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Review

Targeting the WNT/β-catenin/TCF/LEF1 axis in solid and haematological cancers: Multiplicity of therapeutic options

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ABSTRACT

Among aberrantly regulated signalling pathways in cancer the *WNT/β-catenin* pathway plays an outstanding role, since it was shown to be critically involved in a wide range of neoplasias. While the underlying mechanisms vary, overexpression of WNTs was found to mediate active signalling in some of these diseases. Other cancers show a mutation in pathway members further downstream, such as *APC*, *Axin* or *β-catenin*, leading to aberrant signalling activation. Another mechanism initiating activation of *WNT/β-catenin* signalling is the silencing of expression of negative *WNT/β-catenin* regulators, such as *DKK* and *WIF1*, by, for example, promoter hypermethylation. All these mechanisms result in a common consequence, the activation of *TCF/LEF1* transcription factors and subsequent target gene expression. Several target genes are known to be key players in tumourigenesis, such as *c-myc*, *cyclin D1* or *survivin*. The variety of possible underlying mechanisms leading to *β-catenin/TCF/LEF1* activation offers multiple options to target the aberrantly activated pathway in order to prevent target gene expression and/or their gene products to exert their tumourigenic function.

Here, we summarise the physiological role of *WNT/β-catenin* signalling and the consequences of its aberrant activation during tumourigenesis. Furthermore, we discuss the possible strategies to target this pathway and their potential importance in cancer treatment.

1. Physiological and pathophysiological functions of WNT/β-catenin signalling

1.1. General background

WNT proteins are secreted, lipid-modified, glycoproteins which activate cell surface receptor-mediated signal transduction pathways to regulate a range of cellular activities, including cell fate determination, proliferation, migration, polarity and gene expression mostly during embryogenesis and early development.[1]

Wnt ligands trigger at least three distinct intracellular signalling cascades: the *WNT*/Ca^{2+} pathway, the *WNT*/planar cell polarity pathway and the canonical *WNT/β-catenin* pathway. The latter is well characterised and of severe importance in embryogenesis and essential in some processes in the adult organism, such as the regulation of epithelial cell proliferation and haematopoiesis. Furthermore, the pathway has been implicated in various cancers.[2–4] In the following, we will exclusively focus on the canonical *WNT/β-catenin* pathway.

WNT proteins bind to a cell surface receptor complex comprised of a member of the frizzled (*FZD*) receptor family and its

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coreceptor *LDL* receptor-related protein 5/6 (*LRP5/6*), leading to activation of a dishevelled protein family member (*DSH*). Active *DSH* inhibits a complex consisting of glycogen synthase kinase 3β (*GSK3β*), *Axin* and adenomatous polyposis coli (*APC*). In its active state, this complex leads to phosphorylation of *β-catenin* via GSK3β. Phosphorylated *β-catenin* is a target of the proteasome machinery and becomes degraded. Inhibition of the *GSK3β/Axin/APC* complex prevents phosphorylation, hence degradation of *β-catenin* does not take place. It accumulates in the cytoplasm and translocates into the nucleus, where it binds and activates a member of the T-cell factor (*TCF*)/lymphoid enhancer binding factor 1 (*LEF1*) transcription factor family (Fig. 1). This leads to the expression of the target genes, which are involved in the regulation of cellular processes, such as proliferation and differentiation.[4]

In addition to activation through *WNT* signalling β-catenin was described to be also activated via alternative stimuli. Insulin signalling, for example, leads to activation of *PKB/AKT*, which initiates phosphorylation and inactivation of *GSK3β*, subsequently resulting in *β-catenin* accumulation.[5] Lu and colleagues worked with tumour cells expressing the epidermal growth factor receptor (*EGFR*). In these cells *β-catenin/TCF/LEF1* activity can be enhanced by epidermal growth factor (EGF) stimulation independent of the activity status of *GSK3*.[6]

1.2. *Physiological role of WNT/β-catenin signalling*

While inactive in most adult tissues, *WNT* signalling has been shown to be critically involved in embryogenesis and during organ development.[1–3] Mutations in *WNT* genes were shown to lead to remarkable phenotypes in mice and drosophila. For example, mice with *WNT4* mutations failed to develop kidneys. It was shown that *WNT4* might be essential in the mesenchymal–epithelial transition occurring during the formation of this organ. *WNT7a* inactivation was shown to result in mice with ventralised limbs, whereas absence of *WNT2* resulted in placental defects (summarised by Cadigan and colleagues[2]). Loss of particular cells or tissues in *WNT* mutants could possibly result from the perturbations in the cell fate specification. An alternative explanation may be a failure of progenitor cells to expand. A general function of *WNT* signalling during developmental stages may therefore be to regulate

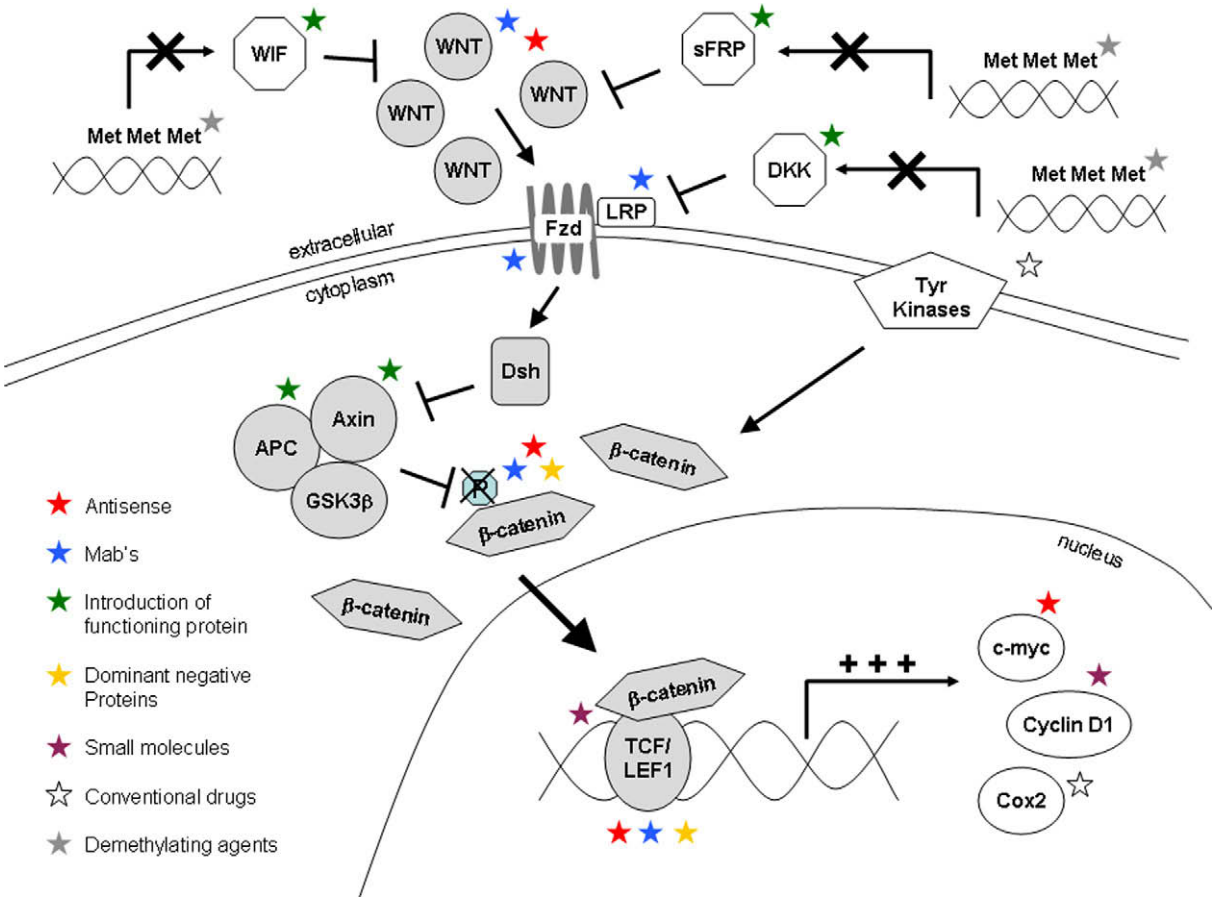

Fig. 1 – Active *WNT/β-catenin* signalling cascade: *WNT* proteins bind the receptor complex and activate *DSH*, which inhibits the *APC/Axin/GSK3β* complex, preventing phosphorylation of *β-catenin*, which accumulates in the cytoplasm, translocates into the nucleus and activates *TCF/LEF1* family transcription factors. Stars indicate possible intervention targets and approaches following shown colour scheme. *APC*: adenomatous polyposis coli; *Cox2*: cyclooxygenase 2; *DKK*: Dickkopf; *DSH*: Dishevelled; *FZD*: Frizzled; *GSK3*: glycogen synthase kinase 3; *LEF1*: Lymphoid enhancer-binding factor 1; *LRP*: low-density lipoprotein receptor-related protein, Mabs: Monoclonal antibodies, Met: hypermethylation; P: phosphorylation, *sFRP*: secreted frizzled related protein; *TCF*: T-cell factor; Tyr kinase: Tyrosine kinase; *WIF1*: *WNT* inhibitory factor 1.

cell proliferation by direct induction of cell cycle regulators, such as *c-myc* and *cyclin D1*.

Also in humans *WNT* proteins could be shown to be crucial during a variety of developmental processes. Tetra amelia is a severe syndrome, which is characterised by the absence of all four limbs. In addition, other malfunctions in head, heart, lungs, skeleton, nervous system and genitalia were described. Patients were found to exhibit a common mutation in both alleles of the *WNT3* gene, which is exclusively responsible for the patient's phenotypes.[7] It is supposed that other malformation syndromes with unclear molecular background, at least in parts, arise from the defects in *WNT* proteins in humans.

Besides its role during development, *WNT/β-catenin* signalling has also been implicated in several processes in the adult organism, such as epithelial cell proliferation, lymphocyte development and regulation of haematopoietic stem cell (HSC) fate.

Reya and colleagues showed that *LEF1*- and *FZD9*-knockout mice exhibit perturbation of B-cell development at the pro/pre-B-cell stage. *LEF1* null mice demonstrated defects in the pro-B-cell proliferation, whereas in *TCF1* null mice the thymocyte differentiation was defective.[8]

The role of *WNT* proteins in haematopoietic stem cell (HSC) renewal is not surprising, since *WNT* proteins are important in mesoderm patterning and the first HSCs during development arise from mesoderm.

Austin and colleagues found *WNT-10b* expression in a haematopoietic cell progenitor population ($AA4^+cKIT^+SCA1^+$) from murine foetal liver. Further, *WNT-5a* was found to be expressed in the local microenvironment of haematopoietic stem cell progenitors in the murine foetal liver, suggesting *WNTs* to be involved in the regulation of haematopoietic stem/progenitor cell fate. When mouse foetal liver progenitor cells (($AA4^+cKIT^+SCA1^+$) were cultured in conditioned medium from 293T cells transfected with *WNT*-1, *WNT*-5a or *WNT*-10b cDNAs plus addition of Kit ligand, cell proliferation was increased 7-, 8- and 11-fold, respectively. When *WNTs* were removed from supernatants by precipitation with a monoclonal antibody, cell expansion was reduced to control values, clearly indicating the role of the *WNT* protein.[9] Thereupon, several studies could demonstrate the involvement of *WNT/β-catenin* signalling in HSC commitment, differentiation and renewal. Van den Berg and colleagues demonstrated expression of *WNT-5A*, *WNT-2B* and *WNT10B* in foetal bone marrow stromal cells, adult bone marrow and haematopoietic cell lines. They further proofed the biological activity of those *WNTs* on haematopoietic stem/progenitor cells. When CD34+ haematopoietic progenitors were cultured on a *WNT* producing feeder layer the number of less differentiated haematopoietic cells increased. Accordingly, fewer mature cells, relative to control, were found. The biological activity of the *WNT* proteins was comparable to those initiated by stem cell factor (SCF) or IL-3 as assessed by their effect on haematopoietic colony formation, CD34 cell numbers and cellular morphology.[10]

In another study, the transduction of mouse HSCs with S33-mutant *β-catenin*, which is constitutively active, lead to the maintenance of their immature phenotype in long term culture, further demonstrating the role of *WNT* signalling in the maintenance of the immature phenotype of haematopoietic progenitors.[11]

In summary, *WNT* signalling plays a significant role during embryogenesis, shown by the development of severe malformation or pre-birth lethality in mice and humans lacking major *WNT*-signalling components, such as *WNTs*, *LEF1* and *β-catenin*. In addition, *WNT/β-catenin* signalling is involved in haematological processes such as haematopoiesis and HSC regulation. Due to these crucial roles, it stands to reason that uncontrolled signalling activity influences the cell to an extent which potentially leads to initiation and progression of diverse malignancies.

1.3. *Alterations of WNT/β-catenin signalling in solid tumours*

The *WNT* proteins were the first players of the *WNT/β-catenin* signalling pathway to be shown being involved in tumourigenesis. Thus, *WNT1* was demonstrated to contribute to tumourigenesis in the mammary gland,[12] and in breast cancer tissue a correlation between *WNT2*, *WNT4* and *WNT7* and abnormal proliferation of the malignant cells was described.[13] In addition, mutations of several other *WNT/β-catenin* pathway members were shown in different cancers, resulting in constitutive pathway activation. Major players of *WNT/β-catenin* signalling, such as *APC*, *Axin*, *FZD*, *LRP* and *β-catenin* itself, have been studied in this regard and are discussed in the following passage.

APC was originally discovered as the genetic cause for familial adenomatous polyposis (FAP). FAP is characterised by the development of colorectal polyps in early adulthood, which can over time mutate into carcinomas and metastatic colorectal cancer. Nearly 80% of all the sporadic colorectal cancers exhibit an inactivation of both *APC* alleles, making the *APC* gene a tumour suppressor in colorectal tumourigenesis. Loss of *APC* function leads to accumulation of *β-catenin*, its translocation into the nucleus and initiation of target gene expression by activating *TCF/LEF1*. *APC* mutations seem to be an exclusive phenomenon in colon cancer cells since they have not been found in any other cancer.[4,14] Colorectal cancer patients with intact *APC* genes were shown to exhibit a mutation in the *β-catenin* gene instead, having the same effect as APC mutation, a constitutive activation of *β-catenin/TCF/LEF1* target gene expression.[15]

Besides colorectal carcinoma, *β-catenin* mutation was detected in a variety of cancers, for example, melanoma, hepatocellular carcinoma, medulloblastoma, ovarian, pancreatic and prostate carcinomas. For a summary see Polakis.[4] As mentioned earlier, most *β-catenin* mutations lead to enhanced *β*-catenin signalling activity. This can be due to a mutational change in the *GSK3β* phosphorylation side, preventing *β-catenin* phosphorylation and therefore, its proteasomal degradation. The contrary would be a mutation preventing binding of *β-catenin* to *TCF/LEF1*, which would impede target gene expression. To our knowledge such mutations have not been found in any cancer yet.

Another member of the *WNT/β-catenin* pathway associated with malignant disease is *Axin*. It binds *APC* and *GSK3β* to form a destruction complex, leading to phosphorylation and subsequent degradation of *β-catenin*. *Axin* mutations and/or

a decreased or absent *Axin* expression lead to *β-catenin* stabilisation and increased expression of *β-catenin/LEF1* target genes. *Axin* defects could be connected to a large number of human neoplasms including colorectal cancer,[16] medulloblastoma[17] or hepatocellular carcinomas.[18]

Besides *WNT* proteins themselves, further extracellular proteins are known to manipulate *WNT* signalling. Secreted frizzled related protein 1 (*sFRP1*) functions as modulator of *WNT* signalling through direct interaction with *WNT*s. Amongst others, downregulation of *sFRP1* was reported in bladder cancer,[19] pancreatic carcinoma,[20] hepatocellular carcinoma[21] and breast cancer.[22] This was in part due to silencing of the *sFRP* promoter by hypermethylation.

As another member of the WNT pathway, the *LRP* coreceptor was linked to the development of cancer. Thus, *LRP5* was identified as a possible marker for disease progression in high-grade osteosarcoma.[23]

LEF1 itself was shown to be a direct target of the *TCF/LEF1/β-catenin* transcriptional complex.[24] Aoki and colleagues demonstrated the significance for *LEF1* in the initiation of tumourigenesis. They replaced the activation domain of *β-catenin* with a chimeric protein consisting of the *LEF1* binding sequence which was fused to the transcriptional activation domain of the oestrogen receptor. The expression of this chimeric protein leads to neoplastic transformation in the chicken embryo fibroblasts, verifying the oncogenecity of transactivating *LEF1*.[25]

Summing up, in a wide range of different cancer types mutations in genes of *WNT/β-catenin* pathway members, such as *β-catenin*, *APC* and *Axin* could be detected. A common consequence is the stabilisation of *β-catenin*, leading to induction of increased expression of the pathwayś target genes, such as *c-myc* and *cyclin D1*, known for their role in initiation and progression of cancer.

1.4. WNTs and their downstream proteins in haematological malignancies

Due to the role of *WNT/β-catenin* signalling in haematopoiesis, it suggests itself to potentially assume a role of this pathway in haematological malignancies. In fact, several leukaemias and myelomas have been associated with aberrant *WNT/β-catenin* activity.

Acute lymphoblastic leukaemia (ALL) cells were shown to express several *WNT*s and *FZD*s. *WNT3a* stimulation led to increased *β-catenin* accumulation and translocation into the nucleus, which was accompanied by an increased proliferation and enhanced survival of ALL cells under starvation conditions *in vitro*.[26] An ALL cell line, which expresses the oncogenic transcription factor E2A-PBX1 due to a t(1;19) translocation, exhibits increased *WNT16b* levels. Downregulation or blocking of *WNT16* by either a neutralising antibody or a siRNA approach led to induction of apoptosis in this E2A-PBX1 positive cell line. This effect was further accompanied by a downregulation of expression of *LEF1/β-catenin* target genes *cyclin D1* and *survivin*, indicating constitutive signalling of the canonical *WNT/β-catenin* pathway in this ALL cell line.[27] Furthermore, in B-cell ALL the promoter of the *DKK3* gene was shown to be hypermethylated, hence *DKK3* expression is attenuated. *DKK3* is an antagonist of *WNT/β-catenin* signalling and supposed to be a tumour suppressor. *DKK3* hypermethylation was shown to have prognostic significance regarding disease free survival in ALL. Thus, patients showing an hypermethylated *DKK3* promoter region exhibit an overall survival of 10.5% after 10 years while in patients without hypermethylation the survival rate was 49.8%. This clearly indicates the influence of active *WNT/β-catenin* signalling in this disease.[28]

Primary acute myeloid leukaemia (AML) cells express *β-catenin* at the mRNA and the protein level to a significantly higher extent than healthy haematopoietic progenitors.[29] Simon and colleagues could verify the presence of *β-catenin* in the nucleus in AML cells, resulting in associated *TCF/LEF1* reporter activity. In addition, AML cells showed an aberrant expression scheme of *WNT1, WNT2b* and *LEF1* transcripts.[30] Additionally, elevated protein levels of *β-catenin, cyclin D1* and *c-myc* are linked to the presence of the AML fusion proteins *AML-1/ETO*, promyelocytic leukaemia/retinoic acid receptor alpha (*RARα*) and promyelocytic leukaemia zinc finger/*RAR*.[31] Luo and colleagues showed a stimulation of myeloid progenitor cell growths initiated by c-myc expression in the bone marrow of mice, which led to the development of AML in these animals.[32] Taking this into account, *c-myc* can be understood as a critical downstream effector of myeloid leukaemogenesis and thus as a potential target for anticancer therapy.

In B-cell chronic lymphocytic leukaemia (B-CLL) several *WNT* proteins and *FZD3* mRNA were markedly elevated compared to normal peripheral blood lymphocytes (PBLs) and B-cells. In contrast, *β-catenin* protein was not detected, but its expression could be induced by *GSK3* inhibition, going along with increased survival, whereas inhibition of *WNT/β-catenin* signalling by an analogue of a non-steroidal antiinflammatory drug (R-etodolac) enhanced cell death.[33] A global gene expression array revealed a high overexpression of *LEF1* in B-CLL cells when compared to normal B-lymphocytes.[34] Furthermore, it could be shown that the expression of known *WNT* inhibitors, such as soluble *FZD*-related proteins (*sFRP*), was silenced through promoter hypermethylation.[35] Also *WNT* inhibitory factor 1 (*WIF1*), a soluble negative regulator of *WNT/β-catenin* signalling, was shown to be silenced by promoter hypermethylation in around 11% of B-CLL patients. In this study, a significant positive correlation between the extent of *WIF1* promoter hypermethylation and age was found.[36]

In chronic myeloid leukaemia (CML) the expression patterns of the members of the *WNT/β-catenin* pathway, such as *FZD2* and *LRP6*, were shown to change with regard to disease progression.[37] Granulocyte–macrophage progenitor pool from patients with CML in blast crisis and imatinib-resistant CML was shown to exhibit elevated levels of nuclear *β-catenin* as compared to the levels in progenitors from normal marrow.[38] Furthermore, *BCR-Abl* initiated CML in mice could be abrogated by *β-catenin* deletion, clearly indicating its importance in self-renewal of neoplastic stem cells in CML.[39] Also multiple myeloma (MM), a B-cell malignancy of mature plasma cells, was associated with *WNT/β-catenin* signalling, since *β-catenin* overexpression was found. The cells responded to *WNT3a* stimulation by increased proliferation, whereas blocking of *WNT* signalling, by, for example, dominant negative *TCF*, influenced cell growth negatively.[40] Kaiser and colleagues

showed that serum concentrations of the *WNT* antagonist *DKK1* correlate with the extent of bone disease in multiple myeloma patients.[41] It can be suggested that both *WNT* antagonists may contribute to osteolytic lesions in MM by suppressing the normal osteoblast function.

Although no concrete role of *WNT/β-catenin* signalling in onset and progression of lymphomas has yet been demonstrated, several facts suggest an involvement of this pathway. Qiang and colleagues showed that *FZD3* is existent in lymphoma cell lines, whereas *LRP5/6* could not be detected. Since they found high levels of several *FZDs* and *LRP5/6* in myeloma cells, which represent a later stage of B-cell development, they concluded that *WNT* receptors might be expressed stage specific during B-cell development.

Also in less common haematological malignancies such as precursor T-lymphoblastic leukaemia/lymphoblastic lymphoma (T-ALL/LyL) and peripheral T cell lymphoma (PTCL) *WNT/β-catenin* was implicated. Dorfmann and colleagues could show *TCF* and *LEF1* expression in 9/10 T-ALL/LyL patients and 39/81 of PTCL patients. These data are descriptive though and so far no prognostic relevance or information about disease state could be associated.[42] Also in cutaneous lymphoma of T- and B-cell origin a mutation-independent deregulation in *β-catenin* expression was shown.[43] Whether this deregulation leads to aberrant *WNT/β-catenin* signalling and whether this is involved in disease onset and/or progression remains to be determined.

Table 1 summarizes the potential causes of deviant *WNT/β-catenin* signalling activation and examples for their cancer involvement.

2. Targeting WNT/β-catenin signalling as therapeutic approach in cancer

Despite the high number of findings linking the activation of *WNT/β-catenin*-signalling in the adult organism to cancers, not very many attempts have been made to manipulate this pathway for therapeutic purposes. Therefore, in this section we summarise the existing studies concerning intervention into *WNT* signalling in the different cancer backgrounds. Possible targets and approaches are further displayed in Fig. 1.

2.1. *Extracellular targets*

DKK, *WIF1* and *sFRP* are the known negative regulators of *WNT* signalling. *WIF1* and *sFRP* directly bind *WNT* ligands, therefore preventing them from binding to their receptors. *DKK* binds the *LRP* coreceptor, hence blocking the intracellular signal

Table 1 – Aberrant *WNT/β-catenin* Aberrant signalling can be provoked by a variety of causes. Here we list the possible causations leading to an activation of the *WNT/β-catenin* cascade and specify examples of cancer involvement.

Aberrant feature	Pathway member	Examples for associated cancers	Reference
Overexpression of pathway activator	*WNTs*	Acute lymphoblastic leukaemia	[26,27]
		Acute myeloid leukaemia	[30]
		Breast cancer	[12,13]
		Chronic lymphocytic leukaemia	[33]
	FZD	Acute lymphoblastic leukaemia	[26]
		Chronic lymphocytic leukaemia	[33]
		Synovial sarcoma	[47]
	LEF1	Chronic lymphocytic leukaemia	[36]
	β-Catenin	Acute myeloid leukaemia	[29,30,32]
		Chronic myeloid leukaemia	[38]
		Multiple myeloma	[40]
	LRP5	Osteosarcoma	[23]
Downregulation of pathway inhibitor	*DKK3*	Acute lymphoblastic leukaemia	[28]
	DKK1	Multiple myeloma	[41]
	sFRP	Bladder cancer	[19]
		Breast cancer	[22]
		Chronic lymphocytic leukaemia	[35]
		Hepatocellular carcinoma	[21]
		Pancreatic cancer	[20]
	WIF1	Chronic lymphocytic leukaemia	[36]
Mutation of pathway member leading to signalling activation	*β-Catenin*	Colorectal cancer	[15]
		Melanoma, medulloblastoma, hepatocellular-, pancreatic-, ovarian-, prostate carcinoma	[4]
	APC	Colorectal cancer	[4,16]
	Axin	Colorectal cancer	[16]
		Hepatocellular carcinoma	[18]
		Medulloblastoma	[17]

APC, adenomatous polyposis coli; *DKK*, Dickkopf; *FZD*, frizzled receptor; *LEF1*, lymphoid enhancer factor 1; *LRP*, low-density lipoprotein receptor-related protein; *sFRP*, secreted frizzled related protein; *WIF1*, *WNT* inhibitory factor 1.

transduction. All the three proteins are commonly downregulated in cancers, due to silencing of their expression through promoter hypermethylation. A reintroduction of these proteins could be useful, in that they could counteract *WNT/β-catenin* signalling activation. This could be mediated by direct introduction of the functioning protein through, for example, virus-mediated gene transfer. Furthermore, demethylating agents might be useful to reinduce gene expression. These agents inhibit methylation, hence expression of previously hypermethylated genes can take place again. Functioning *DKK*, *WIF1* and *sFRP* could fulfil their function and negatively regulate *WNT/β-catenin* signalling, preventing the expression of tumourigenic *β-catenin/TCF/LEF1* target genes.

2.2. Targeting the WNT protein/FZD receptor level

A possible approach for extracellular targeting is diminishing *WNT* ligand activity by an antisense strategy or blockage of their activity by antibodies. Polanec and colleagues studied the effect of *WNT1* antisense RNA on the outgrowth of a *WNT1* expressing mammary adenocarcinoma cell line. They could demonstrate a reduction of *WNT1* proteins going along with significantly decreased tumour outgrowth.[44] He and colleagues tested the effect of *WNT1* abrogation in human non-small cell lung cancer (NSCLC), breast cancer and sarcoma *WNT1* expressing cell lines. They found an induction of apoptosis after both, RNAi targeting of *WNT1* and the incubation with a monoclonal anti-*WNT1* antibody in *WNT1* expressing cell lines, whereas cell lines, which do not exhibit *WNT1* proteins, remained unaffected. Targeting *WNT1* with a monoclonal antibody was also shown to be sufficient to reduce tumour growths in xenotransplanted (H460, NSCLC) female nude mice.[45] Similar results were achieved targeting *WNT2* in human melanoma cell lines using both approaches, antisense targeting and monoclonal antibody treatment. *WNT2* overexpressing cell lines died upon *WNT2* deprivation, whereas cell lines low in *WNT2* did not respond. A nude mouse xenotransplant (LOX, melanoma) model exhibited a decreased tumour growth when treated with a monoclonal antibody targeting *WNT2* compared to untreated control animals.[46]

A potential approach would also be targeting either the *FZD* receptor or the *LRP* coreceptor with antibodies to block further signalling and prevent target gene expression. Nagayama and colleagues used a polyclonal anti-*FZD10* antibody in a synovial sarcoma xenograft model, which lead to attenuation of tumour growth.[47] Synovial sarcomas exhibit a severe overexpression of *FZD10*, whereas other human organs show low levels, making *FZD10* an attractive target in synovial sarcoma. The same group established a monoclonal anti-*FZD10* antibody in subsequent experiments and found it to effectively reduce tumour growths in a synovial sarcoma mouse xenograft model.[48]

Despite these promising results, it has to be kept in mind, that the *WNT/β-catenin* pathway has crucial tasks during several developmental processes also in the adult organism; hence tissue specific side-effects are expected. Thus, targeting players of the signalling cascade, which are located further downstream, might be a way to potentially reduce the side-effects. In this case the option for *WNT* signalling to carry out certain functions through branching of pathways, rather than activation of *LEF1* target gene expression, would still be given.

2.3. Targeting pathway members downstream of WNT/FZD

Many of the cancers shown to be influenced by *WNT/β-catenin* signalling exhibit defects in pathway members, which are negative regulators of the signalling cascade and function as tumour suppressors.

APC has been shown to be mutated in many colon cancers to that regard, that it is not capable of binding *β-catenin* anymore, which therefore is not phosphorylated and not degraded. As a consequence *β-catenin* accumulates in the cytoplasm, translocates into the nucleus, eventually leading to *LEF/TCF/β-catenin* target gene expression. The importance of this fact for tumourigenesis was shown by Shih and colleagues. An *APC* negative colon cancer cell line was transfected with a recombinant adenovirus (Ad-CBR) which constitutively expressed the central third of *APC* including all *β-catenin* binding repeats. The subsequent *β-catenin* decrease resulted in inhibition of *β-catenin/TCF/LEF1* target gene transcription, growth arrest and apoptosis.[49] As *APC*, also *Axin* was shown to function as a tumour suppressor. *Axin* mutations are involved in different kinds of cancer. The introduction of functional wild-type *Axin* in hepatocellular and colorectal cancer cells via adenovirus-mediated gene transfer was shown to induce apoptosis.[50] So, also *Axin* would be a candidate for virus-mediated gene transfer to reconstitute the full function of the protein subsequently preventing *β-catenin/TCF/LEF1*-mediated expression of tumourigenic relevant genes.

2.4. Directly targeting β-catenin/TCF/LEF1

All mentioned defects, such as *WNT* protein over expression, absence of negative regulators through, for example, promoter hypermethylation and mutations in functional sides of pathway members such as *APC* and *Axin*, lead to increased β-catenin levels. So directly targeting *β-catenin* seems to be a consequential approach.

Antisense targeting of *β-catenin* in colon cancer has been done by Roh and colleagues. Decreased *β-catenin* levels lead to decreased *TCF* transcriptional activity and subsequent inhibition of cell proliferation. The same group studied the effect of antisense targeting of *β-catenin in vivo*. Tumour growth was significantly reduced upon *β-catenin* reduction in a colon cancer (SW480) xenotransplant nude mouse model.[51] The same results were achieved in an oesophageal carcinoma cell line. Antisense targeting of *β-catenin* led to decreased *TCF* transcriptional activity, decreased cell proliferation and caspase3-mediated apoptosis.[52] Verma and colleagues showed growth inhibition of colon cancer cells by specific downregulation of *β-catenin* through RNA interference *in vitro* and in a xenotransplant mouse model.[53]

Interfering with *WNT/β-catenin* signalling was also investigated in the haematological background. Dominant negative *β-catenin* and dominant negative *TCF* were shown to reduce signalling activity and subsequently proliferation in Jurkat

(acute T-cell leukaemia) and K562 (myelogenous leukaemia) cells.[54]

Recently, a screening of about 7000 purified natural compounds identified two small molecules, namely PKF115-584 and CGP049090, which specifically inhibit *β-catenin/TCF/LEF1* activation of transcription.[55] This is mediated by disruption of *β-catenin/TCF/LEF1* complexes as could be shown by *in vitro* assays and reporter gene activation studies. In colon carcinoma cells *c-myc* and *cyclin D1* expression as well as cell proliferation was clearly diminished upon treatment and cytotoxicity was induced.[56] We tested both compounds in CLL and found them to disrupt the *β-catenin/LEF1* transcriptional complex, which leads to effective induction of apoptosis (unpublished data).

2.5. *Targeting of β-catenin/TCF/LEF1 target gene*

The final step of the signalling process is the expression of target genes. These genes are the actual executers. Many of the *WNT/β-catenin* target genes, such as *c-myc*, *cyclin D1* and cyclooxygenase 2 (*Cox2*) have been implicated in tumourigenesis of several cancers.

In an *in vitro* experiment the *WNT*-active PC-3 prostate cancer cell line was treated with AVI-4126, an antisense drug targeting *c-myc*. After incubation with AVI-4126, a significant growth inhibition of the cells could be detected, followed by apoptosis of the treated cells. The substance was also tested in a xenograft prostate cancer mouse model. Here AVI-4126 reduced the tumour burden up to 80%. Due to these promising results the first Phase 1 trials were conducted. AVI-4126 was intravenously administered to healthy individuals and no toxicity or serious adverse events could be detected.[57]

Another *WNT/β-catenin* target gene is *cyclin D1*, which functions together with the cyclin-dependent kinases as a regulator of the cell cycle progression and has frequently been involved in tumourigenesis. Whittaker and colleagues examined the effects of a cyclin-dependent kinase inhibitor CYC202 on *WNT*-active colon cancer cell lines and could show a cell cycle arrest of treated cells.[58]

Also *Cox2* has been shown to be a target of the *WNT/β-catenin* pathway.[59] This leads to the hypothesis that non-steroidal anti inflammatory drugs (NSAIDs) may interact with the *WNT/β-catenin* pathway. For this purpose, Gardner and colleagues studied the effects of indomethacin, diclofenac, sulindac sulphide, sulindac sulphone and rofecoxib on SW480 human colorectal cancer cells. All substances, except rofecoxib, lead to a decrease in nuclear *β-catenin* levels going along with reduced *cyclin D1* levels and reduced proliferation.[60] Since cardiovascular adverse advents were reported upon frequent intake of NSAIDs their use as cancer agent is limited. Chemical modifications of the classical substances might make these drugs more attractive though. One such example is NO-acetyl salicylic acid (NO-ASA), which exhibits an NO-releasing moiety, which highly increased the substance's compliance.[61] NO-ASA was shown to inhibit *β-catenin/TCF* signalling in SW-480 (*Cox2*-negative) colon cancer cells and lead to apoptosis. Here, the NO-group, rather than the acetyl group, which is responsible for *Cox* inhibition, was shown to be crucial for *β-catenin/TCF* inhibition.[62] Therefore, NO-ASA acts independent of *COX*. Preliminary results in our research group showed an effective cell kill of primary B-CLL cells upon treatment with NO-ASA (unpublished data).

Other NSAIDs were described to act, independent of their ability to inhibit *COX*, via inhibition of *WNT/β-catenin* signalling. The R-enantiomer of the NSAID Etodolac (R-Etodolac) has no *Cox*-inhibitory activity.[63] Lu and colleagues have shown that R-Etodolac inhibits the transcription of *β-catenin/TCF/LEF1* target genes in human embryonic kidney (HEK) 293 cells and induces caspase-mediated apoptosis in CLL cells *in vitro*.[33] R-Etodolac was tested in primary CLL cells showing a mean IC_{50} of 800μM. Besides this, it could be shown that R-Etodolac has synergistic activity in combination with agents proven to be effective in the treatment of B-CLL: Fludarabine, cladribine, alemtuzumab and rituximab.[63] R-Etodolac was therefore tested for safety and efficacy in a phase II trial. *In vitro* results indicated the need of high concentrations (IC_{50} of 800 μM) in order to efficiently reduce the number of CLL cells. These concentrations could not be reached in the plasma of patients limited by the occurrence of adverse side-effects upon increased dosage. Therefore, R-Etodolac was withdrawn from further studies regarding its function in CLL treatment.

2.6. *Targeting WNT-independent activators of β-catenin/TCF/LEF1*

β-catenin and *TCF/LEF1* carry out the final step of the *WNT* pathway. Induction of target gene expression has been shown to be a crucial factor in the processes of tumour initiation and progression in a variety of cancers. It has been proposed that *β-catenin* signalling can also be activated independently from *WNT* protein binding to *FZD* receptors and the subsequent activation of downstream effectors. This can be mediated by growth factor binding to receptors and subsequent activation of intracellular kinase activity. With this background, researchers have considered tyrosine kinase inhibition as a potential approach to inhibit *β-catenin/TCF/LEF1* activity independent of the actual activity of the *WNT* signalling cascade. One of them is imatinib, which targets *BCR-ABL*, *KIT* and *PDGFR* and is approved to be effective against CML, Ph1 ALL, gastro-intestinal stromal tumours and some solid tumours.[64] Imatinib was shown to downregulate *β-catenin* signalling activity in a colon cancer cell line going along with an effective suppression of proliferation.[65] Due to the known dependence of B-CLL cell survival on *β-catenin/LEF1* activity, imatinib was also tested in B-CLL-like cell lines and primary B-CLL samples. Imatinib was found to sensitise cells to chlorambucil treatment in both, cell lines and primary material. These synergistic effects were observed at imatinib concentrations <10 μM, which are achievable in patients with minimal toxicity.[66]

3. Concluding remarks

To date, the *WNT/β-catenin* signalling pathway forms one of the most attractive targets for anti-cancer strategies. However, although many theoretical interventional approaches exist, their transfer into the human system is rare. This might be due to the risk of potential side-effects. These are likely to occur when the signalling cascade is disturbed too far upstream,

interfering with the physiological role of the pathway. In contrast, several studies have shown that inhibition of components further downstream leads to considerable anti-neoplastic effects while side-effects are reduced. This is especially true for the inhibition of the executing transcription factor family *TCF/LEF*. Thus, we expect that selective interruption of *WNT/β-catenin* signalling at this level will have a high impact for cancer treatment and deserves further investigation.

Conflict of interest statement

None declared.

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