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Review

Dickkopf-3 in Human Malignant Tumours: A Clinical Viewpoint

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Abstract. *Dickkopf-3 (DKK3), also known as REIC, is a secreted glycoprotein. DKK3 is aberrantly expressed in various types of human malignant tumours. Promoter methylation status, intracellular protein expression, and protein expression in tumour vessels are significantly correlated with clinical prognostic factors, including survival. In malignant cells, DKK3 is involved in the induction of apoptosis, inhibition of invasion, and remodelling of tumour vasculature. These activities are carried out via the regulation of the beta-catenin signalling and c-Jun N-terminal kinase-dependent cellular pathway, both of which are critical for carcinogenesis. This review explores the potential value of DKK3 as a clinical biomarker and a therapeutic candidate in human malignancies.*

Dickkopf-3 (DKK3) was discovered to be down-regulated in immortalized cells although, originally, the gene was identified as REIC (1). Since then, the intracellular expression of DKK3 was evaluated in various types of malignant tissues and was found to be down-regulated in cancer cells unlike in normal cells. It has been shown that the reduced expression of *DKK3* is mainly mediated by hypermethylation of the promoter region. This aberrant expression or promoter methylation status was also associated with prognostic clinical factors, including survival, suggesting the potential relevance of DKK3 as a clinical biomarker in human malignancies.

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According to its sequence homology, DKK3 belongs to the DKK family of proteins, which consists of DKK1-4, secreted glycoproteins with two cysteine-rich domains, and Soggy (2, 3). DKK1, -2, and -4 negatively regulate the Wnt signalling pathway by directly interacting with Wnt coreceptors (3, 4), however, the role of DKK3 in Wnt signalling is not clear. It has been shown that DKK3 is associated with β-catenin and c-Jun N-terminal kinase (JNK)-dependent signalling, the pathway critical for the progression of cancer. Furthermore, the tumour-suppressive activities of DKK3 have been demonstrated in a variety of cancers.

In the present review, we summarize the clinical significance, cellular functions, and intracellular signalling pathways related to DKK3, and highlight the potential of DKK3 as a therapeutic target and clinical tumour biomarker.

Aberrant Expression of DKK3 in Malignant Tumours

DKK3 is aberrantly expressed in human malignant cells unlike in normal cells. The intracellular down-regulation of DKK3 has been reported in 40.5 to 76.6% of malignant tumours that originate in the prostate, colon and rectum, breast, uterine cervix, ovaries, endometrium, lungs, stomach, kidney, and bladder, as well as in leukaemias, testicular cancers, and malignant melanomas (5-26) (Table I). These findings indicate that the down-regulation of DKK3 could be an essential event in carcinogenesis. Interestingly, DKK3 can also be over-expressed in a few malignant tumours, such as oesophageal adenocarcinoma with moderate to high DKK3 expression in 46.8% of the cases compared to 20% in Barrett's metaplasia (27), oral squamous cell carcinoma with higher DKK3 protein expression in 84.3% *versus* 22.5±16.5% in normal specimens (28), and hepatoblastoma with DKK3 overexpressed in 11/14 cases compared to non-cancerous parenchyma (29). These differences in aberrant expression patterns may be explained by tumour or tissue context-dependent variations and tissue type-specific biology; however, further studies are required to understand the exact mechanisms.

Table I. *Clinical significance of aberrant expression/promoter hypermethylation of DKK3 in human malignant tissues.*

Human cancer	Down-regulation		Promoter methylation		Clinical significance(s)		Reference
	% (number)	Method	% (number)	Method	Correlated clinical factors	Survival correlation	
Bladder cancer	Lower quantitative level in tumour (N=24)	RT-PCR	70.8 (17/24)	MSP	- Tumour invasion	-	Urakami *et al.* 2006 (23)
Basal breast cancer	Lower median IRS (N=463)	IHC			Triple-negative, HER2 positive, luminal subtype		Lorsy *et al.* 2016 (14)
	Low *vs.* high level of mRNA (N=3554)	*In silico* analysis				Lower recurrence-free survival	
Breast cancer			78 (75/96)	MSP	Higher clinical stage, LN involvement, ER positive	-	Xiang *et al.* 2013 (35)
Breast cancer			61.3 (92/150)	MSP	Older age	Lower overall survival*	Veeck *et al.* 2009 (47)
Cervical cancer	64.8 (57/88)	IHC			Higher FIGO stage	Lower disease-free survival*	Ryu *et al.* 2013 (16)
Cervical cancer			Higher quantitative level in tumour (N=62)	Pyro-sequencing	Higher level of SCC tumour marker, bigger tumour size	-	Kang *et al.* 2013 (43)
Cervical cancer			38 (38/100)	MSP	Higher FIGO stage, LN involvement	Lower overall survival*	Zhang *et al.* 2018 (41)
Colorectal cancer	70.1 (228/325)	IHC			Invasion depth, higher TNM stage, dedifferentiation	No correlation	Wang *et al.* 2012 (6)
Colorectal cancer			52.3 (67/128)	MSP	-	No correlation	Yu *et al.* 2009 (34)
Colorectal cancer			21 (12/58)	MSP	No correlation	-	Sato *et al.* 2007 (48)
Endometrial cancer	Quantitative analysis (N=27)	RT-PCR			Higher FIGO stage, LN involvement, non-endometrioid histology, cytology-positive	-	Dellinger *et al.* 2012 (15)
Gastric cancer	40.5 (64/158)	IHC			Higher TNM stage, LN involvement,	Lower disease-free survival	Park *et al.* 2015 (26)
Gastric cancer	63.9 (236/369)	IHC			Younger age, bigger tumour size, LN involvement, Lauren's classification	Lower survival	Xu *et al.* 2012 (25)
Gastric cancer			67.6 (117/173)	MSP	Higher TNM stage	Lower overall survival, increased risk of cancer-related death*	Yu *et al.* 2009 (34)
Gastric cancer			39 (12/31)	MSP	No correlation	-	Sato *et al.* 2007 (48)
HCC			Quantitative analysis (N=50)	Quantitative MSP	Multicentricity	Lower progression-free survival*	Yang *et al.* 2010 (42)
ALL			33 (60/183)	MSP	No correlation	Lower disease-free survival*	Roman-Gomez *et al.* 2004 (22)
	38.4 (70/183)	RT-PCR					
NSCLC	63 (36/57)	RT-PCR			Higher tumour grade	No correlation	Nozaki *et al.* 2001 (18)
NSCLC	76.6 (72/94)	IHC			Higher tumour grade	-	Yue *et al.* 2008 (17)

Table I. *Continued*

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Human cancer	Down-regulation		Promoter methylation		Clinical significance(s)		Reference
	% (number)	Method	% (number)	Method	Correlated clinical factors	Survival correlation	
NSCLC			16.3 (22/135)	MSP	No correlation	Lower overall survival*	Suzuki *et al*. 2007 (39)
NSCLC			14.2 (22/155)	MSP	No correlation	No correlation	Zhu *et al*. 2012 (40)
Ovarian cancer	66 (37/56)	IHC			No correlation	-	You *et al*. 2011 (19)
Prostate cancer	47.1 (8/17, G3) 52.9 (14/25, G4) 71.4 (5/7, G5)	IHC			High Gleason grade (G)	-	Kawano *et al*., 2006 (79)
Prostate cancer			68 (28/41)	MSP	-	-	Lodygin *et al*. 2005 (37)
Renal cancer	N=64	IHC, RT-PCR			No correlation		Guo *et al*. 2014 (20)
Renal cancer			50 (31/62)	MSP	Higher tumour grade	Lower overall survival	Urakami *et al*. 2006 (24)

Human cancer	Up-regulation		Clinical significance, correlation with		Reference
	% (number)	Method	Correlated clinical factors	Survival correlation	
Oesophageal cancer	Quantitative analysis (n=94)	RT-PCR	Higher stage, LN involvement	-	Wang *et al*. 2015 (27)

RT-PCR: Real time reverse transcriptional polymerase chain reaction; MSP: methylation specific polymerase chain reaction; LN: lymph node; ER: oestrogen receptor; IRS: immunoreactive score; IHC: immunohistochemistry; HER2: human epidermal growth factor receptor 2; FIGO: International Federation of Gynecology and Obstetrics; SCC, squamous cell carcinoma; TNM: tumour node metastasis; HCC: hepatocellular carcinoma; ALL: acute lymphoblastic leukaemia; NSCLC: non-small cell lung cancer. *Shown by multivariate analysis.

DKK3 is identified as a tumour endothelial marker, as is significantly up-regulated in the blood vessels of malignant colorectal tissues (30). Furthermore, DKK3 was found to be up-regulated in tumour endothelial cells (ECs) in 56% of 318 colorectal cancer tissue samples. DKK3-positive colorectal cancer is also significantly associated with higher mean microvessel count than DKK3-negative colorectal cancer samples, as well as with an increase in the number of intra-tumoural microvessels per sample (31). Furthermore, the number of blood vessels expressing DKK3 is increased in glioma, high-grade non-Hodgkin's lymphoma, and melanoma (32). Therefore, these findings suggest that DKK3 is involved in neovascularization and could be used as a marker for neoangiogenesis.

Epigenetic Inactivation by Methylation of the *DKK3* Promoter

Investigation of the mechanisms responsible for the decreased *DKK3* expression in human cancers revealed epigenetic inactivation by hypermethylation of the *DKK3* promoter based on the following findings. 1) the *DKK3* gene is located on chromosome 11p15, a target of methylation-medicated genetic imprinting (33); 2) silenced or reduced mRNA expression of *DKK3* is closely associated with promoter methylation (8); 3) the occurrence of hypermethylation in the promoter leads to the down-regulation of *DKK3*, whereas this down-regulation in the silenced cancer cell lines can be reversed by inhibiting DNA-methyltransferase activity with 5-Aza-dC and trichostatin A (9, 34-36).

As expected, up to 78% promoter methylation of *DKK3* is detected in various malignant tissue samples (Table I) (8, 9, 22-24, 34-41). The quantitative methylation level of *DKK3* in cancer tissues is significantly higher than that in controls, and studies have revealed a threshold of quantitative methylation levels that facilitates prognosis prediction (Table I) (38, 42, 43). Taken together, the promoter methylation of *DKK3* is a highly frequent phenomenon and is causally related to the down-regulation of *DKK3* during carcinogenesis.

DKK3 methylation in circulating free DNA from blood was investigated using methylation-specific polymerase chain reaction (MSP). Interestingly, 37 out of 112 patients

with breast cancer (33% sensitivity in breast cancer) were positive for *DKK3* methylation, while 101 out of 102 normal controls (99% specificity in normal controls) were negative (44). Similarly, *DKK3* methylation was found in 27.3% (9/33) of serum DNA from renal cancer patients, and 100% of normal individuals were negative for methylated *DKK3* in their serum (24). Furthermore, *DKK3* methylation could also be found in urine from patients with bladder tumours: 50% of patient samples were positive (12/24 urine samples), whereas 95% of normal controls (19/20 urine samples) were negative for *DKK3* methylation (23). These findings suggest that DKK3 may be useful as a blood-based or a body-fluid based biomarker in certain types of cancers.

Clinical Implications of *DKK3* Down-regulation or Epigenetic Silencing

The association of DKK3 down-regulation in tumour cells with negative clinical outcomes has been demonstrated in several cancers (Table I). For example, in breast cancer, DKK3 down-regulation is more common in patients with triple-negative or human epidermal growth factor receptor 2-positive breast cancer subgroups. Furthermore, the analysis of a large *in silico* dataset (3,554 cases) showed that low *DKK3* mRNA expression has a significant association with a reduction in recurrence-free survival in luminal and basal-like breast cancer cases (14). In cervical cancer, the down-regulation of DKK3 is associated with higher International Federation of Gynecology and Obstetrics (FIGO) stage and lower disease-free survival rates (16). Patients with down-regulated DKK3 in their colorectal cancer cells show deeper invasion, higher TNM stages, and dedifferentiation, which are poor prognostic factors (6). In endometrial cancer, reduced *DKK3* mRNA expression is associated with higher FIGO stage, lymph node involvement, non-endometrioid histology, and washing cytology positivity (15). Gastric cancer patients with loss of *DKK3* present with aggressive clinical factors, including higher TNM stage, lymph node involvement, and poorer survival than those with normal DKK3 levels (25, 26). Lung cancer patients with loss of DKK3 in cancer tissues exhibit higher tumour grades than patients with no loss of DKK3 (17, 18). On the other hand, protein down-regulation in ovarian and renal cancers has no clinical significance (19, 20) a whereas in oesophageal cancers with increased levels of DKK3 mRNA present has a higher stage and lymph node involvement (27). Taken together, the intracellular aberrant expression of DKK3 can be used as a biomarker to predict poor prognosis, an additional tool for precision medicine before the initiation of treatment.

Interestingly, the clinical significance of DKK3 expression in the tumour endothelium in colorectal, gastric, and pancreatic cancers is not clear. DKK3 positivity in tumour ECs is significantly associated with higher mean microvessel count and worse disease-free survival in colorectal cancer than in DKK3-negative tumours (31). In contrast, in gastric and pancreatic cancers, patients with strong DKK3 expression in the tumour endothelium or with high intratumour microvessel density had significantly better survival (45, 46).

As DKK3 down-regulation has clear clinical significance, hypermethylation of the *DKK3* promoter has potential functional consequences (Table I). In bladder cancer, a high proportion of methylated *DKK3* was found in invasive cancers rather than in superficial cancers (23). In breast cancer, it has been shown that *DKK3* methylation has a significant correlation with a higher clinical stage, lymph node involvement, and oestrogen receptor-positive status, but not with survival (35). At the same time, using multivariate analysis, others reported a significant correlation between *DKK3* methylation and shorter survival rates in breast cancer patients, including disease-free survival and overall survival in patients (47). Comparative quantitative methylation profiling using pyrosequencing or quantitative MSP found a methylation threshold that was associated with survival in patients with cervical cancer and hepatocellular carcinoma; furthermore, this threshold increased the prediction value of disease recurrence when analyzed with clinical factors (42, 43). A multivariate analysis of 104 patients out of 173 with gastric cancers demonstrated that patients with *DKK3* promoter methylation had significantly higher TNM stage and worse survival than patients without *DKK3* methylation (34). Acute lymphoblastic leukaemia patients with methylated *DKK3* also showed lower disease-free survival than patients with unmethylated *DKK3* (22). In non-small cell lung cancer, *DKK3* methylation was significantly correlated with lower overall survival, as determined by multivariate analysis (39). Furthermore, *DKK3* methylation has been found predominantly in higher grade or advanced stages of renal cancer, rather than in lower grade or earlier stages, and was associated with worse overall survival (24). In contrast, no significant differences were observed in two clinical analyses of patients with colorectal cancer (34, 48). Overall, these results suggest that aberrant methylation status and a quantitative threshold of DKK3 have important functional roles in cancer patient prognosis and could, therefore, be used as a clinical biomarker.

Single Nucleotide Polymorphisms (SNPs) in *DKK3*

SNPs are single base changes in a DNA sequence. Germ-line differences in *DKK3* SNPs between cancerous and normal cases have been identified (9, 49-56). The effects of DKK3 SNPs on disease susceptibility have been investigated in several cancers. In a study of 272 cases and 173 controls, SNPs rs12421658, rs11022105, and rs4586138 were found to be associated with prostate cancer risk, whereas SNPs rs2087882 and rs1472190 were associated with prostate cancer aggressiveness, including serum levels of prostate-specific antigen, clinical stage, pathological stage, and Gleason

score (49). In a study of 210 patients with renal cell carcinoma and 200 age- and sex-matched controls, SNP rs3206824 was associated with a decreased risk of renal cancer and cancer-related deaths (50). In a study of 99 patients with breast cancer and 93 controls, women with the GG genotype in the SNP rs6485350 of *DKK3* were found to have a 2-fold reduced risk of breast cancer and protection against oestrogen receptor-positive tumours compared to women with the AA genotype (51). A study of 732 ovarian cancer cases and 765 controls in a Northern Chinese population, the AA genotype, but not the GG genotype, in the SNP rs6485350 of *DKK3* was associated with significantly reduced risk of ovarian cancer (52). Furthermore, in a study of 300 lung cancer cases and 300 controls in north Indians, the genotype combination of *DKK3* rs3206824 and *DKK2* rs419558 was 2-fold higher in lung cancer patients, and was associated with an increased risk of developing lung cancer (53). In contrast, no significant association was observed in patients with uterine cervical, gastric, colorectal and lung cancer (9, 54-56). These findings suggest that *DKK3* SNPs may predict susceptibility to prostate, renal, breast and ovarian cancer; however, further large-scale studies are needed to confirm these results.

DKK3 Is a Tumour Suppressor

As DKK3 expression is typically reduced in cancer cells, the effect of DKK3 on apoptosis, migration, invasion, and angiogenesis in cancer cells has been investigated. Most studies have demonstrated the anti-proliferative activity of DKK3 in osteosarcoma, colon cancer, gastric cancer, glioma, prostate cancer, uterine cervical cancer, malignant melanoma, hepatic, non-small cell lung cancer, and breast cancer (3, 9, 12, 17, 34, 35, 37, 48, 57-60).

Interestingly, normal cells are protected from DKK3-mediated cell death. In a study using adenovirus to express DKK3 in various cancer and non-cancerous cell lines, apoptotic cell death was observed only in cancer cells, and not in normal cells (7, 13), suggesting that DKK3-mediated apoptosis might be a selective cancer-specific feature despite the presence of some resistant tumour cells. Therefore, the knowledge of the selective apoptosis-inducing mechanisms may be useful for identifying DKK3-responsive cancers and could help establish novel pharmacological targets to re-sensitize resistant tumour cells.

It has been reported that the forced expression of *DKK3* in Saos-2 osteosarcoma cells significantly alters their cellular morphology from an irregularly shaped and spread, to a round-shaped, compact, attached, and less motile (61). Ectopic DKK3 expression in melanoma cells and mesenchymal basal breast cancer cells results in changes in cellular morphology facilitating cell-to-cell contact, up-regulation of the epithelial marker E-cadherin, and down-regulation of the mesenchymal marker Snail-1 (12, 14, 35, 62). Through its association with the epithelial-to-mesenchymal transition (EMT), DKK3 expression reduces cellular invasion and motility (12, 61, 63), whereas *DKK3* silencing by small interfering RNA increases invasion capacity (10). Therefore, DKK3 is involved in the EMT and its loss could promote the aggressive properties of cancer cells.

DKK3 has anti-proliferative activity in drug-resistant cancer cells. In cisplatin-resistant lung cancer cells, DKK3 increases cisplatin sensitivity, whereas *DKK3* knockdown decreases the sensitivity of cells to cisplatin. These mechanisms are related to the down-regulation of β-catenin, which has well-documented roles in chemoresistance (64). Doxorubicin-resistant breast cancer cells express abundant P-glycoprotein; in these cells, DKK3 induces apoptosis *via* down-regulation of P-glycoprotein, which plays a role in drug efflux, augmenting the multidrug-resistance of cancer cells and cell survival in response to anticancer drugs (65). Therefore, DKK3 blocks drug resistance mechanisms and its increased expression could enhance drug sensitivity.

DKK3 Is Involved in Tumour Angiogenesis

Growing tumours require blood supply to meet the elevated need for oxygen and nutrients; therefore, tumours stimulate surrounding stromal tissue to produce novel blood vessels (66). Tumour angiogenesis is a hallmark of most malignancies. Because of the differences between the tumour-associated endothelium in abnormal microvessels and the normal endothelium, it is possible to target only the tumour associated endothelium. Unlike in normal blood vessels, DKK3 was positive in tumour vessels of colorectal cancer, glioma, high-grade non-Hodgkin's lymphomas, melanoma, gastric cancer, prostatic cancer, pancreatic cancer, and oesophageal adenocarcinoma (5, 12, 27, 32, 45, 46). DKK3 overexpression has no effect on the proliferation and migration of ECs; however, it enhanced tube formation capacity in Matrigel assays *in vitro* and microvessel density *in vivo*. Conversely, *DKK3* knockout inhibited endothelial tube formation. Furthermore, DKK3 may facilitate the remodelling of tumour vasculature by differentiating tumoural ECs. The overexpression of DKK3 directly promotes the differentiation of human fibroblasts into functional ECs, with fibroblast-derived ECs capable of forming microvascular tubular structures in tissue-engineered vascular grafts *ex vivo* (67). Furthermore, DKK3 may also act as a chemokine by binding to the C-X-C chemokine receptor type 7 and triggering downstream signalling pathways involved in vascular progenitor migration and vascular regeneration (68). Taken together, DKK3 may be a putative pro-angiogenesis factor in vascularization and a potential marker of neo-angiogenesis. However, the exact mechanisms for DKK3 up-regulation in the tumour endothelium remain unclear.

Intracellular Signalling of DKK3

Wnt signalling. Wnt signalling regulates a wide range of physiological processes, including embryonic development, cell proliferation, stem cell maintenance, and epithelial-mesenchymal interactions, and is also linked to human carcinogenesis (69). DKKs are known to antagonize Wnt signalling pathways (4); however, DKK3 has been shown not to affect Wnt signalling in various assays, such as Wnt-dependent secondary axis induction in Xenopus embryos and Wnt/Fz8 signalling in cultured cells (70-73).

To antagonize Wnt signalling, DKKs are known to directly interact with WNT receptors. It has been shown that DKK1, -2, and -4 bind to and internalize LRP5/6, the membrane co-receptors involved in canonical Wnt signalling (3, 71, 74); however, DKK3 does not interact with LRP5/6 (73). Furthermore, DKK1, -2, and -4 directly interact with the transmembrane protein Kremen, expressed on the cell surface (73-75); however, DKK3 does not interact with Kremen either (74). Instead, DKK3 colocalizes with Kremen-1 at the intracellular membranous compartments, such as the Golgi apparatus or the endoplasmic reticulum, leading to potentiation of Wnt-3A signalling (76). Because this interaction occurs in the membrane vesicles, the cellular surface membrane interaction partners of secreted DKK3 still have not been identified, and further studies are needed to elucidate how this type of interaction between DKK3 and Kremen influences the Wnt/β-catenin signalling pathway. In addition, DKK3 inhibits Wnt-7A signalling in pheochromo-cytoma PC12 cells, although this effect is weak and occurs only in the presence of both LRP-5 and -6 (77). Therefore, further studies are needed to elucidate the connection between DKK3 and the Wnt signalling pathway.

DKK3 is involved in the inactivation of β-catenin. Although the effects of DKK3 on the Wnt signalling pathway have not yet been identified, it has been shown that DKK3 facilitates the degradation of β-catenin in glioma, cervical cancer, breast cancer, and osteosarcoma cells (9, 17, 35, 57, 61). For example, DKK3 inhibits Tcf/Lef luciferase activity triggered by β-catenin, whereas β-catenin/TCF-4 signalling is activated in DKK3 knockouts (9, 17). DKK3 is also involved in the proteasomal degradation of β-catenin by direct interaction with β-transducin repeat protein, which first recognizes and targets phosphorylated β-catenin for ubiquitination (78). Moreover, DKK3 inhibits gene expression downstream of β-catenin, including genes encoding VEGF and cyclin D1 (9). Furthermore, there is a statistically significant inverse correlation between DKK3 expression and nuclear β-catenin expression (11). For example, by attenuating β-catenin, DKK3 induces apoptosis in cisplatin-resistant lung adenocarcinoma cells (64). Taken together, these findings suggest that DKK3 plays a role in inhibiting β-catenin signalling, potentially through a Wnt-independent pathway.

DKK3 is involved in caspase-dependent JNK activation. One mechanism of DKK3-mediated apoptosis involves caspase-dependent JNK activation without β-catenin (7, 79). DKK3 induces endoplasmic reticulum stress, which, in turn, triggers the activation of JNK. JNK activation induces mitochondrial translocation of Bax without any changes in protein levels (7). In breast, prostate, and lung cancers, DKK3 induces apoptosis through early JNK phosphorylation and subsequent caspase-9 and -3 cleavage (7, 60, 65). In contrast, in malignant glioma cells, DKK3 induces apoptosis *via* caspase-3 cleavage by caspase-9, not by caspase-8; however, JNK does not appear to play a crucial role (57). Therefore, DKK3 can promote cell type-specific caspase-dependent apoptosis *via* JNK activation.

The upstream regulators of DKK3. The pathways upstream of DKK3 have not been fully elucidated. The knockdown of membrane type-1 matrix metalloproteinase up-regulates *DKK3* mRNA levels *via* transcriptional activation (63). The tumour suppressor GATA4, down-regulated in ovarian cancer, hepatocellular carcinoma, and colorectal cancer (80), directly binds to the promoter region of *miR-125b* and inhibits its expression. *DKK3* is a direct target of *miR-125b*; therefore, GATA4 enhances *DKK3* expression by blocking the transcription of *miR-125b* (81). On the other hand, the tumour-promoting E2 factor transcription factor 3 (E2F3), the transcription factor involved in gastric cancer progression, regulates the *miR-125a*/*DKK3* axis by binding to the promoter of *miR-125*. Overexpressed E2F3 directly augments *miR-125a* expression, resulting in suppression of *DKK3* (82).

Other pathways. DKK3 directly binds to small glutamine-rich tetratricopeptide repeat-containing protein a (SGTA), a component of the androgen receptor (AR) complex and the negative modulator of cytoplasmic AR signalling (83). Cytoplasmic DKK3 inhibits SGTA function by interfering with SGTA dimerization. Furthermore, the suppression of AR signalling by SGTA can be rescued by increased DKK3 expression in prostate cancer cells. Therefore, DKK3 binds to SGTA and plays a physiological role in the regulation of AR signalling. Down-regulation of DKK3 may be associated with decreased androgen insensitivity in prostate cancer cells.

Gene Therapy With DKK3

Since DKK3 acts as a tumour suppressor, augmentation of *DKK3* expression using therapeutic gene approaches has been studied. Adenovirus-mediated induction of *DKK3* (Ad-DKK3) gene therapy can inhibit tumour growth or sensitize cancerous cells to undergo apoptosis in prostate, breast, testicular cancer, malignant mesothelioma, pancreatic cancer, malignant glioma, scirrhous gastric carcinoma, bladder cancer, and biliary cancer cells (7, 84-91), whereas this effect is attenuated in normal cells (7, 85, 86, 92). Furthermore, Ad-DKK3 gene therapy may

enhance anticancer immunity, by activating or differentiating immune cells to induce apoptosis not only at the tumour-treated site, but also at other distant cancer lesions (92, 93). Besides, Ad-DKK3 increases the secretion of IL-7, which activates natural killer cells to induce anticancer immunity (92, 94, 95).

Due to the suppression of DKK3 associated with aberrant promoter methylation, the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system targeting specific epigenetic alterations of *DKK3* has been used in prostate cancer. In these experiments, CRISPR-Cas9 was used to target either the transcriptional activators or catalytic domain of the demethylase Tet1 in the *DKK3* promoter, leading to increased DKK3 mRNA and protein levels, and resulting in decreased prostate cell proliferation and migration (96). Therefore, *DKK3*-based gene therapy could be a potential therapeutic tool for cancer treatment; however, additional experiments are required to further confirm this therapeutic potential.

Conclusion

Here, we summarize the latest findings regarding the potential applications of DKK3 as a clinical biomarker to be used in precision medicine to predict cancer prognosis and drug responsiveness. Furthermore, DKK3 may also act as a biomarker for neovascularization. Further prospective clinical trials using large independent cohorts are necessary to establish DKK3 as an actual biomarker in the clinical setting.

The intrinsic mechanisms of secreted DKK3 still remain elusive, since the putative cell surface receptor has not been identified. Elucidating the specific intracellular signalling cascades activated by DKK3-receptor binding will facilitate the development of DKK3-dependent therapeutics.

Conflicts of Interest

All of the Authors declare that they have no conflicts of interest in relation to this study.

Authors' Contributions

Lee EJ drafted the manuscript; Nguyen Q and Lee M prepared the figures and drafted the figure legends.

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