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Review

Changes in the Wnt signalling pathway in gastrointestinal cancers and their prognostic significance

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Abstract

Many steps in the Wnt signalling pathway may be altered during the process of carcinogenesis. This Review focuses on the changes observed in gastrointestinal cancers. A literature search was undertaken and the currently available data summarised. Understanding the alterations to this signalling pathway may help to reveal future targets for therapeutic agents. In addition, since in some tumours, levels of components of the Wnt pathway have been found to correlate with clinical stage, their potential use as prognostic indicators is highlighted.

Keywords: Wnt pathway; Gastrointestinal cancer; β-Catenin

1. Introduction

Cancer cells are characterised by their ability to proliferate in defiance of normal controls and by the potential to invade other tissues. A series of mutations are needed for a cell to become cancerous and different combinations of mutations are found in different cancers. However, certain alterations are encountered frequently. Changes in concentration, conformation or cellular location of intracellular molecules can affect signalling pathways and lead to cancerous change. Identifying the responsible molecules and defining these sequences of events are important for understanding the disease process and may uncover novel targets for future therapies.

The Wnt pathway (known as the wingless pathway in *Drosophila*) has a role in organ development in a number of species, but when aberrantly activated is associated with carcinogenesis. For example, over 90% of colorectal cancers have a mutation that activates this pathway [1].

The Wnt family of genes code for a group of glycoprotein signalling molecules. The pathway these glycoproteins then activate has been implicated in a number of different human cancers. This Review paper will concentrate on its role in gastrointestinal tumours. Understanding the role of this pathway in carcinogenesis is important, as some of its components have been shown to correlate with the clinical stage of some tumours and may therefore be useful prognostic aids. In addition, targeting inhibitory agents towards steps which are known to be inappropriately activated in carcinogenesis may be useful in the development of chemopreventive or chemotherapeutic agents.

2. β-Catenin breakdown

β-Catenin, coupled with α-catenin, normally connects the cell membrane adhesion molecule E-cadherin to the actin cytoskeleton. Free β-catenin is degraded following

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binding to a complex consisting of the molecules adenomatous polyposis coli (APC), axin, and glycogen synthase kinase-3β (GSK-3β). APC mutation is known to be of great importance in gastrointestinal carcinogenesis. Once bound, β-catenin is phosphorylated. Phosphorylated β-catenin binds to the protein β-TrCP, following which it is tagged by a small protein called ubiquitin, in preparation for degradation by a proteosome (Fig. 1).

3. The effect of Wnt binding on the β-catenin–TCF pathway (the canonical Wnt pathway)

Wnt binds to one of a family of Wnt receptors, proteins that span the cell membrane seven times. These receptors are encoded by the Frizzled genes, (FZD1–10). Binding of Wnt leads to phosphorylation of the cytoplasmic protein Dishevelled (Dsh), which then binds to axin and causes dissociation of the APC/axin/GSK complex. This in turn means that β-catenin is unable to bind and free β-catenin accumulates. This translocates to the nucleus where it binds to T-cell factors and activates transcription of a number of genes, including c-Myc, cyclin D1, matrix metalloproteinase-7 (MMP-7), and immunoglobulin transcription factor 2 (ITF-2). Nuclear β-catenin is therefore the hallmark of an active Wnt pathway.

4. The non-canonical Wnt pathways

Wnt binding may also lead to signal transduction via two other pathways. First, binding to some FZD receptors leads to intracellular calcium release and activation of protein kinase C (PKC). Alternatively, Dsh also activates the jun-N terminal kinase (JNK) pathway. JNK translocates to the nucleus where it regulates the activity

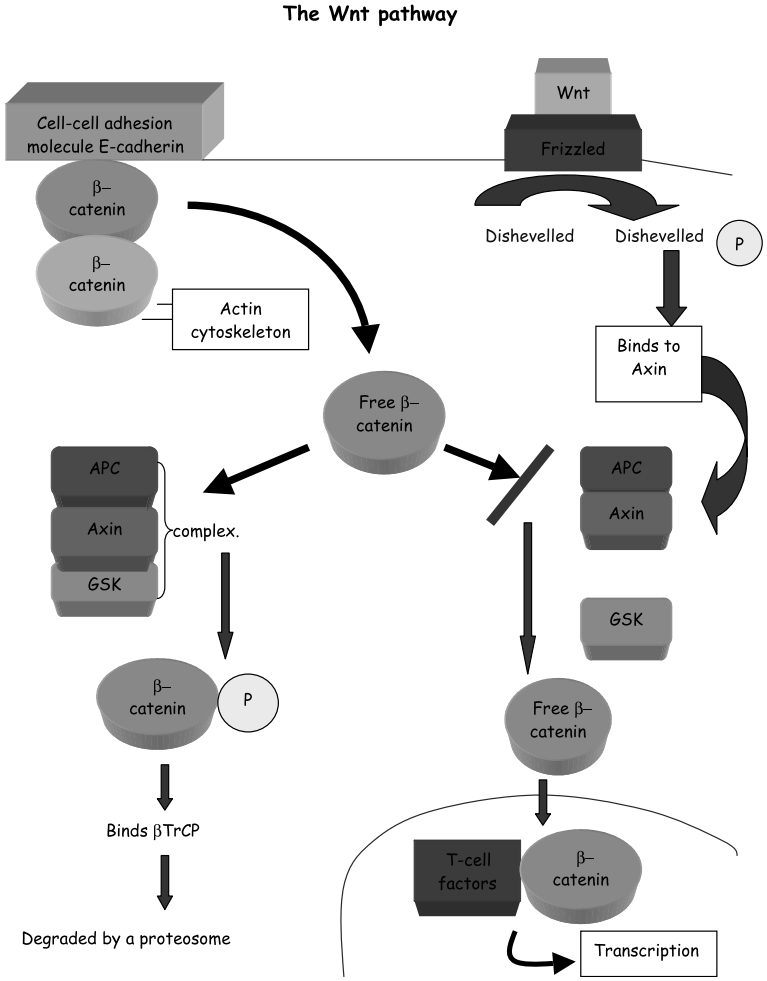

Fig. 1. In the absence of a Wnt signal, β-catenin binds the APC/Axin/GSK complex, is phosphorylated, and subsequently degraded. Wnt binding causes phosphorylation of Dsh, which leads to dissociation of the APC/Axin/GSK complex. Free β-catenin can accumulate, translocate to the nucleus, and after binding to TCF it activates transcription of a number of target genes, including cyclin D1, c-Myc and matrix metalloproteinase (MMP-7).

of transcription factors such as c-jun, ATF 2, Elk1, DPC4, and p53.

The role of these non-canonical Wnt pathways in carcinogenesis is unclear. However, alteration to steps in the canonical Wnt pathway has been directly linked to carcinogenesis. The most important of these changes will be considered below.

5. Wnt genes and their products

The Wnt family consists of at least 19 glycoproteins, which are rich in cysteine. In animal models and tissue cell lines, Wnts have been shown to have a direct involvement in neoplastic transformation. For example, a number of head and neck squamous cell carcinoma cell lines have increased levels of the mRNA of Wnt 1, 7a, 10b, and 13 [2]. Blocking Wnt 1 signalling has been shown to lead to reduced cell proliferation and increased apoptosis. These effects were accompanied by reduced expression of β-catenin and cyclin D1 [2]. Wnt11, a polypeptide containing 354 amino acids, whose mRNA is normally expressed in adult liver, heart, pancreas, and skeletal muscle, is upregulated in the human gastric cancer cell line, MKN45 [3]. Changes in Wnt levels have been described in some tumours. For example, Wnt 2 mRNA is detectable in colon cancer, but is not found in normal colonic mucosa [4]. In most human tumours there is no direct evidence linking the Wnt proteins themselves to carcinogenesis, but mutations mimicking Wnt stimulation lead to activation of the pathway during carcinogenesis.

6. Frizzled receptors

Most Wnt proteins can bind to multiple Frizzled receptors. The co-receptors LRP-5 and LRP-6 are members of the single transmembrane low density lipoprotein receptor related protein (LRP) family [5]. Disruption in the synthesis of either receptor leads to a dramatic reduction in signalling [6]. In *Drosophila*, LRP (known as arrow) is essential for Wnt signalling. It has been shown that canonical Wnt signalling is only initiated when Wnt is complexed with both FZD and LRP [5,7,8]. In *Xenopus* embryos, LRP activates Wnt signalling and induces Wnt-responsive genes [5,7]. Mouse embryos mutant for LRP6 show developmental defects similar to those caused by mutations in Wnt genes [8]. A number of *FZD* genes have been characterised and shown to be expressed in human cancer cell lines (Table 1) [2,9–17]. In cell lines, overexpression of rat FZD 9 induces Dsh to move to the cell membrane and become hyperphosphorylated. This is associated with a rise in cytosolic β-catenin. In this experiment, only Wnt 2 was able to activate FZD 9 [18].

Table 1
Expression of FZD receptors in human tissues and cancer cell lines

	Location on chromosome	Tissues normally expressing receptor								Cancer cell lines in which receptor expressed					Reference
		Pancreas	Brain	Liver	Kidney	Heart	Skeletal muscle	Lung	Prostate	Ovary	CML	Cervical	Head and neck	Oesophageal	Gastric	Lung	
FZD1	7q21	x			x	x		x	x	x							[9]
FZD2						x							10 different cell lines		TMK1, MKN7, 28, 45,&74, KATO-III		[2,9,16]
FZD3	8p21	x	x		x				x								[10,11]
FZD4																	
FZD5	2q33.3-q34	x		x							K-562				MKN45		[12,16]
FZD6																	
FZD7	2q33		x			x	x							TE4, TE5	MKN7		[9,16,17]
FZD8	10p11.2	x			x	x	x					HeLa S3				A549	[13]
FZD9															TMK1, MKN74		[16]
FZD10												HeLa S3, SKG-1, SKG-IIIa			TMK 1, MKN74		[14–16]

CML, chronic myeloid leukaemia.

Some data also exist on the expression of Frizzled receptors in tumour tissues. In colonic tumours, it has been shown that while FZD 1 and 2 are not expressed in normal colonic mucosa or in well differentiated tumours, there is a high degree of expression in poorly differentiated tumours, especially at the margin of cellular invasion [4]. This would suggest that receptor expression may be associated with tumour invasion and may therefore be useful as a prognostic marker. FZD 10 has also been shown to be upregulated in some cases of colon cancer [15]. Similarly, FZD 2 and 3 are upregulated in both oesophageal and gastric tumours, associated with increased nuclear staining of β-catenin and cyclin D1 expression [16,19]. Other FZDs that have been shown to be upregulated in gastric cancer are FZD 7, 8, 9, and 10 [16,17].

7. Glycoproteins similar to Frizzled (Frp)

Frizzled-related proteins (Frp) are soluble forms of Frizzled that compete with FZD to bind Wnt via its cysteine-rich domain and therefore antagonise the Wnt signalling pathway. Analysis of two homologues of human Frp shows that Frp1b is exclusively expressed in the pancreas and Frp2 is found mostly in adipose tissue [20].

The role of Frps in tumour growth is not clearly defined. It would seem logical that increased levels of Frp should be associated with an anti-tumorigenic effect, as Wnt signalling is reduced. It has been shown that Frp is downregulated in some oesophageal and gastric cancers suggesting a potential role as a tumour suppressor, although only a small number of tumours were studied in this case [19]. However, in some tumours Frp appears to be upregulated. In breast cancer, Frp increases as tumours progress and this rise is associated with a fall in Wnt 1 [21]. In addition, Frp seems to promote angiogenesis, being expressed in neovessels, but not in fully mature vasculature [22]. In glioma cells, it promotes tumour growth [23].

8. β-Catenin

Activating mutations in the β-*catenin* gene and subsequent nuclear accumulation of β-catenin are important in the development of many cancers. The association of such mutations with various types of human gastrointestinal cancer is illustrated in Table 2 [24–32]. β-Catenin may be activated by mechanisms other than mutations in its gene, such as alteration to other components of the Wnt signalling pathway, which lead to reduced β-catenin destruction. Therefore changes in cellular levels are seen in many cancers even in the absence of gene mutations. As the results of one study show, changes in β-catenin are found in a wide range of cancers [33].

Table 2
Frequency of β-catenin gene mutations in human tumours

Cancer	Frequency of β-*catenin* gene mutations	Reference
Colorectal	10%	[24,29]
Gastric	8–26%	[25,26]
Gastrointestinal carcinoid	38%	[27]
Oesophageal	0	[28]
Hepatocellular	17–44%	[30,31]
Pancreatic (non-ductal acinar cell)	6%	[32]

Cancerous cell lines in which β-catenin/TCF signalling is upregulated:

- 100% of colorectal (12 of 12).
- 63% of gastric (5 of 8).
- 29% of hepatic (2 of 7).

Human tumours with increased nuclear expression of β-catenin:

- 75% of colonic (15 of 20).
- 56% of gastric (14 of 25).
- 269% of hepatocellular (5 of 19).

8.1. β-Catenin and colorectal cancers

It is probably in this group of gastrointestinal tumours that the role of β-catenin is best understood.

Work on colorectal cancer cell lines suggests that for some β-catenin is a necessary oncogene [34]. If β-catenin activity is disrupted, then there is a rapid arrest in the G1 phase of the cell cycle, resulting in apoptosis [35,36]. In cell lines transfected with small inhibiting RNAs, which decrease β-catenin expression, there is a marked decrease in the expression of genes dependent on β-catenin/TCF activation, leading to a reduction in cellular proliferation [37].

Evidence of the importance of β-catenin in tumorigenesis has been shown in both animal and human studies. In rats, the increase in β-catenin signalling frequently seems to be due to an activating mutation in the gene. The majority of established cancers in rats have such a mutation, which appears to be an early event, as in one study 39% of early dysplastic lesions already had a β-*catenin* mutation [38]. In rats treated with the carcinogenic agent, azoxymethane, crypts with an accumulation of β-catenin develop in the mucosa and seem to be early pre-neoplastic features [39]. The number and size of crypts overexpressing β-catenin increases with duration of azoxymethane treatment [40]. In mice, targeting of the β-catenin pathway leads to reduced tumour growth [41]. By using oligonucleotides directed

against β-catenin mRNA and downregulating its expression, there is a dose-dependent inhibition of tumour growth [42].

β-Catenin has also been implicated in angiogenesis. It upregulates the gene for the growth promoter vascular endothelial growth factor A (VEGF A), which has binding sites for β-catenin/TCF. In addition, the VEGF receptor, Flk-1, is able to phosphorylate β-catenin [43]. If β-catenin is transfected into normal colon cells, VEGF A expression increases. In Min mice (which are mutant for APC), a rise in nuclear β-catenin in colonic cells is associated with increased VEGF A in the vicinity of these cells and a corresponding increase in vessel density [44].

Over 70 β-*catenin* mutations have been reported in colorectal cancers, most of which are mis-sense mutations [45]. However, in humans, mutation of the β-*catenin* gene itself seems to be a less frequent event than in rodents. One study examined 63 sporadic colorectal cancers without finding any β-*catenin* gene mutations [46]. However, there were altered amounts of β-catenin, implying that other elements in the Wnt pathway are responsible for the changed levels of β-catenin.

The change in β-catenin levels in human colorectal tumours has been fairly well documented. Normal localisation of β-catenin at the cell membrane is reduced in 70–84% of established cancers, while nuclear and cytoplasmic β-catenin is increased in 66–79% [47,48]. There is a reciprocal relationship between decreasing membranous and increasing cytoplasmic and nuclear β-catenin during the progression from adenoma to carcinoma. Immunohistochemical studies have shown that as the epithelium changes from normal to dysplastic to cancerous, there is a progressive increase in nuclear β-catenin staining [48]. As nuclear β-catenin can be found in even mildly dysplastic adenomas, this suggests that the rise in its expression is an early event in the tumorigenic process [49]. Two studies have shown that nuclear accumulation of β-catenin occurs diffusely throughout the tumour in 27–44% of colorectal cancers. However, a further 9–26% have nuclear accumulation specifically at the tumour invasion front. This phenomenon is associated with advanced Dukes' stage, tumour recurrence and the presence of metastases [50,51].

A relationship has been found between the presence of cytoplasmic β-catenin and the likelihood of haematogenous metastases, with β-catenin overexpression correlating with venous invasion, depth of tumour invasion and focal dedifferentiation [47]. This suggests that β-catenin expression may prove to be a useful biomarker for colorectal cancers and may also provide an indication of severity.

However, there may be a difference between different morphological types of cancer. Data suggest that nuclear expression of β-catenin tends to be associated with ulcerative tumours, with 72% of these having nuclear expression of β-catenin. This is in contrast to only 27% of polypoid tumours [52]. β-Catenin is overexpressed in ulcerative colitis-related tumours and familial cancers, as well as in sporadic colorectal cancers [53,54].

Combining the analysis of β-catenin with that of other markers may increase its usefulness as a prognostic marker. k-ras is mutated in 42% of colorectal cancers. One study has shown that the majority of tumours (78%), have activation of either β-catenin or k-ras, and those with both have a more advanced Dukes stage and are more likely to develop distant metastases, although the number of cases studied was fairly small [50]. Similarly, a lack of p27 expression, when combined with accumulation of nuclear β-catenin is a marker of poor prognosis. In a study combining analysis of these two factors, none of the patients exhibiting both findings survived more than five years [55].

Having established the existence of deranged β-catenin expression in colorectal cancers, there has been some effort to target therapies at this area of the Wnt pathway. Some agents that are known to be chemopreventive (they suppress or reverse the development of cancer), may exert their effect by inhibiting β-catenin activity. In cell lines, curcumin has been shown to lead to cleavage of β-catenin and so to reduced translocation of β-catenin, and reduced transcription of the target gene, c-Myc [56]. There has also been much interest in the use of non-steroidal anti-inflammatory drugs (NSAIDs), as these have been shown to have a chemopreventive effect on colorectal cancer formation [57,58]. In cell lines treated with various metabolites of sulindac, there is a dose-dependent reduction in β-catenin expression [59]. Similarly, in rats with colorectal tumours, the rate of nuclear β-catenin staining decreases with NSAID treatment, implying an effect on this pathway [60,61]. Various mechanisms by which NSAIDs affect β-catenin have been suggested. In cell lines, aspirin inhibits the association of β-catenin with TCF-4 in the nucleus [62]. In addition, NSAIDs have been shown to cause increased phosphorylation of β-catenin, hence promoting its destruction and downregulating its signalling [63].

Therefore, there is potential for using β-catenin as both a prognostic marker and a target for drug intervention in colorectal cancer. However, it also has a role in other gastrointestinal tumours, although in some cases this role has not been so clearly defined.

8.2. β-Catenin and oesophageal and gastric cancers

β-Catenin is frequently expressed in gastric cancer cell lines [64]. There are mutations of the gene in oesophageal and gastric cancers, although this is not invariably the case, even when β-catenin expression is altered [65–68]. In some human tumours, there is alteration in β-catenin expression similar to that found in colorectal cancers, namely a loss of membranous expression and

an increase in nuclear staining [68–71]. In gastric cancers, this change becomes more marked as the tumour progresses from adenoma or dysplasia to carcinoma, although the percentage of tumours with increased nuclear β-catenin (17–44%) is less than in colorectal cancer [66,71].

β-Catenin expression does seem to differ between different histological types of gastric cancer as some reports suggest that abnormal staining (both reduced membranous and increased nuclear and cytoplasmic) is particularly associated with diffuse tumours [67,71].

These studies also found a correlation between abnormal staining and lymph node metastases [67,71]. However, a large immunohistochemical study failed to show any correlation between the pattern of β-catenin staining and tumour progression or prognosis [72], leaving uncertainty surrounding the association between β-catenin expression and metastatic potential in gastric cancer.

The role of β-catenin in oesophageal tumours and its prognostic significance has not been clearly defined. A study on adenocarcinomas found that patients with increased nuclear β-catenin expression had a better outcome. In this case, it was superficial rather than more invasive tumours that were more likely to have abnormal β-catenin expression [73]. This is in contrast to what is seen in other cancers, suggesting that β-catenin has a different role to play in this tumour. A study on squamous cell carcinomas has shown reduced β-catenin expression at the cell membrane, which correlates with reduced E-cadherin expression, is associated with a worse prognosis, suggesting that the role of β-catenin at the cell membrane may be of significance in this type of tumour [74].

8.3. β-Catenin and pancreatic cancers

There is also evidence that the importance of β-catenin in pancreatic tumours may be due to its role at the cell membrane. In pancreatic cancer cell lines, β-catenin expression is often reduced rather than increased [75,76]. It is thought that the resulting disruption to the E-cadherin adhesion system may confer a growth advantage to cells. This theory is supported by studies showing that transfecting cancer cell lines with both E-cadherin and β-catenin leads to reduced cell growth, tighter adhesion and increased apoptosis [76].

In tumours, mutations in the β-*catenin* gene have occasionally been described [77], but regardless of these, β-catenin expression is often abnormal [78]. Reduced membranous expression is a frequent finding, occurring in 56–58% of tumours, and has been shown to correlate with loss of differentiation, liver metastases and shorter survival time [75,79,80]. Increased expression of cytoplasmic and nuclear β-catenin has also been described, but there are conflicting reports as to the frequency of this finding, with it varying from 4% to 65% [75,80]. Where increased cytoplasmic β-catenin is found, this also correlates with reduced survival time [80].

8.4. β-Catenin and hepatocellular carcinoma

β-*Catenin* gene mutations have been described in hepatocellular carcinomas [30,31,81]. Many tumours (62–70%) have increased nuclear and cytoplasmic β-catenin [31,82]. Abnormal expression is seen even in well differentiated tumours, implying it is an early change, although in large or poorly differentiated tumours there is increased accumulation of nuclear and cytoplasmic β-catenin [82,83]. Some studies have also found a correlation between nuclear β-catenin and high expression of cyclin D1 and c-myc, which are two of its target genes [31,84].

9. APC

As APC forms part of the complex involved in β-catenin destruction, mutations prevent β-catenin binding and subsequent degradation. In cancers where there is a rise in nuclear or cytoplasmic β-catenin without a mutation in the β-*catenin* gene, *APC* mutation may be responsible. The *APC* gene (Fig. 2), which is located on chromosome five, includes regions containing three 15-amino acid (aa) repeats and seven 20-amino acid repeats. Both of these regions bind β-catenin. In colorectal cancer, most mutations are clustered in the central domain of the gene. These mutations result in a protein that is truncated at the c-terminus, eliminating five or more of the 20-aa repeats. As at least three of the 20-aa repeats are needed in order to downregulate β-catenin, so these mutations ensure that β-catenin is able to accumulate.

Within the 20-aa repeat region, there are three SAMP motifs (Ser-Ala-Met-Pro). It is these that mediate APC binding to axin and they are also missing from mutated gene products. In mice with truncating APC lesions, colon cancer will develop. However, if an axin binding site is restored, then cancer development is inhibited, showing that it is not only the loss of β-catenin binding sites that is important in tumour growth following APC mutation [85].

APC is well established as an important gene in the development of colorectal cancers. Its mutation is responsible for familial adenomatous polyposis coli (FAP). In this case, there is a germline mutation such that when a mutation occurs on the remaining allele, adenomas can develop. Further genetic events then allow an adenoma to transform into a carcinoma. *APC* mutation is also found in the majority (60–70%) of sporadic colorectal cancers [86,87]. Most have associated changes in β-catenin levels [88,89]. Patients who have

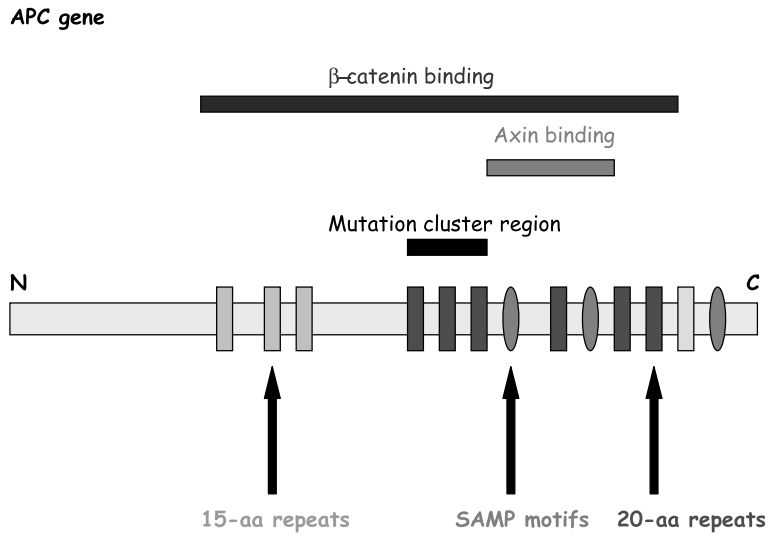

Fig. 2. Structure of the *APC* gene, showing regions encoding binding sites for components of the Wnt pathway. aa, amino acid; SAMP, Ser-Ala-Met-Pro.

lost all β-catenin binding sites on the *APC* gene have been shown to have a shorter survival time than those who retained some binding sites [90].

Only a small number of tumours show altered β-catenin levels in the absence of *APC* gene mutation, suggesting that *APC* dysfunction may be the most important cause of this phenomenon in colorectal cancers. Some colorectal tumours with an intact *APC* gene have been found to have a mutation in β-*catenin* [29,54,91]. The effect of these mutations is functionally equivalent, namely a rise in β-catenin levels.

APC may have a role in other gastrointestinal tumours. Intestinal type gastric tumours may exhibit *APC* gene mutations, although the frequency (20–33%) is much lower than that seen in colorectal carcinoma, with even fewer mutations being seen in diffuse cancers [92–94]. Interestingly, this contrasts with the studies mentioned earlier, which show that β-catenin is more commonly dysregulated in diffuse type cancers. Although the number of cases involved in some of the above studies is quite small, this nevertheless suggests that *APC* mutation is less likely to be responsible for altered β-catenin levels in gastric cancers. When there is an *APC* mutation, the reduction in APC expression does correlate with abnormal nuclear and cytoplasmic β-catenin staining [69]. However, no direct link between *APC* mutation and tumour size, differentiation, or clinical stage has been shown [94]. *APC* mutation has frequently been described in adenomatous polyps, with one study finding a mutation in 76% of adenomas, which is much higher than the rate found in carcinomas [95]. This suggests that while *APC* mutation may have an early role to play in adenoma formation, other factors may be more important in the progression to malignancy.

APC mutations have been described in some pancreatic tumours, such as the rare pancreatoblastoma and acinar cell carcinomas [32]. However, this has not been demonstrated in adenocarcinomas [96].

In hepatic neoplasms, hepatoblastoma is known to be an extra-colonic manifestation of FAP. A case report of a patient with FAP who developed a hepatocellular carcinoma has shown that in the tumour, there was a mutation of the remaining allele [97]. However, in most sporadic hepatocellular carcinomas, no *APC* mutation has been found [98].

Therefore, in some types of cancer, there is not such a clear role for APC as there is in colorectal cancer, suggesting that tumour development may be more affected by other factors.

10. Axin

Axin acts as the scaffold of the APC/axin/GSK complex, binding to all of the other components [99]. It is, therefore, closely linked with the role of each of the other members of the complex and has also been shown to be involved in oncogenesis. *Axin* (Fig. 3) contains a binding site for APC at its N-terminal region, while its central region binds GSK and β-catenin. At the C-terminal end, Dsh can bind and this interaction reduces β-catenin binding.

Some colon cancer cell lines have an *axin* mutation, as do some colorectal cancers without *APC* or β-*catenin* mutations [100]. These mutations result in the elimination of either the Dsh or the GSK-3β-binding site and therefore lead to a rise in nuclear β-catenin [101]. In some cell lines with increased β-catenin expression, transfection with axin leads to reduced cell growth and apoptosis [102]. Axin overexpression results in reduced β-catenin levels and suppression of TCF-dependent gene transcription.

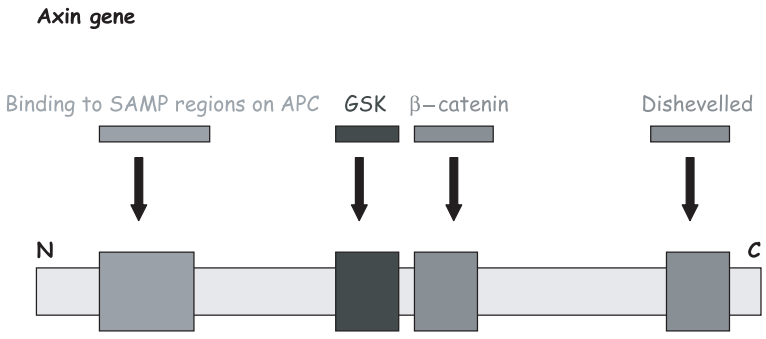

Fig. 3. Structure of the *axin* gene, showing regions encoding binding sites for other components of the Wnt pathway.

Work on oesophageal squamous cell carcinoma has revealed that axin expression is reduced in tumours, and this correlates with depth of invasion, and lymph node metastasis. However, although some cell lines have a mutation in their *axin* gene, the tumours themselves do not, so the mechanism for axin downregulation is unclear [103].

As previously described, in hepatocellular carcinoma there is frequently a rise in β-catenin levels. This is not necessarily accompanied by β-*catenin* mutation, and as *APC* mutations do not seem to play a role in these tumours, there must be another explanation for the rise in β-catenin. Studies on hepatocellular carcinoma cell lines without β-*catenin* mutation have shown that half have an *axin* mutation, associated with nuclear β-catenin [102]. The mutations produce truncated proteins lacking the β-catenin binding site. These same mutations have also been found in hepatocellular tumours.

11. GSK-3β

GSK is an important protein kinase in many biological processes, including regulation of glycogen by insulin, and cell survival. It is implicated in many diseases, including Alzheimer's, psychiatric disorders, and diabetes. GSK-3β has an important role to play in the Wnt pathway, as it phosphorylates and activates several of the other key components. In the absence of Wnt signalling, GSK-3β sequentially phosphorylates β-catenin at the serine and threonine residues Thr41, Ser37, Ser33 [104]. Before this can occur, there first has to be a priming phosphorylation of β-catenin. This occurs at Ser45 by casein kinase 1 (CK1), which acts to switch on the ensuing steps in β-catenin degradation [105,106]. Once all four residues are phosphorylated, the binding site for β-TrCP is created. Many β-*catenin* mutations occur in the region of the gene that codes for the interaction site with GSK-3β (Fig. 4) [107].

In addition, GSK-3β regulates other components of the Wnt pathway. It phosphorylates axin, which enhances β-catenin binding. When Dsh binds to axin, this phosphorylation is inhibited, and β-catenin binding is reduced [108]. GSK-3 also directly regulates the degradation of cyclin D1, one of the β-catenin target genes [109].

In theory, because GSK-3β is involved in β-catenin destruction, it may act as a tumour suppressor and be downregulated during oncogenesis. In some cancers, such as oral squamous cell carcinoma, this does appear to be the case and reduced levels of GSK-3β correlate with increased cyclin D1 expression and with poor survival [110]. In hepatocellular cancer cell lines and tumours, the activity of GSK-3β is often inhibited, as it is persistently phosphorylated. In tumours, this is related to β-catenin accumulation [111,112]. The role of GSK-3β as a tumour suppressor has been investigated by giving lithium, an inhibitor of GSK-3β, to *APC*-mutant mice. This treatment caused no increase in the number of tumours, although there was a small increase in tumour size [113]. No mutation of the *GSK-3β* gene itself has yet been found in any human tumours [31,107]. This may mean that the importance of GSK-3β in intracellular signalling is such that its absence would not be compatible with cell viability [114].

12. TCF

TCF is involved in the final step of the Wnt signalling cascade. Once β-catenin has entered the nucleus, it must bind to TCF in order to initiate transcription of its target genes. Whilst β-catenin has a potent transcription activation domain, it does not have a DNA binding domain. This is provided by TCF, so together they form an effective transcription regulatory complex. The TCF family consists of four different proteins, TCF-1, TCF-3, TCF-4, and LEF. These are normally expressed during embryogenesis, but in most tissues are downregulated once the tissues become terminally differentiated. However, in sites of continual cell growth, such as the bone marrow, skin, and intestinal mucosa, some members of the family continue to be expressed. The N-terminal region of the TCFs contains a binding site for β-catenin. At the C-terminal end, it is possible for each member of the family to have alternative tails. These

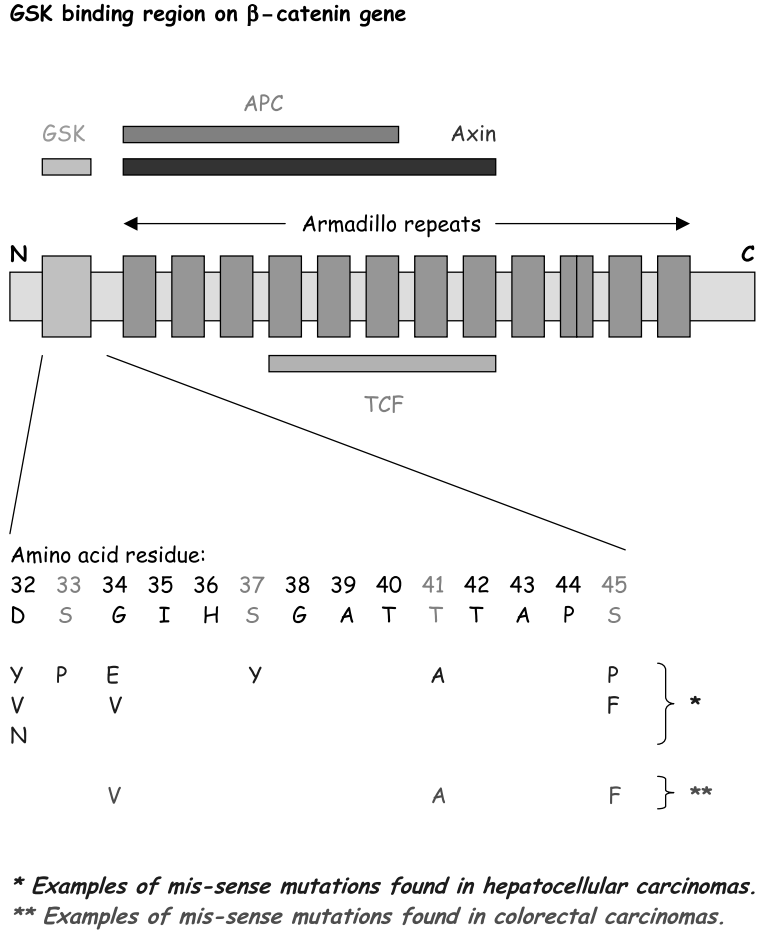

Fig. 4. β-*Catenin* gene, showing GSK binding site and sites of mutations found in cancers.

different forms of TCF may be able to preferentially activate different target genes. In the absence of Wnt signalling, TCFs seem to function to repress transcription of target genes. The genes encoding some of the TCFs (TCF-1 and LEF-1) contain two different promoters (Fig. 5). From one promoter, a full-length protein is produced, but from the other a truncated protein lacking the β-catenin binding domain is formed. These truncated proteins, with the help of other repressor proteins, can occupy regulatory sites and inhibit transcription [115,116]. Therefore, it is possible that cancers may be able to progress if the truncated form of TCF is downregulated and the full-length form is upregulated. In colon cancer cell lines and tumours, it appears that only full-length LEF-1 is expressed [117]. The promoter encoding the full-length protein is activated by TCF-4.

With so many isoforms of the TCF family, and such a wide range of activities, it is not surprising that their exact role in tumour development remains unclear. However, there do appear to be TCF alterations in some cancers. A mutated *TCF*-4 gene, which is found in some colorectal tumours and cell lines is associated with microsatellite instability [118–120]. Approximately 15% of sporadic colorectal cancers show microsatellite instability, mostly due to inactivation of mismatch repair genes, and are less likely to have *APC* mutations [90,121]. Tumours without microsatellite instability rarely have *TCF* mutations [118]. In gastric cancers with microsatellite instability, similar mutations have been described by some authors, but have not been found by all [119]. In some hepatocellular carcinomas, there is also mutation of the *TCF*-4 gene, resulting in its overexpression [31,122]. One study has shown this overexpression to be associated with increased likelihood of intrahepatic metastasis [122].

13. Target genes of the canonical Wnt pathway

The result of overactive Wnt signalling in cancers is the inappropriate transcription of one or more target genes, many of which are involved in control of the cell cycle. Within the same group of cancers, different target genes may be upregulated. In some tumours, the expression of these genes seems to have prognostic significance. The proportion of different types of tumours

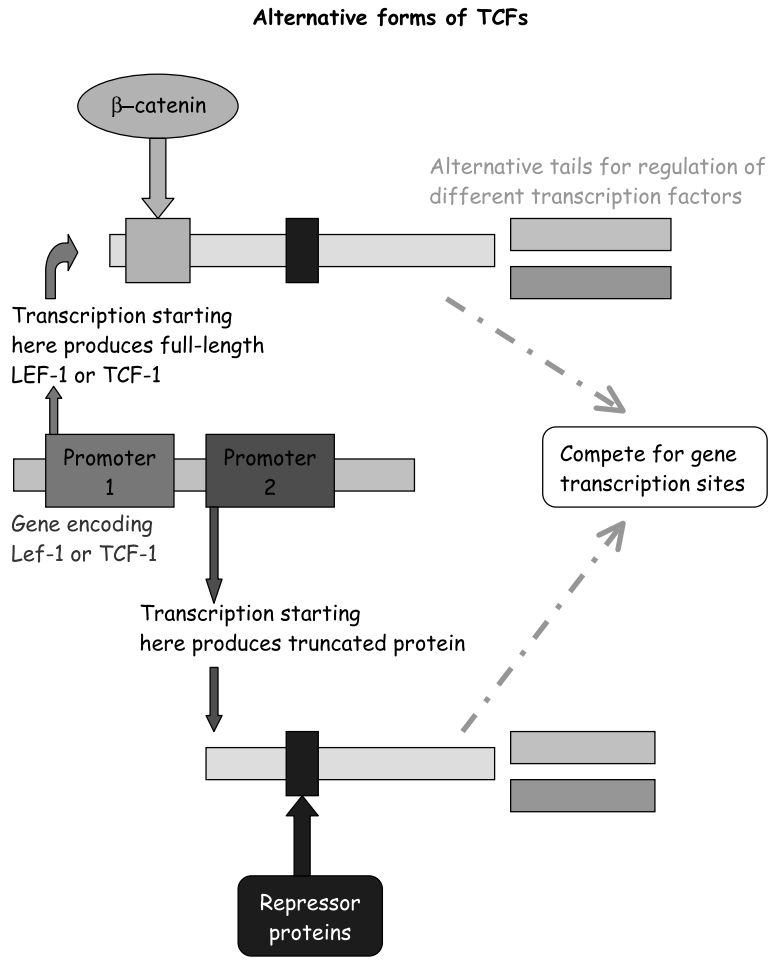

Fig. 5. Different forms of TCFs. Transcription starting at alternate promoters on *TCF* genes can produce either full-length or truncated proteins. The full-length proteins tend to activate transcription of target genes, whereas truncated proteins will block transcription. TCF-4 bound to β-catenin activates transcription of the full-length protein.

which have altered expression of various target genes is shown in Table 3 [68,108,123–138].

13.1. c-Myc

c-Myc regulates transcription, and when overexpressed, prevents apoptosis [108]. Its transcription is activated by the β-catenin/TCF complex and is inhibited by APC [139]. c-Myc is known to be overexpressed in adenomas from FAP patients and in sporadic colorectal tumours [140,141]. In colorectal cancer cells, the rate of c-Myc expression is inversely related to apoptosis [142]. It is expressed in gastric cancer cell lines and antisense oligonucleotides directed against c-Myc will inhibit growth and induce apoptosis in cell lines [143–145]. c-Myc is overexpressed in gastric and oesophageal tumours and is associated with the presence of metastases from gastric tumours [124,125]. It is expressed in pancreatic cancer cell lines and tumours [126,146], and has been linked to advanced tumour grade, although expression has not been found to correlate with survival [126,147]. Overexpression is also found in hepatocellular carcinomas, particularly metastatic or recurrent tumours [148].

Table 3
Human tumours which show alterations in target genes of the Wnt signalling pathway

Tumour	% of tumours in which gene product is inappropriately expressed [Ref]		
	c-Myc	Cyclin D1	MMP-7
Colorectal	44% [123]	33–64% [128,129]	90% [108]
Oesophageal	90% [124]	38–71% [130,131]	21–65% [68,136]
Gastric	26% [125]	22–47% [128,132,133]	71% [137]
Pancreatic	43% [126]	62–68% [134,135]	98% [138]
Hepatocellular	65% [127]	54% [127]	

13.2. Cyclin D1

Cyclin D1 regulates the G1/S transition phase of the cell cycle. There is a TCF binding site within the cyclin D1 promoter region and transcription is activated by

the β-catenin/TCF complex [149]. Adenomas from FAP patients have significantly increased cyclin D1 levels [140]. In sporadic colorectal tumours showing nuclear accumulation of β-catenin, there is also cyclin D1 overexpression, which correlates with advanced Dukes stage and poor survival [128]. Oesophageal cancers, both squamous and adenocarcinomas, show upregulation of cyclin D1 [133]. In gastric cancer cell lines, antisense oligonucleotides directed against cyclin D1 inhibit cell growth and cause loss of tumorigenicity in nude mice [150,151]. Although cyclin D1 is overexpressed in gastric tumours, particularly those of intestinal type, it does not appear to predict survival [132,133]. Pancreatic adenocarcinomas overexpress cyclin D1 and, in this case, there is an association with poor prognosis [135,152,153]. Inhibiting cyclin D1 in pancreatic cancer cell lines leads to growth suppression and loss of tumorigenicity in nude mice [154]. Cyclin D1 has also been demonstrated in hepatocellular carcinomas, although here its expression does not necessarily correlate with the presence of nuclear β-catenin [30,84,127].

13.3. MMP-7

Members of the matrix metalloproteinase family degrade extracellular matrix proteins and are involved in tissue remodelling and angiogenesis. They are implicated in metastasis in a number of cancers. The β-catenin/TCF complex activates the expression of MMP-7 [155], which is frequently upregulated in colorectal cancers [108]. It is also expressed in colorectal liver metastases [156]. In oesophageal cancers, the aberrant nuclear expression of β-catenin correlates with MMP-7 expression and there is an association with tumour invasion and lymph node metastases [68,136]. Similarly, gastric cancers expressing MMP-7 are more likely to be invasive and to have a worse prognosis [137,157–159]. *Helicobacter pylori,*which has been implicated in gastric carcinogenesis, has been shown to increase MMP-7 expression [160–162]. MMP-7 is also expressed in the majority of human pancreatic adenocarcinomas [138].

14. Conclusion

A great deal of research interest is focused on different areas of the Wnt pathway and its role in carcinogenesis. Many alterations to the pathway have been described, but β-catenin would appear to be one of the most important proteins associated with oncogenesis. Generally, the result of any alteration is a rise in nuclear β-catenin. This then results in the inappropriate transcription of various target genes. Occasionally, mutations of the β-catenin gene itself result in the observed rise in nuclear β-*catenin*, but more often it is other components of the pathway, such as *APC*, which are mutated. These mutations affect the binding or phosphorylation of β-catenin and act to increase its stability.

The mechanisms by which alterations in this pathway are linked to carcinogenesis are not always clear. For some cancers, in particular, uncertainty surrounds the implications of the observed changes in Wnt pathway components. In addition, other factors can interact with members of the pathway and affect their stability and actions.

The Wnt pathway has clinical relevance, which lies in its potential first for predicting clinical outcome and second as a target for therapeutic agents. Importantly, in some cancers, the aberrant expression of components of the Wnt pathway correlate with advanced tumours, the probability of metastasis, and survival rate. Therefore, analysis of these components, either alone or in parallel, may offer important prognostic information and therefore also help to direct treatment appropriately. In addition, as this pathway is so important in tumour development, targeting inhibitory agents towards it may help to prevent tumour growth. Some substances which are known to have chemopreventive effects have been shown to act on the Wnt pathway. Therefore, there is much potential to be explored in further uncovering the role of the Wnt pathway in carcinogenesis and utilising it for tumour prevention or treatment.

Conflict of interest statement

None declared.

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