

Rogaczewski, Patryk, Janiak, Michał, Borysewicz, Kamil, Głos, Tadeusz, Ahmadi, Navid, Szyłberg, Łukasz. Clinical significance of WNT pathway inhibition in various cancers. *Journal of Education, Health and Sport*. 2022;12(11):183-191. eISSN 2391-8306. DOI <http://dx.doi.org/10.12775/JEHS.2022.12.11.024> <https://apcz.umk.pl/JEHS/article/view/40485> <https://zenodo.org/record/7277591>

The journal has had 40 points in Ministry of Education and Science of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of December 21, 2021. No. 32343. Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical Culture Sciences (Field of Medical sciences and health sciences); Health Sciences (Field of Medical Sciences and Health Sciences). Punkty Ministerialne z 2019 - aktualny rok 40 punktów. Załącznik do komunikatu Ministra Edukacji i Nauki z dnia 21 grudnia 2021 r. Lp. 32343. Posiada Unikatowy Identyfikator Czasopisma: 201159. Przynależność dyscypliny naukowej: Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).

© The Authors 2022;

This article is published with open access at Licensee Open Journal Systems of Nicolaus Copernicus University in Torun, Poland
Open Access. This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author (s) and source are credited. This is an open access article licensed under the terms of the Creative Commons Attribution Non commercial license Share alike. (<http://creativecommons.org/licenses/by-nc-sa/4.0/>) which permits unrestricted, non commercial use, distribution and reproduction in any medium, provided the work is properly cited.

The authors declare that there is no conflict of interests regarding the publication of this paper.

Received: 13.10.2022. Revised: 20.10.2022. Accepted: 02.11.2022.

Review Article

Clinical significance of WNT pathway inhibition in various cancers

Patryk Rogaczewski^a, Michał Janiak^a, Kamil Borysewicz^a, Tadeusz Głos^a, Navid Ahmadi^b, Łukasz Szyłberg^{a, c}

^a Department of Clinical Pathomorphology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, Poland

^b Department of Cardiothoracic Surgery, Royal Papworth Hospital, Cambridge, UK

^c Department of Tumor Pathology and Pathomorphology, Oncology Centre–Prof. Franciszek Łukaszczyk Memorial Hospital, Bydgoszcz, Poland

Short Title: Clinical significance of WNT pathway inhibition in various cancers

Corresponding Author: Patryk Rogaczewski

Department: ^a Department of Clinical Pathomorphology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, Poland

Institute/University/Hospital: Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, Poland

Tel: +48 791 411 112

E-mail: pat.rogaczewski@gmail.com

ORCID Numbers:

Rogaczewski P. 0000-0003-0919-5695

Janiak M. 0000-0002-9564-4858

Borysewicz K. 0000-0003-4510-2759

Głos T. 0000-0003-0085-7066

Ahmadi N. 0000-0002-0425-2606

Szyłberg Ł. 0000-0003-1349-5906

Number of Tables: 2

Number of Figures: 1

Word count: 3323

Keywords: Wnt, Wnt Pathway, Wnt Pathway Inhibition, Cancer Treatment, Cancer

Abstract

Background. The tumor microenvironment (TME) plays an important role in the cell cycle. There is a correlation between the Wnt/β-catenin signaling pathway and TME. This article reviews methods of inhibiting Wnt Pathway, a useful process in the treatment of various cancers. Compounds of Wnt/β-Catenin Signaling Pathway, such as TCF-1, have an impact on the differentiation and migration of CD8⁺ T cells. CCL4 expression is regulated by the beta-catenin protein to recruit CD103⁺ dendritic cells, which enables CD8⁺ T cell activation. Inhibition of the Wnt/β-catenin pathway has an impact on ovarian cancer patients' prognosis, reducing the development of ovarian cancer. Research shows that inhibition of the pathway with the use of the LGK974 inhibitor may boost immunity, especially when applied with a Paclitaxel mix. After treatment, expression of the inhibitory receptors CTLA-4, TIM3, PD-1 on CD8⁺ T cells decreased. The combination of LGK974 and Paclitaxel can cause the death of tumor cells and significantly inhibit their proliferation. The

application of dose-dense Paclitaxel avoids toxicity related to the maximum dose needed to protect the patient's immune system by increasing CD8⁺ TILs. There are concerns regarding toxicity of the LGK 974, especially for cells dependent on the Wnt/ β -catenin pathway to maintain homeostasis. Many Wnt/ β -catenin pathway inhibitors are tested against colorectal cancer (CRC) with successful results. These include NSAIDs, porcupine inhibitors, tankyrase inhibitors, Wnt5A inhibitors, and disheveled protein inhibitors. The Wnt/ β -catenin pathway, when expressed in Triple Negative Breast Cancer (TNBC), leads to the transition of epithelial to mesenchymal cells. In early clinical development, there are multiple inhibitors (ex. KYA1797K) targeting the Wnt/ β -catenin pathway in TNBC cells, which could become a viable anticancer strategy. **Summary.** The dependency between the TME and the Wnt/ β -catenin pathway plays an important role in cancerogenesis; therefore, these pathways can be a new therapeutic target as antitumor therapy. A promising positive effect is the use of the LGK974 inhibitor together with Paclitaxel. The usage of phytochemicals such as acetylsalicylic acid, curcumin and resveratrol could become standard clinical procedure alongside conventional anti-cancer agents. These phytochemicals are cheap to manufacture, and their side effects are well-known.

Key message. In the case of breast cancer, any form of influence on the Wnt/ β -Catenin pathway may become a prominent factor in forming a sufficient treatment. Recent studies have shown numerous pathway inhibitors and activators, which when combined with conventional treatment may reveal numerous ways of elongating patients lifespan.

Introduction

Cancer has been considered for many years as a near-independent process. It is mostly influenced by mutations of oncogenes and cell cycle malfunctions in a faulty cell, resulting in its immunity to cell death. Modern-day studies show that the development of carcinomas is strongly governed by interactions of malignant cells with non-malignant, albeit altered, elements such as fibroblasts, endothelial cells, white blood cells, and natural anti-inflammatory factors.

The term 'tumor microenvironment' (TME) offers a more holistic approach to carcinomas by taking into consideration not only oncogene-related cell cycle alterations but also cell-cell interactions via signaling molecules and signaling pathways, which affect tumor growth and development. TME consists of fibroblasts, immune cells, endothelial cells, inflammation of CD8⁺ T cell, CD8 alpha/CD103 dendritic cells, T cell (Treg), and high density of FoxP3 [1]. The tumor-intrinsic is controlled by Wnt/ β -catenin (Wnt β c) signaling pathway. TME is responsible for the regulation of key cellular functions such as proliferation, differentiation, migration, genetic stability, apoptosis, and stem cell renewal. There is a correlation between poor spontaneous T cell infiltration in human cancers and the Wnt/ β -catenin signals activation. Data from in-vitro and in-vivo clinical studies have been suggesting that the Wnt/ β -catenin signaling pathway is a potential chemotherapy target [1].

The Wnt/ β -catenin pathway inhibition and modulation have improved Triple-Negative Breast Cancer (TNBC) treatment and opened various pathways in discovering new therapies. Activation of the Wnt is associated with lower overall survival in TNBC patients. The Wnt ligands promote migration and invasion. The Wnt β c is the most commonly overexpressed pathway causing transcriptional factor activation. The Wnt inhibitors and modulators can extinguish TNBC clonal cells and drug-resistant cells. Targeting the Wnt β c pathway is a promising anticancer strategy. Multiple agents targeting the Wnt ligands and/or frizzled receptors are in preclinical and early clinical development.

1. Impact of Wnt/ β -catenin signaling pathway on tumor microenvironment

Tumor-infiltrating CD8⁺ T cell plays important role in antitumor immunity [2]. Regulatory T cells are responsible for the effectiveness of immune checkpoint blockade. The tumor microenvironment is recognized as a main regulator of carcinogenesis. Many studies explore the complex mechanisms underlying tumorigenesis and disease progression, which are important in identifying new biomarkers or targets in stromal components to predict clinical outcomes and guide therapy [3].

Some critical pathways, such as the Wnt signaling, affect the tumor environment. Studies show a correlation between activation of the Wnt/ β -catenin signaling pathway and the absence of T-cell infiltration. Tumors with a high β -catenin expression showed a significant reduction in CD8⁺ T cell infiltration. β -catenin can regulate the CCL4 expression, recruiting CD103⁺ dendritic cells to enable CD8⁺ T cell activation. Study shows that activating oncogenic β -catenin signaling lead to colorectal resistance to immunotherapies when applying the combined PD-1 immunotherapy with targeting β -catenin in colorectal cancer [2]. Wnt family member 3a (Wnt3a) blockade halts the capacity of Wnt/ β -catenin signaling to inhibit the differentiation of T naive cells. This process does not retrieve the dysfunction of differentiated T cells in the tumor setting [4]

The development of tolerogenic dendritic cell (DC) population is promoted by the β -catenin signaling pathway. This tolerogenic DC population can drive differentiation of regulatory T cells. Wnt family member 5a (Wnt5a) ligand from melanoma upregulates activity and expression of indoleamine 2,3-dioxygenase-1 (IDO) enzyme via local DCs pathway. Wnt5a promotes the differentiation of T-regs in an IDO-dependent way, which leads to the suppression of melanoma immune surveillance. Based on

a target gene expression profile, which consists of IDO in human sentinel lymph node–derived DCs, β -catenin signaling activity causes the diminishment of progression–free survival [5].

TME	Compound of WNT/ β -Catenin Signaling Pathway	Result
CD8 ⁺ T Cell	TCF-1	differentiation, effector functions, migration of CD8 ⁺ T cell
CD4 ⁺ T Cell	TCF-1	regulate differentiation of CD4 ⁺ T helper
FoxP3 ⁺ T Cells (tregs)	TCF-1	inhibits the Treg cell-mediated suppression of effector T cell proliferation
CD8 α /CD103-Lineage DCs	co-receptor LRP5	crosstalk between tumor cells and DCs within the TME
Immune Exclusion	ATF3, Snail	<ul style="list-style-type: none"> ● inhibiting the production of CCL4 in Batf3-lineage CD103⁺ DCs by induction of the expression of the transcriptional repressor ATF3 ● increasing interaction between Snail and TAMs ● enhancing Treg survival
Immunosurveillance	CCL4, Foxp3	<ul style="list-style-type: none"> ● accumulation of CD11b+Gr-1+MDSC ● inhibits downstream deletion of Plcg2, Cul4b, Mucl ● increase availability of DKK1

Table. 1 Impact of WNT/ β -Catenin Signaling Pathway on Tumor Microenvironment.

2. Immunotherapy in Ovarian Cancer by use the Wnt/ β -catenin Pathway

2a. Paclitaxel as an Aid to inhibit Wnt/ β -catenin Pathway.

The Wnt/ β -catenin pathway has an impact on ovarian cancer patient’s prognosis. Inhibition of the pathway is beneficial in improving a patient’s prognosis [6,7]. Research shows that inhibition of the Wnt/ β -catenin

pathway with the use of the LGK974 inhibitor may boost immunity, especially when applied with a Paclitaxel mix. The pathway reduces the number of T cells in the cancer microenvironment. The Wnt β c pathway enables tumor growth and also reduces responses of antitumor immunity. Mutations in the Wnt/ β -catenin pathway are responsible for the development of cancer—and activation of this pathway gives a negative prognosis. More importantly, activating this pathway has properties of stem cells and influences proliferation as well as apoptosis in tumor cells. Activating the pathway handicaps treatment because it causes chemoresistance by controlling the epithelial to mesenchymal transition. The study conducted on mice demonstrated that application of the LGK974 increased in CD8:Treg ratio. Moreover, it was noted that the increase in cytokine production was accompanied by a decrease in the expression of the receptor inhibited by CD8 T cells.

2b. Benefits of a LGK974 and Paclitaxel Combination in Cancer Treatment.

Better effects have been obtained when Paclitaxel with LGK974 was served together. This is due to an increase in CD8: Treg ratio and a decrease in tumor size. Therefore, targeted therapy for inhibition of the Wnt/ β -catenin pathway will provide positive effects by increasing antitumor immunity, reducing the development of cancer. The aforementioned theses were checked in research, which shows that mice treated with LGK974 had reduced tumor size, and the infiltration gene of the T cell was higher than mice from the control group. Mice that were treated with Wnt survived longer. However, it has been noted that effects after inhibition of the Wnt β c pathway depend on the functioning of the immune system. The tumor microenvironment was compared before and after treatment with LGK974 in respect to the production of cytokines by T CD4+ cells. It showed that number of cytokine including interferon- γ , tumor necrosis factor- α and T CD8+ cells production of TNF α , IFN γ , and granzyme-B grew significantly after treatment with LGK974. In addition, expression of the inhibitory receptors CTLA-4, TIM3, PD-1 on CD8+ T cells decreased after treatment with LGK974 [8]. Preclinical research has shown that inhibition of the Wnt/ β -catenin pathway with Paclitaxel gave better effects than using them separately [9–11]. Additionally, using dose-dense Paclitaxel avoids dose-related toxicity to the maximum dose needed to protect the patient's immune system by increasing CD8+ TILs [12]. Combination of LGK974 and Paclitaxel showed significantly better effects because the mechanism of action of Paclitaxel in stabilizing β -catenin and microtubules overlap. β -catenin enables separation of centrosomes and also stabilizes mitotic spindles; therefore, inhibiting the Wnt/ β -catenin by LGK974 and action of Paclitaxel can cause the death of tumor cells and significantly inhibit their proliferation. The combination of treatment should be used in cancers that are known to have eminently active Wnt/ β -catenin, for example, colon cancer. Inhibition of this pathway may be used in a dual-target, meaning directly use as an antitumor or for immunostimulatory effects. However, a phase I clinical trial of oral LGK974 is still ongoing to assess its side effects. These are some concerns regarding the toxicity of LGK974, especially for cells dependent on the Wnt/ β -catenin pathway to maintain homeostasis [8].

3. Inhibition of the Wnt signalling pathway in colorectal cancer

Colorectal cancer (CRC) is the second most common cause of cancer-related deaths in the United States [13]. By analyzing 224 Colorectal cancer (CRC) tumors, studies have stated that over 90% of colorectal tumors had mutations in one or more pathways related to the Wnt signaling pathway, predominantly in the APC gene (51% in non-hypermethylated CRCs, 81% in hypermethylated CRCs [14].

Mice with faulty APC genes are more likely to develop colorectal tumors [15]. Functional loss of APC results in constitutive activation of Wnt signaling and chromosomal instability in human colorectal carcinomas. Mutations of the APC gene in the human genome causes Familial adenomatous polyposis (FAP), a condition that results in almost 100% chance of developing CRC [16].

The concept of inhibiting the Wnt cascade to stop the progress of cancer or even disintegrate cancer stem cells present in most types of CRC has been thoroughly researched. However, discoveries regarding either signaling of the Wnt pathway or new molecules and drugs being able to inhibit the mentioned pathway are emerging regularly. The anti-CRC agents can vary in both molecular size, structure and availability, from common substances found in food such as curcumin and vitamin D, over-the-counter drugs, to artificially created protein molecules.

Non-steroidal anti-inflammatory drugs (NSAID) inhibit cyclooxygenase-2 (COX-2) cascade. Prostaglandin E2, one of the products of this cascade, activates the canonical Wnt pathway and induces loss of phosphorylation of β -catenin [17]. Administering high (>500mg) dosage of aspirin daily for 5 years reduced risk of colorectal cancer by about 30% to 40% for up to 20 years. The dosage of 75mg had a similar effect, while lower dosages were deemed ineffective [18]. However, such long usage of aspirin has proven to dramatically increase the risk of cardiovascular-related fatalities [19].

Tankyrase inhibitors, TNKS and TNKS2, have been identified as potent Wnt pathway suppressors. Tankyrase enzymes target specifically the AXIN component of the cytoplasmic β -catenin destruction complex, thus dismantling it. The research on XAV 939, which is approximately 98% purity tankyrase inhibitor, suggested tankyrase inhibitors can increase the sensitivity of CRC to chemotherapy [20,21]. Tankyrase inhibitor JW55 was tested on mice and has been shown to stop Wnt signalling in cancer cells that contained mutations in either the APC locus or in the β -catenin allele [22]. However, the potential of tankyrase inhibition is limited due

to the toxicity and increased proliferation of intestinal crypts. Currently, molecule 2X-121, which is both PARP1/2 and TNKS1/2 inhibitor, is undergoing phase 2 clinical trials for breast cancer treatment (NCT03562832).

The dishevelled protein family is one of the key components of the Wnt-signaling pathway. Those proteins are responsible for interpreting signals from the Wnt receptors in the plasma membrane and conducting them to the correct intracellular pathway [23]. Inhibitors of dishevelled protein-protein interaction can bind both Wnt receptors and AXIN components in non-canonical pathways. Non-protein small molecule component, named FJ9, disrupts cancer cell proliferation and induces apoptosis in human cancer cells of CRC by binding to the PDZ domain of DVL. Since cancer cells are interconnected through a protein-protein interaction network rather than enzyme signalling, disruption of the DVL PDZ domain can interrupt the Wnt and Hedgehog pathways. While the molecule CalBioChem-322338 is the most commercially available DVL inhibitor, five new DVL inhibitors show greater potential than CalBioChem-322338- namely NPL-4001, 4004, 4011, 4012, and 4013- were recently invented [24].

MODELS OF WNT-PATHWAY INHIBITION FOR TNKS/TNKS2 AND FJ9

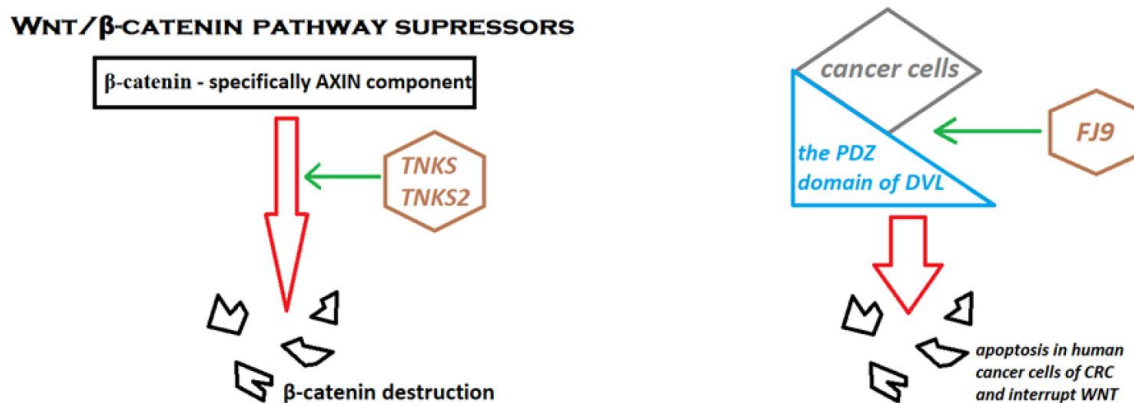


Fig. 1. Models of WNT-pathway inhibition for TNKS/TNKS2 and FJ9. Source: own elaboration

An alternative route of inhibiting the Wnt pathway is through porcupine (PORCN) inhibitors. Acylation of Wnt ligands is crucial to their biological activity. It requires three enzymes- stearyl CoA desaturase (SCD), porcupine (PORCN), and Notum. At the endoplasmic reticulum (ER), SCD transfers monounsaturated fatty acid to PORCN. PORCN further adds the palmitoyl group to the Wnt protein, which is later separated by Notum in the extracellular matrix [25]. The addition of the palmitoyl group by PORCN is mandatory for the Wnt ligands secretion from the ER.

LGK974, an inhibitor of PORCN, has a cytotoxic effect on CRC cell lines and has shown an ability to initiate apoptosis and to disintegrate CRC cell masses when paired with Aspirin and when IC50 drug is administered [26]. LGK974 is currently in phase I clinical trial (NCT01351103).

Dickopf-1 (DKK-1) is a secretory antagonist in the canonical Wnt pathway. However, its influence on cancer cell development remains unclear- depending on the type of cancer, a high expression of DKK-1 can either promote or inhibit metastasis of cancer cells [27]. While methylation of DKK-1, which leads to a low level of DKK-1 in tumor cells, is present in only 13% of CRCs, CRCs exhibiting DKK-1 methylation tend to have a worse prognosis [28]. Past study on the population of nude mice has shown that gene therapy increasing the DKK-1 expression targeting CRC resulted in a decrease in cancer cells density and inhibited tumor growth [29]. Gene therapy targeting specific genes coding protein products that inhibit DKK-1, such as the CSN5 gene, could theoretically increase levels of DDK-1 in tumor cells and hinder cancer's further development [27].

Agonist of the Wnt5A and FOXY5 has shown very promising results in terms of treating colorectal cancer. The Wnt5A protein is one of the non-β-catenin signaling ligands of the Wnt pathway, and reduced Wnt5A expression is directly linked to an increased mortality rate in cancer patients. A high Wnt5a expression, on the contrary, reduces cancer cell metastasis and proliferation [30].

In-vitro experiments on colonic cancer cells have shown that administration of FOXY5 inhibits both β-catenin and COX-2 signaling and reduces the number of cancer stem cells, which are usually resistant to conventional therapies. FOXY5, being a hexapeptide, is easier to manufacture than a complex Wnt5A molecule and shows no signs of toxicity [31]. FOXY-5 has currently three ongoing clinical trials (NCT02655952, NCT02020291, NCT03883802).

Resveratrol, present in grapes, has proven to have an anti-cancerous effect against CRC and underwent clinical trial (NCT00256334). It has been hypothesized in the research that it targets the Wnt pathway; however, the precise mechanism was not discovered until recently. Not only it inhibits the Wnt-2 and β-catenin

expression, suppressing the Wnt pathway in the process, but also downregulates the O-6-methylguanine-DNA methyltransferase (MGMT). MGMT causes resistance to tumor cells to Telozolomide by inducing the expression of a suicide DNA enzyme. By pairing Resveratrol with Telozolomide, the Telozolomide-based chemotherapy could be more effective [34]. R-spondin (RSPO) pathway secretes proteins that activate and promote the Wnt signaling pathway. Translocations of the RSPO2 and RSPO3 genes have been prevalent in cancer stem cells (CSC). These translocations, notably PTPRK-RSPO3 translocation, lead to overexpression of the RSPO3 in the malignant intestinal epithelium. RSPO3 protein, as an agonist of the Wnt pathway; therefore, contributes to further development of CRC [32].

Rosmantuzumab, development code OMP-131R10, is an IgG1 molecule, dubbed 'a first-in-class anti-RSPO3 antibody' in the overview of its clinical test. It inhibits the binding of the RSPO3 to leucine-rich repeat-containing G-coupled receptors (LGRs), suppressing the RSPO-LRG pathway. Rosmantuzumab has exhibited anti-tumor effects both as standalone and in combination with traditional chemotherapeutics on patient-derived xenograft animal models and can damage CSCs [33]. Rosmantuzumab is currently undergoing phase I clinical trials (NCT02482441).

4. Inhibition and effects of stimulation on the Wnt/ β -catenin pathway in Triple Negative Breast Cancer (TNBC)

Approximately half of the breast cancers have activation of the Wnt that correlates with a higher death rate [35]. The Wnt/ β -catenin pathway overexpression in the canonical and non-canonical molecules of the Triple Negative Breast Cancer (TNBC) predominantly causes transcriptional factor activation that stimulates epithelial to mesenchymal cell transitions. The Wnt5A, Wnt11, and Wnt3A ligands promote invasion and migration. β -catenin protein causes resistance in the TNBC cells as a result of overexpression and accretion of protein inside the cells [36]. Expression of β -catenin is associated with CD8+ T cells infiltration; therefore, inhibition of the β -catenin should increase T cell infiltration and positively increase the activity of immune checkpoint inhibition [37]. Inhibitors and modulators of the Wnt are capable of extinguishing the TNBC clonal and drug-resistant cells; however, its safety is not yet to be specified [36]. Considering the Wnt β c pathway as a target might be a promising anticancer strategy. Multiple agents targeting the Wnt ligands are in preclinical and early clinical development [37]. There is evidence that the Wnt is capable of inhibiting tumorigenesis in breast cancer SHH-mediated. Breast cancer stem cells have a visible increase in active Wnt signaling. It was confirmed by increased expression of activated the β -catenin protein and decreased expression of DKK1 protein. The tumor resistant stem cell can be eradicated with Wnt inhibitors, overcoming the conventional therapy. β -catenin, RAS, and epidermal growth factor receptors are overexpressed and correlated with each other in tumor tissues of TNBC [38].

KYA1797K is an axin-binding small molecule that reduces β -catenin and RAS expression through degradation and suppressing epidermal growth factor receptor expression through transcriptional repression. It inhibits the proliferation and the metastasis of stable cell lines as well and TNBC cells acquired from cancer patients. Targeting the Wnt β c and RAS-ERK pathways through KYA1797K simultaneously reduces the levels of β -catenin, RAS, and epidermal growth factor receptor. It possibly can be a therapeutic approach for TNBC [38].

Bevacizumab is a recombinant humanized monoclonal antibody that inhibits the VEGF-A (stopping the angiogenesis process) and is capable of activating the Wnt β c signaling pathway excessively. Patients with TNBC were tested with Bevacizumab in combination with the cytotoxic chemotherapy treatment. The result showed that a combination regime increased progression-free survival. Recent studies have shown that Bevacizumab can increase the invasive and metastatic capacity and induce epithelial-mesenchymal transition in breast cancer cells. Angiogenesis inducing factor VEGF is a target gene of β -Catenin, which is up-regulated by the activated Wnt β c signaling. It possibly leads to the incomplete blocking of tumor angiogenesis [39-41].

5. Wnt/ β -Catenin pathway in other tumors

The Wnt/ β -Catenin pathway is also involved in other cancers, including dysregulation of this pathway involved in gastric cancer (GC) progression. Studies have found that upstream regulator UBE2T, which is responsible for the hyperactivation of the Wnt/ β -Catenin pathway, offers hope for new possibilities in the treatment of GC patients by inhibiting the M435-1279 [42]. Hepatocellular carcinoma is another cancer associated with the Wnt/ β -Catenin pathway, where Wnt β c signaling is crucial for tumorigenesis in HCC. Unfortunately, the molecular mechanisms are insufficiently understood [43]. The next cancer is prostate cancer, where inhibition of this scallum together with inhibition of the androgen receptor shows promise in the treatment of this cancer [44]. In head and neck squamous cell carcinoma, the Wnt β c pathway is an important pathway that may be a therapeutic target as more and more evidence is found that it is a key pathway in the development of head and neck squamous cell carcinoma [45].

Compound	Effect/Mechanism	Type of cancer	Trial Identifier (if applies)
LGK974	PORCN Inhibitor	Ovarian Cancer Colorectal Cancer	NCT01351103
Acetylsalicylic Acid	COX-2 cascade inhibition	Colorectal Cancer	-
XAC 939	Tankyrase inhibitor	Colorectal Cancer	-
2X-121	Tankyrase inhibitor	Breast Cancer	NCT03562832
FJ9	DVL inhibitor	Colorectal Cancer Hepatocellular Carcinoma Triple-Negative Breast Cancer	-
DKK-1	Canonical WNT Pathway Antagonist	Colorectal Cancer	-
FOXY-5	WNT5A Agonist	Colorectal Cancer Breast Cancer Prostate Cancer	NCT02655952 NCT02020291 NCT03883802
Rosmantuzumab	RSPO3-binding inhibition	Colorectal Cancer	NCT02482441
Resveratrol	WNT-2 inhibitor	Colon Cancer	NCT00256334
KYA1797K	β -catenin expression inhibitor	Triple-Negative Breast Cancer	-
Bevacizumab	VEGF-A Inhibitor	Triple-Negative Breast Cancer	NCT01722968

Table. 2. Impact of WNT/ β -Catenin Pathway Inhibitors in cancer treatment.

Statements

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

No funding was used to write this review article.

Author Contributions

Patryk Rogaczewski- Chapter 1., Table 1.

Michał Janiak- Chapter 2., Figure 1.

Kamil Borysewicz- Chapters 3., Table 2.

Tadeusz Głos- Chapter 4.

Navid Ahmadi- supervision, editing, language checks

Łukasz Szyłberg- supervision, editing

References:

- Li X, Xiang Y, Li F, Yin C, Li B, Ke X. WNT/ β -catenin signaling pathway regulating T cell-

- inflammation in the tumor microenvironment. *Front Immunol.* 2019;10(SEP):1-12. doi:10.3389/fimmu.2019.02293
2. Emma M. Schatoff, Benjamin I. Leach, Lukas E. Dow, Wnt Signaling and Colorectal Cancer, *Curr Colorectal Cancer Rep.* 2017 April ; 13(2): 101–110. doi:10.1007/s11888-017-0354-9
 - 3 Xue J, Yu X, Xue L, Ge X, Zhao W, Peng W. Intrinsic β -catenin signaling suppresses CD8+ T-cell infiltration in colorectal cancer. *Biomed Pharmacother.* 2019;115. doi:10.1016/j.biopha.2019.108921
 - 4 Schinzari V, Timperi E, Pecora G, et al. Wnt3a/b-Catenin signaling conditions differentiation of partially exhausted T-effector cells in human cancers. *Cancer Immunol Res.* 2018;6(8):941-952. doi:10.1158/2326-6066.CIR-17-0712
 - 5 Holtzhausen A, Zhao F, Evans KS, et al. Melanoma-derived Wnt5a promotes local dendritic-cell expression of IDO and immunotolerance: Opportunities for pharmacologic enhancement of immunotherapy. *Cancer Immunol Res.* 2015;3(9):1082-1095. doi:10.1158/2326-6066.CIR-14-0167
 - 6 Goldsberry WN, Meza-Perez S, Londoño AI, et al. Inhibiting WNT ligand production for improved immune recognition in the Ovarian tumor microenvironment. *Cancers (Basel).* 2020;12(3). doi:10.3390/cancers12030766
 - 7 Wu G, Liu A, Zhu J, et al. MiR-1207 overexpression promotes cancer stem cell-like traits in ovarian cancer by activating the Wnt/ β -catenin signaling pathway. *Oncotarget.* 2015;6(30):28882-28894. doi:10.18632/oncotarget.4921
 - 8 Soiza RL, Donaldson AIC, Myint PK. Vaccine against arteriosclerosis: an update. *Ther Adv Vaccines.* 2018;9(6):259-261. doi:10.1177/https
 - 9 Flores ML, Castilla C, Gasca J, et al. Loss of PKC δ induces prostate cancer resistance to paclitaxel through activation of wnt/ β -catenin pathway and Mcl-1 accumulation. *Mol Cancer Ther.* 2016;15(7):1713-1725. doi:10.1158/1535-7163.MCT-15-0951
 - 10 Fischer MM, Cancilla B, Yeung VP, et al. WNT antagonists exhibit unique combinatorial antitumor activity with taxanes by potentiating mitotic cell death. *Sci Adv.* 2017;3(6). doi:10.1126/sciadv.1700090
 - 11 Chikazawa N, Tanaka H, Tasaka T, et al. Inhibition of Wnt signaling pathway decreases chemotherapy-resistant side-population colon cancer cells. *Anticancer Res.* 2010;30(6):2041-2048.
 - 12 Chang CL, Hsu YT, Wu CC, et al. Dose-dense chemotherapy improves mechanisms of antitumor immune response. *Cancer Res.* 2013;73(1):119-127. doi:10.1158/0008-5472.CAN-12-2225
 - 13 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin.* 2020;70(1):7-30. doi:10.3322/caac.21590
 - 14 McCullough A. Comprehensive molecular characterization of human colon and rectal cancer. *Yearb Pathol Lab Med.* 2013;2013(7407):295-296. doi:10.1016/j.ypat.2012.11.050
 - 15 Gaspar C, Fodde R. APC dosage effects in tumorigenesis and stem cell differentiation. *Int J Dev Biol.* 2004;48(5-6):377-386. doi:10.1387/ijdb.041807cg
 - 16 Campos FG, Sulbaran M, Safatle-Ribeiro AV, Martinez CAR. Duodenal adenoma surveillance in patients with familial adenomatous polyposis. *World J Gastrointest Endosc.* 2015;7(10):950. doi:10.4253/wjge.v7.i10.950
 - 17 Martin GS. Fly Src: The Yin and Yang of tumor invasion and tumor suppression. *Cancer Cell.* 2006;9(1):4-6. doi:10.1016/j.ccr.2005.12.025
 - 18 Rothwell PM, Wilson M, Elwin C-E, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. *Lancet (London, England).* 2010;376(9754):1741-1750. doi:10.1016/S0140-6736(10)61543-7
 - 19 Cuzick J, Thorat MA, Bosetti C, et al. Estimates of benefits and harms of prophylactic use of aspirin in the general population. *Ann Oncol.* 2015;26(1):47-57. doi:10.1093/annonc/mdl225
 - 20 Frank S Lieberman, MD1, Meihua Wang, PhD2, H Ian Robins, MD3, Christina I Tsien, MD4, Walter J Curran Jr, MD5, Maria Werner-Wasik, MD6, Ryan P Smith, MD7, Christopher Schultz, MD8, Alan C Hartford, MD9, Peixin Zhang, PhD10, Minesh P Mehta M. 乳鼠心肌提取 HHS Public Access. *Physiol Behav.* 2019;176(3):139-148. doi:10.1158/1541-7786.MCR-17-0205.Chemoradiotherapy
 - 21 Wu X, Luo F, Li J, Zhong X, Liu K. Tankyrase 1 inhibitor XAV939 increases chemosensitivity in colon cancer cell lines via inhibition of the Wnt signaling pathway. *Int J Oncol.* 2016;48(4):1333-1340. doi:10.3892/ijo.2016.3360
 - 22 Waaler J, Machon O, Tumova L, et al. A novel tankyrase inhibitor decreases canonical Wnt signaling in colon carcinoma cells and reduces tumor growth in conditional APC mutant mice. *Cancer Res.* 2012;72(11):2822-2832. doi:10.1158/0008-5472.CAN-11-3336
 - 23 Mlodzik M. The Dishevelled Protein Family: Still Rather a Mystery After Over 20 Years of Molecular Studies. Vol 117. 1st ed. Elsevier Inc.; 2016. doi:10.1016/bs.ctdb.2015.11.027
 - 24 Hori K, Ajioka K, Goda N, et al. Discovery of potent Dishevelled/Dvl inhibitors using virtual screening optimized with NMR-based docking performance index. *Front Pharmacol.* 2018;9(SEP):1-14. doi:10.3389/fphar.2018.00983

- 25 Torres VI, Godoy JA, Inestrosa NC. Modulating Wnt signaling at the root: Porcupine and Wnt acylation. *Pharmacol Ther.* 2019;198:34-45. doi:<https://doi.org/10.1016/j.pharmthera.2019.02.009>
- 26 Bagheri M, Tabatabae Far MA, Mirzaei H, Ghasemi F. Evaluation of antitumor effects of aspirin and LGK974 drugs on cellular signaling pathways, cell cycle and apoptosis in colorectal cancer cell lines compared to oxaliplatin drug. *Fundam Clin Pharmacol.* 2020;34(1):51-64. doi:10.1111/fcp.12492
- 27 Zhu G, Song J, Chen W, et al. Expression and role of dickkopf-1 (Dkk1) in tumors: From the cells to the patients. *Cancer Manag Res.* 2021;13:659-675. doi:10.2147/CMAR.S275172
- 28 Rawson JB, Manno M, Mrkonjic M, et al. Promoter methylation of Wnt antagonists DKK1 and SFRP1 is associated with opposing tumor subtypes in two large populations of colorectal cancer patients. *Carcinogenesis.* 2011;32(5):741-747. doi:10.1093/carcin/bgr020
- 29 Aguilera O, Fraga MF, Ballestar E, et al. Epigenetic inactivation of the Wnt antagonist DICKKOPF-1 (DKK-1) gene in human colorectal cancer. *Oncogene.* 2006;25(29):4116-4121. doi:10.1038/sj.onc.1209439
- 30 Dejmek J, Dejmek A, Säfholm A, Sjölander A, Andersson T. Wnt-5a protein expression in primary Dukes B colon cancers identifies a subgroup of patients with good prognosis. *Cancer Res.* 2005;65(20):9142-9146. doi:10.1158/0008-5472.CAN-05-1710
- 31 Osman J, Bellamkonda K, Liu Q, Andersson T, Sjölander A. The WNT5a agonist FOXY5 reduces the number of colonic cancer stem cells in a xenograft mouse model of human colonic cancer. *Anticancer Res.* 2019;39(4):1719-1728. doi:10.21873/anticancer.13278
- 32 Gupta R, Bhatt LK, Johnston TP, Prabhavalkar KS. Colon cancer stem cells: Potential target for the treatment of colorectal cancer. *Cancer Biol Ther.* 2019;20(8):1068-1082. doi:10.1080/15384047.2019.1599660
- 33 Bendell J, Eckhardt GS, Hochster HS, et al. Initial results from a phase 1a/b study of OMP-131R10, a first-in-class anti-RSPO3 antibody, in advanced solid tumors and previously treated metastatic colorectal cancer (CRC). *Eur J Cancer.* 2016;69:S29-S30. doi:10.1016/S0959-8049(16)32668-5
- 34 Ashrafizadeh M, Ahmadi Z, Farkhondeh T, Samarghandian S. Resveratrol targeting the Wnt signaling pathway: A focus on therapeutic activities. *J Cell Physiol.* 2020;235(5):4135-4145. doi:<https://doi.org/10.1002/jcp.29327>
- 35 Nithya Krishnamurthy, Razelle Kurzrock Targeting the Wnt/beta-catenin Pathway in Cancer: Update on Effectors and Inhibitors. doi:10.1016/j.ctrv.2017.11.002.
- 36 Medina MA, Oza G, Sharma A, et al. Triple-negative breast cancer: A review of conventional and advanced therapeutic strategies. *Int J Environ Res Public Health.* 2020;17(6). doi:10.3390/ijerph17062078
- 37 Capasso A, Bagby SM, Dailey KL, et al. First-in-class phosphorylated-p68 inhibitor RX-5902 inhibits b-catenin signaling and demonstrates antitumor activity in triple-negative breast cancer. *Mol Cancer Ther.* 2019;18(11):1916-1925. doi:10.1158/1535-7163.MCT-18-1334
- 38 Ryu WJ, Lee JD, Park JC, et al. Destabilization of β -catenin and RAS by targeting the Wnt/ β -catenin pathway as a potential treatment for triple-negative breast cancer. *Exp Mol Med.* 2020;52(5):832-842. doi:10.1038/s12276-020-0440-y
- 39 Schmidt, B., Roberts, R. S., Davis, P., Doyle, L. W., Barrington, K. J., Ohlsson, A., Solimano, A., and Tim W. New England Journal NFL.pdf. *N Engl J Med.* Published online 2003:1695-1702.
- 40 Xie W, Zhang Y, Zhang S, et al. Oxymatrine enhanced anti-tumor effects of Bevacizumab against triple-negative breast cancer via abating Wnt/ β -Catenin signaling pathway. *Am J Cancer Res.* 2019;9(8):1796-1814. <http://www.ncbi.nlm.nih.gov/pubmed/31497360> <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC6726986>
- 41 Diéras V, Campone M, Yardley DA, et al. Randomized, phase II, placebo-controlled trial of onartuzumab and/or bevacizumab in combination with weekly paclitaxel in patients with metastatic triple-negative breast cancer. *Ann Oncol.* 2015;26(9):1904-1910. doi:10.1093/annonc/mdv263
- 42 Yu Z, Jiang X, Qin L, et al. A novel UBE2T inhibitor suppresses Wnt/ β -catenin signaling hyperactivation and gastric cancer progression by blocking RACK1 ubiquitination. *Oncogene.* 2021;40(5):1027-1042. doi:10.1038/s41388-020-01572-w
- 43 Xuejiao Chen, Hongbo Huan, Chungang Liu, Yongli Luo, Junjie Shen, Yue Zhuo, Zhixin Zhang, Cheng Qian, Deacetylation of β -catenin by SIRT1 regulates self-renewal and oncogenesis of liver cancer stem cells, *Cancer Letters*, Volume 463, 2019, Pages 1-10, ISSN 0304-3835, <https://doi.org/10.1016/j.canlet.2019.07.021>.
- 44 Patel R, Brzezinska EA, Repiscak P, Ahmad I, Mui E, Gao M, Blomme A, Harle V, Tan EH, Malviya G, Mrowinska A, Loveridge CJ, Rushworth LK, Edwards J, Ntala C, Nixon C, Hedley A, Mackay G, Tardito S, Sansom OJ, Leung HY. Activation of β -Catenin Cooperates with Loss of Pten to Drive AR-Independent Castration-Resistant Prostate Cancer. *Cancer Res.* 2020 Feb 1;80(3):576-590. doi: 10.1158/0008-5472.CAN-19-1684. Epub 2019 Nov 12. PMID: 31719098.
- 45 Alamoud KA, Kukuruzinska MA. Emerging Insights into Wnt/ β -catenin Signaling in Head and Neck Cancer. *J Dent Res.* 2018 Jun;97(6):665-673. doi: 10.1177/0022034518771923. PMID: 29771197.