Papers

Immunolocalisation of β catenin in intestinal polyps of Peutz–Jeghers and juvenile polyposis syndromes

Walter Back, Steffan Loff, Dieter Jenne, Uwe Bleyl

Abstract

***Aim*—To examine the membranous and nuclear distribution of β catenin in the epithelial cells of gut polyps from Peutz–Jeghers syndrome and juvenile polyposis in comparison with other types of polyps and tumours.**

***Methods*—Immunohistochemistry for β catenin and proliferation markers was performed on conventional paraffin sections. Immunohistological staining was carried out on Peutz–Jeghers syndrome polyps from four different families, on juvenile polyposis polyps from two different families, on solitary juvenile polyps, and on hyperplastic polyps. The immunohistochemistry was evaluated qualitatively in relation to defined areas of the polyps.**

***Results*—All polyps from the hamartomatous polyposis syndromes (Peutz–Jeghers syndrome and juvenile polyposis) showed nuclear localisation of β catenin in some epithelial cell nuclei. In Peutz–Jeghers syndrome polyps β catenin positive nuclei were seen at the base of the deep crypt infoldings. In juvenile polyposis polyps and in some solitary juvenile polyps they were found in irregularly distributed cryptal epithelial cells corresponding to the proliferative compartments. Normal mucosa of the gut and hyperplastic polyps of the colon do not show nuclear staining for β catenin.**

***Conclusions*—The dysregulation of cellular β catenin distribution is not only a phenomenon of adenoma formation and adenoma progression in the colon—it is at least focally present in polyps of the hamartomatous type and is related to the proliferation zones of these polyps. The nuclear translocation of β catenin most probably reflects a disturbed β catenin metabolism. In view of the different functions of β catenin during development and cell differentiation, the nuclear translocation of β catenin is likely to be an important factor in enhanced cell proliferation which escapes local control mechanisms.**

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Keywords: β catenin, Peutz–Jeghers, juvenile polyposis

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Peutz–Jeghers syndrome and juvenile polyposis syndrome are two relatively rare hereditary polyposis syndromes affecting the gut and to some extent also affecting other organs, giving rise to the characteristic clinical syndromes. Histomorphologically, Peutz–Jeghers polyps show heavily arborised fronds of tall stroma projections which contain smooth muscle cells and are covered by a goblet-cell-rich gut epithelium. On the other hand in juvenile polyposis the polyps contain dilated crypts in a loosely packed oedematous stroma of lamina propria, and there is often a superimposed erosive inflammation.[1] The two types of polyp can be readily distinguished by histological examination. An increased cancer risk has been claimed in both syndromes.[2 3] The genetic basis of the autosomal dominant Peutz–Jeghers syndrome has recently been discovered on the short arm of chromosome 19, affecting the serine/threonine kinase 11 (STK11) gene.[4 5] Several candidate genes are still under investigation for the autosomal dominant juvenile polyposis syndrome,[3] and mutations of the SMAD4 gene have been reported very recently to be detectable in a subset of juvenile polyposis cases.[6]

β Catenin is a multifunctional cytoplasmic protein with a molecular weight of 92 kDa. It is present in epithelial cells in an E-cadherin–catenin complex together with α and γ catenin. There is evidence that the β catenin in the E-cadherin–catenin complex connects the cytoplasmic domains of the adhesion molecules associated in the zona adherens with other catenins and with the actin cytoskeleton of the cytoplasm.[7] Strong amino acid homology for β catenin is found in a member of the "wingless" pathway in *Drosophila* homeobox genes called Armadillo. It has therefore been concluded that β catenin plays a crucial role as a part of the "Wnt" cascade, the mammalian homologue of the wingless signalling pathway.[8 9] As a part of this signal transduction pathway, β catenin can bind to certain transcription factors and can be transferred to the nucleus.[10] Recently it was shown that β catenin is translocated to the nucleus and accumulates in the nucleus during the development of colorectal adenomatous tumours and is detectable in relatively large amounts in the tumour cell nuclei of most colorectal adenocarcinomas.[11 12] There is some

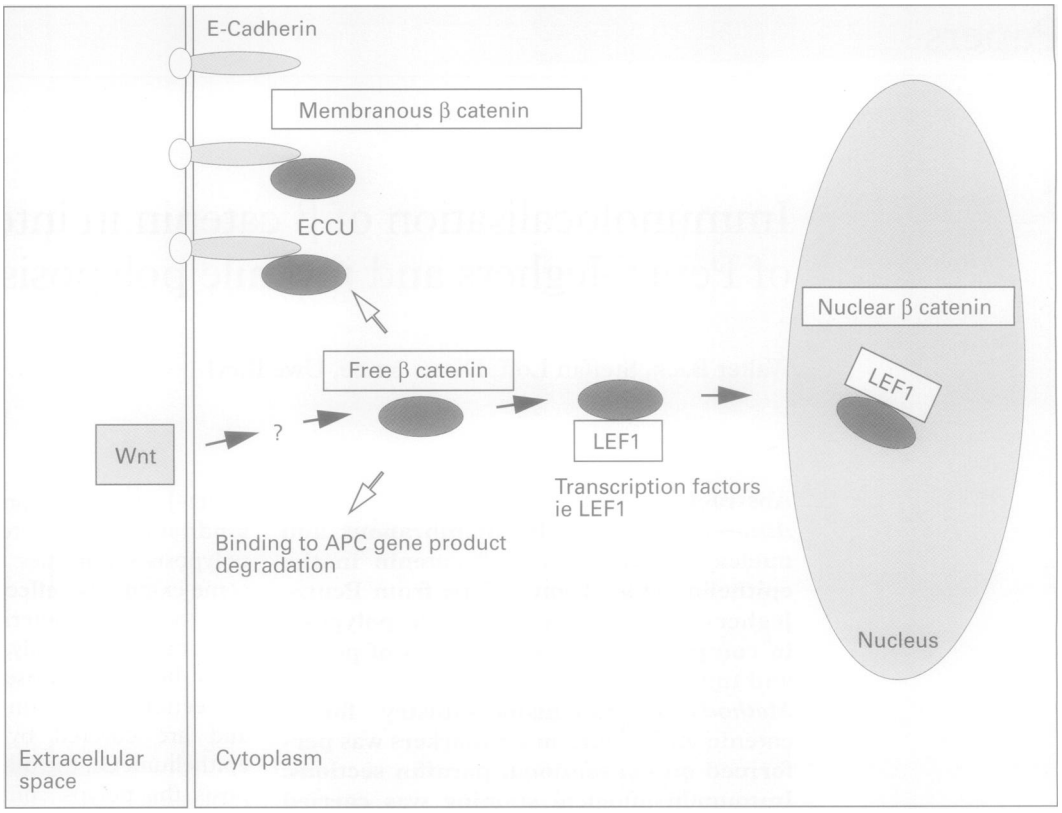

Figure 1 Schematic β catenin actions in epithelial cells. ECCU, E-cadherin–catenin unit (complex of E-cadherin, α, β, and γ catenin); LEF1, lymphocyte enhancer binding factor 1 (transcription factor).

experimental evidence that the gene product of the adenomatous polyposis coli gene (APC) is involved in the degradation and turnover of cytoplasmic β catenin (fig 1).[13–15] So far it is concluded that the altered β catenin metabolism in adenomatous lesions of the colonic mucosa, with nuclear localisation of β catenin, leads to a stimulatory effect on cell proliferation.[16] We studied the localisation of β catenin in intestinal hamartomatous polyps of Peutz-Jeghers and juvenile polyposis syndromes, which are characterised by the proliferation of a non-adenomatous mucosa.

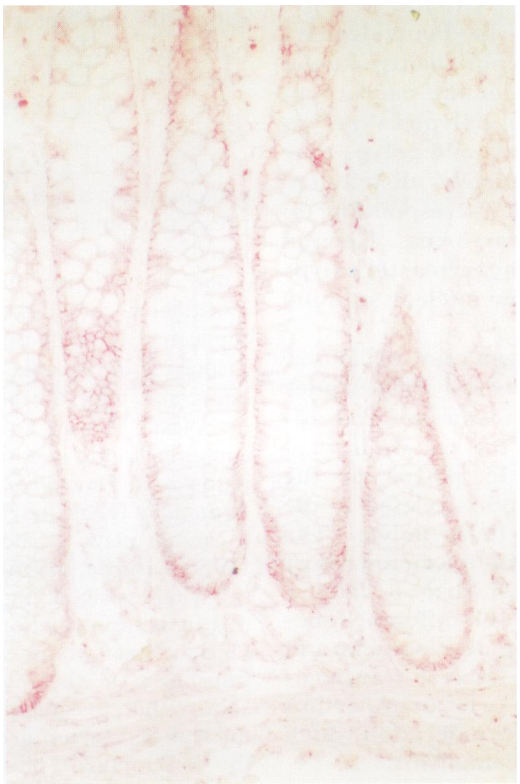

Figure 2 Normal colon mucosa: membranous immunostaining for β catenin (alkaline phosphatase detected by Fast Red).

Methods

Twelve Peutz–Jeghers polyps taken from the files of the department of pathology consisted of nine polyps from the colon and three from the ileum. These 12 Peutz–Jeghers polyps were resected from several patients who are members of four different Peutz–Jeghers families. Germline mutations of the STK11 gene, which are specific for Peutz–Jeghers syndrome, have been investigated and could be found in all these patients.[4]

Six colonic polyps from patients with juvenile polyposis syndrome were examined. They originated from several members of two non-linked juvenile polyposis families.

We also included seven solitary juvenile polyps from the colon, 10 hyperplastic polyps from the colon, and for comparison three colorectal carcinomas from patients with familial adenomatous polyposis.

Normal colonic mucosa was also immunostained as a control. The control materials consisted of unaffected mucosa from Peutz–Jeghers syndrome patients (2), unaffected mucosa from juvenile polyposis patients (2), unaffected mucosa from patients with familial adenomatous polyposis (2), and normal colonic mucosa resected for other reasons (8).

The immunohistological investigations were performed on paraffin sections from archival

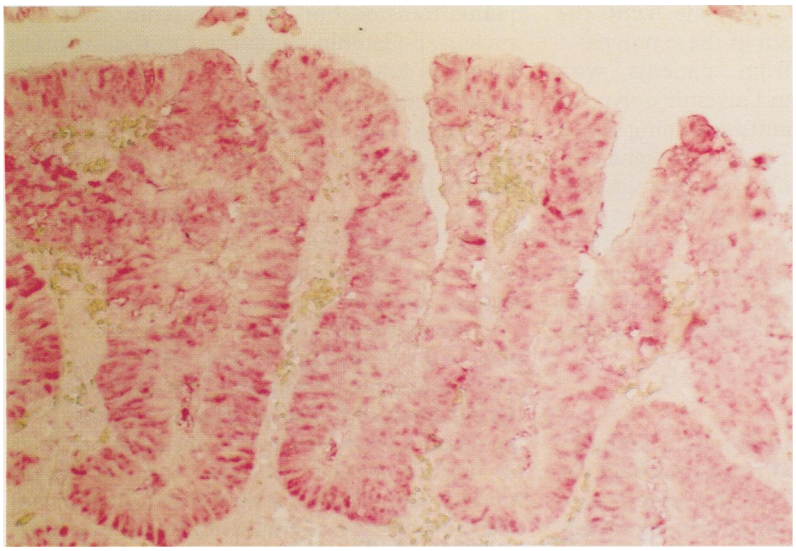

Figure 3 Adenocarcinoma (colon) in familial adenomatous polyposis syndrome: nuclear immunostaining for β catenin (alkaline phosphatase detected by Fast Red).

paraffin wax embedded material from the files of the department of pathology. Before the incubation steps of the immunohistochemical staining protocol, the deparaffinised tissue sections were pretreated with microwave heating for 10 minutes in citrate buffer. Primary antibodies were then applied for overnight incubation at 4°C. The primary antibodies were monoclonal antibodies against β catenin (Transduction Laboratories), PCNA (Camon), and Ki67 (Dianova). A three step immunohistochemical protocol was used, following the streptavidin labelled biotin method (Zymed). The immunolocalisations were visualised with alkaline phosphatase and Fast Red as chromogen. For evaluation of cytoplasmic and nuclear staining no counterstain was applied. Control experiments with preabsorbed antibodies and negative controls were carried out for the immunohistological staining procedure used in this study and proved to be negative. The evaluation qualitatively recorded the localisation of the immunostaining in relation to the different compartments of the polyps.

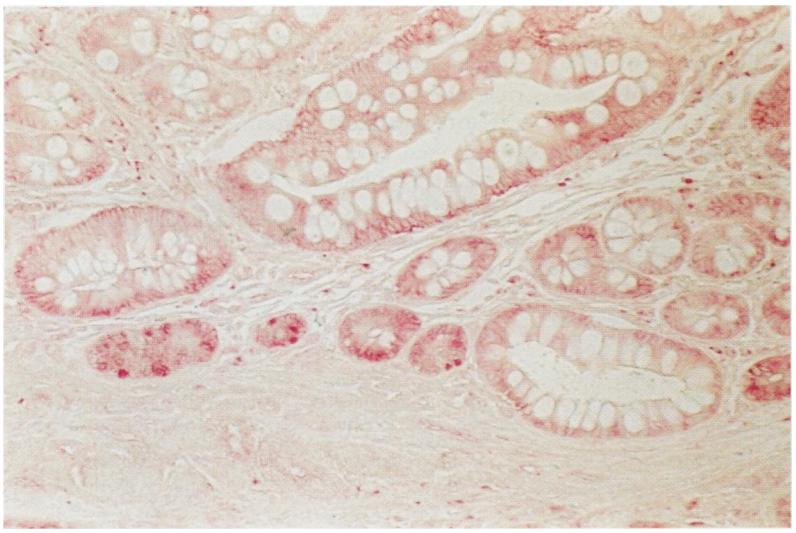

Figure 4 Peutz–Jeghers polyp (colon): some nuclear immunostaining for β catenin in basal crypts (alkaline phosphatase detected by Fast Red).

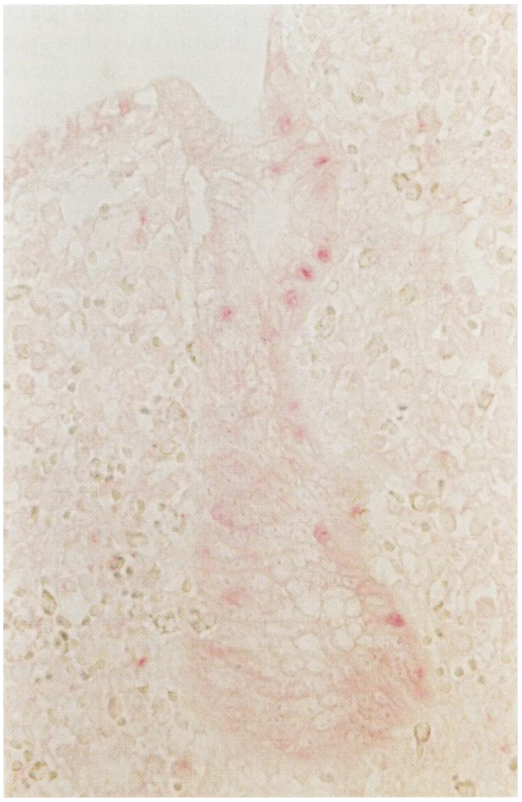

Figure 5 Juvenile polyposis polyp (colon): faint nuclear immunostaining for β catenin (alkaline phosphatase detected by Fast Red).

Results

In normal colonic epithelium there was a delicate membranous staining for β catenin, especially of the basolateral cell membranes (fig 2). On the other hand in all three cases of colorectal adenocarcinoma from patients with familial adenomatous polyposis with known APC gene mutations, which were used as positive control, nuclear immunoreaction for β catenin could be seen (fig 3).

Membranous localisation of immunostaining for β catenin was present in Peutz–Jeghers polyps, especially in the intermediate and superficial villous parts. In co-localisation to the basally located proliferating compartments of the deep cryptal infoldings (as shown on parallel sections with proliferation markers PCNA and Ki67), there was an increased cytoplasmic immunoreaction for β catenin. In the basal epithelium there were multiple, but mostly single, epithelial cell nuclei staining positively for β catenin (fig 4). All 12 Peutz–Jeghers polyps in our study showed positive nuclear immunoreactions for β catenin in some epithelial cells in the basal proliferation zone.

A similar immunoreaction can be seen in polyps of the juvenile polyposis syndrome. These polyps showed relatively small and quite irregularly distributed proliferative compartments in the lower and upper parts of the polyps. In these areas β catenin positive nuclei could be found lying singly or in small groups (fig 5). Surface areas of the polyps from Peutz–Jeghers syndrome and juvenile polyposis showed only membranous staining and not nuclear staining for β catenin. The nuclear β catenin signals in polyps from the juvenile

polyposis cases did not correlate with the inflammatory hot spots seen in these polyps.

Unaffected mucosa from patients with Peutz–Jeghers syndrome and juvenile polyposis did not show nuclear immunostaining for β catenin, nor could nuclear β catenin signals be seen in the mucosal crypts in the other samples of normal colonic mucosa. In all 10 hyperplastic colonic polyps there was only a membranous distribution of β catenin staining on immunohistology. Nevertheless in four of seven solitary juvenile polyps focal nuclear β catenin staining was also observed.

Discussion

Cellular β catenin is regulated by a complex interaction of different effector mechanisms (fig 1). It is presently believed that progression of colonic adenomatous tumours is characterised by a lack of the normal APC gene product or a mutated APC gene product, which interferes with β catenin metabolism in the cytoplasm of the epithelial tumour cells.[16] Altered β catenin metabolism in adenomatous colonic tumours results in the translocation of β catenin to the tumour cell nuclei and an increased transcriptional activity.[12]

Our findings of nuclear β catenin in hamartomatous polyps, localised to some epithelial cell nuclei in the proliferative compartments of these polyps, lead us to speculate about the reasons for the impaired β catenin metabolism in these epithelial cells. About half of the solitary juvenile polyps show this focal dysregulation of β catenin in the epithelial cells, very like the polyps in juvenile polyposis syndrome. Most probably the translocation of β catenin in solitary juvenile polyps and in juvenile polyposis is caused by the same regulatory defects. Polyps in juvenile polyposis syndrome have sometimes been reported to harbour somatic APC gene mutations, especially when containing so called "dysplastic foci," but mutations have not been found in solitary juvenile polyps.[3] Theoretically, APC gene mutations could explain our observations of β catenin positive nuclei in polyps from patients with juvenile polyposis and Peutz–Jeghers syndrome. However, up to now no somatic APC mutations have been reported in Peutz–Jeghers syndrome polyps.

On the basis of our results alone we cannot definitely exclude an involvement of APC mutations in the nuclear translocation of β catenin seen in the polyps from juvenile polyposis and Peutz–Jeghers syndrome. However, epithelial cells from Peutz–Jeghers polyps do not show morphological signs of adenomatous transformation or aneuploidy like the polyps in familial adenomatous polyposis syndrome. On DNA cytometric investigation of the polyps used in this immunohistological study, neither the Peutz–Jeghers polyps nor the juvenile polyposis polyps showed aneuploid stem cell lines (data not shown). Therefore, our data lead us to suggest that the Peutz–Jeghers syndrome gene product STK11 could function as a negative regulator of the β catenin/APC signalling cascade. Loss of STK11 in epithelial stem cells from Peutz–Jeghers polyps may result in an impaired catabolism of β catenin, with subsequent translocation of β catenin into the nucleus. β Catenin complexed to transcription factors is known to be involved in transcriptional regulation of various genes. Thus it is reasonable to assume that intranuclear β catenin seen in epithelial cells from Peutz–Jeghers polyps leads to altered differentiation and growth programme in the absence of STK11 alone.[17] Accumulation of β catenin in the nuclei of proliferating cells to various degrees may have quite different causes.

In adenomatous tumours of the colon, mutations of the β catenin gene are relatively rare and are therefore not a common cause of the nuclear translocation of β catenin in tumour cells.[18] On the paraffin sections used for this study, samples of normal colonic mucosa and unaffected mucosa from patients with Peutz-Jeghers, juvenile polyposis, and familial adenomatous polyposis syndromes did not show any detectable nuclear β catenin staining in crypt cell nuclei. We conclude that the disturbed β catenin homeostasis is most probably an important mediator of the polypoid mucosal proliferation present in polyps from patients with Peutz–Jeghers syndrome and juvenile polyposis, as well as in adenomatous polyps. This could be due to the lack of β catenin in the E-cadherin–catenin complex on one hand, or on the other hand to its transcriptional activity in the nucleus upregulating growth relevant genes. Both mechanisms eventually result in an overflow proliferation. The deep pseudoinvasion of enterocolic mucosa occasionally seen in Peutz–Jeghers syndrome may be promoted by the same dysregulation of β catenin, which is present in crypt cells at the base of Peutz–Jeghers polyps. In particular, the lack of membranous E-cadherin–catenin complex could be involved in this abnormal mucosal growth into the intestinal wall, leading to a functional alteration of cell–cell contacts and cellular mobility.

Up to now these conclusions remain speculative because the exact mechanisms of polyp formation in the hereditary polyposis syndromes of the hamartomatous type are not fully understood, and judging by the morphological differences they unlikely to be the same in the two syndromes. Future investigations must focus on the interactions between the mutated gene products (STK11 in Peutz-Jeghers syndrome, SMAD4 and others in juvenile polyposis syndrome) and the cellular β catenin regulatory system.

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