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The Wnt connection to tumorigenesis

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ABSTRACT Wnt signaling has been identified as one of the key signaling pathways in cancer, regulating cell growth, motility and differentiation. Because of its widespread activation in diverse human tumor diseases, the Wnt pathway has gained considerable and growing interest in tumor research over recent years. Evidence that altered Wnt signaling is important for human tumor development came from three major findings: (i) the tumor suppressor adenomatous polyposis coli (APC) binds to the Wnt pathway component β-catenin and is involved in its degradation, (ii) mutations of *APC* in colon tumors lead to stabilization of the β-catenin protein and (iii) tumor-associated mutations of *β-catenin* in colorectal cancer as well as in other tumor types lead to its stabilisation, qualifying *β-catenin* as a proto-oncogene. Here we will describe the biochemical interactions which shape the Wnt pathway and focus on its role in tumorigenesis.

KEY WORDS: *wnt, tumor, β-catenin, APC, mutation*

Introduction

In mammals, the first *Wnt* gene to be identified was called *int-1* because it was activated by integration of the LTR of the mouse mammary tumor virus resulting in the development of mammary tumors in mice. In *Drosophila* the homologous gene of *int-1* is called *wingless*, and the combination of both names led to the term Wnt (Rijsewijk *et al.,*1987a). In the so called canonical Wnt signaling pathway the cytoplasmic protein β-catenin becomes stabilized and then associates with transcription factors of the LEF/TCF family (hereafter collectively referred to as TCF). The TCF/β-catenin complexes regulate expression of specific target genes thereby transmitting the Wnt signal to the cell nucleus. Besides canonical signaling there are two more main branches of the Wnt pathway: the planar cell polarity pathway, which controls cytoskeletal rearrangments, involving RhoA and Jun Kinase, and the Wnt/Ca^{2+} pathway, which acts via calmodulin dependent kinase, calcineurin and the transcription factor NF-AT (Veeman *et al.,* 2003, Kuhl 2004). In the following we will focus on the canonical Wnt pathway and its implication in cancer development and progression. The possible roles of the planar cell polarity and Ca^{2+} pathways in tumorigenesis are less defined and will not be discussed here.

Wnt signaling has been identified and studied extensively in embryonal development. In *Xenopus* embryos ectopic expression of Wnts induces dorsalization of the embryos and formation of a secondary body axis, resulting in double-headed embryos. This phenomenon is also observed with other activating components of the Wnt pathway while inhibitors lead to defects in axis formation. Thus, if the amount of β-catenin is increased, e.g. by injecting β-catenin mRNA a secondary body-axis forms, whereas if negative regulators such as axin are expressed axis formation is disturbed and ventralization of the embryos occurs. In addition to Xenopus embryology, studies in *Drosophila melanogaster* and *Caenorhabditis elegans* have been most powerful in identifying and charaterizing new components of the pathway. Mice mutated in Wnt pathway components have contributed to our understanding of the physiological role of the Wnt pathway in mammals, and more recently, zebrafish genetics has also entered the Wnt field.

Molecular mechanisms of the canonical Wnt signal transduction pathway

A combination of biochemical and developmental studies together with tumor genetics have contributed to the model of molecular interactions of the Wnt pathway that will be described here. Our model is simplified as it contains only a selection of components for which an essential role has been suggested by different functional studies (Fig. 1).

Abbreviations used in this paper: APC, adenomatous polyposis coli tumor suppressor; FAP, familial adenomatous polyposis.

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Signaling at the plasma membrane

Wnts are secreted glycoproteins which are produced by different cell types and are thought to act mostly in a paracrine fashion (Cadigan and Nusse 1997). There are 19 Wnt proteins known only some of which, such as Wnt-1, Wnt-3a and Wnt-8 activate the canonical Wnt/β-catenin pathway. Wnts bind to seven transmembrane receptors called frizzelds and to co-receptors LRP-5 and LRP-6, which are essential for signal transmission (Bhanot *et al.,* 1996, Tamai *et al.,* 2000, Wehrli *et al.,* 2000, Mao *et al.,* 2001a). The interaction of Wnts with frizzled receptors can be modulated by secreted factors, which act as direct or indirect antagonists. While WIF-1, Cerberus and FrzB bind to Wnts and thereby directly prevent its interaction with frizzleds (Miller 2002), members of the Dickkopf family (Dkk-1, DKK-2, but not DKK-3) prevent Wnt binding in an indirect fashion by reducing availability of the LRP co-receptors. Dkk was shown to promote endocytosis of LRP by interacting with the transmembrane protein Kremen (Mao *et al.,* 2001a, Mao *et al.,* 2002).

Signaling in the cytoplasm

The molecular events taking place immediately after activation of frizzleds remain largely elusive. It is clear from most of the genetic as well as biochemical data that the cytoplasmic phosphoprotein dishevelled plays a crucial role (Wharton 2003). After activation of Wnt signaling dishevelled becomes recruited to the plasma membrane possibly by interaction with phospholipids (Capelluto *et al.,* 2002). It is thought that this step leads to activation of dishevelled which can then interfere with β-catenin degradation. The precise nature of the activation mechanism of dishevelled is not known although phosphorylation of dishevelled might be important. A number of dishevelled interacting proteins have been identified, such as Dapper, Frodo, Daam1, Stbm, PAR-1, and Nkd,

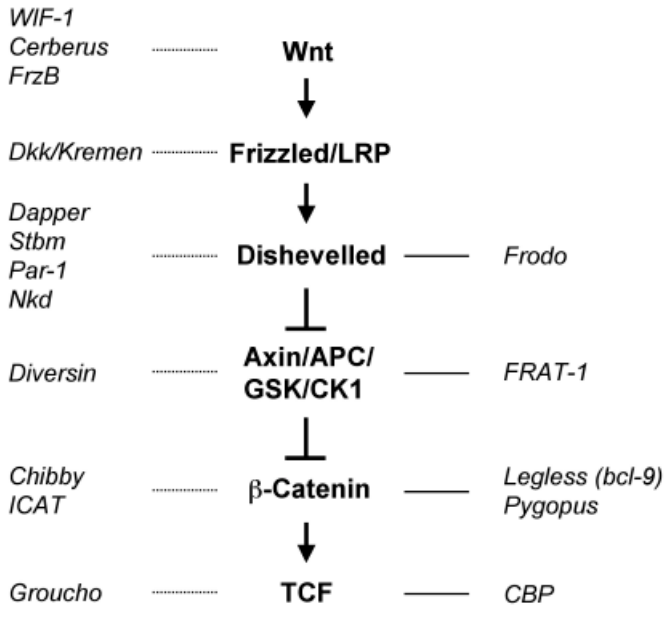

Fig. 1. Overview of the Wnt pathway. *The central components of the canonical Wnt signaling cascade are shown in bold type. Factors which modulate Wnt signaling at different levels of the cascade are shown in italics. Inhibitors are depicted to the left (stippled lines) and activators to the right (straight lines).*

which can either activate or inhibit Wnt signaling in different experimental settings through binding to distinct domains of dishevelled (Habas *et al.,* 2001, Rousset *et al.,* 2001, Sun *et al.,* 2001, Cheyette *et al.,* 2002, Gloy *et al.,* 2002, Heisenberg 2002). The functional role of most of these components in mammalian development or in cancer has not been analysed yet. An alternative, more direct way for cytoplasmic propagation of the Wnt signal is the binding of axin to LRP receptors, which blocks function of the β–catenin destruction complex (see below, (Mao *et al.,* 2001b)).

The levels of cytoplasmic β–catenin are normally controlled by a multiprotein destruction complex which targets β–catenin for degradation in proteasomes (Polakis 2000, Seidensticker and Behrens 2000). This complex is assembled over the scaffold component axin or its homologue conductin, which contain binding domains for β–catenin, the tumor suppressor APC (adenomatous polyposis coli), the serine/threonine kinases GSK3β and casein kinase 1α/ε (Zeng *et al.,* 1997, Behrens *et al.,* 1998, Hart *et al.,* 1998, Ikeda *et al.,* 1998, Kishida *et al.,* 1998, Liu *et al.,* 2002). The main function of the destruction complex is to promote phosphorylation of β–catenin which is required to trigger ubiquitination of β–catenin and its subsequent degradation in proteasomes. The phosphorylation sites are contained in the N-terminal domain of β–catenin and are hotspots for mutations in tumors (see below). Phosphorylation of β–catenin occurs in a two step mechanism: first, serine residue 45 is phosphorylated by the "priming kinase" casein kinase 1, and then the Ser 33, Ser 37 and Thr 41 are phosphorylated by GSK3β (Amit *et al.,* 2002, Liu *et al.,* 2002). Phosphorylated β–catenin is recognized by the ubiquitin E3 ligase β–TrCP which induces its ubiquitination (Kitagawa *et al.,* 1999, Winston *et al.,* 1999). The consecutive phosphorylation mechanisms implies that all four phosphorylation sites are essential for efficient degradation of β–catenin and explains why single mutations in any of these sites lead to stabilization of the β–catenin protein. While CK1α can bind directly to axin, CK1ε is recruited to the degradation complex via its binding to the ankyrin repeat protein diversin which acts as a negative regulator of the Wnt signaling pathway. Diversin binds to axin or conductin at the same binding sites as does GSK3β. However, diversin and GSK3β do not compete for interaction indicating that they might coexist in a common complex with axin (Schwarz-Romond *et al.,* 2002). Axin also facilitates the phosphorylation of APC by direct binding to CK1. GSK3β is a multitasking protein responsible for phosphorylation of multiple Wnt signaling components including β–catenin, APC, and axin (Rubinfeld *et al.,* 1996, Yamamoto *et al.,* 1999). The activity of GSK3β can be blocked by GBP (GSK3 binding protein) in *Xenopus* or its orthologue Frat-1 in the mouse (Yost *et al.,* 1998). Mutations or changes in gene expression of GSK3β have not been shown in tumors so far. Probably this is due to the essential role GSK3β in several cellular signaling which exceeds by far its role in Wnt signaling.

The phosphorylation of β–catenin by GSK3β is far more efficient in the presence of axin than in its absence, and overexpression of axin/conductin promotes significantly the degradation of β–catenin (Behrens *et al.,* 1998, Ikeda *et al.,* 1998). Thus it appears that by approximating the members of the multiprotein complex the phosphorylation of β–catenin is facilitated. Axin and conductin (also called axin2) share an overall identity in amino acids of 45 % and seem to have similar biochemical functions, but they differ in their regulation. Not only do both proteins show a different expression

pattern in embryonic development with a ubiquitous expression of axin and a very defined expression pattern of conductin, but conductin in contrast to axin is upregulated in colon, liver, and ovarian tumors (Jho *et al.,* 2002, Leung *et al.,* 2002, Lustig *et al.,* 2002). The promoter of the conductin/axin2 gene contains functional TCF binding sites and biochemical experiments demonstrate that conductin is a direct target of the Wnt pathway that acts in a negative feedback mechanism.

APC has first been identified as the responsible gene mutated in familial adenomatous polyposis (FAP), an inherited disease characterized by the development of thousand of polyps in the colon, eventually leading to colon cancer (Kinzler and Vogelstein 1996). The elucidation of the role of APC as a negative regulator of β–catenin in the Wnt pathway has greatly helped in understanding its function as a tumor suppressor (Polakis 2000). The *APC* gene is verly large containing 21 exons that encode a 2843 amino acid protein. The central region of APC is most directly implicated in Wnt signaling. It contains three 15-amino-acids repeats and seven 20-amino acid repeats which all bind to β–catenin. Unlike the 20-amino-acid repeats the 15-amino acid repeats are not essential for downregulation of β–catenin and are retained in most APC-truncated tumors (Polakis 2000). Three so-called SAMP repeats containing the core sequence serine-alanine-methionine-proline interact with the RGS domain of the axin proteins (Behrens *et al.,* 1998, Spink *et al.,* 2000). Thus APC, like axin/conductin can be considered a scaffold protein associating with different components of the β–catenin destruction complex. In colorectal tumors mutations of the *APC* gene occur mostly in a mutation cluster region which is located closely 5´ to the sequence encoding the first SAMP repeat. These mutations generate stop codons or frameshifts leading to the deletion of the C-terminal half of the APC protein and thereby removing the interaction sites with axin/conductin (Polakis 2000). In addition, the truncated APC also lacks several nuclear export sequences (NES) which are thought to be important for the distribution of APC between cytoplasm and the nucleus. Besides its function in regulating β–catenin degradation APC is also involved in shuttling β–catenin from the nucleus to the cytoplasm thereby interfering with its transcriptional activity. It has been speculated that this function of APC is important for its role as a tumor suppressor and that lack of β–catenin shuttling contributes to the aberrant activation of the Wnt pathway in tumors (Rosin-Arbesfeld *et al.,* 2003).

β–Catenin has a core domain of twelve so-called arm-repeats which were first identified in the Drosophila homologue armadillo (Peifer *et al.,* 1994). Structurally the arm repeats are build up by three a-helices, which arrange in a superhelical fashion to generate a rod-like structure (Huber and Weis 2001). Arm repeats are also found in several other proteins including APC and have a widespread function as protein interaction domains. In β–catenin different segments of the arm domain bind to cadherins, APC, axin/conductin, and TCFs (von Kries *et al.,* 2000). Most of these interactions appear to be mutually exlusive, in particular the associations with cadherins and components of the destruction complex (Hulsken *et al.,* 1994).

Signaling in the nucleus

Stabilized β–catenin enters the cell nucleus and associates with TCF/LEF transcription factors, leading to the transcription of Wnt target genes. TCFs bind to DNA via an HMG domain and to β–catenin with a short stretch of amino acids at their N-terminus. TCFs lack transactivation function, however, in β–catenin, the N- and in particular the C-terminal regions that flank the arm repeat domain exhibit transcriptional activation function (Behrens *et al.,* 1996, Huber *et al.,* 1996, Molenaar *et al.,* 1996, van de Wetering *et al.,* 1997, Hsu *et al.,* 1998).

The interaction between stabilized β–catenin and TCFs can be modulated by various direct and indirect mechanisms (Fig.1). In the absence of β–catenin, TCFs can repress gene transcription either as naturally occurring dominant-negative variants or in association with transcriptional repressors such as groucho (Roose *et al.,* 1998). Transcriptional activity of TCF/β–catenin complexes is increased by activators such as p300/CBP, a histone acetyl transferase, and by the chromatin-remodeling SWI/SNF complex (Hecht *et al.,* 2000, Barker *et al.,* 2001). A pair of factors containing DNA-induced ATPase and DNA helicase activity, pontin52 (also called TIP49) and reptin52 (TIP48) was shown to bind to β–catenin and modulate its activity in an opposite fashion, i.e. pontin52 activates while reptin52 represses activity (Bauer *et al.,* 2000). A dominant-negative mutant of TIP49 interfered with oncogenic transformation (Feng *et al.,* 2003). Legless/bcl-9 recruits the nuclear protein pygopus to β–catenin thereby activating the complex by a yet unknown mechanism (Kramps *et al.,* 2002, Thompson *et al.,* 2002). Several nuclear effectors of the TCF/β–catenin interaction have been identified. ICAT is a small protein that binds to the central and C-terminal segments of the arm repeat domain with different parts and thereby blocks interaction of β–catenin with both TCFs and CBP (Tago *et al.,* 2000). Similarly, the nuclear factor Chibby binds to the β–catenin arm repeats and blocks its association with TCF thereby interfering with Wnt signaling in mammalian cells and in *Drosophila* (Takemaru *et al.,* 2003). Taken together the transcriptional control exerted by TCF/β–catenin complexes is under stringent positive and negative regulation which might determine cell type specificities of Wnt signaling under physiological conditions and in tumor development. The various interaction partners of β–catenin in the nucleus are promising candidates to be tested for a putative role in tumorigenesis.

Other tumor-related functions of Wnt pathway components

APC has been shown recently to be associated with multiple other functions. The N-terminal part of APC harbors an oligomerization domain and a so called armadillo domain. This domain interacts with the APC-stimulated guanine nucleotide exchange factor (ASEF) thereby activating Rac (Kawasaki *et al.,* 2000), with the kinesin superfamily protein KAP3A (Jimbo *et al.,* 2002), and with the regulatory subunit of the phosphatase 2A (Seeling *et al.,* 1999). The interaction of mutated but not wild-type APC with ASEF was shown to promote migration of epithelial cells, suggesting a role of ASEF in tumor invasion and metastasis (Kawasaki *et al.,* 2003).

Recently, several components of the Wnt pathway, in particular those involved in degradation of β–catenin were shown to be associated with microtubules and the mitotic spindle apparatus. Endogenous APC was located at the distal ends of microtubules in migrating mammalian tissue-culture cells and in epithelial inner ear cells (Nathke *et al.,* 1996, Mogensen *et al.,* 2002). Exogenously expressed wild-type APC stabilises microtubules *in vitro* and *in*

vivo (Zumbrunn *et al.,* 2001). APC has also been localized to the mitotic spindle and to kinetochores, and mutation of APC in embryonic stem cells was associated with defects in chromosome segregation (Fodde *et al.,* 2001, Kaplan *et al.,* 2001). Thus loss of APC could lead to chromosomal instability and thereby promote cancer progression. Similar to APC, axin was detected at microtubules and shown to protect microtubules against depolymerization by nocodazole. Furthermore, dishevelled seems to cooperate with axin in the control of microtubule stability (Ciani *et al.,* 2004). Also, both GSK3 and β–catenin were found to be associated with the mitotic spindle (Olmeda *et al.,* 2003, Kaplan *et al.,* 2004), and interference with β–catenin through RNAi led to disturbance of centrosome separation and formation of monoastral spindles (Kaplan *et al.,* 2004), while inhibition of GSK activity with specific drugs led to disturbances in chromosome movements and development of astral microtubules (Wakefield *et al.,* 2003). Taken together it appears that the members of the β–catenin destruction complex reunite at microtubules and in particular at the mitotic spindle. Whether this reflects a new functional properties of the complex connected to the Wnt pathway remains to be determined. Obviously, localization of these components at a delicate structure such as the mitotic spindle might have functional consequences for cancer development.

β–Catenin is also involved in the control of cell-cell adhesion by binding to cadherin cell adhesion molecules. It is not yet clear as to which degree the cell adhesion function of β–catenin plays a role in Wnt signaling, but it is well established from many studies that disturbances of cell junctions is a prerequisite for tumor invasion and metastasis (Behrens 1999, Christofori 2003).

Wnt target genes and cancer development

Meanwhile a large list of Wnt target genes has been compiled by investigations in model organisms and cellular systems (see http://www.stanford.edu/~rnusse/wntwindow.html for an updated list). Wnt targets include genes regulating cell proliferation and developmental processes as well as tumor progression (Fig. 2). Here we will highlight only some of those targets for which a role in tumorigenesis was shown, or can be assumed from their known function. The promoters of the *c-myc* and *cyclin* D1 genes contain TCF binding sites and are controlled by TCF/β–catenin complexes (He *et al.,* 1998, Shtutman *et al.,* 1999, Tetsu and McCormick 1999). Through both targets Wnt signaling may promote progression of cells through the cell cycle. While upregulation of c-myc leads to repression of the cyclin-dependent kinase (CDK) inhibitor $p21^{CIP}$ and thus stimulates G1/S progression (van de Wetering *et al.,* 2002), cyclin D1 can directly activate G1 phase CDKs. Obviously, regulation of cell cycle by Wnt might play a role not only in tumorigenesis but also during normal tissue regeneration and stem cell proliferation as seen in the gut (Fig. 2). Another way by which Wnt signaling could lead to accumulation of transformed cells is inhibition of apoptosis. Expression of the anti-apoptotic gene *survivin* was downregulated by APC and analysis of its promoter revealed TCF-4 binding sites (Zhang *et al.,* 2001a, Kim *et al.,* 2003). Survivin is mainly expressed at the base of the crypts of normal colon epithelium and upregulated in colon tumors, again reflecting similar roles for Wnt signalling in the normal epithelium and tumors (Fig. 2). Wnt signaling also interferes with apoptosis induced by chemotherapeutic drugs, such as vinblastine and vincristine in Rat-1 fibroblasts, and inhibition of TCF/β–catenin increased the sensitivity of colorectal tumor cells to these compounds (Chen *et al.,* 2001).

Wnt signaling might also affect cell proliferation by induction of expression of growth factors and their receptors. For instance, FGF18 was found to be upregulated in colorectal cancer and its promoter shown to be activated by TCF/β–catenin (Shimokawa *et al.,* 2003). The tyrosine kinase c-met is upregulated in polyps of FAP patients and could be down-regulated experimentally by dominant-negative TCF in colorectal tumor cells (Boon *et al.,* 2002). c-Met is the receptor for the mesenchyme-derived Scatter Factor/HGF which is an epithelial growth factor but also promotes cell motility and invasion. Thus, upregulation of c-met could allow a cross-talk of tumor cells with the surrounding stroma and thereby promote cell proliferation and tumor cell invasion. A similar cross-talk, albeit in the opposite direction, might be generated by the upregulation of VEGF in colorectal tumor cells by TCFs, which could lead to stimulation of tumor-induced angiogenesis in the stroma and thus provide the basis for tumor growth and metastasis (Zhang *et al.,* 2001b). These examples show that aberrant Wnt signaling is not only important for the initial expansion of the transformed cell compartment as implied by the loss of the "gatekeeper" function of APC, but might also be related to the acquisition of properties required for tumor progression. In this respect it is of significance that several proteases capable of degrading extracellular matrix such as *matrilysin/MMP7* and *MMP-26* were identified as Wnt targets (Brabletz *et al.,* 1999, Crawford *et al.,* 1999, Marchenko *et al.,* 2002). The matrix metalloproteinase matrilysin has an impact on cancer progression since deficiency of matrilysin in the APC-deficient *min* mice resulted in a decrease of total tumor number and size (Crawford *et al.,* 1999). Interestingly a strong nuclear accumulation of β–catenin occurs at the invasive front of colorectal carcinomas (Fig. 2). Moreover nuclear accumulation of β–catenin together with MMP-7 expression at the invasion front was related to unfavourable outcome in colon cancer (Ougolkov *et al.,* 2002).

The motility of tumor cells and their capacity to metastasize might also be stimulated by the Wnt-induced expression of cell adhesion and extracellular matrix proteins. For instance, CD 44, a protein implicated in metastasis formation through interaction with proteoglycans was shown to be strongly overexpressed already in aberrant crypt foci, and its expression was lost in TCF-4 knockout mice (Wielenga *et al.,* 1999). Similarly, Nr-CAM a member of the Ig superfamily of adhesion receptors was shown to be induced by either β–, or γ–catenin (plakoglobin) in colon and melanoma cell lines. Nr-CAM can transform fibroblasts and increase their motility. Finally, the γ2 chain of laminin is also upregulated in the invasive areas of colon cancer and its gene promoter contains functional TCF binding sites (Hlubek *et al.,* 2001).

TCFs control cell differentiation in the gut as revealed by the finding that EphB2 and EphB3 receptors were downregulated by dominant-negative TCF while their ligand ephrinB1 was upregulated. Further analysis in *EphB* knock-out mice showed that this differential regulation served to prevent the intermingling of cells within the intestinal epithelium (Batlle *et al.,* 2002). This role in sorting out of cells could be of relevance for the development of intestinal adenomas as seen in the *min* mouse because mutant APC cells invaginate around the crypt-villus junction and avoid migration into the area of high ephrinB expression at the top of the crypt. Whether

this is also important for human colon tumor formation remains to be determined.

The Wnt pathway, similar to other signal transduction cascades, includes mechanisms for negative feedback control. The scaffold component of the β–catenin destruction complex, conductin/axin2 is a direct target gene of Wnt signaling and might be induced to attenuate the Wnt signal in normal embryonic development (Jho *et al.,* 2002, Leung *et al.,* 2002, Lustig *et al.,* 2002). Conductin is massively over-expressed in early colorectal adenomas and in carcinomas as well as in other tumors. Whether this upregulation has a functional role in the tumors in which β–catenin degradation is apparently prevented by mutations of APC or β–catenin remains to be determined. Negative control of the pathway is also achieved through the induction of dominant-negative forms of TCF-1 (Roose *et al.,* 1999).

In the future, more candidate Wnt target genes will be deciphered from gene expression profiling studies of cellular systems and from comparisons of tumors with normal tissues. An example of such an analysis is given by Schwartz *et al.,* who could identify Wnt targets by microarray analysis of ovarian endometrioid adenocarcinomas (Schwartz *et al.,* 2003). Since direct targets are likely to be of particular functional significance, detailed analysis of their promoter sequences for functional interaction with TCFs by reporter assays, chromatin IP, and footprinting methods will be one criterion to sort out "key players" from "bystanders" in such studies.

Effects of Wnt signaling on transformation in cell culture

Cell culture experiments have proven useful in establishing the biochemistry of the pathway as well as determining the contribution of individual components for oncogenic transformation. Given the potential to interfere with aberrant Wnt signaling for therapeutic purposes the establishment of Wnt-dependent cellular transformation systems is also of importance for drug screening purposes. Since the discovery of *int-1* as a protooncogene a number of cell biological studies have shown that Wnts can induce transformation *in vitro* depending on the cell culture system and target cells. For instance, transfection of int-1 in cuboidal RAC mammary cell line resulted in morphological transformation and tumorigenicity (Rijsewijk *et al.,* 1987b). Following the discovery of the other Wnt family members differences in their capacity to induce transformation were revealed which could be related to the activity in inducing stabilisation of β–catenin (Shimizu *et al.,* 1997). Transformation was also achieved by directly activating the Wnt signal in the nucleus. For instance, fusion of LEF-1 to β–catenin or to transcriptional activation domains from other transcription factors was sufficient to transform chicken embryo fibroblasts (Aoki *et al.,* 1999). Expression of the dominant-active version of β–catenin mutated in the GSK phosphorylation sites led to anchorage–independent growth and suppression of anoikis (apoptosis after loss of cell-substrate adhesion) in MDCK cells (Orford *et al.,* 1999), although it should be noted that in a similar experimental setting expression of β–catenin in an inducible system did not lead to anchorage-independent growth of MDCK cells (Barth *et al.,* 1999). β–Catenin was unable to transform 3T3 cells, while it was a potent oncogene for the RK3E epithelial cell line, indicating cell type specificity (Kolligs *et al.,* 1999). In addition, while exogenous expression of Wnts was able to transform Rat-1 fibroblasts, expression of activated β–catenin was not sufficient, possibly because Wnts have additional activities required for transformation in these cells (Young *et al.,* 1998). Collectively these results suggest that the oncogenic capacity of the Wnt pathway *in vitro* depends on the target cell as well as the read out used to determine transformation.

Several studies have shown that interference with TCF/β–catenin signaling leads to inhibition of cell proliferation in tumor cells, in particular in colorectal cancer cell lines. For example, the expression of E-cadherin was sufficient to arrest cell growth,

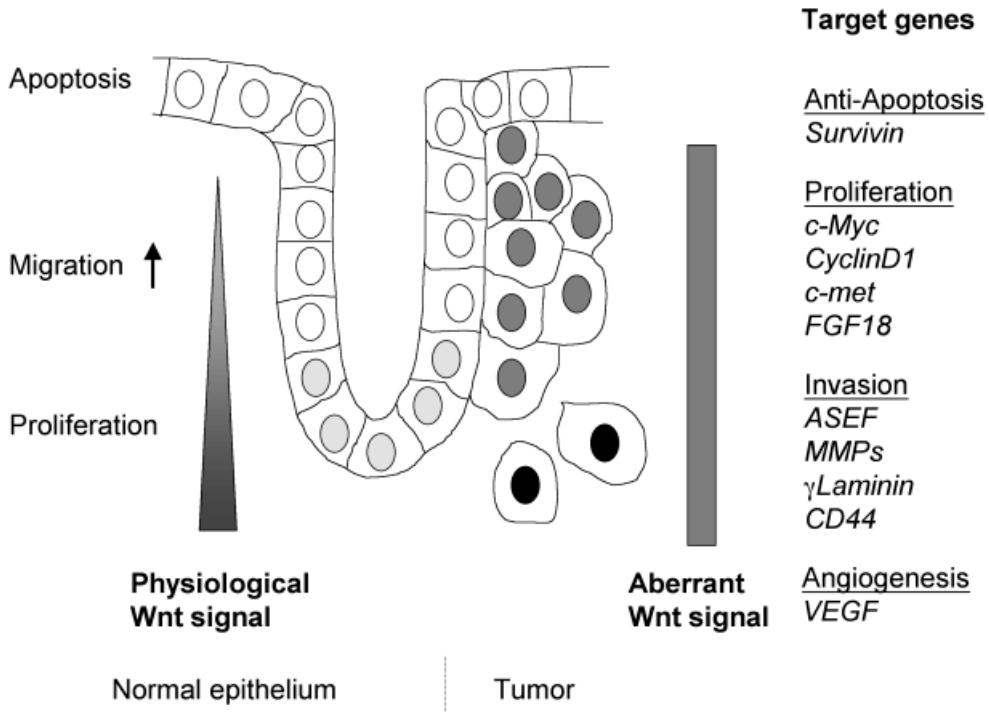

Fig. 2. Role of Wnt signaling in normal homeostasis of the colonic epithelium and in cancer. *A scheme of a colon crypt with an adjacent invasive tumor is shown. The left part schematically depicts the normal sequence of epithelial proliferation, migration and apoptosis which occurs from the bottom to the top of the crypt. A gradient of physiological Wnt signalling, presumably originating from secreted Wnt factors of the surrounding stroma, is indicated. The right part shows development of a colon tumor by aberrant Wnt signaling i.e. after mutations in either* APC *or* β-catenin. *A selection of* Wnt *target genes with their presumed role in proliferation, cell survival and invasion is shown (see text for details). Shaded nuclei indicate increasing levels of nuclear β-catenin in the normal crypt epithelium (probably reflecting the stem cell compartment), in the central tumor areas and at the invasive front (cf. Brabletz et al., 2002).*

apparently by interfering with β–catenin signaling (Stockinger *et al.,* 2001). Expression of a dominant-negative mutant of TCF, or knock-down of β–catenin by antisense or RNAi strategies blocks colon cancer cells in G1, providing proof of principle that interference with aberrant Wnt signaling could be of therapeutical value (van de Wetering *et al.,* 2003). Recently, low molecular weight compounds were found in high throughput screens that interfere with TCT/β–catenin complex formation and activation of target gene transcription. In addition these compounds blocked proliferation of colorectal cancer cell lines (Lepourcelet *et al.,* 2004).

Mutations of Wnt signaling components in tumors

As mentioned above the initial clue for a role of Wnt signaling in cancer came from the discovery of the *Wnt-1* (*int-1*) gene being transcriptionally activated in mouse mammary tumors. Whether upregulation of Wnts in human cancer plays a functional role is not known, although there are occasional reports that Wnts were found to be expressed in certain tumors. The essential finding that led to the addition of the Wnt pathway to the list of cancer relevant signaling pathways was the discovery of mutations of *APC* or *β–catenin* in colorectal cancer and later in other tumor types. Mutations of *APC* occur in a high proportion of colorectal carcinomas, while *β–catenin* is mutated in a small percentage of colorectal carcinomas and to a higher ratio in a variety of other tumors. Mutations in the *APC* gene are nonsense or frame shift mutations leading to a truncated APC protein. About 60 % of these mutations are clustered in a 700 bp "mutation cluster region" corresponding to the β–catenin/axin binding domain. Hypermethylation of the wild-type *APC* allele are also found in some sporadic CRC and may constitute an alternative mechanism for *APC* inactivation (Hiltunen *et al.,* 1997, Esteller *et al.,* 2000). Most of the *β–catenin* mutations are activating mutations, mainly occurring in exon 3 at one of the 4 phosphorylation sites (Polakis 2000).

In most cases *APC* and *β–catenin* mutations are linked to an increase of transcriptionally active β–catenin and are mutually exclusive, reflecting their role in a common pathway (Korinek *et al.,* 1997, Morin *et al.,* 1997). For instance colon tumors with mutations in *APC* have a wild-type *β–catenin* gene, and vice versa, tumors with mutations in *β–catenin* are wild-type for *APC*. In the following we will briefly summarize some of the mutational analyses that have been performed so far in tumors from different tissues. We will concentrate on frequent tumor diseases in which a high proportion of samples show evidence of Wnt pathway activation (for more detailed reviews see (Giles *et al.,* 2003, Lustig and Behrens 2003))

Mutations of *APC* were first identified in the germline of FAP (familial adenomatous polyposis) patients. These patients develop hundreds of polyps in the colon after loss of the remaining wild-type allele demonstrating that *APC* behaves as a classical tumor suppressor and follows the Knudsen two-hit hypothesis. There are different phenotypes and thus subclasses of FAP associated tumors which correlate with the position of the mutations within the *APC* gene (for details on the genetics of APC see the article by Fodde in this issue). Since *APC* mutations are detected very early in the adenoma-carcinoma sequence, the APC protein has been suggested to act as a gatekeeper of colorectal carcinogenesis, which means that functional loss of APC is a prerequisite for the further progression towards malignancy (Kinzler and Vogelstein 1996). Importantly, about 80% of the non-inherited, sporadic CRC carry mutations of *APC*.

Colorectal adenomas have been the first tumors in which nuclear localization of β–catenin was demonstrated (Inomata *et al.,* 1996). Interestingly, the nuclear staining for β–catenin often shows a heterogenous pattern with strong nuclear enrichment at the invasion front and mainly cytoplasmic and membrane staining in the central tumor area. This indicates that high levels of nuclear β–catenin play a role in the transition to the invasive state of tumor cells. The molecular basis for this differential distribution of β–catenin is not known. Obviously, one possibilty is that signals coming from the mesenchyme that surround the invasive tumor cells might lead to additional stabilisation and nuclear enrichment of β–catenin (Brabletz *et al.,* 2002).

Mutations of *β–catenin* have been mainly detected in microsatellite instabile colorectal tumors (Kitaeva *et al.,* 1997, Sparks *et al.,* 1998). These tumors are characterized by either sporadic or, as in the case of HNPCC patients, inherited mutations in DNA mismatch repair components. It appears that mutations in *APC* occur less frequently in these tumors than in mismatch repair proficient cases although numbers vary between different studies (Miyaki *et al.,* 1999, Domingo *et al.,* 2004). Mismatch repair-deficient CRC also show heterozygous mutations in the *axin2* gene which resulted in truncated protein with a dominant-negative action (Liu *et al.,* 2000, Domingo *et al.,* 2004). However, mutations in error-prone nucleotide repeats are frequent in mismatch repair deficient tumors and may not always be of functional relevance. For instance, mutations in the *TCF-4* gene found in microsatellite-instabile tumors had no effect on transcriptional activity, suggesting that they are not relevant for tumor formation (Ruckert *et al.,* 2002).

A plethora of studies of the recent years have shown that aberrant Wnt signaling is not restricted to colorectal tumorigenesis but might play a role in a variety of other tumor types of gastrointestinal origin. Liver tumors seem to be particularly prone to harbor mutations in the *β–catenin* gene. Up to 70% of hepatoblastomas, which are early childhood liver tumors, and about 25% of hepatocellular carcinomas contain activated β–catenin (de La Coste *et al.,* 1998, Koch *et al.,* 1999, Park *et al.,* 2001, Taniguchi *et al.,* 2002). In addition, mutations in the *axin* and *axin2* genes resulting in truncated proteins were also found although at a lower frequency (Satoh *et al.,* 2000, Taniguchi *et al.,* 2002). *APC* mutations are present in 76% of sporadic gastric adenomas (Groves *et al.,* 2002, Lee *et al.,* 2002), while gastric carcinomas showed only few *APC* mutations (Lee *et al.,* 2002). Gastric adenomas also occur in FAP patients (Groves *et al.,* 2002). Activating *β–catenin* mutations were found in about one third of gastric tumor samples (Park *et al.,* 1999, Clements *et al.,* 2002).

In endometrial carcinomas *β–catenin* mutations were found in about 40% of cases and were associated with the endometrioid phenotype (Mirabelli-Primdahl *et al.,* 1999, Moreno-Bueno *et al.,* 2002). In ovarian carcinomas *β–catenin* mutations were found in 7 of 11 cases (Gamallo *et al.,* 1999). In another study 14 of 45 ovarian endometrioid carcinomas carried mutations in *β–catenin*. Mutations in *APC*, *axin*, and *axin2* were also found in a few cases (Wu *et al.,* 2001).

Prostate cancer has been shown to carry mutations in *β–catenin*, *APC*, and the E3 Ubiquitin ligase of β–catenin, *βTrCP* in

total in about 30% of cases (Voeller *et al.,* 1998, Chesire *et al.,* 2000, Gerstein *et al.,* 2002). Functional interaction of β–catenin with the androgen receptor was also shown indicating a cross-talk of both pathways in the development of prostate cancer (Chesire *et al.,* 2000, Truica *et al.,* 2000, Mulholland *et al.,* 2002, Yang *et al.,* 2002). In transgenic animal models stabilized β–catenin induced lesions reminiscent of prostatic intraepithelial neoplasia (Gounari *et al.,* 2002).

In sporadic anaplastic thyroid carcinomas immunofluorescence staining showed nuclear localization of β–catenin in 42% and mutations in 61% of the analyzed samples. In a further study exon 3 mutations and nuclear β–catenin localization were restricted to poorly differentiated (25%) or undifferentiated (66%) carcinomas (Garcia-Rostan *et al.,* 1999, Garcia-Rostan *et al.,* 2001). Wilms tumor as one of the most common childhood renal malignancy, has been shown to harbor *β–catenin* mutations with a preference for mutation of *β–catenin* at codon 45, which occurred in more than 90% of the cases (Koesters *et al.,* 1999, Maiti *et al.,* 2000, Kusafuka *et al.,* 2002).

In melanomas immunohistochemistry showed increased β–catenin levels, however, mutations in β–catenin were rarely detected, indicating that other components of the pathway might be affected (Rimm *et al.,* 1999). The Microphtalmia associated trancription factor (MITF), a factor that is involved in melanocyte differentiation is a direct target of TCF/β–catenin complexes and can rescue suppression of melanoma growth by dominant-negative TCF (Widlund *et al.,* 2002).

There is evidence that the Wnt pathway plays a role in several tumors of mesenchymal origin. *β–Catenin* mutations associated with nuclear localization of β–catenin are found in desmoid tumors**,** which represent an infiltrative form of fibromatosis; these tumors also occur in a subgroup of FAP partients. Furthermore, transgenic expression of β–catenin in mice led to development of aggressive fibromatosis (Cheon *et al.,* 2002). Analysis of osteosarcomas revealed a cytoplasmic/nuclear accumulation of β–catenin in 33 cases out of 47 samples, but *β–catenin* mutations were not detected (Haydon *et al.,* 2002).

Finally, various mutations of Wnt pathway components including *APC*, *β–catenin*, and *axin* have been found in medulloblastomas (Zurawel *et al.,* 1998, Huang *et al.,* 2000, Dahmen *et al.,* 2001, Koch *et al.,* 2001, Baeza *et al.,* 2003).

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