Short communication

Are Intestinal Tumours in Apc+/-mice a Suitable Model of Colorectal Carcinoma in Man?

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Abstract. In snap frozen sections of the duodenum, jejununum, ileum, the righ and left colon of APC+/- mice mucosubstances, activities of brush border glycosidases and proteases, immunoreactivity of sucrase and activities of some enzymes of pericellular proteolysis were studied. Multiple adenomas (tubular or tubulovillous) the numbers of which decreased in the aboral direction occurred in the small intestine. Two tubulovillous adenomas with dysplastic nuclei but with no invasion were found in the right colon. The morphological and histochemical findings resembled those of human colorectal tumours. Activities of brush border enzymes and sucrase immunoreactivity were decreased to various extent or were not present at all. The findings fluctuated even within the same section. Activities of enzymes of pericellular proteolysis were slightly increased in comparison with non affected mucosa. This model is suitable and deserves further studies.

Key words: Histochemistry — Brush border enzymes — Bowel tumours — APC+/-mice

Colorectal cancer is one of the most frequent human tumours with a still high mortality. It is thus not surprizing that its origin, properties, diagnosis and prognosis are intensely studied using various approaches. Mutations of the APC (adenomatous polyposis coli) gene were observed (together with mutations of other genes) not only in patients with familial adenomatous polyposis but also in patients with sporadic cancer (Fodde et al. 1994, 1996; Shoemaker et al. 1997; Yang

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et al. 1997). The generation and analysis of inbred mouse models represent a very important experimental approach (Fodde et al. 1996; Shoemaker et al. 1997; Yang et al. 1997). Because histochemical data of experimental tumours which could show similarities with human colorectal cancer are missing studies have been undertaken in our laboratories to fill this gap. Some results are presented in this communication.

Four 8-month old and thirteen 12-month old Apc+/-mice supplied from Institute of Molecular Genetics breed were used in this study. After 24 hour fasting the animals were anaesthetized and killed, the bowel dissected, duodenum, jejunum, ileum, right and left colon separated, placed on a cork plate covered with parafilm and opened longitudinally. After careful removal of the content with a tampon moistened with PBS the mucosal surface was inspected with a magnifying glass. Sites with tumours were excised together with a sufficient amount of unchanged surrounding mucosa, stretched carefully mucosal surface up on a gelatin leaflet and quenched in heptane cooled with acetone-dry ice mixture. They were then frozen to a brass cube and cut in the cryostat longitudinally to the axis of the intestine and perpendicularly to the surface. Cryostat sections were transferred to non-precooled slides or semipermeable membranes and subjected unfixed or appropriately fixed to the following histological or histochemical reactions: Azur A, hematoxylin-eosin, high iron diamine-alcian blue (HID-AB), alcian blue-PAS (Pearse 1985), trehalase (trehalose-GO-PO-DAB), sucrase-isomaltase (sucrose-GO-PO-DAB), α-glucosidases (2-naphthyl- or 5-Br-4-Cl-3-indolyl-α-D-glucoside), lactase (5-Br-4-Cl-3-indolyl-β-D-fucoside) (Lojda et al. 1979), dipeptidyl peptidase (DPP) IV (Gly-Pro-MNA + FBB), γ-glutamyl transpeptidase (GGT, γ-Glu-MNA + FBB + Gly-Gly), DPP I (Gly-Arg-MNA + NSA), cathepsin B (Z-Ala-Arg-Arg-MNA + NSA) (Lojda et al. 1991) and urokinase (Z-Gly-Gly-Arg-AFC) (Lojda 1996). (Naphtholic substrates and diazonium salts were purchased from Sigma-Aldrich, Praha, Czech Republic; MNA-substrates from Bachem, Bubendorf, Switzerland; AFC substrates and NSA from Enzymes Systems Products, Dublin, Calif. USA and other chemicals from Serva, Heidelberg, Germany.) Sucrase was also demonstrated immunohistochemically using an antibody raised in guinea pigs against isolated purified rabbit sucrase (a kind gift of dr. Kolínská, Institute of Physiology, Academy of Sciences of the Prague, Czech Republic) and FITC labeled pig antiguinea pig γ-globulin (Sevac, Prague, Czech Republic).

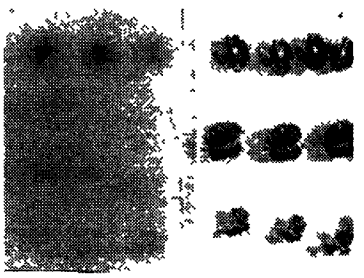

Figure 1. Macrophotograph of cryostat sections of tumours stained by hematoxylin and eosin (right panel) and after the demonstration of sucrase-isomaltase (SI) activity in serial sections of the same tumour (left panel). Note the different levels of activity in different adenomas

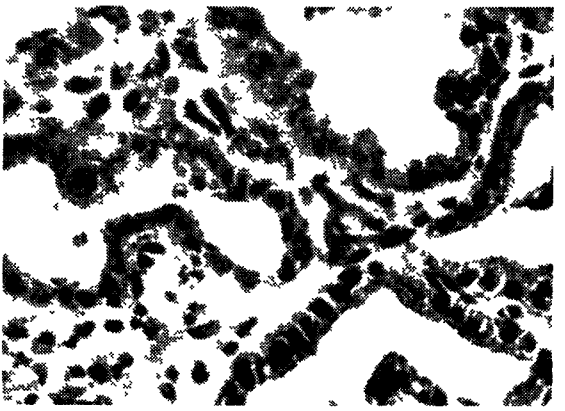

Figure 2. Part of a section of tubulovillous adenoma with severe dysplastic changes (called Ca in situ by some authors) HE

Tumours found in these mice were adenomas. They were multiple and located predominantly in the small intestine. Their numbers decreased in the aboral direction. Only in 2 animals tumours were found in the colon ascendens. Thirteen of these adenomas were tubular, 2 tubulovillous. In some cases their epithelium displayed dysplastic changes (Fig. 2) so that they could be considered as carcinomas "*in situ*". However, no invasion into the submucosa was observed. The quantity of the mucus was diminished and "sialomucins" surpassed "sulphomucins". Lactase was totally absent. Among other disaccharidases, trehalase activity was higher than that of sucrase (Fig. 3). None of these disaccharidases was present in all tumours, however. Similar results were obtained with the immunohistochemical demon-

Figure 3. Macrophotograph of serial sections of two tumours after the reaction for SI (left) and trehalase (right). Trehalase activity is higher

stration of the sucrase-isomaltase protein (Fig. 4). The localization and reaction intensity varied even within the same section. With synthetic naphtholic (Fig. 5) and indoxyl (Fig. 6) substrates for α-glucosidases, which demonstrate in the normal intestine sucrase and glucoamylase activities, the reaction in tumours was the weakest and in the majority of sections gave negative results. DPP IV activity was present in the apical region of some tumour cells in several places only. Varying number of T-lymphocytes reacted in the stroma (Fig. 7). GGT activity was weak. In some adenoma cells DPP I activity localized normally near the apical surface was seen at the basis of cells. Cathepsin B activity was low. Both enzymes were present also in stromal macrophages. Urokinase activity fluctuated in various sites

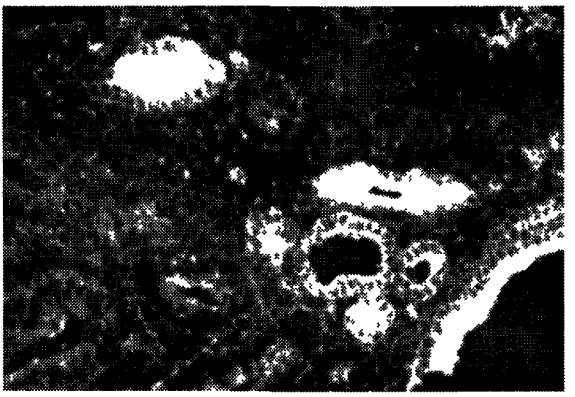

Figure 4. Varying degree of SI immunoreactivity in the apical portion of the lining of the tubuli of a tubular adenoma

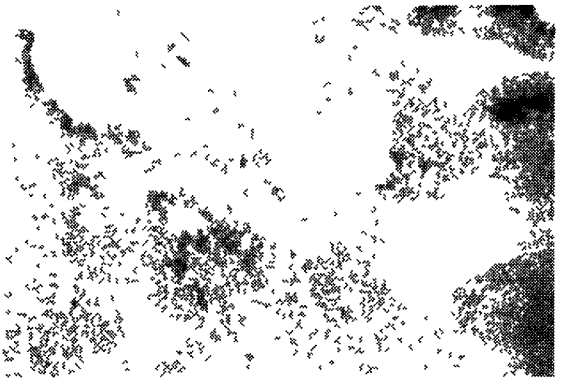

Figure 5. Azo-coupling reaction for SI and glucoamylase in a tubulovillous adenoma The reaction is weak and is present in some cells only

of the tumours and was present in epithelial cells and in macrophages. Our results are very similar to those reported for human colorectal tumours (Lojda and Frič 1996). It is concluded that the mouse model is worth studying. It enables us to vary genetic, epigenetic and enviromental factors and to analyze their influence on the tumour pattern.

Figure 6. Indigogenic reaction for SI and glucoamylase in a tubulovillous adenoma Nuclei counterstained with nuclear fast red The reaction is weak and is present in some cells only

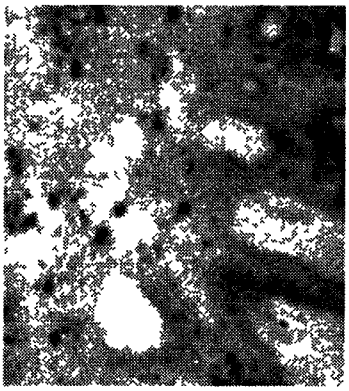

Figure 7. DPPIV activity in a tubular adenoma Strongly reacting T-lymphocytes in the stroma The reaction in the brush border is mostly negative Only in some tubules (right upper corner) a weak reaction is apparent

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