Advances in immunotherapy targeting WNT signaling pathway-mediated cancer stem cells

Yang Zhang[1, ¶], Zhining Zhang[2, ¶, *], Xijun Zhou[3, ¶]

[1]School of Life Science, Shanghai University, Shanghai, China
[2]College of Science, The University of Western Australia, Perth, Australia
[3]College of Pharmacy, Heilongjiang University of Chinese Medicine, Harbin, China
[¶]These authors contributed equally.
*Corresponding author: 23257169@student.uwa.edu.au

Keywords: Cancer stem cells (CSCs); WNT signaling pathway; immunotherapy.

Abstract: Cancer is the leading cause of death and one of the major obstacles to improving life expectancy in countries around the world. Currently, the discovery of cancer stem cells (CSCs) has led to a new direction in tumor treatment. Tumor stem cells can self-renew and generate heterogenous tumor cells, which participate in the process of tumor genesis, recurrence and metastasis. Immunotherapy against tumor stem cells is the current direction of human tumor therapy, and many studies have linked alterations in WNT signaling to the development of multiple tumor stem cells, evasion of immune detection, and resistance to therapy. The literature demonstrates a close association between tumor stem cells and the immune microenvironment through targeting the wnt pathway. Wnt signaling can affect cancer immune surveillance through molecular and cellular mechanisms, and therapeutic cancer immunotherapy using Wnt modulator blocking signaling pathway approaches may be the cancer stem cells to cure the disease. Herein, we review recent studies on the mechanisms of CSC and immune microenvironment and the feedback effects of the wnt signaling pathway on CSC and immune cells, as well as current clinical data on Wnt pathway blocking agents. The implementation of cancer stem cell immunotherapy by blocking the Wnt pathway has great therapeutic potential.

1. Introduction

Cancer is a chronic disease that poses a major threat to human life and is one of the top causes of death. Despite breakthroughs in medical technology, there are still a limited number of effective cancer treatments, and the cost of cancer therapy is still considerable [1]. Due to their remarkable self-renewal capacity and multifunctionality, cancer stem cells (CSCs) make a vast difference in carcinogenesis, growth, resistance, recurrence, and post-treatment metastasis [2-4]. These cells have the unusual capacity to resist many forms of anticancer therapy, spawn recurring malignancies, and propagate and transplant to distant organs due to their intrinsic tumorigenic features [5].

In cancer, there is a shift between CSCs and cancer cells and some other non-CSCs, which may be mediated by CSC signals and the tumor microenvironment (TME) [4]. TME protects CSCs from genotoxicity, enhances CSC self-renewal, stimulates angiogenesis, attracts cells, and facilitates tumor invasion and metastasis [6]. CSC interacts with TME through a variety of regulatory channels, suppressing developmental signaling pathways such as Notch, Wnt, Hedgehog, and Hippo hierarchical linkage signaling, which are important for stem and progenitor cell homeostasis and function [2]. There is a clear link between the Wnt pathway's function and the development of cancer among them.

Cell proliferation, cell polarity, cell motility, differentiation, survival, self-renewal, and calcium homeostasis are all dependent on Wnt signaling. The Wnt pathway's function is important in cancer, and abnormal regulation of the Wnt pathway causes tumor proliferation in the same organs. Wnt signaling is also involved in the control of cancer stem cells (CSC), which is an important mechanism in their growth [7]. Changes in Wnt signaling have been related to a range of carcinogenesis, disease

progression, and medication resistance in numerous studies [6–8, 10]. Oncogenic mutations activate pro-tumorigenic functions not only by providing malignant properties to altered cells, but also by compromising anti-cancer immune surveillance, according to emerging data [4].

The importance of immune cells in the tumor microenvironment (TME), which have shown unique properties in constructing the TME, has garnered increased attention in recent years. Tumor cells have been shown to regulate certain immune subpopulations, thereby transforming the surrounding TME into an immunosuppressive environment and causing immune evasion, which results in the immune system's inability to recognize and eliminate CSC. As CSC modify the TME around them, the immune cells they attract can influence CSC features, enhancing tumorigenicity, treatment resistance, and metastatic potential while also encouraging angiogenesis and cancer cell dedifferentiation [4]. Cancer treatment becomes much more difficult as a result of this. As a result, inhibiting signaling pathways that favor immune cell recruitment in TME and reverse control of CSC by recruited immune cells has emerged as a novel cancer therapeutic strategy.

Based on the importance of the WNT signaling route in both CSC and the immune milieu, this review discusses progress in immunotherapy targeting WNT signaling pathway mediated cancer stem cells. Although Wnt modulators appear to be interesting candidates for cancer immunotherapy development, further preclinical and clinical research is needed to fully understand their potential.

2. Therapeutic significance of WNT signaling pathway

2.1 Overview of WNT signaling

The WNT signaling cascade consists of three main pathways: the typical WNT pathway activates catenin, T-cell-specific transcription factor (TCF), and lymphoid enhanced binding factor (LEF) transcriptional reactivation complexes; Atypical (-catenin independent) planar cellular polarity pathways mainly regulate the cytoskeleton; And noncanonical WNT [2].Furthermore, Wnt/-catenin signaling pathways coordinate multiple cellular signaling cascades, including EGFR, Notch, Sonic Hedgehog, and PI3K/Akt signaling pathways, which are important molecular mechanisms in carcinogenesis [8].

2.2 Role of wnt pathway in stem cell therapy

The function of the WNT pathway is closely related to cancer. Wnt signaling is upregulated in most colorectal cancers (CRC), most commonly due to loss of adenomatous colonic polyposis protein (APC) function, of which approximately 50% is due to APC mutations. APC interferes with ubiquitination and degradation of the -catenin complex. APC deficiency leads to accumulation of -catenin in cells and up-regulation of target genes of -catenin- TCF-LEF complex. Endogenous dicKKOPF-associated protein 1 (DKK1) inhibitors that induce WNT signal transduction, or anti-FZD antibodies have been shown to slow progression of pancreatic duct adenocarcinoma (PDAC). It has been found that when catenin is mutated, it may lead to abnormal WNT signaling expression in hepatocellular carcinoma (HCC), activation of the classical WNT signaling pathway is thought to be caused by mutations in CTNNB1(encoding catenin), leading to overexpression of target genes in the WNT pathway. In addition, -catenin mutations have also been found in endometrioid ovarian cancer, colorectal cancer and melanoma.

Dysregulation of WNT signaling is also vital in blood cancers. Dysregulation of WNT signaling is a secondary event of leukemia stem cell (LSC) precursor transformation in a mouse acute myelogenous leukemia (AML) model. In AML cells and AML cell lines, chromosomal translocation products such as AML1-Eto, MLL-AF9 and PML-RAR fusion proteins activate classical WNT signaling pathways. There is evidence that endogenous WNT pathway inhibitors may also be silenced through epigenetic silencing, such as DKK1 and DKK2, leading to increased WNT pathway activity. Defects in WNT signaling pathway are also associated with the pathogenesis of multiple myeloma (MM). Interestingly, mutations in the core components of the WNT pathway are not common in MM, and abnormalities in the MM pathway seem to be associated with WNT regulation.

Abnormal MM pathways appear to be associated with genetic and/or epigenetic changes in WNT regulatory components that lead to increased sensitivity of MM cells to WNT ligands secreted by bone marrow microenvironments. By physical interaction, the BCR-ABL1 fusion protein stabilizes -catenin and improves its nuclear localization in chronic myelogenous leukemia (CML).

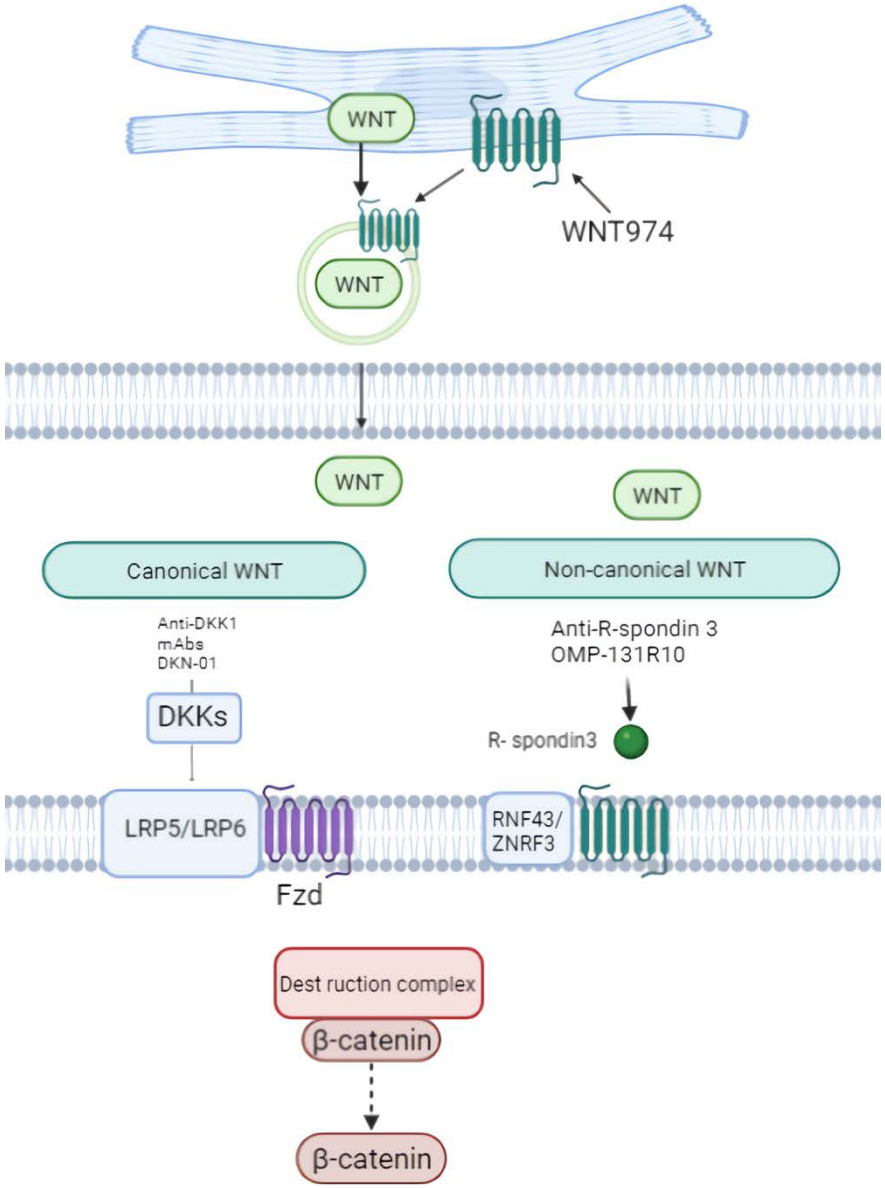

Figure 1. The canonical and non-canonical WNT signaling pathway and related pharmacological inhibitors that are under investigation in oncology. The secretion of WNT proteins is regulated by several other proteins, including Porcupine and Wntless.

3. Cancer stem cells

3.1 Biological characteristics of CSCs

CSCs, like regular stem cells, have a strong ability for self-renewal and are directly responsible for cancer [9]. Important signals for supporting CSC self-renewal include WNT/β-catenin, transforming growth factor-β, Hedgehog and Notch [4]. The key to carcinogenesis is CSC self-renewal, which has crucial implications for tumor and cancer treatment. Self-differentiation is also a strong suit of CSC. In general, numerous signaling pathways maintain normal stem cell self-renewal and differentiation in a somewhat balanced manner; once this regulatory balance is broken, uncontrolled CSCs eventually

lead to cancer. CSCs can also transdifferentiate into a variety of other cells, which can lead to tumor formation [6].

For cancer treatment and recurrence prevention, understanding the mechanism of CSC drug resistance is very crucial. CSCs express ATP-binding cassette (ABC) transporters, which are multidrug resistance proteins that shield leukemia and some solid tumor cells from drug damage and generate drug resistance [10]. Aldehyde dehydrogenase (ALDH), a hallmark in many CSCs, has been demonstrated to reduce oxidative stress and improve chemotherapeutic treatment resistance. ALDH also eliminates free radicals produced by radiation and boosts radiation resistance. By boosting DNA repair capacity, CSCs can successfully preserve cancer cells from apoptosis caused by chemotherapy and radiotherapy. [11].

3.2 The microenvironment of CSC

CSCs' microenvironment functioned in tumor self-renewal and metastasis. The microenvironment provides a safe environment for CSCs to self-renew and differentiate while also protecting them from genotoxicity. At the same time, the milieu encourages CSC self-renewal, angiogenesis, immune cell and stromal cell recruitment, and tumor invasion and metastasis [6]. Immune-related cells like macrophages are part of the CSC milieu, and they can respond to stimuli like hypoxia by generating growth factors and cytokines that govern CSC proliferation via signaling pathways like Wnt and Notch.

CSC has been demonstrated to be involved in the recruitment of macrophages as well as the conversion of macrophages to the anti-inflammatory M2 type, which promotes tumor growth [12]. The removal of these macrophages may prevent early carcinogenesis and is crucial for cancer treatment. CSC can also change the makeup and function of tumor-specific effector T cells, encouraging them to differentiate and proliferate into immunosuppressive tumor-promoting T cells (Treg) [4]. Treg is notably linked to cancer treatment's poor prognostic characteristics. Glioblastoma CSCs have been demonstrated to produce TGF-, which aids in the transformation of Tregs [13].

An immunosuppressive environment can form surrounding CSCs as they attract immune cells, and these immune cells can influence CSC characteristics. TAMs produce IL-6, which converts non-cancer stem cells into CSCs and increases their drug resistance. This shows that CSCs and various immune cell subpopulations have a strong bidirectional interplay that promotes cancer progression.

3.3 Wnt signaling pathway in CSCs

TME has several signaling pathways that regulate CSC self-renewal, dedifferentiation, apoptosis suppression, and metastasis (e.g., Notch, WNT, Hedgehog, Hippo hierarchical linkage signaling). Furthermore, these signaling channels do not exist in isolation, and there is crosstalk between them. NF-B and Wnt/-catenin pathway synergistically promote the survival and proliferation of CSCs cells, and there is also negative regulation between them. Wnt/-catenin and Hh signaling pathways are involved in embryogenesis, stem cell maintenance and tumorigenesis.[6]. When considering the treatment of CSCs that target the Wnt pathway, this also enlightens us to evaluate the effects of medicines on other pathways.

Cells can release cytokines such as Wnt protein, bone morphogenetic protein (BMP) secretion inhibitors and Delta in the microenvironment to stimulate signal cascades and allow CSCs to self-renew [14].As a direct target of -catenin/TCF in the intestinal tract, MYC can help Wnt to indirectly inhibit the proliferation of cancer cells.Lgr5, a member of the G protein-coupled receptor (GPCR) protein family, inhibits the differentiation of esophageal SCC stem cells downstream of the Wnt signaling pathway[6] .Wnt signaling is also involved in the regulation of CSC apoptosis. Dickkopf-related protein 2 suppresses -catenin activity in breast CSCs, causing G0/G1 arrest and cell death. DACT1, a Dapper homolog found at 14q23.1 on chromosome 14, causes apoptosis in breast CSCs by inhibiting the Wnt/-catenin signaling pathway [15]. CSC-mediated metastasis has been linked to Wnt/-catenin signaling. Wnt/-catenin and AKT/RhoA signaling are inhibited by CDH11, which prevents colorectal CSCs from migrating and invading [16]. Wnt signaling promotes CSC metastasis by lowering HOXA5 expression [17].

4. Wnt signaling pathway-mediated immunotherapy

4.1 The mechanism of wnt signaling pathway in the immune system

Wnt/ -Linked protein Pathway promotes self-renewal and pluripotency of hematopoietic stem cells by limiting the proliferation and differentiation of hematopoietic stem cells. However, its role in the generation and maintenance of memory T cells is unknown. Wnt/- Linked protein signal transduction inhibited the development of CD8 T cells into effector cells. By inhibiting T cell differentiation, Wnt signaling pathway enables CD8 memory stem cells with low CD44+, high CD62L, high SCA-1, high CD122 and high Bcl-2 to have self-renewal, multipotent proliferation and anti-tumor ability compared with central T cell subsets and effector T cell subsets. These suggest that the Wnt signaling pathway involved in maintaining stem cells of mature memory CD8 T cells, which has important implications for the development of new vaccination strategies and over-the-counter immunotherapies [18].

T cell proliferation and differentiation are also assisted by Wnt signaling. TCF and LEF are expressed dynamically during T cell maturation, and constitutive activation of Wnt/ -Linked protein pathway inhibits the growth of mature cytotoxic T cells. Stable junction proteins in T cells reduce phospholipase C-1 activity and IL-2 production, inhibiting maturation, differentiation, and activation, thereby promoting cancer progression. However, elevated expression and release of the Wnt antagonist DKK2 in tumor cells reduced T cell activity and enhanced tumor growth in colorectal cancer. This suggests that inhibition of the Wnt pathway in a variety of cells may affect T cell activity and anticancer immunity in different ways.

NKT cell is a kind of specialized T cell, which has the characteristics of natural NK cell and adaptive T cell. Immune cells (NK cells, T cells, dendritic cells, etc.) in TME are regulated by cytokines produced by activated NKT cells, which release IFN and IL4, leading to a variety of anti-tumor responses. In addition, NKT cells recognize glycolipid antigens through CD1D and directly destroy tumor cells by releasing perforin. NKT cell maturation is achieved through WNT signal transduction, and LEF-1 regulates the expression of CD1D gene. The expression of transgenic -Connexin increased the frequency and quantity of more type 2 cytokines released by NKT cells, suggesting that -Connexin functioned in the development and differentiation of NKT. Although studies have shown that the Wnt/β -connexin signaling pathway is beneficial and necessary for NKT maturation, its abnormal activity is also associated with poor differentiation and dysfunction of NKT cell terminals. [19]

Table.1. Evidence for the involvement of the Wnt/β-linked protein pathway in the regulation of immunosuppression and immune cell exclusion [20].

Observation
Wnt signaling regulating DC function
Wnt/β-catenin signaling regulates differentiation, maturation, and activation of DCs
Like tumor DCs, Wnt-conditioned DCs are programmed to a regulatory state to induce Tregs
Tumor DCs-deficient in LRP5/6 or β-catenin is more potent in capturing and cross-presenting TAAs to CD8 T cells
Tumor DCs lacking LRP5/6 or β-catenin are programmed to induce Th1/Th17 cells
Active Wnt/β-catenin signaling affects trafficking of DCs to tumors and TDLNs
Active Wnt/β-catenin signaling in tumor DCs regulates metabolic pathways involving FAO, vitamin A. and tryptophan to induce regulatory T cell (Treg) response
Wnt-signaling in tumor DCs suppresses chemokines that are critical of recruitment and accumulation of CTL in the TME
Wnt signaling in T cells
Wnt/β-catenin signaling in Tregs promotes its survival, activity and infiltration
Wnt3a/β-catenin signaling suppresses effector T cell differentiation

Wnt/β-catenin-signaling limits the expansion of tumor-antigen specified CD8 T cells and is important in the maintenance of stemness of memory CD8 T cells++

Wnt signaling in CD4 T cells favors Th17 cell differentiation+

Wnt signaling in macrophages

Wnt-β-catenin signaling regulates macrophages functions, such as adhesion, migration and tissue recruitment

Wnt-β-catenin signaling promotes M2-like polarization of TAMs resulting in tumor growth. migration, metastasis, and immunosuppression

Wnts produced by macrophages drive contribute to tumor cell invasiveness and tumor growth

Wnt signaling in MDSCs

The MUC1-β-catenin pathway regulates MDSC-mediated immune suppression in the TME

The PLCγ2-β-catenin pathway in MDSCs promotes tumor progression

Wnt signaling in NK cells

Wnt signaling in NK cells regulates maturation and effector functions

Wnt signaling in tumor cells

Tumor growth, migration, and metastasis

Immune cell exclusion

4.2 Application of wnt in cancer immunotherapy

Tumor cells, fibroblasts, stromal cells, the vascular system, immune cells, and the extracellular matrix make up the tumor microenvironment (TME). Infiltration of immune cells serves many functions in the TME, which is a distinct ecological niche [19]. Wnts are lipid-modified cysteine-rich glycoproteins that are secreted, and TME contains a lot of Wnt family ligands [20].

In the tumor microenvironment, Wnt signaling is also important for inhibiting antitumor immune responses. Wnt signaling has been proven in several studies to increase tumor growth by increasing tolerance and immune escape mechanisms [21].

Immune checkpoint inhibition has been found to be particularly effective in the treatment of melanoma and other tumor types, and overcoming cancer cells' immune evasion is a viable therapeutic approach. Wnt signaling regulates T and dendritic cell proliferation, maturation, and differentiation, although the involvement of tumor-intrinsic Wnt signaling in immune evasion has just recently been discovered [22].

4.3 Frontier findings on immune system treatment of cancer via WNT pathway

Expression of Wnt signaling enhancer R-spondin genes is associated with good prognosis and positively correlates with the genetic profile of NK and T cells. Wnt signaling plays a crucial role in modulating the anti-tumor action of NK cells, according to several recent studies. Inhibition of GSK3 increases the maturation and activity of NK cells through activating the Wnt/B-catenin pathway. The release of the Wnt signaling antagonist Dickkopf 1/2 (DKK1/2) by cancer cells has been shown to aid cancer cells resist NK cell-mediated antitumor responses in some situations. Excessive stimulation of the Wnt-p catenin pathway, on the other hand, is a hallmark of cancer cells and is required for tumor formation. Across malignancies, evidence suggests that the immune cell rejection phenotype is linked to inherent abnormal catenin signaling activity in tumor cells. Thus, TME is controlled by intricate interactions of Wnt agonists, antagonists, and anti-antagonists and there may be certain components of the Wnt signaling pathway that play a key role in regulating NK cell activity and infiltration in TME [23]. Melanoma cells can shield themselves from the immune system's front-line weapon against cancer, T cells, by producing large amounts of beta-catenin. beta-catenin prevents T cells from infiltrating tumor cells, thus diminishing the effect of immunotherapy.

The researchers found that activated beta-catenin signaling showed the greatest difference between the two groups. 49% of the samples without T-cell infiltration showed high levels of beta-catenin activity, and six beta-catenin target genes were also significantly elevated. Melanoma cells can shield themselves from the immune system's anticancer front-line weapon, T cells, by producing large amounts of beta-catenin [24].

5. Preclinical Development Research

Intrinsic treatment of drug-resistant CSCs with novel pharmacological targets has the potential to lower cancer recurrence rates and boost oncologic therapeutic efficacy. Multiple new CSC-targeted medicines have been created and are in clinical trials, and several drugs have already won regulatory approval, as our understanding of CSC biology continues to grow [2].

Among the antagonists that target the WNT pathway are the following classes : agents targeting ligands or receptor involved in WNT signaling; porcupine inhibitors that prevent Wnt ligands from being processed and secreted; agents that activate caspases or inhibit tankyrase, promoting -catenin breakdown by maintaining the multiprotein destruction complex and downstream β-catenin-TCF-LEF-dependent transcription inhibitors (listed in Table2) [2].

Table.2. Targeting CSC-associated Wnt signaling pathways in ongoing clinical trials

Drug name	Target	Condition	Phase	Sample size	NCT number	Current status
DKN-01	Wnt Ligand or receptor	Advanced-stage DKK1-positive oesophageal cancer or gastroesophageal junction tumors	I	52	NCT04166721	Recruiting
WNT974 (LGK-974)	Porcupine	Metastatic colorectal cancer	I	20	NCT02278133	Completed
		Pancreatic cancer	I	170	NCT01351103	Recruiting
CWP232291	β-Catenin	Acute myeloid leukemia	I	69	NCT01398462	Completed
PRI-724	β-Catenin/CBP	Colorectal adenocarcinoma	II	0	NCT02413853	Withdrawn
		Acute myeloid leukemia		49	NCT01606579	Completed
		Solid tumors		23	NCT01302405	Terminated
		Advanced pancreatic cancer		69	NCT01764477	Completed

5.1 Ligand or receptor targeting agents

Monoclonal antibodies, adc, decoy receptors, and small molecule inhibitors of diverse targets, of which DKN-01 can be utilized in various combinations, are all experimental antagonists of WNT pathway-related transmembrane proteins or ligands. DKN-01 is a human-derived monoclonal antibody that neutralizes the extracellular protein DKK1, which promotes LRP5/6 co-receptors and thereby antagonizes the WNT signaling pathway. It's increased in a variety of solid tumors, keeping the immunosuppressive TME active, and it's linked to a poor prognosis. DKN-01 can be used to treat a wide range of advanced solid tumors, including esophageal cancer, uterine cancer, ovarian cancer, and others. DKN-01 has been clinically efficacious and well-tolerated when combined with the chemotherapeutic drug paclitaxel [25].

5.2 Porcupine inhibitors

The acyltransferase Porcupine palmitoylates WNT proteins, which is critical for WNT secretion and signaling. Porcupine inhibitors prevent WNT proteins from becoming palmitoylated and retaining them in the endoplasmic reticulum, affecting WNT signaling and further modulating TME [26].

WNT974, also known as LGK-974, is a first-class Porcupine inhibitor that inhibits WNT signaling and tumor growth in vivo [27]. LGK-974 has proven in phase I and II clinical studies that it can treat rectal and head and neck cancers caused by mutations in the WNT system, however there is currently a dearth of meaningful clinical evidence (NCT02278133, NCT01351103) [28].

5.3 Agents that promote β-catenin degradation

CWP232291 is a first-of-its-kind peptidomimetic medication that activates caspases to destroy -catenin, reducing the expression of -catenin target genes such as survivin and cyclin D1. The medicine is also just in phase I and II clinical trials, mostly for the treatment of multiple myeloma, and existing evidence suggests that it could produce side effects like fever and nausea, allergies, or severe acute respiratory distress syndrome [2].

The end-anchored polymerase regulates AXIN, which is a key component of the -catenin degradation complex (tankyrase, TANKS). Through the ubiquitin-proteasome pathway, end-anchored polymerases control the stability of AXIN1 and AXIN2. TANKS inhibitors (AXIN stabilizers), on the other hand, block -catenin signaling by stabilizing AXIN. The end-anchored polymerase inhibitors XAV939 and IWR-1 block end-anchored polyI and end-anchored polyII, which regulate Axin [29]. In research in which 13 of 40 patients had high nuclear-linked protein levels and were receiving prior PI3K/AKT/mTOR inhibition [30].

5.4 Antagonists of β-catenin-mediated gene expression

Because WNT pathway mutations are so diverse, medicines that target downstream effectors are needed [31]. PFK115-584, CGP049090, and PKF222-815 all inhibited the complex's binding, while PKF118-310 and NPDDG39.024 interfered with -catenin precipitation and showed dose-dependent inhibition [32]. PRI-724 inhibits the interaction between AMP response element binding protein and -catenin (CBP). PRI-724 in combination with gemcitabine was well tolerated but clinically poor in a Phase Ib dose-increase study involving patients with advanced PDAC112, 40% of whom had SD and a median PFS duration of 2 months. (NCT02413853, NCT01606579, NCT01302405, NCT01764477).

The aberrant activation of the WNT signaling system promotes tumor stem cell proliferation, and anti-cancer medicines that target the WNT pathway and CSC's immunological microenvironment have become a hot study issue in tumor biology. Anti-tumor medicines that suppress aberrant activation of the WNT signaling pathway, on the other hand, are still a work in progress. The discovery of a pathway antagonist opens up a new avenue of tumor research, which will definitely usher in a new era of study and provide new hope for human anti-tumor efforts. In terms of the immune system's interaction with tumor stem cells, we may be able to develop better and more direct techniques for immune destroying tumor stem cells in the future.

6. Conclusion

The Wnt pathway's function is linked to cancer in a substantial way. In these tissues, aberrant Wnt pathway regulation leads to tumor proliferation. Although the Wnt signaling pathway is a key regulator of CSCs, there are still significant challenges in treating cancer stem cells. Wnt signaling has recently been discovered to have a role in immunological escape, and Wnt markers have been linked to T cell rejection. The technique of inhibiting T cell differentiation and promoting Wnt signaling to retain the stem cell activity of memory CD8 + T cells can be employed to produce memory T cell populations and plan anti-tumor T cells for adoptive immunotherapy. By combining the abilities of tumor related antigens to mediate the buildup of anticancer immune responses, Wnt signals discriminate healthy cells from pathogens or tumor cells, combine all signals from microorganisms and cells, and adjust the balance to activate or suppress immune responses. When immune effector cells penetrate the tumor, tumor cells either prevent them from reaching the tumor or cause them to become inactive and die. The aberrant activation of the Wnt signal promotes not only the growth of tumor stem cells (CSCs), but also tumor treatment resistance. The discovery of a route antagonist opens up a new sector of tumor

research, one that will definitely usher in a new era of research and provide new hope for the human anti-tumor cause.

References

[1] Cao M, Li H, Sun D, Chen W: Cancer burden of major cancers in China: A need for sustainable actions. Cancer communications (London, England) 2020, 40(5):205-210.

[2] Clara JA, Monge C, Yang Y, Takebe N: Targeting signalling pathways and the immune microenvironment of cancer stem cells - a clinical update. Nat Rev Clin Oncol 2020, 17(4):204-232.

[3] Najafi M, Farhood B, Mortezaee K: Cancer stem cells (CSCs) in cancer progression and therapy. Journal of cellular physiology 2019, 234(6):8381-8395.

[4] Müller L, Tunger A, Plesca I, Wehner R, Temme A, Westphal D, Meier F, Bachmann M, Schmitz M: Bidirectional Crosstalk Between Cancer Stem Cells and Immune Cell Subsets. Frontiers in immunology 2020, 11:140.

[5] van Stijn A, van der Pol MA, Kok A, Bontje PM, Roemen GM, Beelen RH, Ossenkoppele GJ, Schuurhuis GJ: Differences between the CD34+ and CD34- blast compartments in apoptosis resistance in acute myeloid leukemia. Haematologica 2003, 88(5):497-508.

[6] Yang L, Shi P, Zhao G, Xu J, Peng W, Zhang J, Zhang G, Wang X, Dong Z, Chen F et al: Targeting cancer stem cell pathways for cancer therapy. Signal transduction and targeted therapy 2020, 5(1):8.

[7] Takahashi-Yanaga F, Kahn M: Targeting Wnt signaling: can we safely eradicate cancer stem cells? Clinical cancer research : an official journal of the American Association for Cancer Research 2010, 16(12):3153-3162.

[8] Zhang Y, Wang X: Targeting the Wnt/β-catenin signaling pathway in cancer. J Hematol Oncol 2020, 13(1):165.

[9] Bjerkvig R, Tysnes BB, Aboody KS, Najbauer J, Terzis AJ: Opinion: the origin of the cancer stem cell: current controversies and new insights. Nature reviews Cancer 2005, 5(11):899-904.

[10] Gottesman MM, Fojo T, Bates SE: Multidrug resistance in cancer: role of ATP-dependent transporters. Nature reviews Cancer 2002, 2(1):48-58.

[11] Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, Qian D, Lam JS, Ailles LE, Wong M et al: Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature 2009, 458(7239):780-783.

[12] Jinushi M: Role of cancer stem cell-associated inflammation in creating pro-inflammatory tumorigenic microenvironments. Oncoimmunology 2014, 3:e28862.

[13] Wei J, Barr J, Kong LY, Wang Y, Wu A, Sharma AK, Gumin J, Henry V, Colman H, Priebe W et al: Glioblastoma cancer-initiating cells inhibit T-cell proliferation and effector responses by the signal transducers and activators of transcription 3 pathway. Molecular cancer therapeutics 2010, 9(1):67-78.

[14] Reya T, Morrison SJ, Clarke MF, Weissman IL: Stem cells, cancer, and cancer stem cells. Nature 2001, 414(6859):105-111.

[15] Yin X, Xiang T, Li L, Su X, Shu X, Luo X, Huang J, Yuan Y, Peng W, Oberst M et al: DACT1, an antagonist to Wnt/β-catenin signaling, suppresses tumor cell growth and is frequently silenced in breast cancer. Breast cancer research : BCR 2013, 15(2):R23.

[16] Li L, Ying J, Li H, Zhang Y, Shu X, Fan Y, Tan J, Cao Y, Tsao SW, Srivastava G et al: The human cadherin 11 is a pro-apoptotic tumor suppressor modulating cell stemness through Wnt/β-catenin signaling and silenced in common carcinomas. Oncogene 2012, 31(34):3901-3912.

[17] Ordóñez-Morán P, Dafflon C, Imajo M, Nishida E, Huelsken J: HOXA5 Counteracts Stem Cell Traits by Inhibiting Wnt Signaling in Colorectal Cancer. Cancer cell 2015, 28(6):815-829.

[18] Gattinoni L, Zhong XS, Palmer DC, Ji Y, Hinrichs CS, Yu Z, Wrzesinski C, Boni A, Cassard L, Garvin LM et al: Wnt signaling arrests effector T cell differentiation and generates CD8+ memory stem cells. Nature medicine 2009, 15(7):808-813.

[19] El-Sahli S, Xie Y, Wang L, Liu S: Wnt Signaling in Cancer Metabolism and Immunity. Cancers (Basel) 2019, 11(7).

[20] Suryawanshi A, Hussein MS, Prasad PD, Manicassamy S: Wnt Signaling Cascade in Dendritic Cells and Regulation of Anti-tumor Immunity. Frontiers in immunology 2020, 11:122.

[21] Patel S, Alam A, Pant R, Chattopadhyay S: Wnt Signaling and Its Significance Within the Tumor Microenvironment: Novel Therapeutic Insights. Frontiers in immunology 2019, 10:2872.

[22] Zhan T, Rindtorff N, Boutros M: Wnt signaling in cancer. Oncogene 2017, 36(11):1461-1473.

[23] Tang Y, Xu Q, Hu L, Yan X, Feng X, Yokota A, Wang W, Zhan D, Krishnamurthy D, Ochayon DE et al: Tumor Microenvironment-Derived R-spondins Enhance Anti-Tumor Immunity to Suppress Tumor Growth and Sensitize for Immune Checkpoint Blockade Therapy. Cancer Discov 2021.

[24] Spranger S, Bao R, Gajewski TF: Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. Nature 2015, 523(7559):231-235.

[25] Pai SG, Carneiro BA, Mota JM, Costa R, Leite CA, Barroso-Sousa R, Kaplan JB, Chae YK, Giles FJ: Wnt/beta-catenin pathway: modulating anticancer immune response. J Hematol Oncol 2017, 10(1):101.

[26] Martin-Orozco E, Sanchez-Fernandez A, Ortiz-Parra I, Ayala-San Nicolas M: WNT Signaling in Tumors: The Way to Evade Drugs and Immunity. Frontiers in immunology 2019, 10:2854.

[27] Esmaeili SA, Sahranavard S, Salehi A, Bagheri V: Selectively targeting cancer stem cells: Current and novel therapeutic strategies and approaches in the effective eradication of cancer. Iubmb Life 2021, 73(8):1045-1059.

[28] Wang B, Tian T, Kalland KH, Ke X, Qu Y: Targeting Wnt/β-Catenin Signaling for Cancer Immunotherapy. Trends Pharmacol Sci 2018, 39(7):648-658.

[29] Le PN, McDermott JD, Jimeno A: Targeting the Wnt pathway in human cancers: therapeutic targeting with a focus on OMP-54F28. Pharmacology & therapeutics 2015, 146:1-11.

[30] Fischer MM, Cancilla B, Yeung VP, Cattaruzza F, Chartier C, Murriel CL, Cain J, Tam R, Cheng CY, Evans JW et al: WNT antagonists exhibit unique combinatorial antitumor activity with taxanes by potentiating mitotic cell death. Science advances 2017, 3(6):e1700090.

[31] Wang L, Shalek AK, Lawrence M, Ding R, Gaublomme JT, Pochet N, Stojanov P, Sougnez C, Shukla SA, Stevenson KE et al: Somatic mutation as a mechanism of Wnt/β-catenin pathway activation in CLL. Blood 2014, 124(7):1089-1098.

[32] DeBruine ZJ, Ke J, Harikumar KG, Gu X, Borowsky P, Williams BO, Xu W, Miller LJ, Xu HE, Melcher K: Wnt5a promotes Frizzled-4 signalosome assembly by stabilizing cysteine-rich domain dimerization. Genes & development 2017, 31(9):916-926.