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Review

Dkk1 involvement and its potential as a biomarker in pancreatic ductal adenocarcinoma

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ABSTRACT

Dickkopf-1 (Dkk1)'s dysregulation has been implicated in the pathogenesis of a variety of cancers. It is part of the Dkk family of proteins that includes Dkk2, Dkk3 and Dkk4. This family of secreted proteins shares similar conserved cysteine domains and inhibits the Wnt/b-catenin pathway by causing proteasomal B-catenin degradation, inducing apoptosis, and preventing cell proliferation. Pancreatic ductal adenocarcinoma (PDAC) is the 4th leading cause of cancer mortality in the United States due to the late stage of diagnosis and the limited effectiveness of current therapy. Dkk1 is found increased in PADC patients' specimens and serum. Dkk1 can be a promising biomarker specific to PDAC, which has the potential to increase PDAC survival rates through improving early stage detection and monitoring progression compared to current biomarker gold standards. In addition, recent studies suggest that Dkk1 could be an excellent target for cancer immunotherapy. Interestingly, Dkk1-CKAP4-PI3K/AKT signal pathway also plays role in pancreatic cancer cell proliferation. In this review, we present the multiple mechanisms of Dkk1 in PDAC studied thus far and explore its function, regulation, and clinical applications in gynecological cancers including pancreatic ductal adenocarcinoma (PDAC), breast, ovarian, cervical, and endometrial cancer. Further research into Dkk1's mechanism and use as a diagnostic tool, alone or in combination with other biomarkers, could prove clinically useful for better understanding the pathology of PDAC and improving its early detection and treatment.

1. Introduction

Dickkopf-1 (Dkk1) is part of the Dkk family of proteins that includes Dkk2, Dkk3 and Dkk4. This family of secreted proteins share similar conserved cysteine domains and inhibits the Wnt/β-catenin pathway [1,2]. Structurally, Dkk1, −2, and −4 are more similar to each other than to Dkk3, due to the shorter linker region between the two cysteine domains of Dkk3 than in the others (Fig. 1) [1]. Dkk1, −2 and −4 also map to chromosomal regions in the same genetic paralogy group while Dkk3 does not. Dkk1 maps to 10q11, Dkk2 maps to 4q25 and Dkk4 maps 8p11, while Dkk3 maps to 11p15 [3]. The major form of Dkk1 has been shown to contain N- and O-linked glycosylations with a molecular weight of approximately 29.5 kDa while the minor form only has an O-linked glycosylation with a molecular weight of 27 kDa [4]. Dkk2 has a molecular weight of approximately 15–17 kDa and Dkk3's is near 38 kDa [1]. Dkk4 has been shown to have 3 different forms, the first with a molecular weight of 40 kDa, the second with molecular weight between 30 and 32 kDa, and the third with a molecular weight between 15 and 17 kDa [1].

Dkk1 was initially studied for its role in *Xenopus* head development by Glinka et al. in 1998 [5]. This group discovered Dkk1 was secreted by the Spemann organizer, an embryonic cluster of cells responsible for directing neural plate and head formation from nearby ectodermal cells, to inhibit the Wnt signaling pathway in *Xenopus* embryos [6]. The Spemann organizer is initially activated by the Wnt signaling pathway during early embryogenesis, and during late embryogenesis it is deactivated by Wnt inhibitors such as Dkk1 [6,7]. Krupnik et al. later isolated a cloned sequence of Dkk1 from a human fetal kidney cDNA library [1]. Dkk2 has been observed to be both an activator and inhibitor of the Wnt pathway with the Kremen2 receptor acting as its on/off switch [2]. It promotes invasiveness and metastatic spread in Ewing sarcoma as well as plays a role in osteoblast differentiation [8,9]. Dkk3

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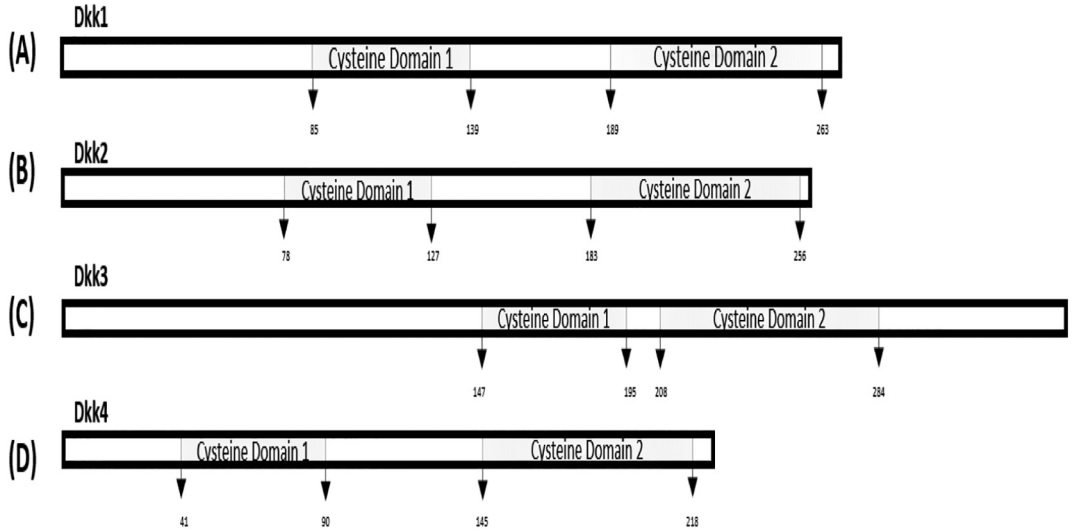

Fig. 1. Dkk family of proteins: (A) Dkk1 has 266 amino acids with its first Cysteine domain from amino acid 85–139 and the second Cysteine domain from amino acid 189–263. (B) Dkk2 has 259 amino acids with its first Cysteine domain from amino acid 78–127 and the second Cysteine domain from amino acid 183–256. (C) Dkk3 has 350 amino acids with its first Cysteine domain from amino acid 147–195 and the second Cysteine domain from amino acid 208–284. (D) Dkk4 has 224 amino acids with its first Cysteine domain from amino acid 41–90 and the second Cysteine domain from amino acid 145–218. *Edited from Krupnik et al., 1999 [1].

was not initially thought to affect Wnt signaling, however, more recent studies have demonstrated a regulatory role [10]. Dkk3 knockdown mice have been observed to have to decreased hemoglobin and hematocrit levels, decreased lung ventilation tidal volumes, as well as increased levels of Immunoglobulin M and natural killer cells [11]. In addition, Dkk3 regulates cell proliferation, apoptosis, collagen synthesis and T-cell responses [12–14]. Dkk4 is the least studied protein of this family and has been shown to have a smaller inhibitory effect on the Wnt pathway [15].

Out of the Dkk protein family, Dkk1 has been studied the most and has been implicated in Alzheimer's disease, bone formation and cancer [5,15,16]. In Alzheimer's disease, Dkk1 has been shown to be overexpressed in the brain and induced by β-amyloid peptide toxicity [16,17]. It regulates bone formation by blocking osteoblast bone maturation [18,19]. Expression of Dkk1 varies in cancerous tissues, increasing in some due to positive feedback, while decreasing in others due to epigenetic silencing [20,21]. Here we provide a review of current research findings regarding Dkk1's function, mechanism, regulation, and clinical applications in gynecological cancers including breast, ovarian, cervical, and endometrial cancer, as well as in pancreatic ductal adenocarcinoma (PDAC).

2. Mechanism/function of Dkk1

Dkk1 is involved in cell apoptosis through the Wnt signaling pathway. The Wnt signaling pathway is important in many cellular processes and implicated in various activities including skeletal development, cell differentiation, cell polarity, bone metabolism, and myogenesis [22–25]. There are three forms of the Wnt signaling pathway: the non-canonical, Wnt/Ca^{2+}, and the canonical pathway. The Wnt non-canonical pathway, also known as the β-catenin-independent pathway, is involved in regulating actin cytoskeleton and cell polarity [25–28]. The Wnt/Ca^{2+} pathway, a branch of the non-canonical β-catenin independent pathway, has a variety of roles in zebrafish and *Xenopus* embryogenesis, such as in regulation of dorsal axis and heart formation [26,29–31]. While the roles of the Wnt non-canonical and Wnt/Ca2+ pathways are diverse and at present are not fully understood, the Wnt canonical pathway, also known as the β-dependent or Wnt/β-catenin pathway, has been studied more in relation to cancer and the Dkk1 protein.

In the active Wnt canonical pathway, secreted Wnt glycoprotein binds to a co-receptor complex consisting of LDL receptor-related protein 5 (Lrp5), Lrp 6 and Frizzled (Fz) (Fig. 1) [32–36]. Wnt proteins bind the cysteine-rich N-terminal of the Fz receptor to recruit and activate the Disheveled (Dvl) protein [35–37]. The binding of Wnt, through conformational changes from an oligomeric to an active monomeric state, causes phosphorylation of a serine residue on the intracellular PPPSP motif of the Lrp5/6 receptors by G-protein coupled receptor kinases 5/6 (GRK5/6) [32,38–41]. Actived Dvl binds Fz through its PDZ domain, disrupting a protein destruction complex that includes Axin, Adenomatosis Polyposis Coli (APC), Casein kinase 1 (Ck1), and Glycogen Synthase Kinase 3 (GSK3) [34,42–44]. Axin is a scaffolding protein that has been shown to bind phosphorylated Lrp5/6, by using its phosphorylated PPPSP motif as a docking site [32,39,45]. Separation of Axin from the destruction complex allows it to bind to Dvl through the DIX domains on both proteins, which increases the stability of its downstream effector, β-catenin [43]. With the destruction complex inactivated, β-catenin remains active and is translocated into the nucleus, where it binds a complex of proteins (TCF, BCL9, C13P & Pygo) to activate the transcription of oncogenes, c-myc, cyclin D1 and Axin 2 (Fig. 2).

Dkk1 participates in the canonical Wnt (β-catenin dependent) pathway by acting as a competitive inhibitor of Wnt [1,46]. Dkk1 binds to, stabilizes and accumulates Lrp6 on cell surfaces [47]. The C-terminal of Dkk1 contains a colipase fold and is essential for the interaction between Dkk1 and Lrp6, Kremen1 and Kremen2 co-receptors [2,48–53]. Kremen2 binding initiates formation of a ternary complex with Dkk1 and Lrp6 that induces endocytosis or clearance of Lrp6 from the cell membrane, preventing further Wnt-Lrp5/6 interaction [47,54]. In the absence of Wnt, the destruction complex remains intact and Ck1, stimulated by Axin, phosphorylates β-catenin at its serine 45 initiating further phosphorylation of β-catenin by GSK3 [55,56]. This facilitates ubiquitin binding to β-catenin by Skp and β-TrCP and, therefore, subsequent degradation of β-catenin by a proteasome (Fig. 3) [57,58]. This pathway is significant to cancer development because in the absence of Dkk1, the canonical Wnt pathway is unregulated and leads to excessive cell proliferation through the upregulation of β-catenin.

Dkk1 has also been shown to affect the RANK/RANKL/OPG pathway involved in bone formation and resorption. Receptor activator of nuclear factor kappa-B ligand (RANKL), also known as Osteoprotegerin ligand (OPGL), is a cytokine that regulates bone resorption. The binding of RANKL to its receptor, RANK, causes

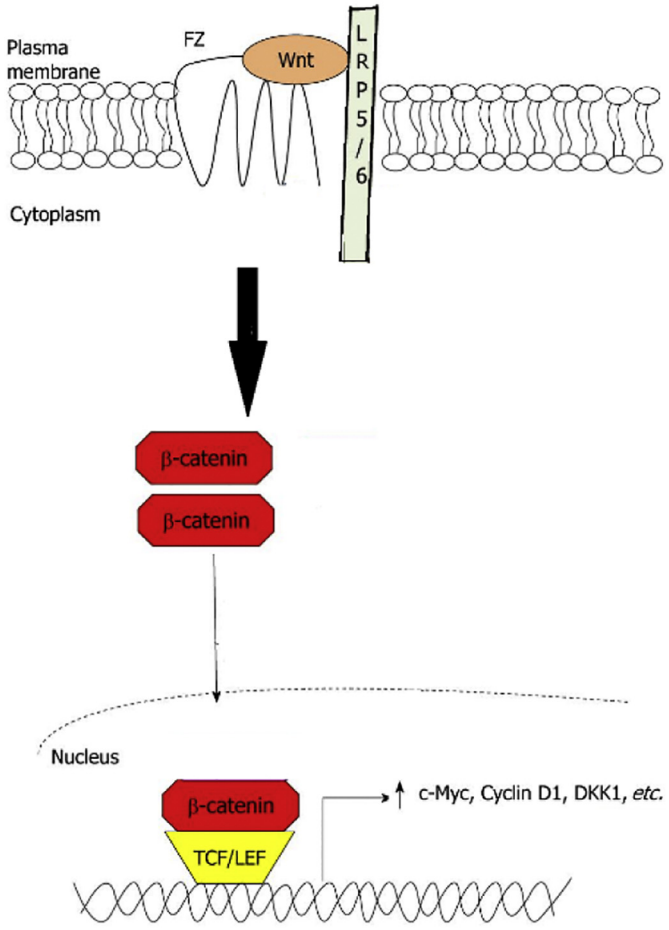

Fig. 2. Wnt canonical pathway: The binding of the Wnt ligand leads to disruption of the APC destruction complex, allowing β-catenin to remain active. Active β-catenin is translocated into the nucleus causing upregulation of proteins such c-Myc, Cyclin D1 and Dkk1. *Edited from Fatima et al. [129].

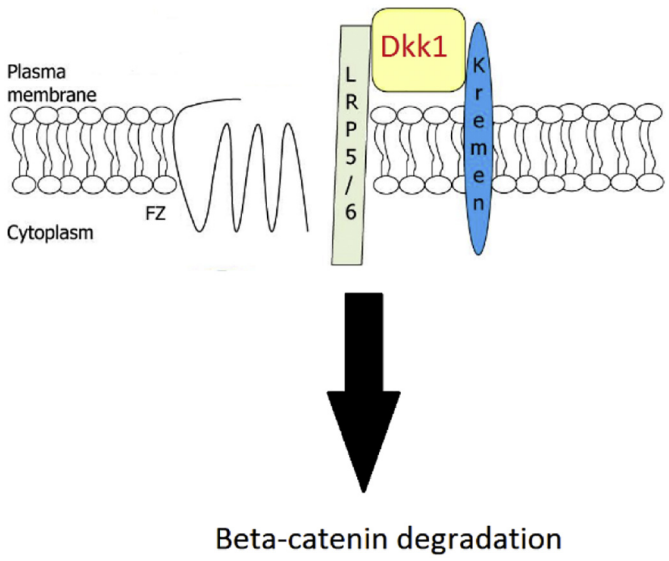

Fig. 3. Wnt canonical pathway: Dkk1 antagonizing and binding to the LRP5/6 receptors leads the destruction complex to phosphorylate and ubiquinate β-catenin for degradation. *Edited from Fatima et al. [129].

multinucleation and maturation of osteoclast precursors into osteoclasts, leading to increased bone resorption [59]. Osteoprotegerin (OPG) inhibits the RANK/RANKL interaction, thereby preventing osteoclast formation, maturation, and activation. The Wnt/β-catenin pathway has been shown to cross-talk to with the RANK/RANKL/OPG pathway by upregulating osteoblasts and OPG and downregulating RANKL [60–62]. Dkk1 increases bone resorption by inhibiting Wnt [15,63,64], and has also been shown to decrease OPG and osteoblast levels as well as increase expression of RANKL [18,65,66].

3. Dkk1 in cancers

Dkk1 dysregulation has been implicated in the pathogenesis of a variety of cancers. Upregulation of Dkk1 contributes to the development of cancer in prostate tissue, where it is regulated by p38 MAPK, and in non-small cell lung carcinoma, where it is regulated by p53 [20,50,67–69]. On the other hand, in gastric and colorectal cancer, Dkk1 has been shown to be under-expressed [21]. In these cancers, Dkk1 is regulated by miR-493 and by epigenetic silencing, respectively [21,70]. In chronic lymphocytic leukemia (CLL) it is expressed at normal levels, but unable to affect the Wnt/β-catenin pathway, while in multiple myeloma, Dkk1's role is shown to change from tumor suppressor to a stress responsive gene involved in the JNK pathway [71,72]. As all these studies have shown, the activity and expression levels of Dkk1 varies in different cancers and needs to be further investigated, in a specific manner to better understand its mechanism and possible use as a predictive marker.

Pancreatic ductal adenocarcinoma (PDAC) currently accounts for 3% of all cancer diagnoses and is often diagnosed during Stage III or IV of the disease. The American Cancer Society estimates that in 2017 there will be approximately 53,670 people diagnosed and 43,090 deaths due to this disease [73]. PDAC survival rates are low, with an average of 13% for Stage I, 6% for Stage II, 3% for Stage III, 1% for Stage IV, and a five-year survival rate of 8% [73]. These low survival statistics are due not only to the late stage of diagnosis, but also to the lack of effective therapies at that point of the cancer's progression. There are no significant early signs and the symptoms are nonspecific, such as back pain, fatigue, weight loss, and jaundice. Current late stage therapy includes chemotherapy, surgery, radiation, and adjuvant therapy, all of which have limited effectiveness. There is a dire need for early detection methods to improve survival and for a better understanding of its pathogenesis to help facilitate creation of effective therapies.

PDAC's pathogenesis involves the activation of various oncogenes, such as KRAS, PAK4 and MYB, as well as the inactivation of tumor suppressors including TP53, SMAD4 and PTEN, in their respective signaling pathways [49,74]. KRAS is a GTPase mutated in over 90% of PDAC cases on codon 12 of the gene [75,76]. It is responsible for relaying cell proliferation signals through the MAPK/ERK pathway wherein activated Raf phosphorylates Mek which, in turn, activates Erk through phosphorylation [77,78]. Active Erk is responsible for phosphorylating and activating various transcription factors, such as c-JUN and c-myc, which are responsible for the progression of the cell cycle [77]. MYB is another oncogene that has been found to be upregulated in PDAC [79,80]. Like KRAS, MYB plays a role in promoting PDAC malignancy through cell growth, proliferation, and metastasis [79]. PAK4 is a serine/threonine-protein kinase that has also been linked to the cell cycle and apoptosis [81]. It is often overexpressed or upregulated in cancers, playing a role in cell proliferation and invasion [82,83]. Specifically, in PDAC, it has been confirmed that mutated KRAS activates PAK4, which aids in the acquisition of invasive properties of PDAC cells [84]. Tumor suppressor TP53 is commonly known to induce apoptosis in damaged cells and is often mutated in cancer cells leading to uncontrolled cell growth [85,86]. It has been shown to be mutated in multiple PDAC cells lines [87]. SMAD4 is another tumor suppressor found to be inactivated in over 55% of PDAC tumors [88]. Many studies

Table 1
Dkk1 in Pancreatic Cancer.

Papers	Dkk1 expression	Pathway	Method	Sample type
Han et al., 2015	Dkk1 is overexpressed in PDAC, and increases with each stage of cancer	Not studied	ELISA; IHC	Serum & tissue
D'Amico et al. (2016)	Overexpressed	Not studied	IHC	TMA (Stage I-II with no metastasis)
Kimura et al., 2016	Overexpressed	Akt/CKAP4	IHC	Tissue
Hallas et al., 2016	Overexpressed	Not studied	RT-PCR; qPCR	Tissue
Zhong et al., 2011	Low expression	Not studied	IHC	PDAC tissues
Zhang et al., 2013	No test on Dkk1 expression. Exogenous Dkk1 used to test inhibition of wnt pathway on pdac initiation	Wnt pathway is required for pancreatic cancer initiation & progression	N/A	N/A
Nagano et al., 2013	No direct test on Dkk1 OE	Not studied	N/A	N/A
Takahasi et al., 2009	No direct test on Dkk1 OE	Not Wnt or JNK Pathway	RT-PCR	Suit-2 cells

have shown a correlation between high SMAD4 levels and extended patient survival [89–91]. Unlike in many other cancers, PTEN is not mutated in pancreatic tumors, though its expression levels are confirmed to be reduced in both mice models and the Panc-1 cell lines by TGF-β1, a growth factor overexpressed in cancer cells [92,93]. Many of these molecules are currently being studied in hopes of better understanding the pathology of PDAC and discovering new biomarkers for early detection and treatment.

Research investigating new methods of early PDAC detection has found many candidate makers. Some of these potential biomarkers include PAM4 (sensitivity = 64%; specificity = 96%), REG4 (sensitivity = 94.9%; sensitivity = 64%), Mesothelin (sensitivity = 68%; specificity = 91%), uPAR (sensitivity = 93.6%; specificity = 53.2%), RCAS (sensitivity = 85%; specificity = 42%), miR-200a (sensitivity = 84.4%; specificity = 87.5%), miR-200b (sensitivity = 71.1%; specificity = 96.9%), glycolytic enzyme enolase (1/2) autoantibody (sensitivity = 62%; sensitivity = 97%) and miR-1246 [94–99]. In addition, Dkk1 is also being studied for use as a potential early detection biomarker [100]. It has been proven to be upregulated in gastric, breast, endometrial, ovarian and lung cancer and downregulated in colorectal cancer [20,67–69]. Recent studies show that Dkk1 is overexpressed in pancreatic cancer tissues and could be a potential biomarker for this disease, with a sensitivity of 89.3% and specificity 79.3% in PDAC [100].

Dkk1 has been studied only in relation to the Wnt/β-catenin pathway and not the RANK/RANKL pathway in PDAC. The Wnt/β-catenin signaling pathway facilitates cell proliferation and has been proven to be required for PDAC progression in a mouse model [101]. Zhang et al. proved this by generating a mouse model that caused Dkk1 expression to be induced by doxycycline, a common antibiotic used to treat bacteria and protozoa infections. This mouse model was then crossed with another model (KC mice) that developed PanIN lesions, and eventually progressed to PDAC. The cross of these two mouse models generated a new model (KDC mice) that developed PanIN lesions and whose Dkk1 expression could be induced by administering doxycycline, an antibiotic. The Wnt/β-catenin pathway in the KDC mice was confirmed to be inhibited. Before PanIN formation, administering doxycycline continuously gave promising results showing that without the Wnt/β-catenin signaling pathway, which was blocked by Dkk1, the progression of PDAC is significantly reduced with higher grade PanIN lesions developing in later months compared to controls. The significance of the Wnt signaling pathway on the progression of PDAC was also confirmed using OMP-18R5, a monoclonal antibody that blocks Frizzled receptor activity [101,102]. Administration of this antibody to KC mice inhibited the Wnt/β-catenin pathway (resulting in specimen and showed the abundance of normal acini and ADM compared to controls. Cross-talk between the Wnt/β-catenin pathway and the MAPK/ERK signaling pathway during PanIN formation and PDAC progression was then confirmed through co-immunofluorescence. Interestingly, Dkk1-expressing cells were found to have lower levels of phosphor-ERK1/2 and CK19, a marker for PanIN and ADM expression in 2 and 9-month-old KDC mice. This confirmed that Dkk1 expressing cells inhibit PanIN formation through the MAPK pathway, but associated mechanism of action is not yet known [101]. These studies have demonstrated the significance of Dkk1 in cancer progression and have identified the need to better understand its mechanism of action through further study.

3.1. Dkk1 as a biomarker in pancreatic cancer

Currently, CA19-9 is the standard biomarker used for detection of pancreatic cancer, but has limited efficacy in detecting the disease at an early stage [103–108]. This is because CA19-9 serum levels not only increases in PDAC, but also in pancreatitis, compromising its specificity for pancreatic cancer and therefore making it a poor biomarker [109–111]. CA19-9 has a sensitivity of 79–81% and a specificity of

82–90% for the diagnosis of PDAC [112–114].

Dkk1 is a promising biomarker for early detection and progression of PDAC [100] (Table 1). Han et al. measured serum Dkk1 levels in 140 PDAC patients at various stages of the PDAC, including before and after surgical treatment using ELISA and Immunohistochemistry. Their data showed Dkk1 had significantly higher levels in PDAC serum compared to healthy controls, benign pancreatic tumors, and even chronic pancreatitis serum samples. Dkk1 serum levels were also shown to increase with the advancement of the disease from Stage 1 to Stage 4 [100]. However, the serum concentration levels of Dkk1 compared to that of CA19-9 was not found to be significantly different. The accuracy and AUC of the Dkk1 serum levels, determined using ROC curve analysis, was greater than that of CA19–9, suggesting that Dkk1 would be an excellent candidate as a biomarker for PDAC. The value of Dkk1 in monitoring prognosis, before and after surgical treatment, was also analyzed by monitoring the serum levels at both time points. They concluded higher Dkk1 serum levels correlated with lower survival, supporting the utility of Dkk1 as a prognostic biomarker [100].

Dkk1 was initially found to be expressed in PDAC in 2010 by Takahashi et al. [115]. Several pancreatic cell lines were tested (such as Suit-2, Suit-4, AsPc-1, MIA PaCa-2, Panc-1, HPAF and BcPC3) and Dkk1 was found to be upregulated in these cell lines and in cells with good, moderate, and poor differentiation [115]. In well-differentiated cells, 50% of cases had high levels of Dkk1 as well as 66% in moderately-differentiated, and 100% of poorly-differentiated cells. While these results are promising, their significance is limited by a small sample size (23 N) it is important to mention that there was only a total of 23 cases. Through Dkk1 knockdown, Matrigel invasion assays, and cell scratch assay, Takahashi et al. demonstrated a possible role for Dkk1 in cellular invasiveness of carcinogenic cells [115]. They also found Dkk1 did not use the JNK pathway to affect changes in cells by knocking down Dkk1 and confirmed beta-catenin, JNK phosphorylation and c-myc mRNA levels were not changed by this knockdown [115]. This left the need for further confirmation of the pathway Dkk1 in affecting cellular invasiveness in PDAC.

In 2011, transcription factor GATA6 was found to regulate Dkk1 in pancreatic cancer [116]. A variety of experiments were done to verify that GATA6 is amplified in the late stages of pancreatic cancer (> 2 copies in PanIN-3 compared to 0 in PanIN-1 and PanIN-2), and that this amplification is highly correlated to increased nuclear β-catenin protein, which enhances cell proliferation [116]. Interestingly, a correlation was found between increased GATA6 copy number (> 2.3) and longer survival in patients with surgically resected PDAC by the Kaplan Meier survival estimate [116]. The role of GATA6 in cell growth was confirmed using lentiviral vectors expressing mock or GATA6-specific shRNA [116], which were then used to infect AsPC1 (found to have 2.3 GATA6 copy numbers) and AI3A (found to have 9.0 GATA6 copy numbers) cell lines. Cell proliferation increased when these cells lines were subjected to GATA6 overexpression, and decreased with GATA6 knockdown (specifically, in the G2/M phase). The increase of the GATA6 gene was also found to be correlated with typical gene mutations found in PDAC on proteins such as KRAS and TP53 [116]. This research group also discovered that GATA6 negatively affects Dkk1 transcription activity by binding to its promoter as shown by a chromatin immunoprecipitation assay and an electrophoretic mobility shift assay. A luciferase reporter controlled by the Dkk1 promoter confirmed the correlation between GATA6 and Dkk1 by showing that Dkk1 mRNA levels were decreased in Panc1 cells with forced expression of GATA6. This was also demonstrated *in vivo,* with 12 PDAC tissues having decreased levels of Dkk1 with over-expression of GATA6, but it was also discovered that without over-expression of GATA6, there were only 3 out of 12 PDAC cases that exhibited over-expressed Dkk1 [116]. While this contrasted from it should be acknowledged that neither of the previous groups verified GATA6 levels in their PDAC tissues. However, the low number of Dkk1 overexpression caused Zhong et al. to verify other possible Dkk1 suppressors besides GATA6. Through a methylation specific PCR of the Dkk1 promoter they confirmed Dkk1 could be epigenetically silenced when GATA6 is not overexpressed. These results are significant as they potentially suggest an alternative pathway for how Dkk1 could possibly be involved in the progression of PDAC.

Kimura et al. also investigated Dkk1's activity in PDAC and found a receptor, cytoskeleton-associated protein 4 (CKAP4), which was upregulated in 66.1% of PDAC cases [117]. CKAP4 is a type II transmembrane protein [118,119] located on the cell surface in a S2-CP8 pancreatic cell line [117]. This group also found that co-expression of Dkk1 and CKAP4 is inversely related to prognosis and relapse-free 5-year survival. This co-expression was also found to have high levels of AKT, a proto-oncogene that has been implicated to be improperly regulated in cancer cells [117]. Knocking down Dkk1 or CKAP4 in the S2-CP8 cell line was found to lead to decreased AKT, cell proliferation in 2D and 3D culture, and cell migration [117]. Their initial experiment tested the efficiency of an anti-CAKP4 antibody on the S2-CP8 cell line and confirmed its effectiveness. The anti-CKAP4 antibody was injected, twice a week, in mice that were implanted with the S2-CP8 cell line. Their results were encouraging as the antibody reduced the volume, weight, Ki-67-positive cell number and AKT activity. This study was helpful in discovering that Dkk1 binds to CKAP4 through its cysteine residues and knowledge that the upregulation of both proteins in PDAC is significant in helping better understand Dkk1's mechanism of action as a biomarker, indicating immunotherapies could be developed to better target its action.

The latest work characterizing Dkk1 in PDAC was published in early 2016 from the D'Amico et al. group [120]. This group discovered a correlation between Dkk1 and myeloid derived suppressor cells (MDSCs) in PDAC [120]. A sub-group of MDSCs are CD15+ leukocytes often upregulated in several cancer tissues [121–123]. CD15+ is increased in the bone marrow and peripheral circulation of PDAC patients [123]. D'Amico et al. used tissue microarray (TMA) slides obtained from patients who were diagnosed with invasive PDAC, had not received any neoadjuvant therapy, underwent a pancreaticoduodenectomy and then followed with adjuvant chemotherapy [120]. CD15+ leukocytes and Dkk1 expression levels were then analyzed and quantified from the TMA slides and the data collected confirmed that there is a correlation between high Dkk1 levels and high CD15+ levels in Stage 1 to Stage 2 PDAC ($p < .05$) [120]. This work showed that Dkk1 regulated myeloid derived suppressor cells (MDSCs) in PDAC tissues, which is insightful as MDSCs have been proven to advance PDAC tumor progression in mice models and patient tissues [124–126].

4. Outlook of PDAC immunotherapy with Dkk1

While studies on Dkk1 and its role in PDAC are still in their early phases, this protein shows potential for being good target for cancer immunotherapy. As of now, there are no full studies testing the effectiveness of targeting Dkk1 in PDAC, and only a few studies of Dkk1 in other cancer subtypes. Some of these studies have shown that anti-Dkk1 antibodies are effective in stopping progression of cancer cells [100]. Sato et al. confirmed anti-Dkk1 antibodies have the ability to decrease cellular invasiveness and growth by inducing apoptosis in A549 lung cancer cell lines in a mouse model [100]. Other studies have begun researching the potential of Dkk1 as an immunotherapy for treating multiple myeloma [127,128]. Park et al. synthesized a cyclized oligopeptide, that acted against the Dkk1 and LRP5/6 interaction, as a possible treatment option for multiple myeloma [127]. This cyclized oligopeptide was found to effective in inhibiting multiple myeloma tumor growth in a mouse model by binding to the E1 site of the LRP5 receptor [127]. Fulciniti et al. analyzed the effect of a Dkk1 neutralizing monoclonal antibody (BHQ880) on multiple myeloma cells *in vitro* and in a mouse model [128]. Their results have been significant in showing that administration of BHQ880 (200μg; 3×/week) in the mouse model inhibited the growth of the multiple myeloma cells [128]. BHQ880 is currently in phase II clinical trials for multiple myeloma. Further

research will be needed to confirm details of the mechanism of the cyclized oligopeptide, but it provides a promising potential treatment for PDAC that should be investigated.

5. Conclusion

Dkk1 is the most studied Dkk protein family member and plays a role in Alzheimer's, bone formation regulation and cancer. Its activity has mostly been linked to the Wnt/β-catenin pathway, but also found to be involved in the RANK/RANKL pathway. Dkk1's expression in cancer has been reported to vary depending on the cancer type, overexpressing in some and under-expressing in others. In breast cancer and PDAC, Dkk1 has been shown to be overexpressed in patient serum and correlated with lower overall survival rates. While not as extensively studied in PDAC, Dkk1 has been shown to be regulated by GATA6. The low survival rates of PDAC makes it imperative for an early method of diagnosis to be found and the high serum Dkk1 make it a promising diagnostic tool for early detection and/or progression of gynecological cancers and PDAC. Studies on Dkk1's immunotherapeutic role in PDAC is still in their early phases, but Dkk1's involvement in cellular invasion, anchorage-independent growth, cell proliferation and apoptosis make it necessary to better understand this glycoprotein.

Additional advances in the early detection and progression of pancreatic cancers are strongly needed. Many approaches have been studied, but none have demonstrated results significant enough to replace current biomarker gold standards such as CA-125 and CA19–9 which have poor sensitivity and specificity. In this review, we presented the multiple mechanisms of Dkk1 in PDAC studied thus far. Further research into Dkk1's mechanism and use as a biomarker alone or in combination with other biomarkers can prove to be useful.

The need for early detection and progression biomarkers for pancreatic cancers is due to the low survival statistics of these diseases. Early detection allows physicians to better assess and plan treatment options that best suit each patient, which would result in higher survival statistics. Increased advances into the potential of new biomarkers and PDAC will be invaluable to the detection and treatment of these cancers.

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