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The *APC* gene in colorectal cancer

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Abstract

Mutations in the *adenomatous polyposis coli (APC)* gene are not only responsible for familial adenomatous polyposis (FAP), but also play a rate-limiting role in the majority of sporadic colorectal cancers. Colorectal tumours are known to arise through a gradual series of histological changes, the so-called 'adenoma–carcinoma' sequence, each accompanied by a genetic alteration in a specific oncogene or tumour suppressor gene. Loss of APC function triggers this chain of molecular and histological changes. In general, an intestinal cell needs to comply with two essential requirements to develop into a cancer: it must acquire *selective advantage* to allow for the initial clonal expansion, and *genetic instability* to allow for multiple hits at other genes responsible for tumour progression and malignant transformation. Inactivation of *APC* seems to fulfill both requirements. In this short review, I will discuss the role played by *APC* in providing, when mutated, selective advantage, through constitutional activation of the Wnt signal transduction pathway, and chromosomal instability to the nascent intestinal tumor cell. © 2002 Published by Elsevier Science Ltd.

Keywords: *APC*; Colorectal; FAP; Review; Gene mutation; Wnt signal transduction pathway

1. Introduction

Colon cancer is one of the most common malignancies among populations in the US and Western Europe, and one of the leading causes of worldwide morbidity and mortality due to cancer. In the US, approximately 140 000 new cases and 50 000 deaths are registered each year [1]. In Europe, each year about 213 000 new cases and 110 000 deaths are reported, respectively [2] (see also: http://www-dep.iarc.fr). The lifetime colorectal cancer risk in the general population is 5%, but this figure rises dramatically with age: by the age of 70 years, approximately half the Western population will have developed an adenoma. In general, the incidence of colorectal cancer is high in developed countries, with incidence rates varying up to 20-fold between high- and low-risk geographical areas throughout the world [2]. These variations in colorectal cancer incidence are likely to result from environmental and mainly dietary modifying factors.

Colorectal carcinomas arise through a series of well-characterised histopathological changes as the result of specific genetic 'hits' at a few oncogenes and tumour suppressor genes. At least four sequential genetic changes need to occur to ensure colorectal cancer evolution (Fig. 1). One oncogene (*KRAS*) and three tumour suppressor genes (*adenomatous polyposis coli (APC), SMAD4* and *TP53*) are the main targets of these genetic changes. In particular, loss of *APC* gene function seems to trigger the cascade of events that eventually leads to malignant transformation in the large bowel. Here, I will focus on the function of the *APC* gene in homeostasis and cancer, as this knowledge is expected to provide the basis for the development of future tailor-made preventive and therapeutic interventions for colorectal cancer.

2. The *APC* gene in homeostasis and cancer

The *APC* gene was initially identified by positional cloning of the FAP (familial adenomatous polyposis) locus [3,4]. Subsequently, the majority of sporadic colorectal tumours were found to harbour mutations in both *APC* alleles [5,6]. Initially, the sequence of the large (312 kDa) APC protein did not allow specific predictions about its intracellular function. The first functional clues were provided by the identification of β-catenin as

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a binding partner of APC [7,8]. β-catenin was originally identified as an essential intracellular component of cadherin adhesion complexes. However, we now know that it also represents a very important component of the Wingless/Wnt signal transduction pathway.

The canonical Wnt signalling pathway has been derived from combined work in flies, frogs and mammals. In unstimulated cells, i.e. in the absence of the extracellular Wnt signal, free β-catenin is bound and phosphorylated to the so-called destruction complex, consisting of the scaffolding protein axin and conductin, glycogen synthase kinase 3 β (GSK3β) and APC [9–12]. Phosphorylation of β-catenin by this complex earmarks it for ubiquitination and subsequent proteolytic degradation.

In the presence of the Wnt signal, this binds to the Frizzled receptors [13] that subsequently inactivate GSK3β in the destruction complex. This inactivation process is not well understood but involves the intracellular protein Dishevelled [12]. As a consequence, β-catenin becomes stabilised and shuttles to the nucleus. Once in the nucleus, β-catenin binds to DNA-binding proteins of the T-cell factor (TCF) family, to serve as an essential co-activator of transcription [14,15].

In addition to controlling the Wnt pathway, APC may perform other cellular functions. In this regard, β-catenin not only functions as a Wnt transducer, but is also an essential component of the adherens junctions, where it provides the link between E-cadherin and α-catenin, binds actin and actin-associated proteins [16]. APC may thus control cell adhesion by regulating the stability and subcellular localisation of β-catenin. In addition, APC directly associates with the microtubule cytoskeleton [17,18]. This function involves its C-terminus and is unrelated to its capacity to regulate the Wnt signalling pathway.

As shown above, the *APC* gene encodes a multifunctional protein that may participate in several cellular processes such as cell adhesion and migration, signal transduction, microtubule assembly and chromosome segregation. However, despite the fact that each of these roles is potentially linked with cancer, it seems that the main tumour suppressing function of APC resides in its capacity to properly regulate intracellular β-catenin levels [19–21]. Moreover, although the vast majority of colorectal tumours carry mutations in APC, those with an intact *APC* gene were found to contain activating mutations in β-catenin that alter functionally significant phosphorylation sites [20,22]. In addition, mutations in other members of the Wnt pathway have been shown to be associated with cancer including conductin [23] and axin [24,25].

If we assume that the tumour suppressing function of APC is indeed its capacity to control β-catenin levels in the cell, which are the Wnt/β-catenin downstream targets responsible for APC-driven tumorigenesis? The first two identified downstream targets of this signal transduction pathway, MYC and cyclin D1, are clearly relevant for tumour formation because of their role in

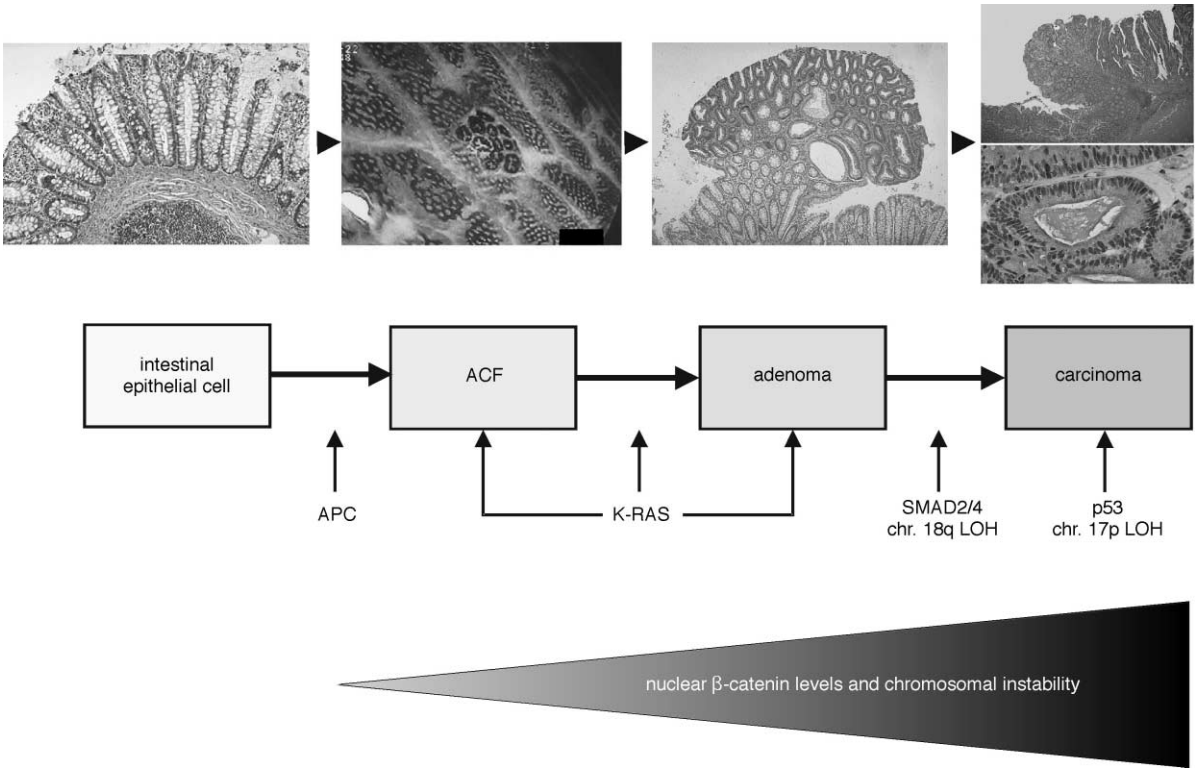

Fig. 1. Sequential genetic changes leading to the evolution of colorectal cancer. chr, chromosome; LOH, loss of heterozygosity.

proliferation, apoptosis and cell-cycle progression [26–28]. Changes in the normal expression pattern of MYC and cyclin D1 are likely to affect normal intestinal epithelial renewal by increasing the overall proliferation rate. In fact, several studies reported increased numbers of cycling cells in colorectal tumours [29–32]. Other Wnt target genes, such as matrilysin [33,34], *CD44* [35], *MYC* [36] and the urokinase-type plasminogen activator receptor [37], appear more likely to play a role in tumour promotion rather than initiation.

In the normal intestinal epithelium, nuclear β-catenin expression is higher in the proliferative compartment, while it is decreased in the upper two-thirds of the crypt. Conversely, cytoplasmic APC staining is markedly increased in post-replicative cells within the upper portions of the crypt, suggesting an increased level of expression with maturation, whereas it is virtually absent in the crypt region where cells are actively dividing [38,39]. This pattern of expression is in agreement with the role of β-catenin signalling in maintaining stem cell properties and controlling differentiation in the intestine [40]. In the bowel, TCF4 is the main transcription factor that transduces β-catenin signals in the nucleus [41]. Tcf-$4^{-/-}$ mice cannot sustain an intestinal stem cell compartment, strongly suggesting that activation of downstream targets such as MYC, TCF1, and cyclin D1 are required to maintain the proliferative capacity [40,42–44]. Moving upward along the crypt–villus axis, an increase of *APC* expression counteracts β-catenin signalling and allows differentiation. Activation of β-catenin signalling by *APC* mutation is therefore likely to result in the enlargement of the stem cell compartment and diminished differentiation.

But the role of APC mutation in deregulating the Wnt pathway does not necessarily need to be limited to the initial stages of the adenoma–carcinoma sequence. Nuclear β-catenin staining strongly correlates with tumour size and dysplasia [36,45], and high levels of nuclear β-catenin have been found at invasion fronts of adenocarcinomas [46]. Thus, the progression from an early adenoma to an invasive carcinoma is associated with a progressive increase of nuclear β-catenin levels. Accordingly, several downstream targets of the APC/β-catenin signalling pathway such as MYC, matrilysin, CD44, and the urokinase-type plasminogen activator receptor, show similar correlations with tumour progression and have been implicated in tumour invasion and metastasis [33–37].

In addition, mutations in other genes not directly involved in the Wnt pathway may also affect signal transduction. For example, loss of E-cadherin is often observed in epithelial tumours and is generally correlated with cell adhesion defects. However, because of its direct interaction with β-catenin, E-cadherin loss is predicted to result in an increase of cytoplasmic β-catenin available for nuclear signalling. Accordingly, both genetic and biochemical evidence has recently been provided to show that the growth suppressor activity of E-cadherin is adhesion-independent and results from an inhibition of the β-catenin/TCF signalling pathway, and that loss of E-cadherin expression can contribute to upregulation of this pathway in human cancers [47,48].

Recent studies have shown that the C-terminus of APC is involved in chromosomal stability at mitosis [49,50]. APC localises at the kinetochore of metaphase chromosomes, and this localisation is likely to be dependent on the interaction between APC and EB1. Accordingly, *Apc*-mutant cells have an abundance of spindle microtubules that fail to connect to kinetochores and are characterised by chromosomal instability. Moreover, $Apc^{-/-}$ cells have supernumerary centrosomes, a defect possibly unrelated to the kinetochore-capture function of APC [49]. Two types of chromosomal abnormalities occur in *Apc*-deficient mouse cells: quantitative changes (near-tetraploidy) presumably arising from non-disjunction defects, and structural rearrangements (chromosomal translocations) resulting from chromosomal breakage and reunion. Notably, both processes seem to occur in most cells. This is in agreement with a dual function for APC in mitosis: the proper attachment of the mitotic spindle to the dividing chromosomes at the kinetochore, and the regulation of centrosome duplication through its interaction with tubulin and the centrosome [50]. While loss of the former function will lead to non-disjunction and tetraploidy, defects of the latter will result in mitotic cells with multipolar spindles that exert multidirectional forces on the kinetochore, resulting in chromosomal breakage and fragmentation [51]. Accordingly, Thiagalingam and colleagues [52] have observed a similar dichotomy in a detailed study of chromosomal losses in human colorectal cancers. While approximately 40% of the losses were predicted to result from mitotic non-disjunction, in more than half of the cases fusions between different chromosomes were observed that are likely to derive from double-strand breaks and inter-chromosomal recombination.

3. A genetic model for *APC*-driven colorectal tumorigenesis

A general picture is emerging from the analysis of the essential roles of the Wnt signal transduction pathway in providing selective advantage to the nascent tumour cell, and in exerting genetic instability to ensure both tumour progression and malignant transformation: the *APC* gene, because it encompasses both functions, plays a central initiating and promoting role in colorectal cancer. Its inactivation and the resulting constitutive activation of the Wnt pathway provides a strong selective advantage by affecting cell proliferation, migration,

apoptosis and possibly differentiation of the intestinal stem cell. Subsequently, other synergistic mutational events may allow the mutant APC to induce CIN (chromosomal instability) and accelerate tumour progression along the adenoma–carcinoma sequence (Fig. 1). In fact, several of the oncogenes and tumour suppressor genes known to be altered along the adenoma–carcinoma sequence cooperate in promoting genomic instability and may represent the synergistic partners of APC in eliciting CIN [53–58]. Thus, CIN will allow a stepwise malignant progression through a gradual increase of numerical and structural chromosomal rearrangements. Notably, Samowitz and colleagues have reported that small adenomas with β-catenin mutations do not appear to be as likely to progress to larger adenomas and invasive carcinomas as adenomas with *APC* mutations [59]. This suggests that, although tumour initiation either by loss of APC or by oncogenic β-catenin mutations is functionally equivalent (by constitutive activation of the Wnt signalling pathway), inactivation of the additional APC functions in chromosomal stability underlies malignant progression in the colorectum.

Based on the above, we have proposed [60] that the multifunctional nature of APC confers a rate-limiting role in tumour initiation and progression to this colorectal cancer suppressor gene. Loss of β-catenin regulation by APC provides the intestinal cell with a selective advantage and allows the initial clonal expansion. At this stage, CIN caused by loss of the C-terminal functional motifs of APC is latent due to surveillance by the cell cycle and mitotic checkpoint machinery. The early activation of the oncogenes *KRAS* (by point mutation) and *MYC* (as a downstream target of the Wnt pathway) will synergise with *APC* in triggering CIN and the subsequent allelic imbalances at 17p and 18q. Additional synergisms between APC and other tumour suppressor genes in eliciting aneuploidy and CIN will progressively lead to malignant transformation and metastasis.

How does the above 'molecular knowledge' affect our diagnostic and therapeutic approach to colorectal cancer? The elucidation of the molecular and cellular mechanisms underlying colorectal tumorigenesis will point out which cellular functions (proliferation, apoptosis, cell migration and differentiation) are affected and which are associated with a poor clinical prognosis. In addition, the identification of downstream targets of the Wnt/β-catenin pathway will open the way for 'tailor-made' preventive and therapeutic interventions.

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