*Chapter 34. The APC story – colon cancer arising in polyps 230127be*

# Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

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CHAPTER 34

## *The APC story: colon cancer arising in polyps.*

The previous chapter discussed a cancer, retinoblastoma, brought about by mutations of a single gene, *RB*. That was a unique case, because the great majority of cancers result only after a series of mutations in different genes. A classic example is colorectal cancer, particularly the common cancers arising in polyps in the descending colon and rectum in the left side of the body. The relatively slow development of those malignancies, typically over a period of about 10 years, during which they progress through the series of mutations or other genetic changes allows time to remove the polyps during colonoscopy before malignant cancers appear (Figure 34.1). A similar process of mutations also occurs in the ascending colon, particularly mutations of DNA mismatch repair genes that were the subject of Chapter 25. But here we focus on cancers that develop in polyps in the descending colon and rectum.

### *Multistep process of mutations leading to cancers in colorectal polyps.*

Colorectal cancer is the third most prevalent cancer in terms of incidence or mortality worldwide, and of these the most common are carcinomas of the descending colon or rectum. A dominant characteristic of these cancers is *chromosome*-instability, which arises when mitosis does not divide the chromosomes equally or when a chromosome is duplicated or deleted. This contrasts with cancers in the ascending colon that are instead characterized by *microsatellite*-instability (Chapter 25). A hallmark of chromosome instability is loss of function of the *APC* (adenomatous polyposis coli) gene, resulting in inactivation of the Wnt pathway (discussed in a later section) and is the first step on the path to cancer in colorectal polyps (Figure 34.2). After *APC*, other genes commonly impaired in the sequence on the way to cancer are *KRAS* (Chapter 18), *SMAD2* and *4* in the

TGF-beta pathway, and *TP53* (Chapter 32) (Figure 34.2). However, there are also variations and subtypes of this major category of cancers (Parmar and Easwaran, 2022).

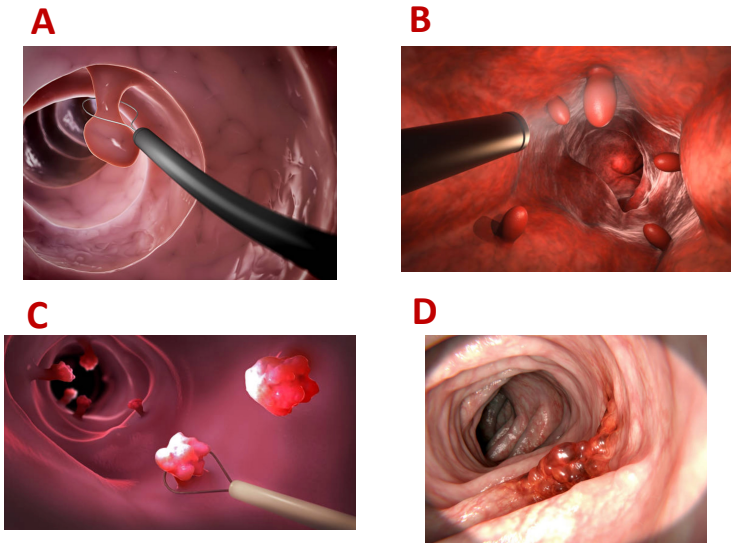


Figure 34.1. Images of benign and early malignant polyps in the descending colon.
**A**. A benign polyp showing how it is removed with a snare during colonoscopy.
**B**. Multiple polyps in the colon.
**C**. Early adenomas growing on polyps.
**D**. An early cancer invading the wall of the colon.
(From Getty Images.)

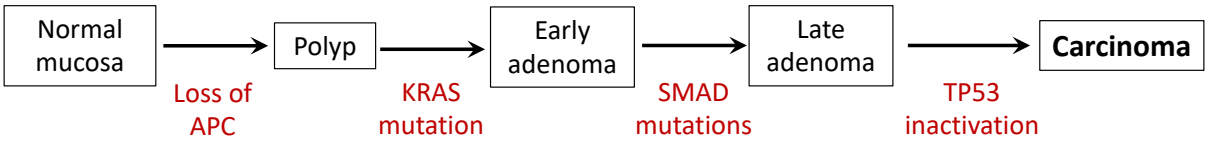


Figure 34.2. Multiple steps of mutations or other genetic changes on the path to malignant carcinoma in polyps in the descending colon and rectum (Parmar and Easwaran, 2022), based on the model proposed by (Fearon and Vogelstein, 1990).

## *Familial adenomatous polyposis colon cancer.*

Although cancer arising over many years from polyps in the descending colon and rectum is common, there is a rare familial form in which affected individuals develop huge numbers of such polyps and cancers at an early age (Figure 34.3). Even though rare, the familial form enabled the discovery also of the causative genes and mechanisms of the common form. That was because the common form can be caused by any of many

mutations in the causative genes, whereas the causative mutation patterns in families was more restricted and hence easier to identify.

The history of familial adenomatosis polyposis and cancer was well told by Buelow and colleagues (Bulow et al., 2006). Among the first cases they cited was that of a woman with polyps all over the colon who died at the age of 32 in 1857 and that of two siblings, ages 17 and 19, with "disseminated polypus of the rectum" who had 20-30 polyps in the rectum and had experienced rectal bleeding before puberty; reported in 1882, it may have been the first familial case. In 1927, E. A. Cockayne concluded that familial polyposis was inherited as a genetically dominant condition. In 1939, a Polyposis Registry at St. Mark's Hospital in London included 10 families and reported that sigmoidoscopy was commonly used as a prophylactic. Of five patients who had their colon removed, four survived the cancer.

On 25 March 1952, Cuthbert E. Dukes, Pathologist and Director of Research Laboratory of St. Mark's Hospital, presented a lecture on Familial Intestinal Polyposis to the Royal College of Surgeons (Dukes, 1952) (Figure 34.4). His attention to this disease was drawn by J. P. Lockhart-Mummery, who in 1925, while senior surgeon to St. Mark's Hospital, reported the history of three families in which polyposis was prevalent and noted that cancer of the colon and rectum was common in these families and occurred at an unusually early age. During the following 12 years, Lockhart-Mummery and Dukes followed these three families, as well as seven others, and confirmed that the disease was inherited as a dominant trait. By 1951, after Lockhart-Mummery's retirement, Dukes had prepared family pedigree charts for 41 polyposis families, some going back four generations.

Dukes showed the pedigree of a family he had kept under observation and charted in 1925 and 1951, which showed the high prevalence of intestinal polyposis and cancers of colon or rectum in unusually young family members (Figure 34.5). Perhaps inspired by family stories like that, he concluded a lecture with following (Dukes, 1952):

*At present surgery is the only reliable remedy but we must all look forward to the day when other means may be found for the treatment of polyposis and the prevention of cancer. I should like to conclude by provoking you to think of the possibilities of new methods of investigation and treatment and it occurred to me that I might achieve this best by means of a short poem, which I hope may be considered both entertaining and provocative to thought. You must imagine a young surgeon addressing an elderly non-affected member of a polyposis family.*

*"You are old, Father William," the young surgeon said,*
*" And your colon from polyps is free.*
*Yet most of your sibling are known to be dead*
*A really bad family tree."*
*"In my youth," Father William replied with a grin,*
*" I was told that a gene had mutated,*
*That all who carried this dominant gene*

*To polyps and cancer were fated.*
*"I sought for advice from a surgical friend,*
*Who sighed and said-' Without doubt*
*Your only escape from an untimely end*
*Is to have your intestine right out.'*
*"It seemed rather bad luck-I was then but nineteen-*
*So I went and consulted a quack,*
*Who took a firm grip on my dominant gene*
*And promptly mutated it back."*
*"This," said the surgeon, " is something quite new*
*And before we ascribe any merit*
*We must see if the claims of this fellow are true,*
*And observe what your children inherit!"*

His poem fit remarkable well the family tree shown in Figure 34.5. In 1951 however, Dukes imagined genes only as solid entities of unknown nature strung along chromosomes. The genes duplicated during mitosis and could be altered by mutation, but his speculations about the physical makeup of the gene were far off the mark. He did of course know that expression of a gene typically followed a dominant or recessive inheritance pattern, and the familial disease appeared to be inherited in a dominant fashion. That was only a year or two before the nature of the gene and the manner of its duplication were revealed by Watson and Crick.

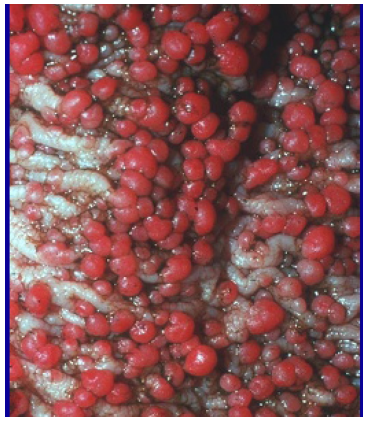

Figure 34.3. Huge numbers of polyps with adenomas in the colon of a teenager who had inherited familial adenomatous polyposis coli (Half et al., 2009).


Figure 34.4. C. E. Dukes and the Old St. Mark's Hospital on City Road, London. Cuthbert E. Dukes (1890-1977) was Pathologist and Director of Research Laboratory at St. Mark's Hospital. In 1932, he originated the famous Dukes staging system for colorectal cancer that was widely used until replaced by the TNM system (Bulow et al., 2006).

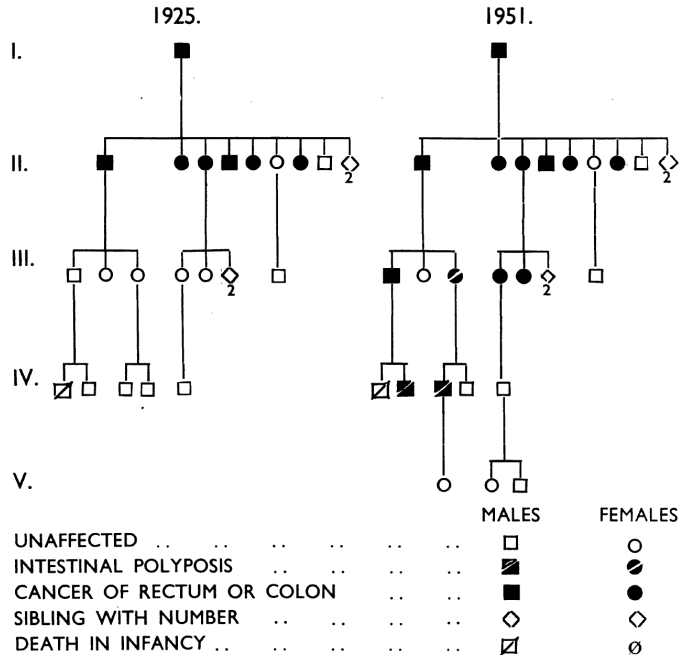


Figure 34.5. An adenopolyposis family charted by C. E. Dukes in1925 and 1951 (Dukes, 1952). During the interval, new family members were born and new cases of intestinal polyposis and colon or rectal cancer appeared.

## *Stages of colorectal cancer.*

In 1932, Dukes proposed the following staging system for colorectal cancer:

- **Dukes A:** invasion into but not through the bowel wall (90% 5-year survival)
- **Dukes B:** invasion through the bowel wall but not involving lymph nodes (70% 5-year survival).
- **Dukes C:** involvement of lymph nodes (30% 5-year survival).
- **Dukes D:** widespread metastases.

By 1954, Dukes staging had been modified and was commonly used for many years, although it was later replaced by the more detailed TNM (tumor, nodes, metastasis) system and was then no longer recommended for clinical practice. In 1954, Astler and Coller related the modified Dukes staging to the 5-year survival of 352 patients who were operated for colorectal cancer at the University of Michigan Medical School between 1940 and 1944 (Astler and Coller, 1954). Most common was stage B2 where the cancer had invaded deep into the bowel wall but had not spread to any lymph nodes; this included 47% of the patients of whom 54% survived 5-years. Next most common was stage C2 where the cancer had invaded deep into the bowel wall and had spread to some lymph nodes; this included 36% of the patients of whom 22% survived 5-years. Of the entire group of 352 patients, 44% survived 5-years. By 5-years, the death rates had leveled off, suggesting that most of the survivors may have been cured. That was the state of the diagnosis and surgical treatment of colorectal cancer in the 1940's: about 40% cure rate among those diagnosed in time. For rough comparison, the latest 5-year survival rates in the United States (as of 2022) once cancer had developed was 64% for colon cancer and 67% for rectal cancer according to the SEER (Surveillance, Epidemiology, and End Results) database maintained by the National Cancer Institute. If the SEER stage was regional (cancer spread to regional lymph nodes but no distant metastases), then 5-year survival for colon cancer was 77%; if there were distant metastases, the 5-year survival was only 14%.

## *Discovery of the APC gene.*

In 1987, researchers in the United Kingdom (Bodmer et al., 1987; Solomon et al., 1987) followed up on a clue from L. Herrera, A. A. Sandberg and coworkers (Herrera et al., 1986) that had reported an altered chromosome 5 in a patient with Gardner's syndrome which included a colon carcinoma as well as several severe genetic defects. It might seem like a nebulous clue but nevertheless led to a major discovery.

Investigators in the UK had a long interest in familial polyposis and carcinoma of the colon and rectum going all the way back to C. E. Dukes and his staging system in 1932. So they decided to take the leap and look to see whether chromosome 5 was involved in the familial disease (Bodmer et al., 1987). They collected DNA from 124 members of 13 families in whom the disease appeared to be inherited and for whom they were able to get clinical and pathology information from the Polyposis Register at St Mark's Hospital or from the Gastroenterology Unit, Broadgreen Hospital, Liverpool. They digested the DNA with restriction enzymes and hybridized the fragments with probes specific to chromosome 5. Figure 34.6A shows an example of a family whose members had their DNA

treated with one of the restriction enzymes and hybridized with one of the probes. We see that the members who had the disease (filled symbols) produced two bands while the unaffected members (open symbols) produced three. Thus, the affected members had lost part of one of their two chromosomes 5. Moreover, the loss was in all of the body cells. However, to produce the disease, both chromosomes 5 would have to be defective, yet one appeared to be normal. Although the vast majority of the cells had a normal chromosome 5, it could take an inactivating mutation in just one cell for it to multiply into a premalignant growth. Since mutations were rare, it could be years before tumors appeared.

They went on to determine where on chromosome 5 most of the hybridization took place and found that it centered in the 5q21-22 region of the long arm of the chromosome (Figure 34.6B). So that was where the gene they were looking for most likely was to be found.

Meanwhile, their colleagues asked whether the familial gene they had localized to chromosome 5q21-22 was also implicated in the sporadic cases (Solomon et al., 1987). They examined DNA from 45 cases and found that 20% of them showed loss in chromosome 5 (Figure 34.6C), from which they inferred that at least some of the sporadic cases likely involved the same gene as in the familial cases.

"The game was afoot," as a well-known British detective might have said, although the next phase of actually finding and cloning the gene was taken up by investigators mainly in the USA. An international team, largely with the inspired direction of Bert Vogelstein, then coordinated efforts and succeeded to identify the causative gene, *APC* (adenomatosis polyposis coli), in chromosome 5q21 (Groden et al., 1991; Kinzler et al., 1991a; Kinzler et al., 1991b; Nishisho et al., 1991).

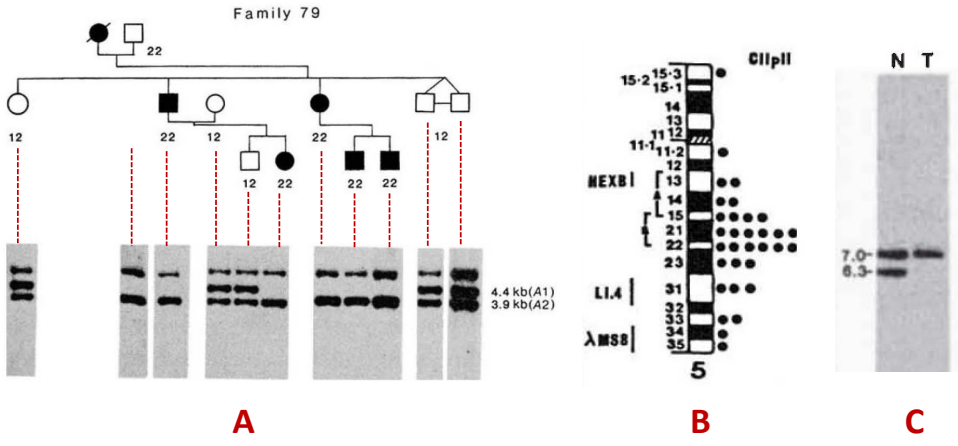


Figure 34.6. How a gene associated with familial and sporadic (non-familial) adenomatous polyposis coli was localized to the 5q21-22 region of chromosome 5 (Bodmer et al., 1987; Solomon et al., 1987).
**A**. Unaffected members of this family (open symbols) showed three DNA restriction fragments of different sizes in their genome (after their DNA was digested with a particular

restriction enzyme and hybridized with a particular radioactively labeled probe). Affected individuals (filled symbols) showed only two fragments, indicating that a relevant part of an inherited chromosome was missing (Bodmer et al., 1987). (Squares, males; circles, females.)
**B**. The probe used in **A** localized most strongly at region 5q21-22 of the long arm of chromosome 5, suggesting that a gene associated with the disease was in or near that region (Bodmer et al., 1987).
**C**. Loss of a restriction fragment from chromosome 5 of a sporadic adenocarcinoma of the sigmoid colon (Dukes stage B) relative to adjacent normal mucosa. About 20% of 45 patients showed loss in a chromosome 5 fragment (N, normal; T, tumor) (Solomon et al., 1987). Thus, at least some of the sporadic cases had the same gene basis as the familial cases.

## *From Wnt to APC.*

That concise title came to me on awaking this morning to embody both information and mystery. APC should be familiar. Wnt you may have heard of. But Wingless (to be explained below) would seem superfluous for us wing-lacking creatures. Mindful of Son-of-Sevenless on the way to the RAS cancer genes in Chapter 18, however, it should not surprise that Wingless is a name given by fruit fly geneticists to a mutation of those normally winged beings (Figure 34.7). A taste for whimsy now delights me to relate how fruit flies with defective wings helped to reveal the pathway leading to polyps and cancer in the colon.

A mutation of fruit flies affecting the development of wings was reported by R. P. Sharma of the Indian Agricultural Research Institute, New Delhi, in 1973. The progeny of wingless flies, however, were not always wingless. In fact, they were wingless, one-winged, or two-winged, always at a ratio of 2:2:1 regardless of the wing status of the parents (Figure 34.7). This indicated to geneticists that the mutated gene had incomplete penetrance and variable expressivity (Sharma and Chopra, 1976). That made sense when later the *Wingless* mutation was found to be in an enhancer region 3' to the gene, not within the gene itself. Note that *Wingless* affects wings while *Frizzled* and *Disheveled* affect hairs *on the wing*. We shall see that downstream relationship preserved in the corresponding mammalian genes: first wings then hairs on the wing; in mammals *Wnt* signals to *Fzd* and then *Dvl*.

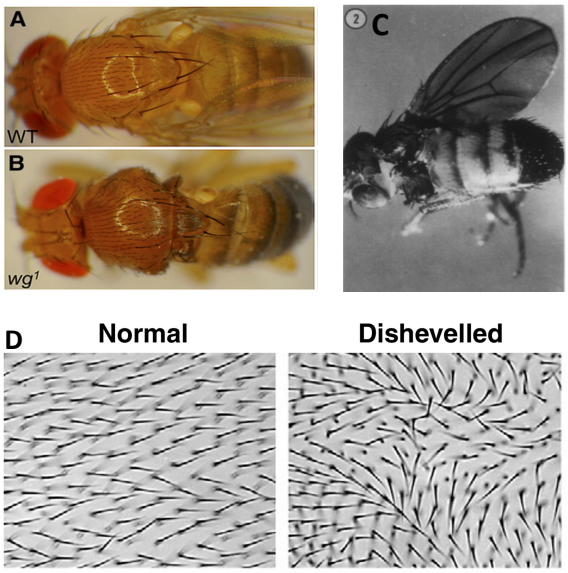


Figure 34.7. *Wingless*, *Frizzed*, and *Disheveled* mutations of fruit flies.
**A**. Normal; **B**. *Wingless* with both wings absent (Bejsovec, 2018). **C**. *Wingless* with one wing absent (Sharma and Chopra, 1976). **D**. Disorganized hairs on a *Disheveled* fruit fly wing (Zhong et al., 2021). The *Frizzled* mutation disrupted the normal pattern of wing hairs similarly to the *Disheveled* mutation.

The story of the mammalian Wnt (homolog of Wingless) pathway began 40 years ago with an investigation by Roel Nusse and Harold Varmus of DNA from tumors induced by mouse mammary tumor virus (MMTV) (Nusse and Varmus, 1982). Several of the tumors had viral DNA inserted into the mouse genome. They then cloned DNA fragments covering the junctions between the viral and mouse genomes in those tumors. The mouse gene into which virus had integrated appeared to be a novel protooncogene that they called *int-1*. Further investigation disclosed that the fruit fly had a gene of similar sequence. Quite remarkably, that fruit fly homolog of the mammalian *int-1* gene turned out to be identical to the fruit fly *Wingless* gene (Rijsewijk et al., 1986). Moreover, the mammalian and fruit fly versions of the gene showed 54% identity of amino acid sequence and the locations of the cysteines in the proteins was preserved. They therefore renamed the novel protooncogene by combining *int-1* and *Wingless* to yield the new name *Wnt*.

The next discoveries of how Wnt relates to adenomatous polyposis colon cancer were the mammalian versions of the fruit fly *frizzled* and *disheveled* genes (Figure 34.7D), their functions, and their potential as targets for therapy. The fruit fly *frizzled* gene was found to code for a membrane protein that looped 7 times through the cell surface membrane, a structure characteristic for a type of receptor protein, a so-called G-protein-coupled receptor, that can bind a growth factor or other signaling molecule at the cell surface. Samuel Chan, Robert Nissenson and their colleagues at the University of California, San Francisco, then, in 1992, discovered in rat osteosarcoma cells two cDNA's that coded for proteins that were homologous to the fruit fly's Frizzled protein (Chan et al., 1992). The proteins coded by the two new mammalian genes, *FZD1* and *FZD2*, like the protein coded

by the fruit fly *Frizzled* gene, resembled G-protein-coupled receptors. But what was the ligand that bound and signaled to FZD-receptors? Next, the ligand – the presumed signaling molecule – that bound to FZD-receptors at the surface of mammalian cells was discovered in fact to be Wnt (Bhanot et al., 1996).

FZD, being a receptor in the cell surface membrane, connects between outside and inside. To do that, it binds something on the outside and something on the inside. On the outside, it bound Wnt. What it bound on the inside was in fact the mammalian disheveled protein, DVL. Thus, signals from outside to inside the cell would go from Wnt to FZD to DVL (Wallingford and Habas, 2005).

Wnt, being secreted by some cells and bound by receptors on other cells, would serve to communicate influences between different cells during development of the embryo and coordinated functions in the adult.

## *From Wnt and APC to adenomatous polyposis.*

Wnt proteins are present in all multicellular organisms and have multiple essential functions. Nineteen Wnt proteins are known in mammals and all have 23-24 conserved cysteines in their primary structure near the N-terminus. Of several pathways from Wnt in mammals, we focus on the so-called canonical pathway that leads to β-catenin binding and activating transcription factors TCF/LEF in the nucleus. The pathway explains how adenomatous polyps form as precursors of colon cancer and shows where drugs could be targeted.

The Wnt's activate the pathway by interacting with a cysteine-rich domain in the Frizzled (FZD) protein in the surface membrane of Wnt-receptor cells. The pathway is diagrammed and explained in Figure 34.8. The end-effect of Wnt in the canonical pathway is to stabilize β-catenin and thereby stimulate the transcription of genes for cell replication. β-catenin levels are kept low by a "destruction complex" consisting of a glycogen synthase kinase (GSK3), a casein kinase (CK1), a scaffold protein (Axin), and adenomatous polyposis coli (APC). GSK3 and CK1 phosphorylate β-catenin, leading to its degradation by proteasomes. Wnt binding to Fzd brings in two additional proteins, LRP5 or 6 and disheveled (DVL) to form a cell surface complex that binds the destruction complex and prevents it from destroying β-catenin. That is essentially how Wnt stimulates the transcription of gene that promote cell proliferation (as summarized by (Mehta et al., 2021)).

The Wnt-FZD combo recruits DVL to the membrane. DVL then recruits the destruction complex by binding the Axin component of the complex. Axin recruits LRP5 or 6, which stabilizes the complex. With the destruction complex bound to DVL at the cell surface membrane, the complex cannot destroy β-catenin. If APC is inactivated by mutation, as in familial or sporadic adenomatous polyposis of the colon, the destruction complex is unable to function, β-catenin accumulates, and the cell is induced to proliferate and to form polyps.

## *From Wnt-secreting cells to Wnt-responsive cells.*

The previous section discussed the pathway from Wnt binding at the cell surface to the transcription of β-catenin-responsive genes that stimulate cell proliferation. But where does Wnt come from? Wnt is produced by Wnt-secreting cells and then moves through the extracellular fluid to binding sites on cells that have Wnt-receptor complexes on their surface (described in the upper part of Figure 34.8) (Mehta et al., 2021). Wnt-secreting cells synthesize Wnt in the endoplasmic reticulum, where Wnt binds the multi-pass transmembrane protein, Porcupine (Porc), that adds a hydrophobic group (palmitate) to the protein. The palmitate group helps Wnt binding to membranes. Wnt-palmitate then transfers to the Golgi membranes, where it is bound by another multi-pass transmembrane protein, Wntless (Wls). The Golgi system secretes vesicles with Wnt-palmitate bound to Wls on the vesicle membrane. Finally, Wnt-palmitate is picked up from the extracellular vesicles by Frizzled receptors on Wnt-responsive cells.

## *The canonical Wnt pathway in adenomatous polyposis and colon cancer.*

The essential outcome function of the canonical Wnt pathway is the induced transcription of genes promoting cell proliferation and stem cell renewal, particularly in colon cancer (bottom of the simplified molecular interaction map in Figure 34.8, which summarizes the essential processes as currently understood (Elez et al., 2022; Giebel et al., 2021; Swoboda et al., 2022; Tewari et al., 2021).

The transcription of these cell-proliferation genes, which include cyclin D1 and Myc, is induced by β-catenin binding to the transcription factor TCF/LEF in the cell nucleus. To avoid excessive cell proliferation, the level of β-catenin is tightly controlled by degradation by means of a destruction complex. This complex includes two kinases -- glycogen synthase kinase (GSK3) and casein kinase (CK1) – that phosphorylate β-catenin. The complex also includes APC that mediates the ubiquitination of the phosphorylated β-catenin and its consequent degradation by the destruction complex and proteasomes (see Figure 34.8). Essential to the function of the destruction complex is the presence also of Axin that holds the complex together. As already noted earlier in this chapter, mutation of APC, which inactivates the destruction complex, is the most common cause of adenomatous polyposis of the colon, the first step of a mutation sequence leading to cancer (Figure 34.2).

Several processes inhibit the function of destruction complex, thereby allowing β-catenin to stimulate the transcription of the cell proliferation genes; some of these processes are controlled and some are uncontrolled.

An important controlled process initiates when Wnt binds to a combination of a receptor of the FZD family and co-receptor LRP5 or 6. The Wnt-FZD-LRP complex then recruits DVL at the cell surface (Figure 34.8). Any combination of the 19 Wnt's, 10 FZD's and 2 LRP's may recruit DVL, although the eventual consequences for different cancer types may differ

(Tewari et al., 2021). The bound DVL then sequesters the destruction complex to the cell surface, where it phosphorylates LRP5/6, resulting in exclusion of Axin from the complex. β-catenin then cannot be destroyed and survives to enhance cell proliferation. This is a direct control by Wnt to inhibit the destruction of β-catenin.

The destruction of β-catenin then is controlled by the amount of Wnt available for binding at the cell surface. The amount of Wnt available depends on how much is secreted by nearby cells, which secrete Wnt attached to a multi-pass protein in the membrane of vesicles that float in the extra-cellular fluid (upper part of Figure 34.8). As already mentioned above, Wnt-secreting cells synthesize Wnt in the endoplasmic reticulum, where it binds to the membrane protein, Porc, which adds a palmitate chain that allows Wnt to bind to membranes (Mehta et al., 2021). The Wnt-palmitate then transfers to Wntless (Wls), a multi-pass protein in the Golgi membranes, an apparatus that secretes molecules in or on vesicles. Those are the essentials of how Wnt extra-cellular vesicles are produced and how one aspect of the canonical Wnt pathway in Wnt-receptor cells could be regulated (Figure 34.8). Additional means of regulation may come from controls on FZD, LRP5/6, and DVL at the surface of those receptor cells.

A third control on β-catenin (added in Figure 34.9) is the E-cadherin surface protein of epithelial cells that binds epithelial cells together. E-cadherin can bind β-catenin and make it unavailable to the TCF/LEF transcription factors (Jeanes et al., 2008; Mendonsa et al., 2018). E-cadherin is also a tumor suppressor gene: its loss promotes tumor progression and metastasis. By binding β-catenin, it reduces cell proliferation. E-cadherin is a single-pass transmembrane protein. The extracellular domains of E-cadherin on adjacent epithelial cells bind each other to hold the cells tightly together; this inhibits their division, migration, and invasion, effectuated largely by β-catenin binding.

A fourth control comes from the extracellular Dickkopf-1 protein (DKK1) that was reported to negatively regulate the Wnt/β-catenin pathway by binding LRP6 and thereby inhibiting the binding between the Wnt core complex and the destruction complex (Aguilera et al., 2015; Chu et al., 2021) (Figure 34.8).

Finally, a general control of the Wnt pathways was recently shown to be provided by RNF43, a transmembrane protein that stimulates the proteasomal degradation of the Wnt core complex (Figure 34.8) (Elez et al., 2022; Mikaeel et al., 2022). Moreover, the action of RNF43 is inhibited by USP42, providing additional complexity to this control (Giebel et al., 2021).

An uncontrolled process stimulating the canonical Wnt pathway is biallelic inactivating mutation of APC (Figure 34.8), the main cause of adenomatous polyposis of the colon. Without APC, the destruction complex cannot function; as a result, β-catenin accumulates and stimulates proliferation of stem cells in the colon crypts, giving rise to polyps that set the stage for other gene mutations resulting in cancer. A less common type of mutation – an activating mutation of β-catenin – also leads to the same effect. Inactivating mutations

of APC and activating mutations of β-catenin together were found in about 80% colorectal cancers (Cancer Genome Atlas, 2012).

## *The puzzle of Axin2*.

In 1998, Jurgen Behrens, Walter Birchmeier and their colleagues in Berlin and Ulm, Germany, reported that a protein they had called conductin interacted with APC, GSK3β and β-catenin to direct the latter to degradation (Behrens et al., 1998). Determination of conductin's amino acid sequence and binding assays showed that it had distinct domains for each of those three interactions. Moreover, the amino acid sequence of conductin was about 50% identical to Axin, which led to conductin being renamed, Axin2. In 2002, they found that Wnt signaling stimulated the *Axin2* gene, resulting in high levels of Axin2 mRNA and protein, and that this stimulation occurred specifically in colorectal and liver tumors but not in other cancers (Lustig et al., 2002). Since most colorectal cancers have APC inactivating mutations, which would inactivate the destruction complex, β-catenin would be available to activate transcription and Axin2 would be transcribed, presumably regardless of Wnt. We will see that canonical Wnt signaling is more complicated and has more connections of consequence than shown in the simplified picture in Figure 34.8.

One complication is that β-catenin-stimulated expression of TCF4 and hence of Axin2 does not occur in all cells of the intestinal epithelium. Rather, it is confined to the stem cells in the epithelial crypts; those are the only cells that divide to produce the other epithelial cells (Figure 34.10) (Lustig et al., 2002).

In 2012, Steven J. Weiss and coworkers at the University of Michigan found a new connection in the function of Axin2 (Wu et al., 2012). They found that Axin2 stimulated the production of Snail1, a protein that was known to convert epithelial cells to a mesenchymal type that tends to migrate, invade, and metastasize. In so doing, Snail1 inhibited the production of E-cadherin, an important protein for epithelia that holds adjacent epithelial cells together. Moreover, E-cadherin was known to bind β-catenin, making it unavailable for LEF/TCF-stimulated transcription of Axin2. These interactions via Snail1 appear to constitute a positive feedback loop that would tend to increase the level of β-catenin (Figure 34.9). However, Behrens, Birchmeier and their colleagues had surmised that Axin2 initiated a negative feedback loop that would moderate β-catenin activity (Lustig et al., 2002), which would be the case if Axin2, upon replacing Axin, would increase the activity of the destruction complex (Figures 34.8 and 9).

Adding to the picture, Moshkovsky and Kirschner at Harvard Medical School recently found interesting time-dependence of the action of Wnt on Axin2 (Moshkovsky and Kirschner, 2022) (Figure 34.11A). From their findings, they deduced that the action of Wnt on Axin2 involved two feedback loops (Figure 34.11B). This proposed network could be consistent with Figure 34.9 if one takes into account the time delays involved in the transcription and degrardation events.

## *Diverse expression of MYC, Cyclin D1, and Axin2.*

However, some findings on the expression of Myc, Cyclin D1, and Axin2 in colorectal cell lines suggest that the picture in Figures 34.8 and 9 is incomplete. According to Figures 34.8 and 9 those three genes should be co-stimulated by TCF/LEF – unless there were other factors controlling the expression of those genes. Examination of the expressions (mRNA levels) in NCI's cellminerCDB indicated that they were not mutually correlated (Figure 34.12). It seems therefore that there may be diversity in the expression of those functionally important genes among the cells of the same or of different tumors. Moreover, the expressions of Cyclin D1 and MYC appeared to be negatively correlated, which would suggest that cell division among the colorectal cell lines was mainly driven by one or the other of those factors. The expressions of one or more of those three genes may be controlled also by factors than only TCF/LEF -- for example, the EGFR-MEK pathway (Figure 34.13C). Cells may differ in regard to which pathway dominates. There are in fact many reported Myc interactions that may affect Myc expression in colorectal cancer (reviewd by (Tan et al., 2022)).

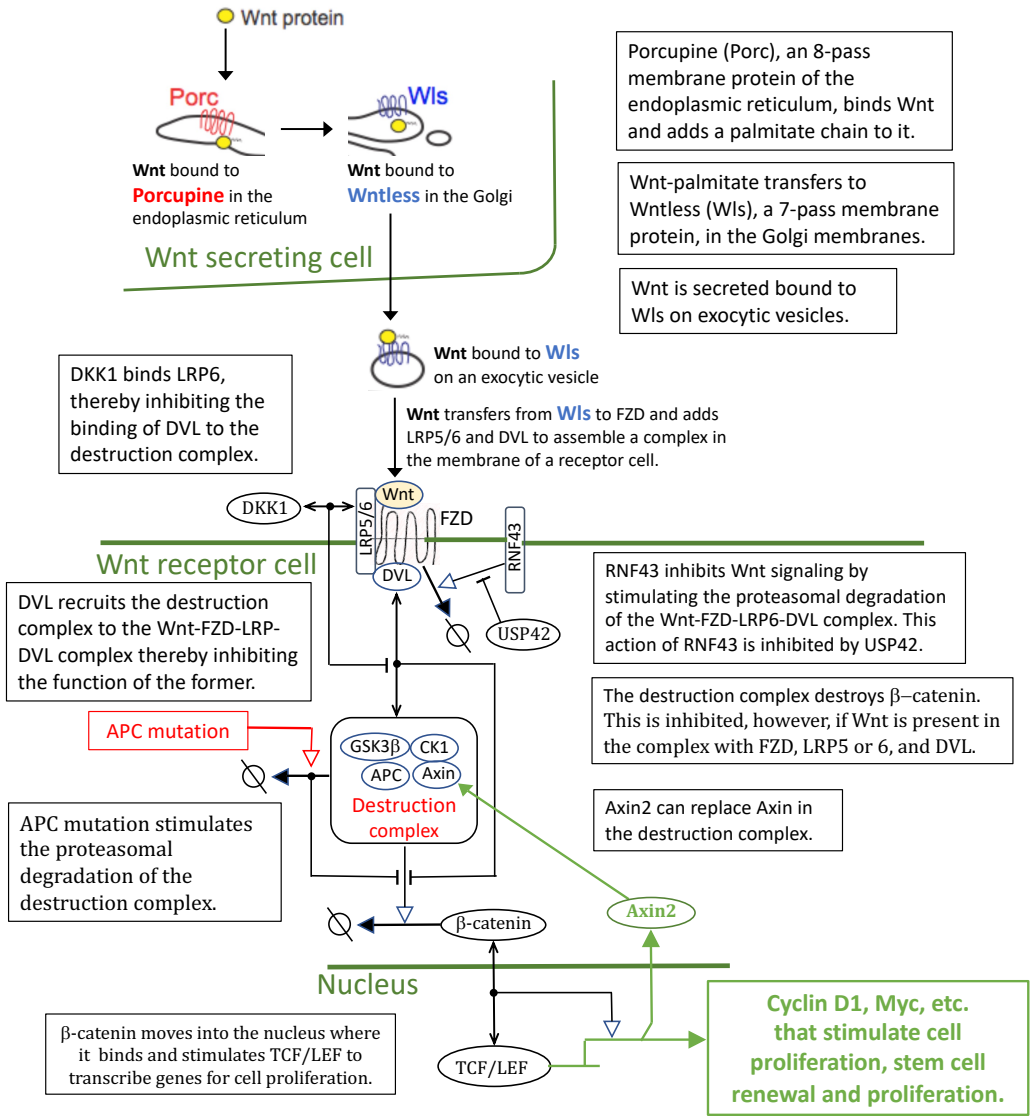


Figure 34.8. A simplified molecular interaction map showing the essentials of the process leading from the production of Wnt in Wnt-secreting cells to the stimulation or inhibition of β-catenin in Wnt receptor cells with consequent effects on proliferation through β-catenin-stimulated transcription by TCF/LEF of relevant genes. (Based on information from (Elez et al., 2022; Giebel et al., 2021; Swoboda et al., 2022; Tewari et al., 2021).)

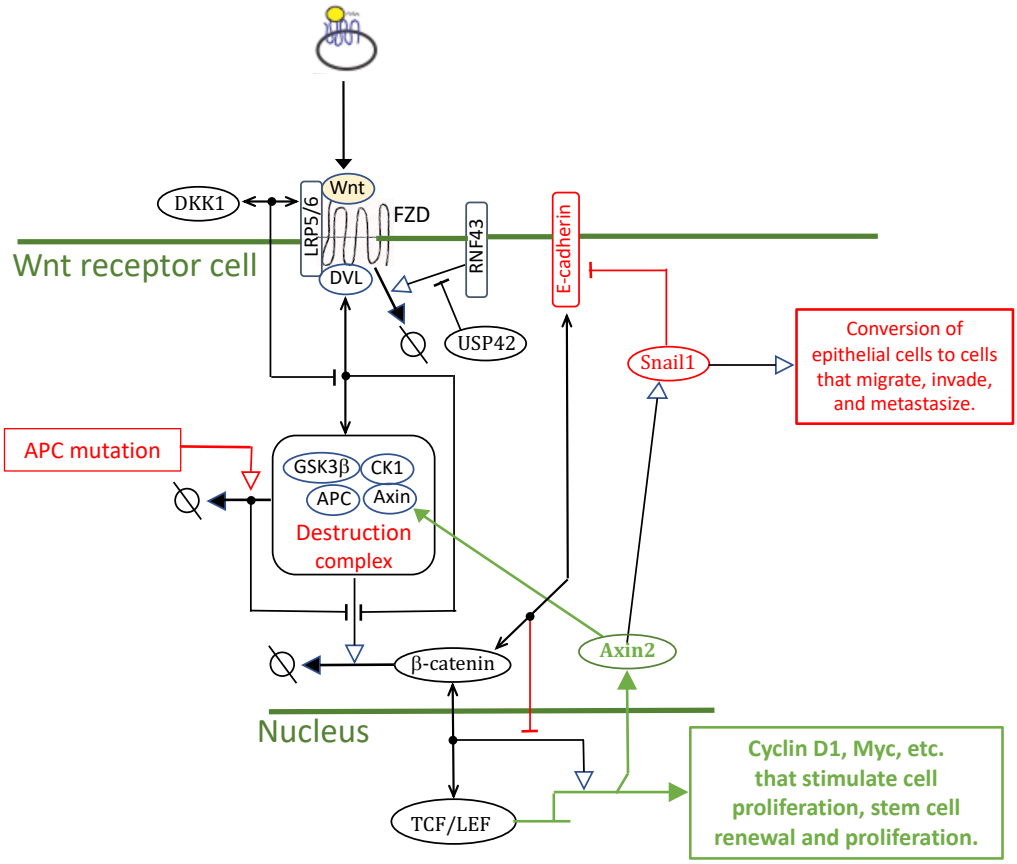


Figure 34.9. How Axin2→Snail1→E-cadherin→β-catenin→Axin2. (Based on findings by (Wu et al., 2012).) Since the loop has two inhibitory steps (red), it is a positive feedback.

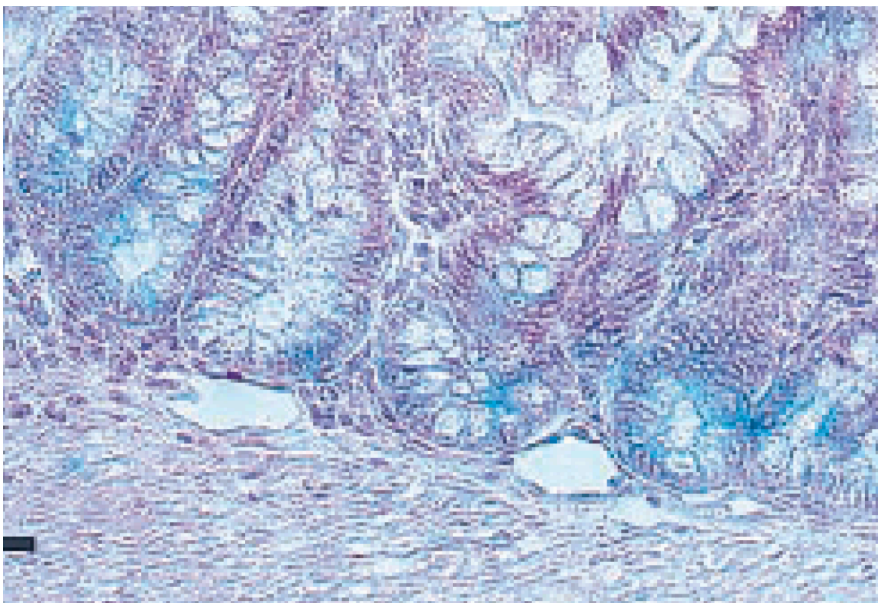

Figure 34.10. Mouse intestinal epithelium showing that Axin2 (blue staining) is expressed selectively in the crypts, which is where cell division for renewal of the epithelium takes place (from (Lustig et al., 2002)). The same was true for expression of TCF4.

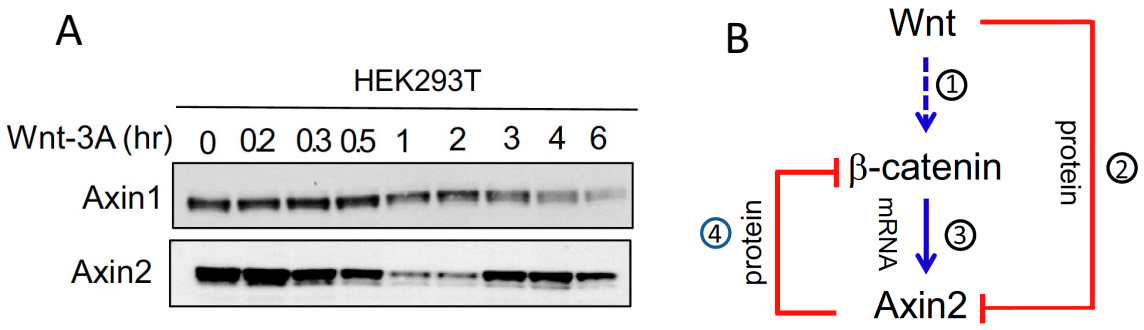


Figure 34.11. **A**. Artificially activating Wnt in a colorectal cancer cell line first inhibited Axin2 but later allowed it to recover. Similar patterns were found in two other colorectal cancer cell lines (Moshkovsky and Kirschner, 2022). **B**. Proposed interaction scheme with two nested feedbacks (Moshkovsky and Kirschner, 2022).

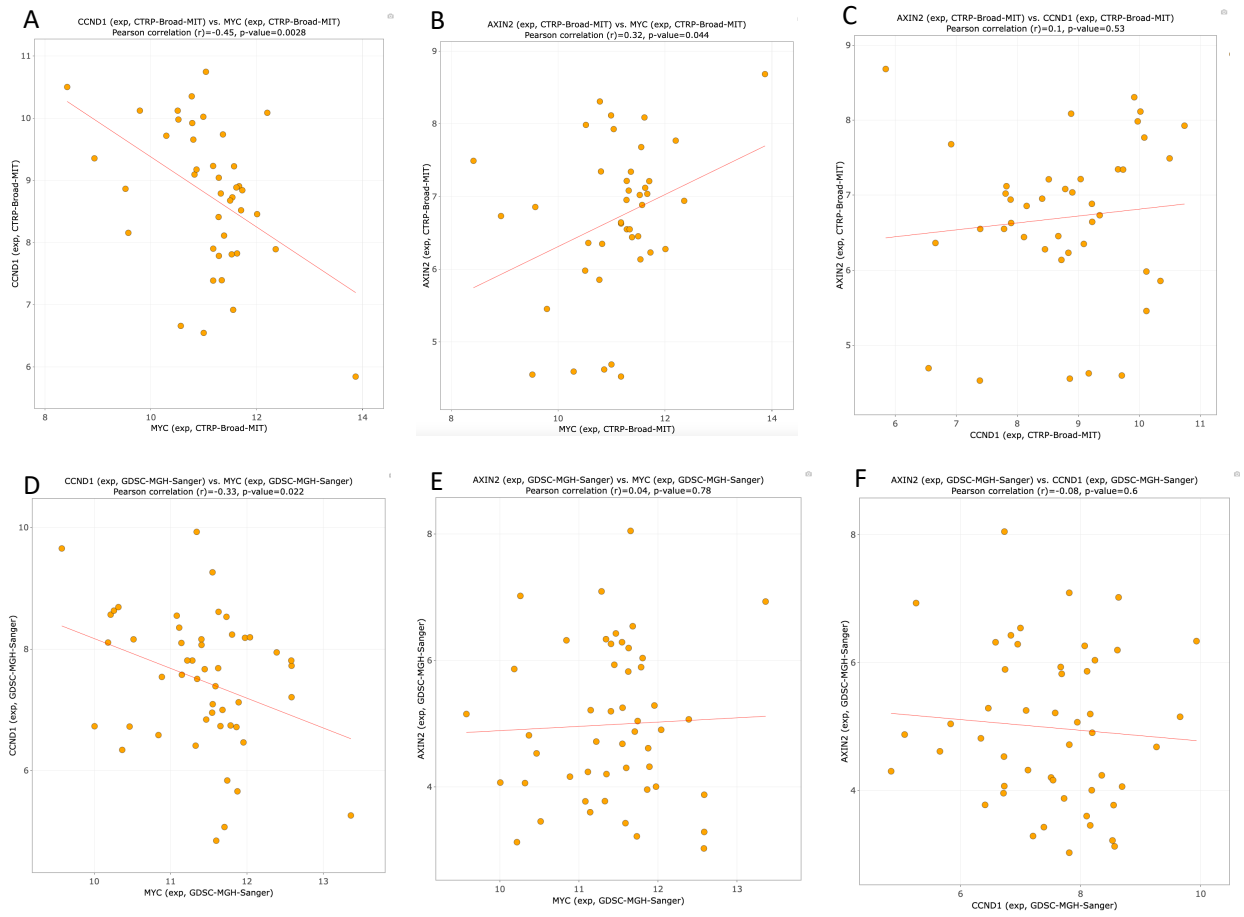


Figure 34.12. Expression levels (mRNA) of MYC, cyclin D1 (CCND1), and Axin2 in colorectal cell lines in the CTRP-Broad-MIT (**A**-**C**) and GDSC-MGH-Sanger (**D**-**F**) databases as displayed by cellminerCDB (version 1.6, release October 2022; https://discover.nci.nih.gov/rsconnect/cellminercdb/). The scales are in factors of 2. **A**,**D**, Cyclin D1 versus MYC; **B**,**E**, Axin2 versus MYC; **C**,**F**, Axin2 versus Cyclin D1. Of the 6 panels, only **B** (Axin2 versus CyclinD1 in the CTRP-Broad-MIT database) showed the expected positive correlation, but this was not confirmed in the GDSC-MGH-Sanger database (**E**). The expressions of MYC and Cyclin D1 appeared to be inversely correlated (**A**,**D**).

## *Therapy of colorectal cancer targeted to the Wnt/β-catenin pathway.*

Before the development of targeted therapies, the usual treatments for colorectal cancer – surgery and chemotherapy – were generally disappointing. Recent therapies giving more promising results mostly targeted processes outside of the Wnt/β-catenin pathway (Xie et al., 2020). A few inhibitors of steps in the Wnt/β-catenin pathway were indeed tested in metastatic colorectal carcinoma but without notable success (Krishnamurthy and Kurzrock, 2018).

5-Fluorouracil (5FU) was long a mainstay for therapy of colon cancer (Chapter 6) but resistance soon developed often associated with overexpression of TCF4 that enhanced the expression of cell proliferation genes (Figure 34.8) (Kendziorra et al., 2011; Zhao et al., 2022). Moreover, inhibitors of β-catenin sensitized colorectal cancer cells to radiochemotherapy (Emons et al., 2017; Zhu et al., 2021). This would be contrary to the usual increased 5FU sensitivity of proliferating cells. More important however may be the better ability of proliferating cells to repair radiation-induced DNA double-strand breaks. Thus proliferating (as opposed to non-proliferating) cells would be better able to survive 5FU treatments with or without radiation.

Drug-resistant cancer stem cells were reported to exist as a small fraction of the cells in colon cancers and were proposed as therapy targets. Colon cancer stem cells, obtained from the tumors based on the cells' CD133 expression, reproduced the tumors when the stem cells were implanted in immune-deficient mice (Prieto-Vila et al., 2017; Ricci-Vitiani et al., 2007). Cancer stem cells were thought protected from the toxic effects of drugs by the stem cells' neighborhood consisting of normal cells and extracellular matrix, which was described as a niche that allowed tumor cells to proliferate. Canonical Wnt/β-catenin signaling was thought to enhance the stemness of cancer stem cells (Xu et al., 2019). Wnt/β-catenin signaling was enhanced by the G-protein-coupled receptor, Lgr5, whose expression was associated with stemness and was considered as a potential target for therapy. Lgr5 was found to be a biomarker for colorectal adenocarcinoma stem cells. These studies were at the forefront of colon cancer therapy investigations, and we could look forward to further developments..

## *Inhibitors of the EGFR-BRAF pathway for treatment of metastatic colon cancers having mutations in the* **RNF43** *gene.*

Cancers arising in the descending colon and rectum differ greatly from those arising in the ascending colon. The latter differ from the former in having mutations of DNA mismatch repair genes and instability of DNA microsatellites and were the subject of Chapter 25. Microsatellite instability was explained in Figure 25.7 and associated text in that chapter. The distinction between those two kinds of colon cancers is important for we say here, because of a recently discovered therapy that works only for colon cancers having *stable* microsatellites, which are in fact the cancers of the descending colon and rectum that are the focus of this chapter.

An international research group led by Roderigo Toledo was investigating therapy of microsatellite-*instability* colon cancers that occasionally had mutations of the *BRAF* gene, a mutation often found in malignant melanomas, which made them amenable for treatment with inhibitors of the EGFR-BRAF pathway (Chapter 19). That treatment however did not work for the colon cancers having that mutation. Investigating further, they discovered unexpectedly that the treatment did work for microsatellite-*stable* colon cancers that, instead of *BRAF* mutations, had mutations of the *RNF43* gene (Elez et al., 2022).

RNF43 is a membrane protein that stimulates the destruction of the core Wnt complex (Figure 34.9), presumably resulting in inhibition of all of the Wnt pathways, including the canonical pathway that is usually blocked by *APC* mutations in cancers of the descending colon and rectum. The situation is rather complicated and not fully understood, but it turned out that inhibitors of the EGFR-BRAF pathway improved the survival of those colon cancer patients whose cancers had *microsatellite-stable* genomes (Elez et al., 2022) (Figure 34.13).

The Wnt and EGFR-BRAF pathways are alternative drivers of cell proliferation. When the Wnt pathways are blocked by *RNF43* mutation and the EGFR-BRAF pathway is inhibited by drugs, the cancer cells would be unable to multiply. The combined inactivation of the Wnt and EGFR-BRAF pathways therefore helps survival in microsatellite-stable metastatic colon cancer, which would be predominantly cancers of the descending colon and rectum.

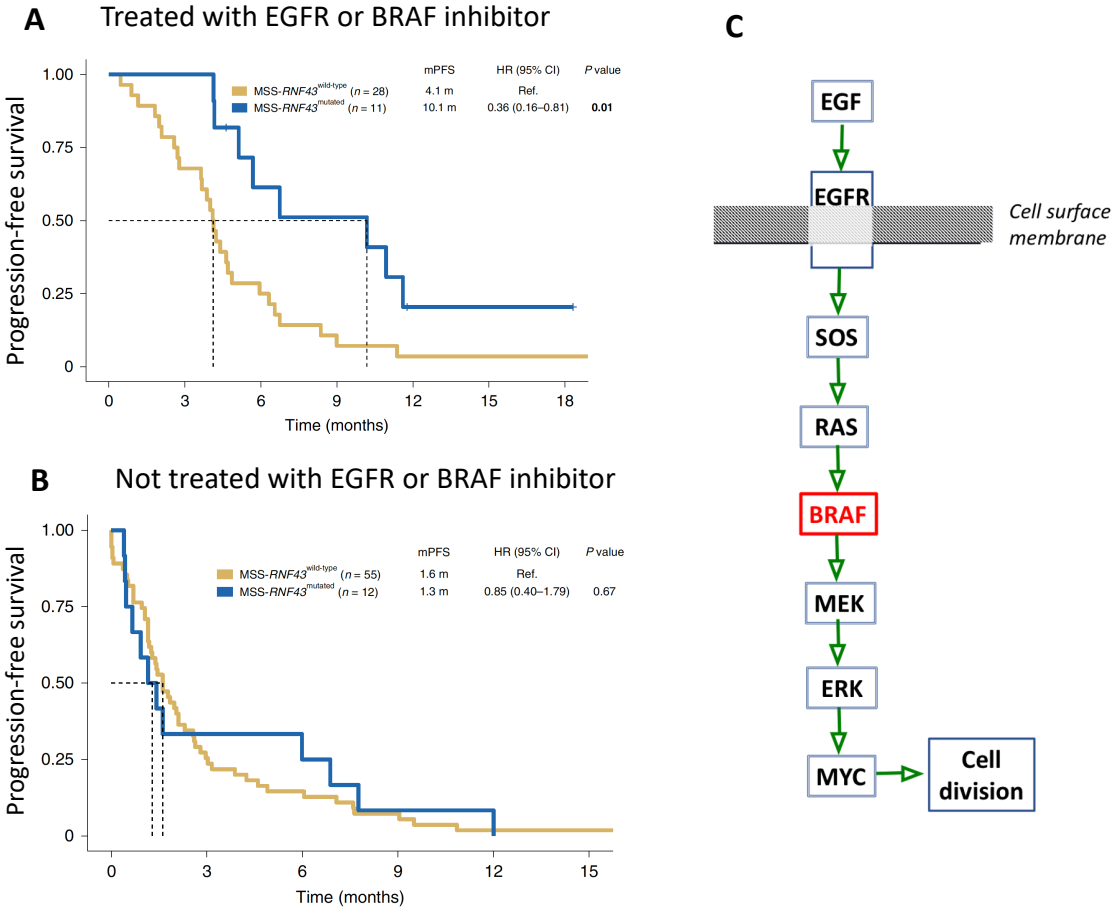


Figure 34.13. **A**, **B**, Progression-free survival (PFS) of microsatellite-stable (MSS) metastatic colorectal cancers. (Months before the cancer progressed.) **A**. Patients treated with an inhibitor of EGFR and/or BRAF. **B**. Patients who did not receive treatment with an EGFR or BRAF inhibitor. *Blue*, mutated *RNF43*. *Yellow, RNF43* not mutated. Cancers with microsatellite-stable genomes most likely arose in polyps in the left side of the colon or rectum. **C**. Pathway from EGFR via BRAF to MYC. (**A** and **B** are from (Elez et al., 2022).)

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