

Review Article

The role of Dickkopf family in cancers: from Bench to Bedside

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Abstract: Numerous epidemiological studies indicate that cancer will be responsible for millions of deaths in one year. Although multiple therapeutic strategies exist, and vast research efforts are being focused on developing newer and better regimens, cancer-related morbidity and mortality remain high. Metastasis and recurrence are prominent causes of treatment failure in cancers. Moreover, early diagnosis and treatment initiation are difficult to achieve in clinical practice. Fortunately, targeted therapy, which exerts its function at the molecular level, has proved to be greatly beneficial in several human diseases including cancers. The Wnt signaling pathway is a crucial regulator of embryogenesis and development in humans, and its dysfunction has been implicated in the incidence and development of cancers and other diseases. The Dickkopf family (Dkks) is a widely studied Wnt signaling pathway antagonist and plays multiple roles in human physiological and pathological process through both Wnt pathway-dependent and -independent manners. However, the precise roles of Dkks in tumorigenesis and the causal mechanisms have not been clearly elucidated. We discuss the pleiotropic roles of Dkks, with a specific focus on the underlying mechanisms, in cancer biology. We review recent literature to explore the potential use of Dkks as a tumor diagnosis biomarker and therapeutic target.

Keywords: Cancer, Wnt, Dkks, mechanism, diagnosis, biomarker

Introduction

Cancer is the second leading cause of death worldwide, second only to cardiovascular diseases. For men, prostate and lung cancer are the most common and the leading causes of cancer death all over the world, respectively, while for women, breast cancer has the highest incidence and mortality rate. Interestingly, although the stomach cancer, esophageal cancer, and chronic myeloid leukemia incidence rates are decreasing rapidly, the corresponding death rates remain increasing [1]. Traditional diagnostic methods, such as α -fetoprotein (AFP), a gold standard in liver cancer diagnosis, are rapidly proving ineffective in the early detection of liver cancer [2]. Moreover, although diverse therapeutic strategies are being employed in the treatment of cancers, patient prognosis remains obscure [3]. Targeted therapy is a new approach to facilitate the treatment of cancer, and is based on the concept of targeting specific cancer-related genes and their products. In addition, targeted therapy is an important advance in the field of modern molecular biology

and has yielded promising results in clinical practice. For instance, Trastuzumab, a monoclonal antibody with a selective effect on the human epidermal factor receptor 2 (HER2), has proved efficacious in the treatment of Her2-positive breast cancer [4, 5]. Collectively, targeted therapy is a crucial driving force and an essential technology for precise tumor treatment.

The Wnt signaling pathway is critical in development and homeostasis of the human body, while its dysfunction has been implicated in the development and progression of cancer [6, 7]. The Dickkopf family (Dkks) is a canonical Wnt signaling pathway antagonist, and play varied roles in human physiology. Notably, aberrant function of the Dkks has been reported in several cancers [8-10]. Herein, we summarize the available research data on the roles of Dkks in cancer biology, delineate the potential mechanisms, and try to explain some of the seemingly contradictory findings. Through this review, we aim to establish a bridge between basic research and clinical practice of cancer therapy.

DKKs and cancer

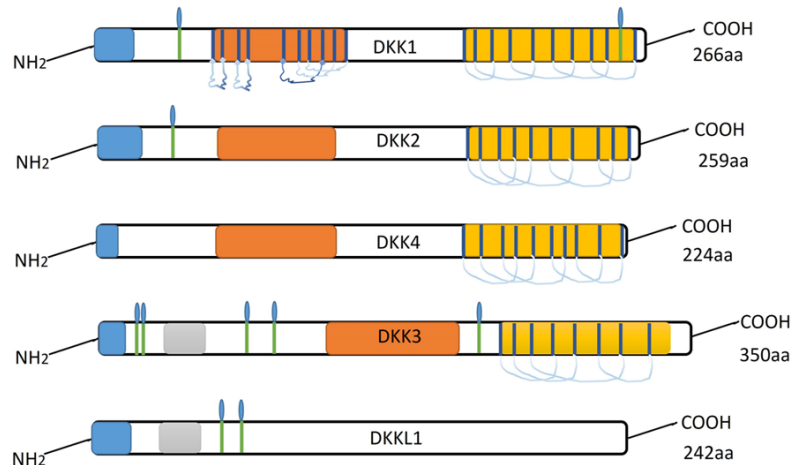


Figure 1. Ideograph of Dkk proteins.

Wnt signaling pathway & Dkks

Wnt signaling pathway is a well-conserved and vital regulatory cascade in human embryogenesis, including growth, differentiation, and apoptosis [11, 12]. Dysfunction of this pathway has been reported in a multitude of human diseases, especially in cancers [13-17]. The Wnt signaling pathway consists of the β -catenin-dependent canonical pathway and the β -catenin-independent non-canonical pathway, which covers the planar cell polarity pathway (PCP) and the Wnt/ Ca^{2+} pathway [18]. The canonical Wnt pathway is more widely studied, and then we have a relatively clear understanding of the ligands and downstream effectors [19]. Wnts comprise a group of secreted lipid-modified cysteine-rich signaling glycoproteins that are 350-400 amino acids in length. And then the Wnt protein family has 19 members and are highly conserved across species [20]. Frizzled proteins, with a conserved cysteine-rich domain and ten known members in the human, are a family of G protein-coupled receptors that function as receptors of the Wnt and other signaling pathways [21, 22]. Wnt proteins activate frizzled family receptors (Frz) on the responder cells and initiate at least three different signaling pathways, including the canonical and the non-canonical β -catenin pathway. The binding of Wnts to a receptor complex composed of members of the seven transmembrane Frz; the serpentine co-receptors; and the low-density lipoprotein receptor-related protein 5/6 (LRP5/6), transduces upstream signaling, which subsequently activates the canonical Wnt signaling pathway [23, 24]. β -catenin is the core signaling ele-

ment in this cascade, and its activation and nuclear translocation play an essential role in the canonical Wnt signaling pathway. Several Wnt antagonists, such as the Wnt inhibitory factor (WIF), Cerberus, and the secreted frizzled-related protein (sFRP), block the binding of Wnt to the frizzled receptors, while Dkks inhibit the Wnt/LRP5/6 interaction [25]. Mutations in the Wnt signaling elements have been linked to a range of human diseases, and the balance between

antagonistic and agonistic effectors play a crucial role in Wnt signaling pathway regulation [26-28].

The Dkks family has rarely been studied systematically in relation to cancer research. The gene family encode secreted glycoproteins that are hallmark antagonists of the canonical Wnt signaling pathway, and is composed of five members: Dkk1-4, and DkkII (soggy) [29]. The Dkks glycoproteins, except for soggy, contain an N-terminal signal peptide and two conserved cysteine-rich domains separated by a linker region. The amino-terminal cysteine-rich domain (Cys-1) is unique to each Dkks, whereas the carboxyl-terminal cysteine-rich domain (Cys-2) is highly conserved among all Dkks members. Additionally, the sgy-domain is found only in Dkk3 and DkkI1. The function of these structural domains vis-à-vis their role in the mechanism of Dkks action have not been clarified in details and will be important to further our understanding of the Dkks (**Figure 1**). Not all members of the Dkks family, however, antagonize the Wnt/ β -catenin signaling. While Dkk1, Dkk2, and Dkk4 have been shown to antagonize Wnt-mediated β -catenin stabilization by binding to Wnt co-receptors, Dkk3, in contrast, does not bind cell surface co-receptors [30]. Although Dkks members play diverse roles in the Wnt signaling pathway, several studies have noted that other pathways may also be involved in the role of Dkks [9, 31].

Dkks gene expression pattern in cancer

Although an increasing number of studies are being conducted to elucidate the roles of Dkks

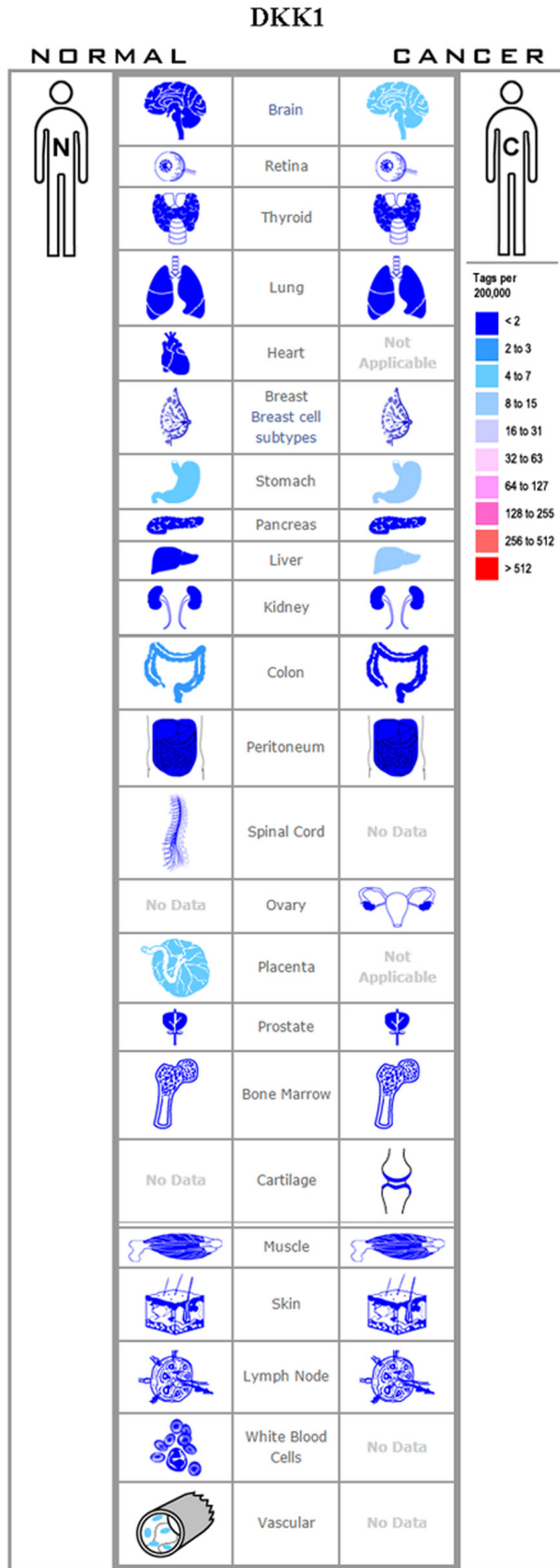


Figure 2. Expression profile for Dkk1 in human cancers.

in cancer, contradictory reports can be noted. Inconsistencies exist in the reported expression levels of Dkks members in similar tumors. The expression of Dkks gene family is tightly regulated during embryogenesis, both spatially and temporally. Furthermore, interactions between the encoding gene products also play crucial roles in an organism's development.

Dkk1 has been demonstrated to inhibit Wnt signal transduction through two potential mechanisms: (1) Dkk1 can restrain Wnt signaling by binding to and antagonizing LRP5/6, and (2) Kremen1/2 (Krm1/2) can form a ternary complex with Dkk1 and LRP6, leading to a rapid internalization and clearance of the Wnt co-receptor LRP6 from the cell membrane, consequently blocking Wnt signaling [32]. Dkk1 upregulation has been reported in human hilar cholangiocarcinoma (HCCA) [33]; intrahepatic cholangiocarcinoma (ICC) [34]; chondrosarcoma [35]; hepatocellular carcinoma [36]; bladder cancer [37]; breast cancer [10, 28]; pancreatic cancer [38]; multiple myeloma [39]; prostate cancer [40]; esophageal squamous cell carcinoma [41]; and laryngeal squamous cell carcinoma [42], while its downregulation has been noted in papillary thyroid cancer [43]; colorectal adenocarcinoma [44]; cervical squamous cell carcinoma [45]; and lung cancer [46] (Figure 2). Interestingly, Shi et al. [33] have shown that a higher expression of Dkk1 in HCCA was correlated with hilar lymph node metastasis, and have further noted that a loss of Dkk1 expression significantly inhibited cancer cell proliferation; colony formation; and migration, compared to controls. Zhao et al. [43] report that serum Dkk1 level was associated with various clinical features of papillary thyroid cancer, including tumor size, lymph node metastasis, and tumor-node-metastasis stage. Jiang et al. [47] reported significantly higher serum Dkk1 level in cervical cancer patients compared with healthy women. They also reported that the high serum Dkk1 was associated with lymphatic metastasis and tumor diameter in cervical carcinoma, and was associated with poor patient prognosis. However, Hou et al. [45] noted significantly lower Dkk1 expression in carcinoma tissue compared to normal cervical tissue, with the Dkk1 expression level

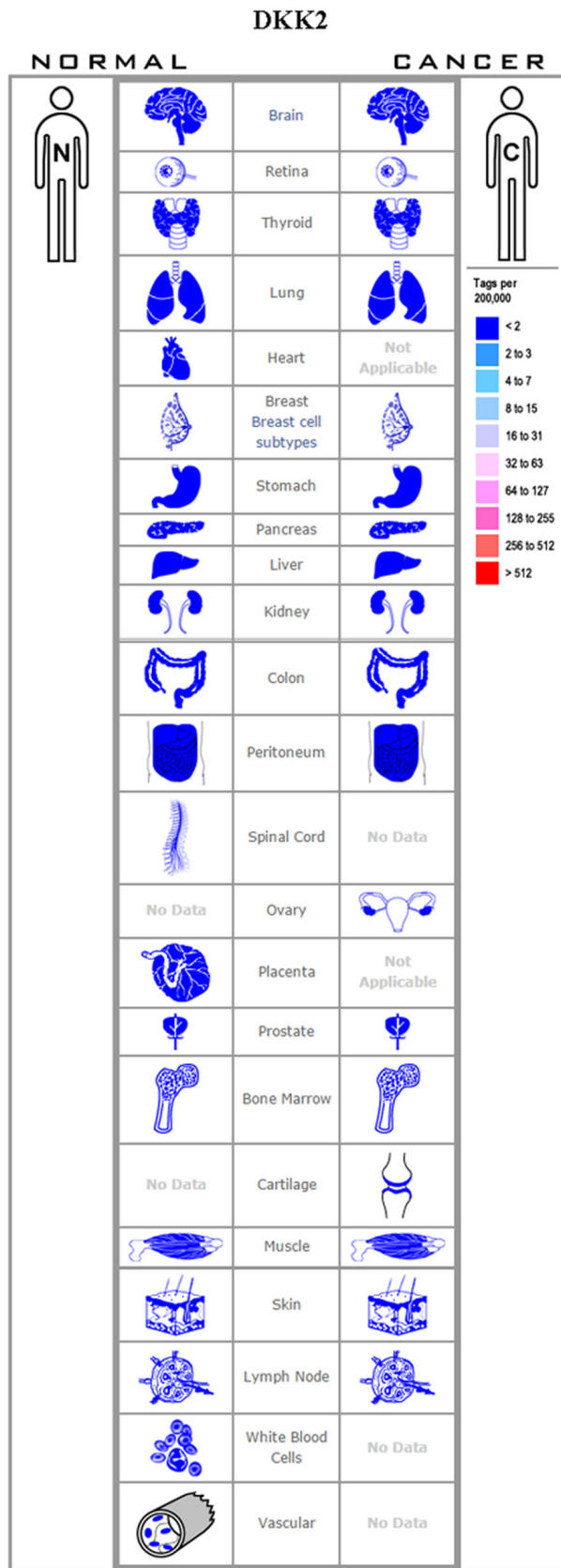


Figure 3. Expression profile for Dkk2 in human cancers.

being correlated with clinical stage, tumor differentiation, depth of invasion, and lymph node metastasis of the tumors. These contrasting reports evidence the need for further elucidation of the role of Dkk1 in cancer.

Unlike Dkk1, Dkk2 can function as an LRP6 agonist or an antagonist, the particular role being dictated by the cellular context. The Krm2 serves as a co-factor that regulates Dkk2 activity in the Wnt/LRP6 signaling. Mao and Niehrs [48] have confirmed that Dkk2 can function as a Wnt/LRP6 signaling agonist or antagonist and that both contradictory functions are mediated via Krm2. Furthermore, as Dkk2 alone cannot activate the Wnt/ β -catenin signaling pathway, the LRP6 level is vital to the Dkk2-mediated activation of the Wnt pathway. Like Dkk1, aberrant expression of Dkk2 has been detected in many tumor tissues. Several epigenetic alterations leading to a decline in the expression of Dkk2 have been reported in tumors (Figure 3). RNA interference-mediated silencing of Dkk2 has been observed in oral tongue squamous cell carcinoma [49]; esophageal adenocarcinoma [50]; and frequent epigenetic Dkk2 silencing has been found in ovarian carcinoma [51]; renal cancer [52]; and hepatocellular carcinoma [8]. In the abovementioned studies, Dkk2 played an inhibitory role in the development of the various tumors. However, increased Dkk2 expression can promote proliferation and invasion via the Wnt signaling in prostate cancer [53], Ewing sarcoma [54], and colorectal cancer [55]. These examples exhibit that even as the role of Dkk2 is closely related to the tumor environment, its function in the cellular context is incredibly complex.

The role of Dkk3 in Wnt/ β -catenin signaling has remained unclear. Although previous studies have stated that Dkk3 does not influence Wnt signaling, recent studies indicate that Dkk3 is capable of interacting with Krm1/2 and LRP5/6 with comparable binding energies, thereby disrupting downstream signaling [56, 57]. There are numerous pieces of evidence reflecting the inhibitory effect of Dkk3 on tumor growth and development via the Wnt pathway. However, the mechanism of this inhibitory effect is not clear, and only a few studies have reported a putative mechanism for the Dkk3-mediated tumor

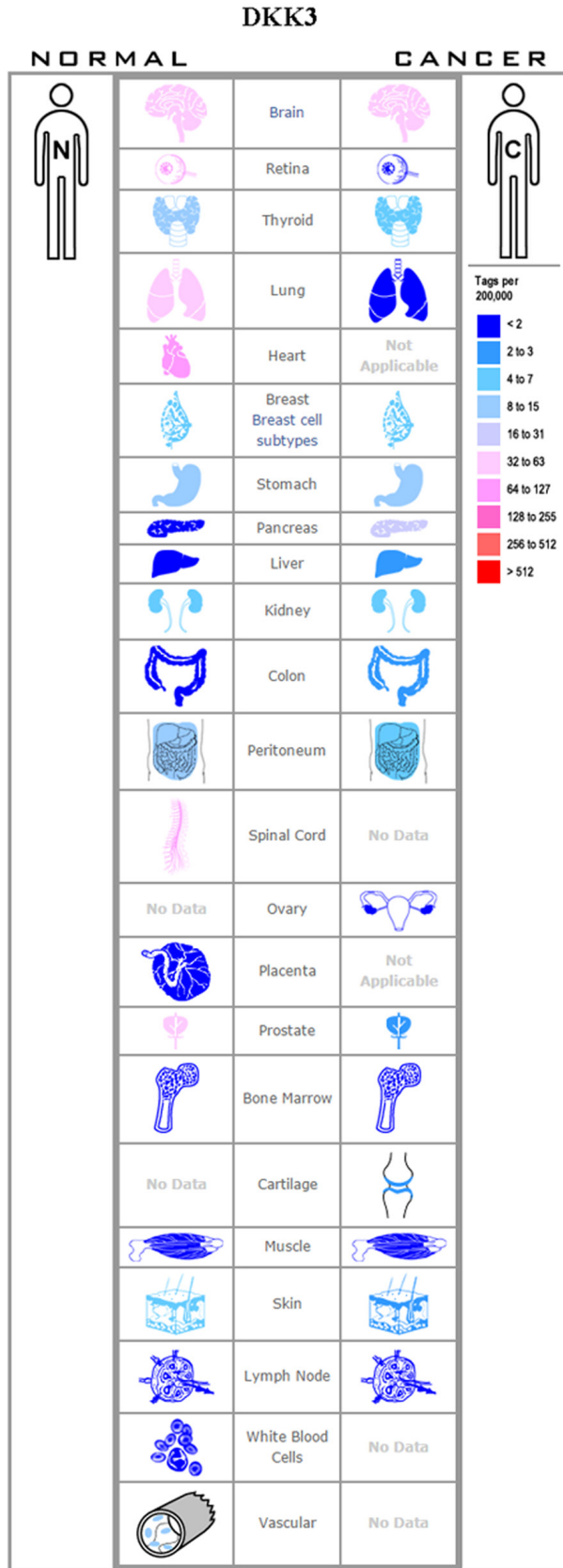


Figure 4. Expression profile for Dkk3 in human cancers.

suppression. One such study reported that while Dkk3 could interact with Krm1/2, it could not interact with, or affect, the expression of LRP6 [57]. Dkk3 downregulation has been detected in melanoma [58]; basal breast cancer [59]; gastric cancer [60, 61]; clear cell renal cell carcinoma [62]; and pancreatic cancer [63], while its overexpression has been reported in hepatoblastoma [64] and esophageal adenocarcinoma [65] (Figure 4).

The Dkk4 binds LRP5/LRP6 and Krm1/2 and demonstrates a Wnt/ β -catenin antagonistic activity similar that of Dkk1. Thus, the downregulation of Dkk4 can promote tumor progression. However, its upregulation may also contribute to tumor progression. Pan et al. [66] observed that Dkk4 expression was high in pancreatic cancer tissues, and was associated with tumor and organ development, and with inflammation. Immunohistochemical and immunofluorescence studies indicated that Dkk4 was co-expressed with the mitogen-activated protein kinase 3 (MAPK3) and guanine nucleotide exchange factor (VAV3) in pancreatic cancer tissues, suggesting that in pancreatic cancer, Dkk4 may function through other signaling pathways such as the MAPK pathway. Hu et al. [67] reported that the Dkk4 mRNA and protein expression levels were significantly upregulated in clear cell renal cell carcinoma along with the downregulation of cyclin D1, c-myc, and β -catenin. Additionally, Hinoda et al. [52] reported that Dkk4 expression was increased in renal cancer tissues wherein it could activate the non-canonical c-Jun N-terminal kinase (JNK) signaling pathway while inhibiting the canonical Wnt pathway. Dkk4 expression was reportedly also upregulated in colorectal tumors and could promote tumor progression [55, 68]. Contrary to these reports, however, a short hairpin (shRNA)-mediated knockdown of Dkk4 was noted to promote cell proliferation in hepatocellular carcinoma [69] (Figure 5).

Role of Dkks in the biological phenotype of cancer cells

Dkks and cancer cell proliferation

In normal tissue, cellular proliferation is strictly controlled to maintain homeostasis. Tumor cells, however, amass the ability to

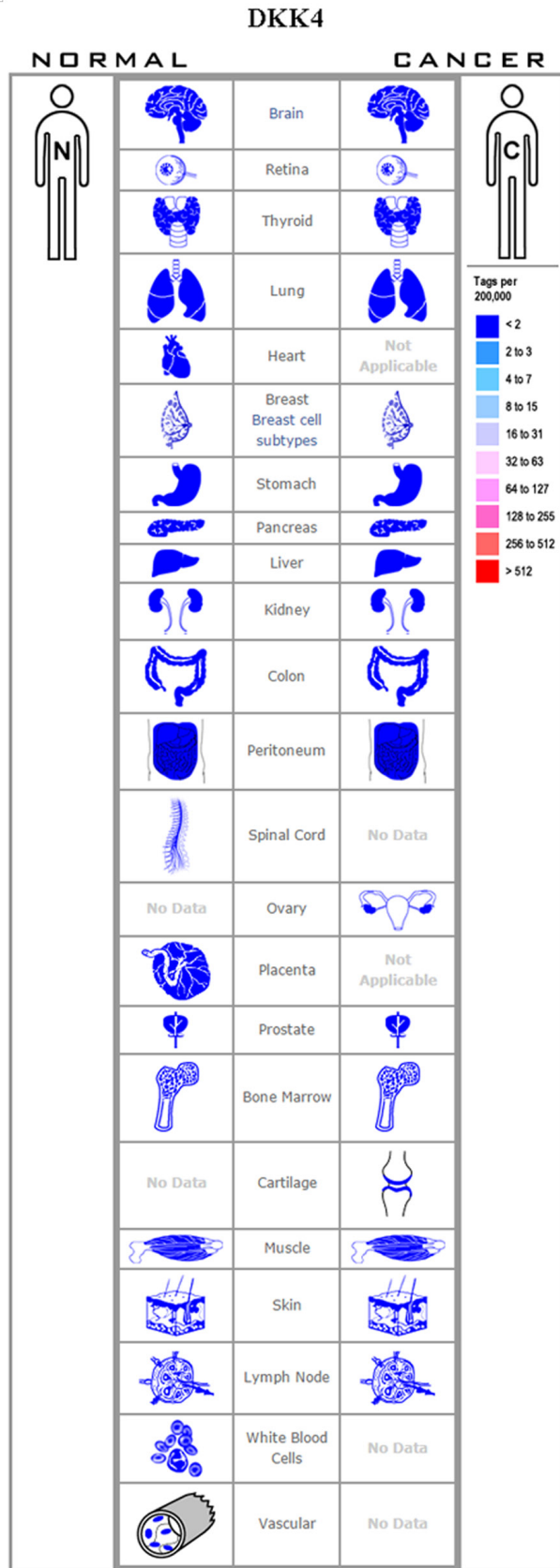


Figure 5. Expression profile for Dkk4 in human cancers.

propagate indefinitely. Cellular proliferation is controlled through the regulation of cell cycle, growth factors, and their receptors, and oncogenes/tumor suppressor genes [70]. Dkk1 could inhibit the proliferation of MCF-7 breast cancer cell line, whereas LM-MCF-7 cells, where Dkk1 expression was knocked down via siRNAs, exhibited increased proliferation. The high Dkk1 level can downregulate the expression of β -catenin, c-Myc, cyclin D1, and Survivin by accelerating their phosphorylation-dependent degradation, thereby blocking the heightened proliferation of breast cancer cells [71]. Conversely, Dkk1 can promote cancer cell proliferation independent of the Wnt signaling pathway [14]. Cytoskeleton-associated protein 4 (CKAP4) is a receptor for Dkk1, and the expression of Dkk1 and CKAP4 are frequently dysregulated in human pancreatic and lung cancer lesions. Binding of Dkk1 to CKAP4 activates the PI3K/AKT pathway, resulting in cellular proliferation [72].

The Dkk2 expression is significantly upregulated in prostate cancer, and its knockdown can suppress cell proliferation and invasion. Downregulation of Dkk2 can decrease the expression of β -catenin, cyclin D1, and c-Myc in prostate cancer cells through Wnt signaling inhibition [53]. An *in silico* study has shown that Dkk3 could efficiently interact with LRP, Krm, and epidermal growth factor receptor (EGFR) with comparable binding energies. Dkk3 can inhibit cancer cell growth by blocking Wnt and EGFR downstream signaling [56]. Dkk4 is frequently downregulated in colorectal cancer cell lines and primary tumors, and the ectopic overexpression of Dkk4 or treatment with recombinant Dkk4 could both inhibit the growth of colorectal cancer cells. Cell cycle regulation, specifically a G_0/G_1 arrest, may partially explain this inhibitory effect of Dkk4 on tumor proliferation [73].

Dkks and cancer cell apoptosis

Controlled cellular proliferation and apoptosis ensure normal growth and development of the body and normal physiological functions. Apoptosis is regulated by a balance of anti-apoptotic and pro-apoptotic factors, and tumor cells have gained the ability to avoid apoptosis [70].

The role of Dkks in apoptosis is well described in renal cell carcinoma (RCC). The expression of Dkk1 is significantly lower in the RCC tissue compared with the adjacent normal tissue. However, Dkk1 overexpression in cells could induce apoptosis and inhibit proliferation. Interestingly, while T-cell factor/lymphoid enhancer factor (TCF/LEF) activity; the expression of nuclear β -catenin; cyclin D1; and c-Myc remained unchanged in Dkk1 transfectants, the expression levels of cleaved caspase 3; p53; p21; and p53 upregulated modulator of apoptosis (puma) were significantly upregulated. These results indicate that the pro-apoptotic effect of Dkk1 is independent of Wnt signaling [74].

Like Dkk1, the expression level of Dkk2 is decreased in RCC cell lines. However, unlike Dkk1, the downregulation of Dkk2 in RCC cell line A498 could inhibit RCC progression by inducing apoptosis and G₁ phase cell cycle arrest [52]. The expression of Dkk-3 is downregulated by histone modification. RCC proliferation was significantly inhibited, and apoptosis promoted, in Dkk3-overexpressing RCC cell lines. Further, the expression levels of p21, MDM-2, and Puma genes were increased in the Dkk3-overexpressing cells. Thus, Dkk3 upregulation in renal cancer cells can induce apoptosis via the non-canonical JNK pathway [75]. Besides RCC, Dkk3 exhibits tumor-suppressive and pro-apoptotic effects, inducing apoptosis through mitochondrial and Fas death receptor pathways [76]. Pro-apoptotic effect of Dkk1 has also been reported in lung cancer; however, the mechanism remains unclear. Dkk3 gene knock-out in the non-small cell lung cancer cell line H460 increased the expressions of cyclin-dependent kinases D1 and E, p53, p21, and Bax, thereby activating the apoptotic pathway [77]. In contrast, Dkk3 overexpression in lung cancer cell line resulted in significant upregulation of E-cadherin, while the expression levels of matrix metalloproteinase-7 (MMP7), survivin, c-myc, and cyclin D1 were downregulated. In cisplatin-resistant lung adenocarcinoma cell lines, Dkk3 overexpression induced cell cycle arrest and apoptosis [78]. Lastly, Terauchi et al. [79] have reported that the downregulation of Dkk4 expression could suppress apoptosis in osteoblasts via the Wnt signaling pathway.

Dkks and cancer angiogenesis

Angiogenesis is critical to provide the body with sufficient nutrients and oxygen and to elimin-

ate metabolic wastes. Angiogenesis is strictly and precisely regulated to maintain homeostasis. Tumor angiogenesis or neovascularization, however, is a result of a loss of angiogenic control. Tumor angiogenesis is essential for invasive growth and metastasis and consists of two patterns: general angiogenesis and vasculogenic mimicry [70, 80]. Emerging reports indicate that the Wnt signaling pathways and their antagonists modulate vessel neogenesis during both developmental and pathological angiogenesis [29].

Dkk2 can promote angiogenesis in murine and human endothelial cells, and the Dkk2-mediated angiogenesis is distinct from VEGF-mediated angiogenesis [81]. Compared to VEGF-induced blood vessels, Dkk2-induced vessel displays closer interconnections. Additionally, Dkk2-induced vessels consistently show higher coverage of endothelial cells (ECs) by pericytes and smooth muscle cells (SMCs), which in turn plays an important role in vessel maturity and stability. Dkk2-mediated angiogenesis involves a signaling cascade induced through LRP6-mediated APC/Asef2/Cdc42 activation.

Dkk1 reportedly suppresses angiogenesis, and its expression is downregulated upon induction of morphogenesis [81, 82]. In non-small cell lung cancer, Dkk1 promotes vasculogenic mimicry by inducing the expression of epithelial-mesenchymal transition (EMT)-related and cancer stem cell (CSC)-related proteins [83].

Dkks and cancer invasion & metastasis

Invasion and metastasis are the most prominent biological characteristics of cancer and are also the most important causes of cancer-related deaths. Tumor metastasis is a continuous and dynamic process, wherein tumor cells translocate from their primary position via local invasion, blood vessels, and lymphatic tracts.

Recent studies have identified a role of Dkk1 in tumorigenesis and the invasiveness of several tumors. Dkk1 is upregulated in human HCCA tissues and increases tumor cell invasion and metastasis. The high Dkk1 level in HCCA correlates with metastasis to the hilar lymph nodes. Downregulation of Dkk1 in HCCA cells, however, significantly inhibits proliferation, colony formation, and migration. Dkk1 exerts its pro-invasive effect, at least in part, through the β -catenin/MMP-7 signaling pathway [33]. Similarly,

β -catenin/MMP-7 signaling, a pathway independent of the canonical Wnt signaling pathway, is one of the pathways responsible for the pro-invasive effect of Dkk1 in hepatocellular carcinoma [84].

Expression of Dkk1 is elevated in an intrahepatic cholangiocarcinoma (ICC) cell line and promotes migration and invasion via elevated MMP-9 and VEGF-C [34]. Conversely, Dkk1 overexpression inhibits the proliferation and migration of human retinal pigment epithelial cells by suppressing the Wnt/ β -catenin signaling pathway [85]. The expression of Dkk1 is markedly decreased in gastric cancer (GC) tissue and serum samples, and an upregulation of Dkk1 in chemo-resistant GC cells inhibits their proliferation and invasion [86].

Interestingly, there are several hormone response elements in the Dkks genes, which allow these hormones to regulate important physiological processes by modulating the expression of Dkks genes. Thyroid hormone receptor (TR) binds to nucleotides -1645 to -1629 of the Dkk4 gene promoter and induces its expression in HepG2-TR cells at the transcriptional level. Dkk4 overexpression suppresses hepatoma cell invasion *in vitro* and reduces metastasis in SCID mice via decreased MMP-2 expression [87]. The $1,25(\text{OH})_2\text{D}_3$ induces an early and transient binding of the vitamin D receptor (VDR) and the Silencing mediator for retinoic acid and thyroid hormone receptor (SMRT) corepressor to a region adjacent to the transcription start site of Dkk4. Ectopic Dkk4 expression increases the migration and invasion of colon cancer cells, which can be reversed by $1\alpha,25$ -dihydroxyvitamin D3 [88].

Dkks and cancer diagnosis

Even as a growing body of literature confirms the essentiality of early diagnosis in improved patient prognosis, there is a dearth of early diagnostic markers for a vast number of cancers. In a previous study, Dkks were reported to be a highly sensitive and specific early detection markers for hepatocellular carcinoma (HCC) [89]. The role of Dkk1 in the diagnosis of HCC is relatively clear. In a large multicenter study conducted by Fan et al. [90], Dkk1 exhibited multiple advantages in the diagnosis of HCC: (1) Serum Dkk1 levels were significantly higher in HCC patients compared to unaffected

controls; (2) Dkk1 level retained diagnostic accuracy in AFP-negative HCC patients, as well in patients with early-stage HCC; (3) Elevated serum Dkk1 concentration could differentiate between HCC, chronic HBV infection, and cirrhosis; and (4) A combination of Dkk1 and AFP level measurement greatly improved HCC diagnostic accuracy than when either test was used separately. Dkk1 can thus serve as a potential biomarker for HCC diagnosis, and subsequent studies have further confirmed the usefulness of a combined Dkk1-AFP measurement in improving early diagnostic efficacy [2].

In cervical cancer patients, serum levels of Dkk1 were associated with the histological type and lymphatic metastasis [45]. Serum levels of Dkk1 were also significantly higher in gastric adenocarcinoma patients. More importantly, a Dkk1 cutoff level of 25 U/mL could discriminate between gastric cancer patients and unaffected controls with 100% specificity and sensitivity. Thus, serum Dkk1 level may serve as a potent, novel serological marker for gastric cancers [91]. Similar to Dkk1, the serum level of Dkk3 was also significantly associated with lymphatic metastasis and tumor diameter in cervical cancer patients [92]. We also found that the serum Dkk3 level was significantly higher in healthy individuals than the gastric carcinoma patients and inversely linked to tumor size [61].

Dkks and the treatment of cancer

In assimilating the various roles played by the Dkks family in tumor development and metastasis, Dkks emerge as potential therapeutic targets for cancer treatment. In this regard, researchers have extensively studied Dkk3 as a genetic therapeutic target, and have demonstrated its tumor-suppressive effects. In one such study, Shiraha et al. [63], using intratumoral injections of an adenovirus vector carrying the human Dkk3 gene (Ad-REIC), showed that Dkk3 expression could be a potential therapeutic tool for pancreatic cancer. They further reported that Ad-REIC could induce apoptosis and inhibit the growth of pancreatic cancer cell lines, which were the underlying mechanisms for Dkk3-mediated tumor suppression. In another study, Kurozumi et al. [93] reported that in malignant glioma, Dkk3 expression could regulate cell growth through caspase-dependent apoptosis. Dkk3 expression was also reported

Dkks and cancer

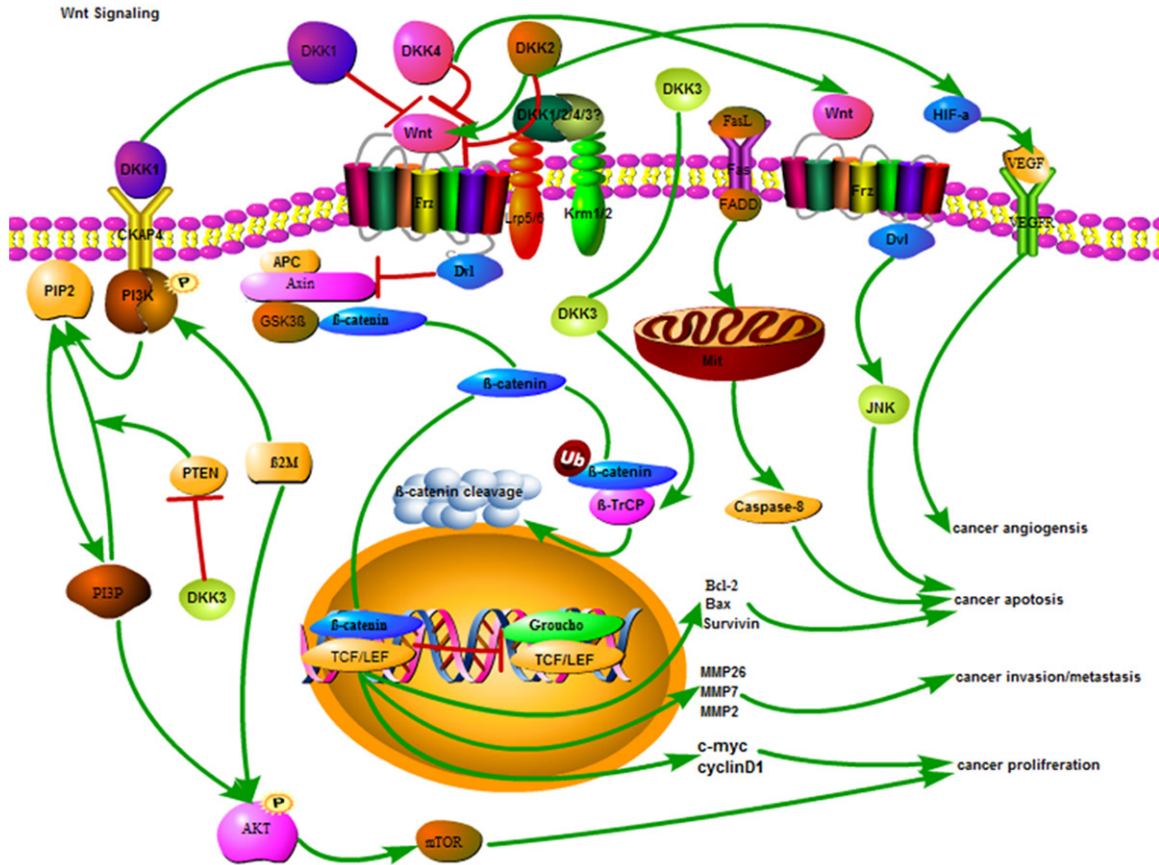


Figure 6. Schematic representation of the antitumor mechanisms of Dkks in cancer cell.

to induce apoptosis in human prostate cancer cells through the activation of c-Jun-NH2-kinase [94].

Dkks and cancer molecular mechanism

With the rapid development of modern molecular biology, genetic and epigenetic alterations have been identified as key players in cancer initiation and progression. In the Dkks gene family, methylation is the most commonly identified epigenetic hallmark associated with tumorigenesis.

Dkks methylation in cancer

DNA methylation is a crucial epigenetic modification with key functions during development. Moreover, aberrant DNA methylation has been linked to many human diseases, particularly cancer. Sites of differential and aberrant DNA methylation include regulatory DNA sequences, such as CpG islands in promoters; and distal cis-regulatory elements, such as enhancers and promoters [95]. Recent studies have sho-

wn that Dkks gene methylation is involved in tumorigenesis and cancer progression. Furthermore, the Dkks family members exhibit different methylation status in different cancer types [96].

Dkks related miRNAs in cancer

MicroRNAs (miRNAs) are a subset of highly conserved, small noncoding RNAs that are approximately 18-22 nucleotides in length. miRNAs bind to the 3'-untranslated regions of mRNAs and bring about their degradation or translational repression, thereby acting as vital post-transcriptional regulators of gene expression [97]. miRNAs play important regulatory roles in many biological processes, including cellular differentiation, proliferation, and apoptosis of cancer cells [98]. Dysregulated miRNAs expression has been reported in a large number of human cancers.

Ren et al. [98] noted that miR-501-5p was markedly upregulated in gastric cancer cell lines and affected tissues and that its expres-

sion level was significantly correlated with a more aggressive phenotype in gastric cancer patients. They further demonstrated that miR-501-5p could directly target and suppress multiple repressors of the Wnt/ β -catenin signaling cascade, including Dkk1. Yu et al. [99] demonstrated that miR-522 overexpression could promote cell proliferation, colony formation, and cell cycle progression, whereas a knockdown of miR-522 could attenuate these effects.

Dkks gene polymorphisms

In a recent study, Singh et al. [100] showed that Dkk3 rs7396187 exhibited a protective effect on lung cancer patients. Subjects with a heterozygous genotype of Dkk2 rs17037102 and rs419558 were at an increased risk. Subjects with the variant genotypic combination of Dkk3 rs3206824 and Dkk2 rs419558 showed a two-fold higher risk of developing lung cancer. Additionally, subjects with all three Dkk2 genotypic variants had a four-fold higher risk of developing lung cancer.

Other mechanisms

In addition to the regulatory mechanisms noted above, other mechanisms have been noted through which Dkks expression levels are modulated in certain cancer subtypes. In HBV-expressing liver cancer cell lines, HBV could bind to the Dkk1 promoter region and trigger its increasing expression at mRNA and protein level [101]. $1,25(\text{OH})_2\text{D}_3$ promotes the up-expression of Dkk1 mRNA and protein through an indirect transcriptional mechanism, which act as a tumor suppressor in colon cancer. In contrast, $1,25(\text{OH})_2\text{D}_3$ blocks Dkk4 transcription by targeting its promoter via VDR binding [102]. Additionally, thyroid hormone (T3) can induce Dkk4 mRNA and protein expression in HCC cells.

Perspective

The Dkk proteins have lately attracted a wide range of attention in cancer research as both a diagnostic biomarker and a potential therapeutic target. A large number of studies report the pleiotropic roles of Dkk proteins in physiological processes, while Dkks dysfunctions have been implicated in many diseases. In this review, we summarized the different functional pathways of Dkks gene family, in addition to the

canonical and widely-studied Wnt pathway (Figure 6). Of note, even as the underlying mechanisms remain unclear, the growing body of literature provides irrefutable evidence of the important role played by Dkks in the diagnosis and treatment of tumors.

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Disclosure of conflict of interest

None.

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