectal carcinoma cell line. We have investigated the expression and function of individual members of the β1 integrin subfamily in SW1222 colon carcinoma cells. The methods used have been ELISA, indirect immunoperoxidase staining and immunoprecipitation. Although many of the β1 receptors were expressed, only α2β1, when blocked by monoclonal antibodies to its subunits, was found to be a functional regulator. This was measured in terms of cell adhesion assays and glandular differentiation in collagen gels.

We have expanded this work further to consider changes in integrin receptor expression in CAM expression in vivo. 28 colorectal carcinomas and normal colonic mucosa were stained by an indirect immunoperoxidase technique utilizing monoclonal antibodies against α1, α2, α6, αv, αvβ3, E-cadherin and CEA. Significant down-regulation of α2 (p<0.01) and αvβ3 (p<0.05) were seen in tumour when compared to normal tissue. This was not a finding that was consistently seen in the other integrin subunits. No correlation to Dukes’ stage was found.

Loss of expression of α2β1 in moderate and poorly differentiated carcinomas in vivo and the observation that it is the key integrin that modulates differentiation in vitro are strong evidence for the role of this receptor in the biological behaviour of colorectal tumours.
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**EXPRESSION OF IMMUNOREGULATORY CYTOKINES AND EPITHELIAL CELL GROWTH FACTOR BY NATIVE AND ACTIVATED HUMAN INTESTINAL INTRAEPITHELIAL LYMPHOCYTES. K. Schölßhorn, S. Daum, F. Wagner, M. Classen, and K. Deusch.** Dpt of Medicine, Technical University, Munich, Germany.

Little is known about the function of the lymphocyte population residing in the epithelial layer. Human intestinal intraepithelial lymphocytes (IEL), which are predominantly CD4-8- subset of T cells are the first cells to encounter the multiple antigens present in the gastrointestinal tract. Previously, we have reported that IEL constitutively express mRNA for a variety of predominantly proinflammatory cytokines despite proliferative unresponsiveness towards conventional T cell specific stimuli. In this study we investigated the further role of IEL in regulation of immuneresponses in the gut and in maintaining epithelial function. Therefore IEL were isolated from human normal colonic mucosa obtained from patients who underwent surgical resection because of colorectal carcinoma by repeated density gradient centrifugation. Subsequently, total cellular RNA was extracted and cytokine expression analyzed in a semi-quantitative polymerase chain reaction (PCR). As results we found that human IEL produce readily detectable amounts of mRNA encoding for cytokines exerting trophic effects on epithelial and mucosal mesenchymal cells such as epithelial cell growth factor (EGCF), GM-CSF, IL-1, IL-6 and Interferon-γ, that has been shown to upregulate epithelial antigen presentation. Additionally mRNA transcripts for IL-4 and IL-10 and IL-5 mRNA upon in vitro stimulation with phytohaemagglutinin (PHA) were detectable. The specificity of these cytokine gene primers had been validated by southern blot analysis and radioactive labelling with internal probes. Upon in vitro stimulation with pokeweed mitogen (PWM) IL-4 mRNA expression was increased. After one day of culture without additional stimulation IL-4 mRNA was no longer detectable. Taken together, our data suggest that IEL have the functional capacity to support actively the maintenance of epithelial growth and function. Moreover, despite the lack of proliferation towards mitogens our results give evidence that IEL possess an immunoregulatory function after stimulation.

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**OVEREXPRESSION OF p53 IN RELATION TO LONG-TERM PROGNOSIS IN COLORECTAL CARCINOMA. T. Starzyńska, K. Marlicz, P.L. Stern, Dept of Gastroenterology, Medical Fomerian Academy, Szczecin, Poland; Dept of Immunology, Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Manchester M0 9BX, England.

Overexpression of p53 protein in the cell nucleus as a result of mutation is very common in human neoplasia; but any relation to prognosis remains to be completely defined. In breast tumours p53 expression is related to established prognostic factors but in colorectal cancer there are conflicting claims. The objective of the present study was to investigate the relationship between p53 expression in colorectal cancer and the outcome of the disease. The expression of p53 protein was analysed immunohistochemically in 122 colorectal carcinomas by use of a polyclonal antibody CM-1 and routinely fixed tissue. 44% of these carcinomas expressed elevated level of p53 protein in the cell nucleus. A statistically significant association was found between overexpression of p53 and high risk of local recurrence. p53 overexpressing-tumours showed a local recurrence rate of 18%, compared with 4% in p53-mutant negative carcinomas. However, there was no significant effect of p53 expression on a long-term prognosis. The proportions of patients with 5-year survival with or without p53 overexpressing tumours was 45% and 50% respectively. The results suggest that in colorectal carcinoma immunohistochemical detection of p53 can be helpful to identify patients with high risk of local recurrence but expression of p53 in this malignancy is not associated with a different long-term prognosis.

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Although it is accepted that a family history of colorectal cancer increases the risk of developing the disease, it is still unclear whether the presence of only one affected first degree relative is sufficient to identify high risk subjects. The role of total colonscopy as appropriate first step in screening those subjects is also controversial. The aim of this study was to determine whether subjects with only one first degree relative with colon cancer are at increased risk of developing premalignant lesions such as adenomatous polyps. II) to determine whether total colonscopy is the appropriate first step in screening subjects with simple family history of colon cancer. METHODS: the frequency of positive family history of colorectal cancer was evaluated, by personal interview, in 155 subjects with colorectal polyps (95 males, 60 females; mean age 55.7 ± 11.1) and in a comparable group of 156 subjects without colorectal polyps (73 males, 83 females; mean age 52.7 ± 16.6). In all patients and control subjects the following conditions were excluded: symptoms or signs of colorectal neoplasia, prior polyps or cancer, hyperplastic polyps or polyps with invasive cancer, inflammatory bowel disease. The presence or absence, respectively, of adenomas polyps was established in all subjects by total colonscopy. For the purpose of the present study only simple primary family history, i.e. one affected first degree relative, was considered. Polyps were grouped into distal and proximal lesions using the junction of descending-sigmoid colon as a landmark. RESULTS: the frequency of primary family history of colon cancer was 17.4% (27/155) in patients with adenomatous polyps and 7.8% (12/156) in controls (O.R. = 2.53; 95% C.L. = 1.253 to 5.118; chi-square 6.70; p = 0.01). Subject with adenomatous polyps in the descending colon or proximally, without adenomas in the rectum sigmoid were 52% (142/275) in the group with positive family history, and 25% (32/128) in those without affected relatives (p = 0.006). The frequency of polypoid lesions characterized by severe dysplasia was 30.4% (7/23) and 12.6% (15/119), respectively, in the two above considered groups (p = 0.04). CONCLUSIONS: the present case-control study show a significant increased risk of developing premalignant lesions such as adenomatous polyps in subjects with even one first degree relative with colon cancer; in these subjects there is a higher frequency of negative morphological abnormalities. Furthermore, since in these subjects the majority of premalignant lesions is located in the proximal colon without sentinel lesions in the rectum and sigmoid, total colonscopy should be considered as the initial screening tool.

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**LONG STANDING HYPERGASTRINEMIA IN MAN: EVIDENCE FOR A MITOGENIC BUT NOT TUMORIGENIC EFFECT ON THE COLONIC MUCOSA. I. Sobhani, T. Lehy, G. Cadot, Ph. Ruszniewski, M.A.M. Lewin* and M. Migone, Dept of Hepato-Gastroenterology and *UNEDR U 10, CHU Bichat - Claude Bernard, 75877 Paris Cedex 18, France.

Gastrin is known to be a trophic factor for the gastrointestinal tract. However informations concerning the influence that it may exert on the colon mucosa is fragmentary and controversial. The aim of the present study was to analyze the effect of chronic endogenous hypergastrinemia on cell proliferation and tumor occurrence in the human colonic mucosa.

Material and Methods. Twenty-three consecutive hypergastrinemic patients presenting with the Zollinger-Ellison syndrome and 18 normogastrinemic subjects were studied. All had fasting serum gastrin-determination, colonscopy and cell kinetic measurement in two colonic sites using in vitro Sß-bromodeoxyuridine incorporation in biopsies performed for 1 hr. For each subject, 20 up to 36 histocrypt columns were analyzed per site for labeling index (LI) calculation.

Results. Macroscopic tumors, one endocrine and 5 adenomas, were found in 5/23 hypergastrinemic patients, 48 to 61 year-old, without apparent relationship with the level of gastrin. The labeling indices were significantly higher in hypergastrinemic than in normogastrinemic individuals in the right and left colon, p < 0.002 to p < 0.001, without resulting in colonic cell hyperplasia. There was no correlation between labeling indices and serum gastrin concentrations or duration of hypergastrinemia. A DNA labeling distribution along the crypt was normal in the two groups, without expansion of the proliferative zone towards the surface.

Conclusion. These results showed for the first time that long-lasting endogenous hypergastrinemia is accompanied by increased in vivo cell proliferation in the human colonic mucosa but would seem to play a rather indirect role on tumorigenesis.