The molecular basis of colorectal cancer

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Abstract

Colorectal cancers, whether sporadic or hereditary, are caused by a defined set of molecular events. The genes and pathways involved in the earliest steps of tumorigenesis have crucial functions in the regulation of normal crypt homeostasis. Further insight into these pathways can lead to the development of useful prognostic indicators, and target preventive and therapeutic strategies in the management of colorectal cancer. Mutations in the APC/ β-catenin/Tcf-4 pathway lead to important changes in stem cell dynamics, before clinically identifiable lesions appear. Preventive strategies aimed at reversing these changes or therapeutic interventions targeting cell populations with these alterations should be most efficacious. (Acta gastroenterol. belg., **2001**, 64, **249-254**).

Key words : colon cancer, APC, β-catenin.

Introduction

Tumorigenesis is a multistep process, each step reflecting genetic alterations that drive the progressive transformation of normal human cells into highly malignant derivatives (1). E. Fearon and B. Vogelstein proposed this multistep model for colorectal tumorigenesis in 1990 (see figure 1) (2). This hypothesis has since been confirmed and expanded for colorectal cancer, but has also served as a paradigm for tumorigenesis in many other organs (3).

In colorectal cancer the progressive evolution of cells from normalcy via a premalignant state to an invasive cancer is reflected by the distinct entities of adenomas and carcinomas. The existence of such distinct histopathological lesions has greatly contributed to the molecular understanding of colorectal cancer. Intensive molecular analysis of the earliest detectable lesions (Aberrant Crypt Foci, ACF) to adenomas, carcinomas and invasive carcinomas led to the identification of the necessary molecular genetic events and their possible sequence during tumorigenesis (4).

A cancer cell must accumulate alterations that allow it to become self-sufficient in growth signals, insensitive to growth-inhibitory signals, evade programmed cell death and modulate it's environment allowing sustained angiogenesis, invasion and metastasis. Although a definite sequence of molecular genetic events is known in colorectal tumorigenesis (see figure 1), the exact contribution of each of these events is not as clear. Recent research has focused on the earliest events, that might be more specific to the gut epithelium, whereas later events such as k-ras and p53 mutations might be more common in the later stages of many types of malignancies.

This review will focus on the earliest genetic events thought to initiate the transition from normal epithelium to beginning adenoma.

The clues to understanding cancer often rely on the knowledge of the normal regulation of its tissue of origin. Every tissue or organ has a very own specific set of genes that regulate its homeostasis. The gut epithelium for example is a highly proliferative organ, with high rates of cell loss that need to be continuously replaced. Correct homeostasis in the gut epithelium is achieved by a fine balance between cellular proliferation, differentiation and cell death. It is no surprise that the genes involved in this delicate balance, are also the genes involved in the first steps of deregulation or tumorigenesis. Thus, combining two fields of research allows powerful insights into the normal function of some known oncogenes in colorectal cancer (5).

Biology of the normal intestinal crypt

The intestine is lined by a simple columnar epithelium, which is continuously replaced as cells are shed into the gut lumen (see figure 2). It is estimated that the normal adult colon contains 5×10^{10} epithelial cells, 1/6 to 1/3 of which are shed into the lumen every 24 hours. The small intestinal villi and colonic intercrypt plates receive a constant supply of enterocytes from stem cells located in the lower poles of the crypts of Lieberkuhn (6). In the small intestinal crypts the proliferating cells are located at positions 4 to 6 above the Paneth cells, whereas in the colon they originate from the very base of the crypt. Under normal conditions stem cells are thought to undergo "asymmetrical" division, producing one stem cell and one daughter cell that will differentiate and migrate. Each migrating cell will undergo 4 to 6 rounds of cell division on its way out of the crypt to the mucosal surface. Under stress conditions it is likely that stem cells can switch to "symmetrical" division, leading to two daughter stem cells, and thus increasing the pool of stem cells. The unknown mechanism governing this switch, as well as the mechanisms leading to correct differentiation and migration of daughter cells, are possible targets in tumorigenesis.

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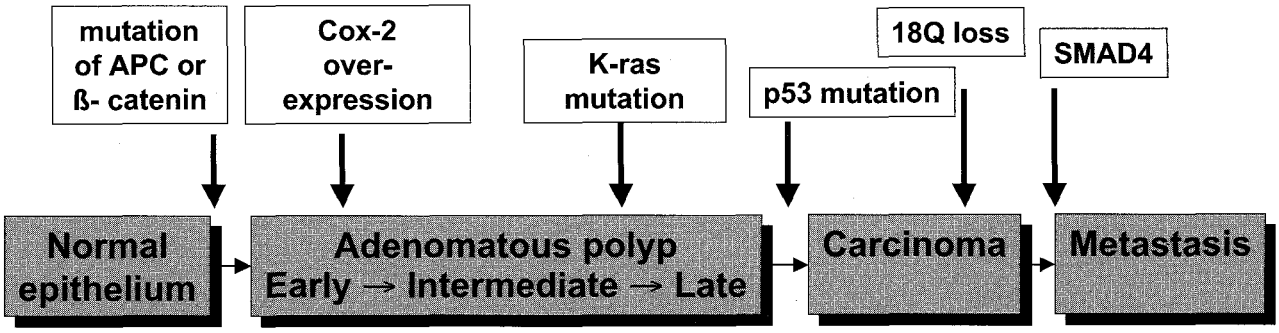

Fig. 1. — Correlation between histological progression and molecular changes in colorectal cancer. The APC tumor suppresor gene has a key function in tumor initiation. Tumor progression is dictated by a series of alterations in different genes involved in cell proliferation.

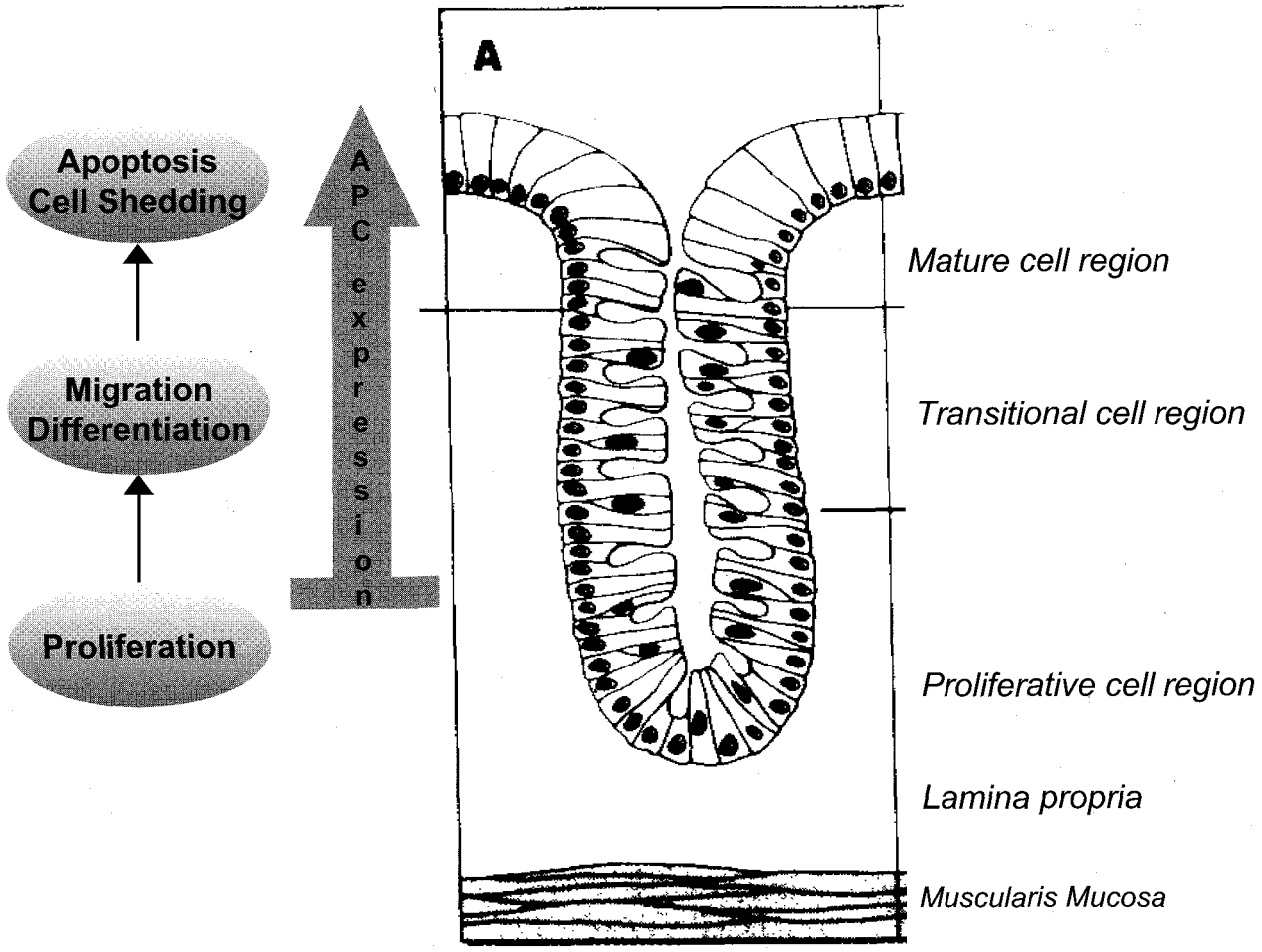

Fig. 2. — The intestine is lined by a simple columnar epithelium, which is continuously replaced as cells are shed into the gut lumen. APC is expressed in cells migrating along the crypt-villus axis. The cells expressing APC in the luminal half of the crypt are the cells that will be shed into the lumen after programmed cell death or apoptosis.

Hyperproliferation is believed to precede adenoma formation in pre-neoplastic epithelium in both hereditary and sporadic colorectal cancer. It is unclear if this is mainly due to an upward extension of proliferation towards the crypt orifice, or because of the development of new intestinal crypts through a process of longitudinal crypt fission (7). A gene functionally implicated in both these processes is the APC (Adenomatous Polyposis Coli) gene.

APC and colorectal cancer

Some strong epidemiological evidence already existed in favour of a fundamental role for APC in colorectal tumour initiation. Patients with FAP (Familial Adenomatous Polyposis), an inherited syndrome characterised by hundreds of adenomatous polyps in the gastro-intestinal tract, carry germline mutations in the APC gene. Transgenic mice, engineered to carry a single germline

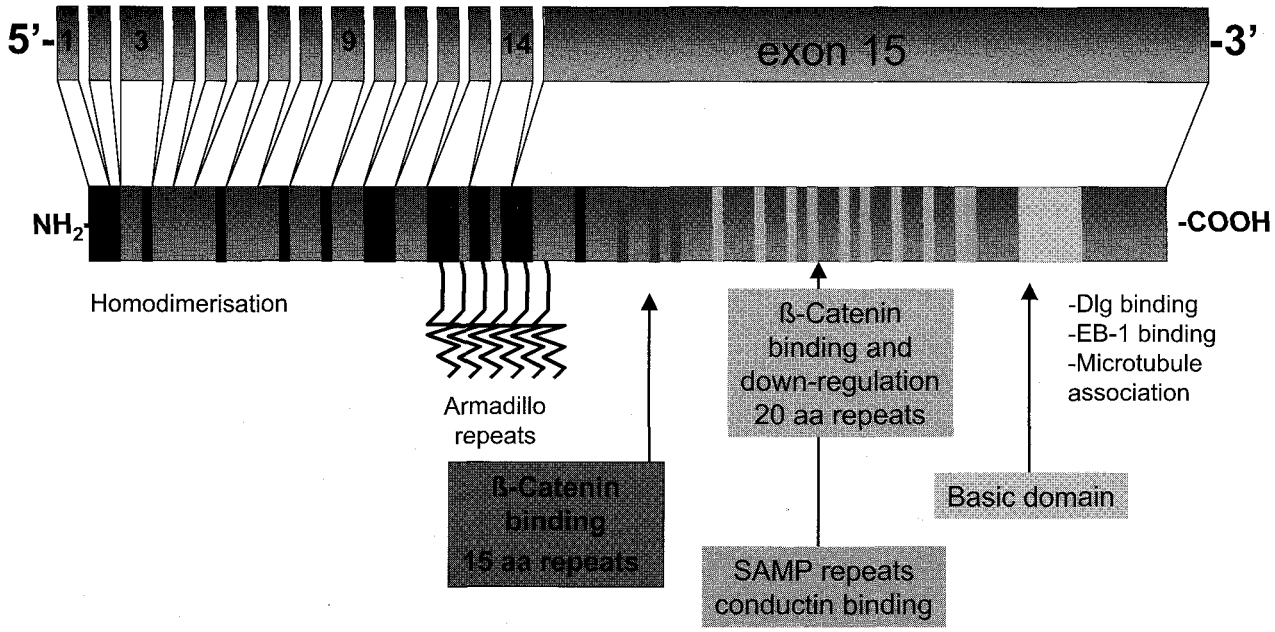

Fig. 3. — Schematic representation of the APC gene and the corresponding APC protein. Known functional domain are indicated.

mutation in their APC gene, similarly develop adenomatous polyps in their small and large intestine (8). Mutations in APC are found in over 80% of sporadic colorectal cancers and adenomas, and moreover these mutations are found in the earliest detectable lesions (ACF), implicating this gene in the earliest steps of tumorigenesis (9).

The APC gene was first discovered 10 years ago through linkage analysis in FAP pedigrees (10). It behaves as a tumour supressor gene meaning both copies have to be functionally inactivated for tumours to appear. In FAP one mutant copy is inherited, leading to 50% reduction of the normal gene product in all cells. Additional loss of the remaining normal allele in a single epithelial (stem ?) cell is necessary for adenoma formation. In sporadic adenomas, both copies have to undergo mutational inactivation in the same epithelial cell, a process statistically less likely to occur.

The APC gene product

The APC gene encodes for a 312 kDa protein, comprising 2843 amino acids. This large protein contains many putative functional motifs (see figure 3) (11). From N- to C-terminus we recognise a homodimerisation domain and a functional nuclear export signal (NES). The first third of APC contains seven Arm repeats. These Arm repeats are present in a number of other proteins such as b-catenin, desmosomal proteins, the importin protein family and are implicated in mediating protein-protein interactions. The central third of APC contains three 15-amino acid and seven 20-amino acid repeats. These 15-amino acid and 20-amino acid repeats are involved respectively in the binding and degradation of other proteins such as β-catenin and plakoglobin. The carboxy-terminus of APC contains basic residues that might confer binding to microtubules. The C-terminus of APC can also associate with EB1, a tubulin binding protein and with DLG, a protein localising to cell-cell contacts.

APC function in tumorigenesis

There are multiple ways in which the APC protein could be involved in tumorigenesis, as can be expected from a protein with that many known and yet unknown functional domains and binding partners. There are as many processes in crypt homeostasis that can be affected during tumorigenesis, including proliferation, migration, differentiation and apoptosis. An important function of the APC gene is to regulate β-catenin mediated transcription, providing an additional way of influencing each of these processes.

APC, β-catenin and transcription

One function of the APC gene product is to regulate the cellular levels of β-catenin, a mediator in the Wnt/wingless signalling pathway (12).

Wnts are secreted glycoproteins that act as ligands to stimulate receptor-mediated signal transduction pathways in both vertebrates and invertebrates. Wnt signalling pathways often modulate gene expression and cell fate in a combinatorial manner with other pathways, notably FGF and TGF β pathways. The Wnt/β-catenin pathway is the best understood Wnt signalling pathway, and is highly conserved during evolution.

In the absence of Wnt signalling (figure 4a and b), a cytoplasmic degradation complex (consisting of at least APC, Axin, GSK-3β and β-catenin) leads to the

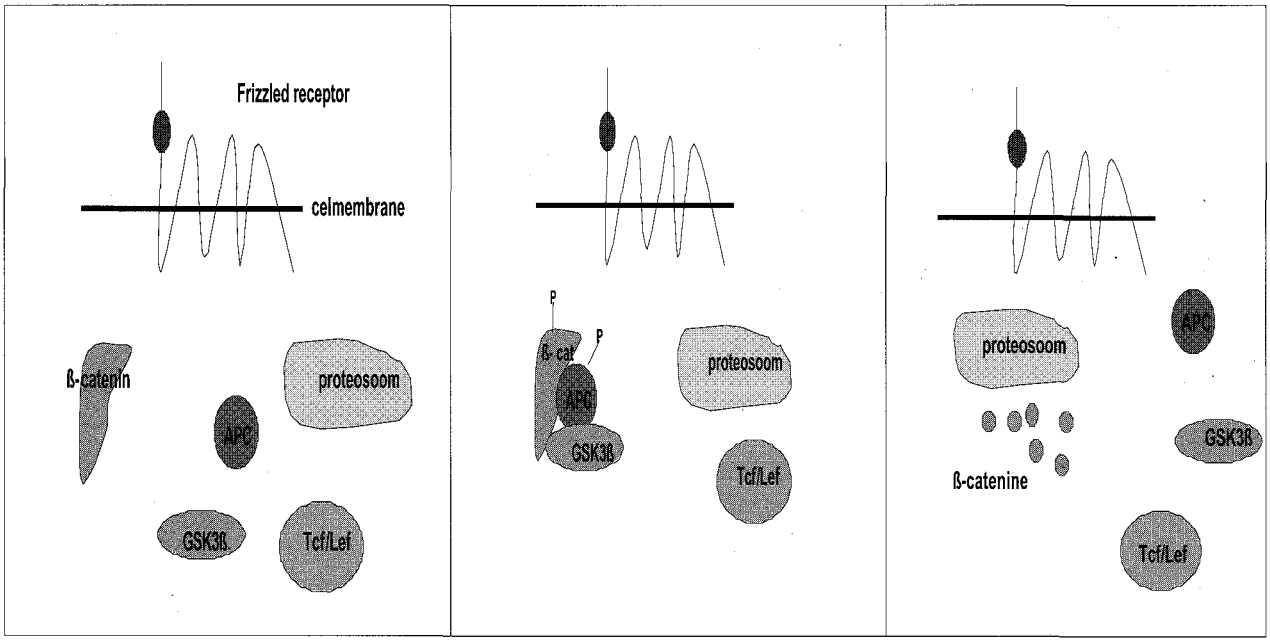

Fig. 4a. — *Absence of Wnt signal.* If no Wnt signal is present, cytoplasmic β-catenin will be phosporylated within a multiprotein complex. Phosphorylated β-catenin will be degraded through the ubiquitin/proteosome pathway.

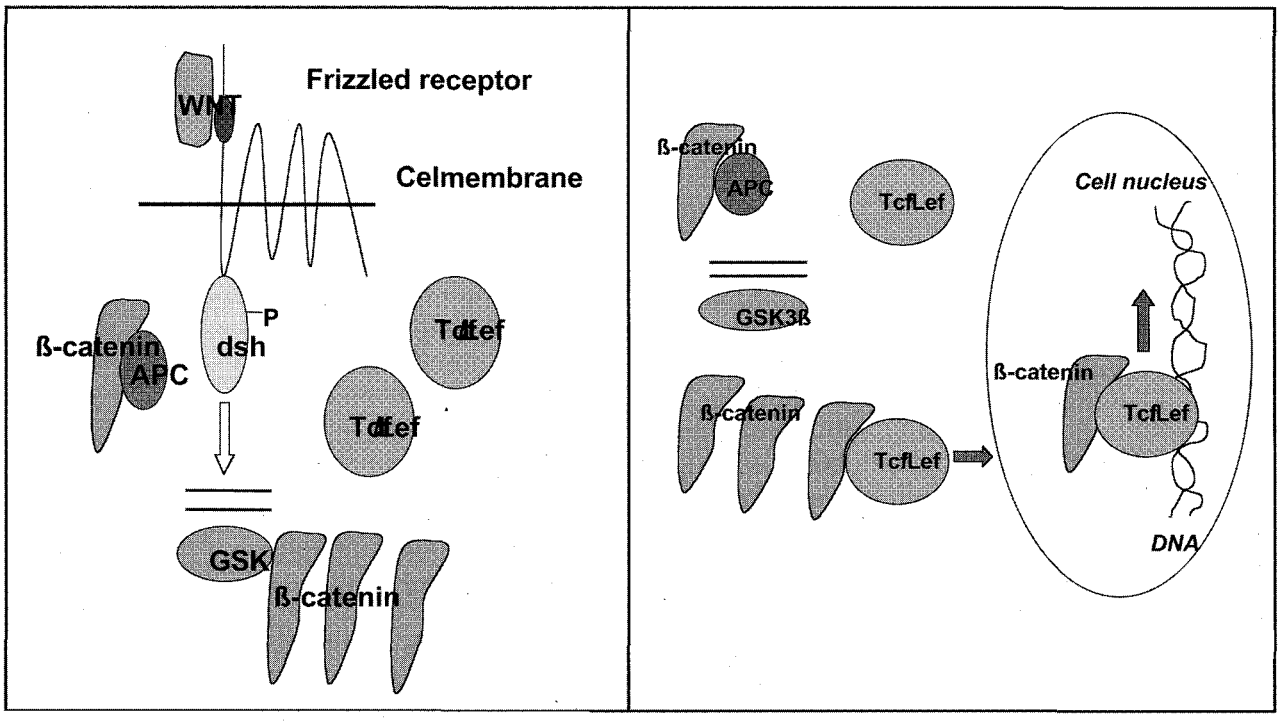

Fig. 4b. — *Presence of Wnt signal.* In the presence of a Wnt signal, an activated Frizzled receptor will lead to activation of the disheveled protein. Disheveled will inhibit the phosphorylation of β-catenin, leading to its cytoplasmic accumulation. Cytoplasmic β-catenin translocates to the nucleus and will activate gene transcription through its association with Tcf/Lef transcription factors.

phosphorylation of both β-catenin and Axin by GSK-3β. This promotes interaction of β-catenin with β-TrCP, leading to the ubiquitination of β-catenin and its degradation by the proteosome (13). Thus, at steady state in the absence of Wnt signalling, β-catenin is rapidly degraded in the cytoplasm.

To activate the pathway, secreted Wnts are thought to interact with serpentine receptors of the Frizzled gene family. Activation of some Frizzled homologs by Wnt ligands leads to activation of the modular protein Dishevelled, through a process likely involving phosphorylation. Dishevelled functions through binding

components of the degradation complex to reduce the function of GSK-3β. This reduces the phosphorylation and degradation of β-catenin, generally leading to its accumulation in the nucleus (14).

The mechanism of nuclear accumulation of β-catenin occurs without a nuclear localisation sequence, and occurs by direct binding to the nuclear pore machinery. β-Catenin is also rapidly transported from the nucleus, thus its nuclear levels are determined by both import and export. In the nucleus β-catenin forms a complex with HMG box transcription factors of the LEF and TCF classes leading to activation of expression of target genes (15). In the nucleus prior to Wnt signalling, LEF and TCF transcription factors bind to DNA with sequence specificity in promoter/enhancer regions of target genes, and along with Groucho and CTBP often function to repress gene expression. Elevation of β-catenin levels by Wnt signalling leads to binding of β-catenin to TCF/LEF, promoting changes in the transcriptional machinery that lead to activation of target genes. Known target genes include c-Myc, cyclin D1, metalloproteinase-7, the nuclear receptor PPARdelta, T (brachyury) and the homeobox proteins engrailed-2, siamois and twinned (16,17,18,19).

In summary, the Wnt signalling pathway is a conserved embryonic developmental pathway that regulates the expression of certain developmental genes via β-catenin. In normal adult tissue this pathway is not active and β-catenin is tightly downregulated. Constitutive reactivation of the Wnt/β-catenin pathway has been observed in colorectal neoplasia, due to mutations in APC or β-catenin that render β-catenin stable.

APC, β-catenin and transcription applied to colorectal tumorigenesis

Although Wnt/β-catenin signalling is a normal developmental pathway, and although its deregulation might be found in different tumour types, it seems a fundamental event in the initiation of colorectal cancer.

No other cancer to date has been found to harbour such high rates of mutational deregulation of this pathway. More than 80% of colorectal adenomas have mutations in APC, and more than half of those with intact APC, harbour activating β-catenin mutations. Both these mutational events lead to an accumulation of β-catenin protein and are found in the very earliest lesions.

In addition adenoma formation can be elicited in transgenic mice by the simple deregulation of either APC or β-catenin (20).

Some recent studies have focused on the potential downstream targets of Tcf/Lef/β-catenin transcriptional complexes in the gut. First it was shown that the expression of Tcf-4, a member of the Tcf/Lef family, was restricted to the gut epithelium and mammary glands in mice (21). Since different Tcf can show different target specificity, this could imply the existence of Tcf/β-catenin target genes, only activated in cells expressing Tcf-4 and high levels of β-catenin.

To address this question a specific Tcf-4 knockout mouse was made. This mouse, lacking Tcf-4 during embryogenesis and adult life, had only one major abnormality : loss of proliferating stem cells in the gut, resulting in death 3 days postnatal, when the villi became depleted of cells (22).

Taken together, this data point to a major role of β-catenin/Tcf-4 in normal gut development, homeostasis and tumorigenesis. Some β-catenin/Tcf-4 target genes have been identified in the gut epithelium, such as c-Myc, cyclin-D1 and matrilysin. c-Myc and cyclin-D1 are potent cell cycle regulators, but it is not yet clear what their exact role is in the initiation and maintenance of colorectal tumours.

Other roles for APC in tumorigenesis

It is clear that APC/ β-catenin/Tcf-4 signalling is involved in normal gut homeostasis and the earliest stages of tumorigenesis in colorectal cancer, but APC might have additional roles besides it's modulation of β-catenin signalling. As shown in figure 2, APC is expressed in cells migrating along the crypt-villus axis. APC is known to localise to the leading edges of actively migrating epithelial cells (23), in association with the microtubules. A possible function for APC in cell motility or adhesion through its association with microtubules is currently under investigation.

The cells expressing APC in the luminal half of the crypt are the cells that will be shed into the lumen after programmed cell death or apoptosis. Inducing normal APC expression in colorectal cancer cell lines that carry only mutant APC, can increase apoptosis about ten-fold (24). How APC might regulate cell death is another area currently under investigation.

Additional genetic alterations found in colorectal cancer

During the progression from adenoma to invasive colorectal carcinoma (see figure 1) some consistent molecular genetic alterations have been identified, leading to the deregulation of at least four signalling pathways (4). These include the Wnt signalling pathway, the K-ras pathway, the p53 pathway and the TGF-β pathway.

Mutations in K-ras are found in nearly 50% of colorectal carcinomas and are already present in the earlier adenoma stages (25). The ras family of oncogenes is very important in human tumorigenesis in general and is not restricted to colorectal cancer since ras mutations are found in about 30% of any human malignancy. A major function of the ras protein is that it can activate the MAP kinase signal transduction pathway, in turn leading to nuclear expression of several genes.

Mutations in p53 are found in the majority of colorectal tumours, and are probably involved in the conversion from a benign to a malignant lesion.

Alterations of the TGF-β pathway are also a frequent finding in colorectal cancer, either by inactivation of SMAD 4 (18Q loss) or by mutations of the TGF-β type II receptor. A lot of the data on these recurrent genetic alterations remained descriptive until mouse models allowed further study of genetic interactions between these alterations, and their further positioning in the sequence of events.

Using crosses between APC mutant mice (sufficient to induce gastro-intestinal adenomas) and for example SMAD 4 mutant mice, SMAD 4 could be positioned as late event, involved in tumour progression and invasion (26). Another example relates to the cyclooxygenase-2 (COX-2) expression found in about 90 % of sporadic colon carcinomas and 40 % of colon adenomas but not in normal colonic epithelium (27). This finding has raised a lot of interest since selective COX-2 inhibition could be a potential pharmacological target in the prevention of colorectal adenomas. The molecular mechanism linking COX-2 expression to adenoma formation is yet unclear, but compelling genetic evidence was provided by a mouse experiment in which crossing the APC mutant mice with COX-2 deficient mice led to a substantial reduction in adenoma formation (28).

Summary

Colorectal cancers, whether sporadic or hereditary, are caused by a defined set of molecular events. The genes and pathways involved in the earliest steps of tumorigenesis have crucial functions in the regulation of normal crypt homeostasis. Further insight into these pathways can lead to the development of useful prognostic indicators, and target preventive and therapeutic strategies in the management of colorectal cancer. Mutations in the APC/ β-catenin/Tcf-4 pathway lead to important changes in stem cell dynamics, before clinically identifiable lesions appear. Preventive strategies aimed at reversing these changes or therapeutic interventions targeting cell populations with these alterations should be most efficacious.

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