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REVIEW ARTICLE

Rationale for targeting the Wnt signalling modulator Dickkopf-1 for oncology

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Wnt signalling is a fundamental pathway involved in embryonic development and adult tissue homeostasis. Mutations in the pathway frequently lead to developmental defects and cancer. As such, therapeutic intervention of this pathway has generated tremendous interest. Dickkopf-1 (DKK1) is a secreted inhibitor of β-catenin-dependent Wnt signalling and was originally characterized as a tumour suppressor based on the prevailing view that Wnt signalling promotes cancer pathogenesis. However, DKK1 appears to increase tumour growth and metastasis in preclinical models and its elevated expression correlates with a poor prognosis in a range of cancers, indicating that DKK1 has more complex cellular and biological functions than originally appreciated. Here, we review current evidence for the cancer-promoting activity of DKK1 and recent insights into the effects of DKK1 on signalling pathways in both cancer and immune cells. We discuss the rationale and promise of targeting DKK1 for oncology.

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Abbreviations

APC, adenomatous polyposis coli; CK1, casein kinase 1; CKAP4, cytoskeleton-associated protein 4; Cys, cysteine-rich; DKK1, Dickkopf-1; FZD, Frizzled; GSK3, glycogen synthase kinase 3; LCC, latency competent cancer; LRP5/6, low-density lipoprotein receptor-related proteins 5 and 6; MDSCs, myeloid-derived suppressor cells; NK, natural killer; PCP, planar cell polarity; Rac, Ras-related C3 botulinum toxin substrate; RNF43, ring finger protein 43; ROR, receptor tyrosine kinase-like orphan receptor; ZNRF3, zinc and ring finger 3

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Introduction – an overview of Wnt signalling and cancer

Wnt signalling is a multifaceted pathway that regulates stem cell maintenance, cell fate decisions, cell proliferation, survival, migration and polarity determination during development and adult tissue homeostasis (Logan and Nusse, 2004; MacDonald *et al.*, 2009; Clevers and Nusse, 2012; Clevers *et al.*, 2014; Sedgwick and D'Souza-Schorey, 2016). Given the diverse cellular outcomes mediated by Wnt signalling, it is not surprising that it is exceedingly complex, involving 19 Wnts, 10 **Frizzled (FZD) Wnt receptors**, other classes of receptors including low-density lipoprotein receptor-related proteins 5 and 6 (LRP5/6), agonists and antagonists. Wnt signalling is classified into two main branches: **β-catenin**-dependent and β-catenin-independent. The β-catenin-dependent Wnt pathway is better characterized and understood and is mediated by the tight regulation of β-catenin stability (MacDonald *et al.*, 2009). In the absence of Wnt, signalling is kept off through β-catenin degradation by the action of the 'destruction' complex which consists of axin, adenomatous polyposis coli (APC), casein kinase Iα and glycogen synthase kinase 3 (Figure 1A). Through phosphorylation of β-catenin, the 'destruction' complex targets β-catenin for ubiquitin-mediated proteasomal degradation. The β-catenin-dependent Wnt signalling pathway is initiated by Wnt binding to FZD receptors and the LRP5/6 co-receptor (Figure 1B). This begins a signalling cascade that inhibits the 'destruction' complex and leads to the stabilization of β-catenin. The β-catenin protein then translocates to the nucleus and interacts with DNA-binding T-cell factor/lymphoid enhancer factor family members, thereby activating a Wnt-responsive transcriptional programme. Mutations in β-catenin-dependent Wnt signalling components occur frequently in cancer and result in constitutive β-catenin accumulation and signalling (Polakis, 2012; Zhan *et al.*, 2017). For example, loss-of-function *APC* mutations are prevalent in colorectal cancer, and *CTNNB1* (β-catenin) stabilizing mutations have been identified in colorectal cancer and a high percentage of liver and endometrioid tumours (Kwong and Dove, 2009; McConechy *et al.*, 2014; Zucman-Rossi *et al.*, 2015). Additionally, loss-of-function alterations in zinc and ring finger 3 (ZNRF3)/ring finger protein 43 (RNF43), which are ubiquitin ligases promoting FZD degradation, or translocations involving R-spondin proteins, which are secreted Wnt agonists by inhibiting ZNRF3/RNF43, are also found in colorectal cancer and other malignancies (Seshagiri *et al.*, 2012; Assie *et al.*, 2014; Giannakis *et al.*, 2014; Hao *et al.*, 2016). As such, targeting/inhibiting β-catenin-dependent Wnt signalling has garnered much attention and there are multiple oncology candidates in preclinical and clinical development (Anastas and Moon, 2013; Lu *et al.*, 2016). Although targeting the β-catenin-dependent Wnt pathway is attractive, caution is warranted due to the ubiquitous nature of the pathway and the possibility for serious side effects (Kahn, 2014).

The β-catenin-independent Wnt signalling pathway regulates cell motility and polarity throughout development (Wang, 2009; Sedgwick and D'Souza-Schorey, 2016). It is not nearly as well characterized as β-catenin-dependent Wnt signalling and involves multiple overlapping pathways, of which the Wnt/planar cell polarity (PCP) pathway is best

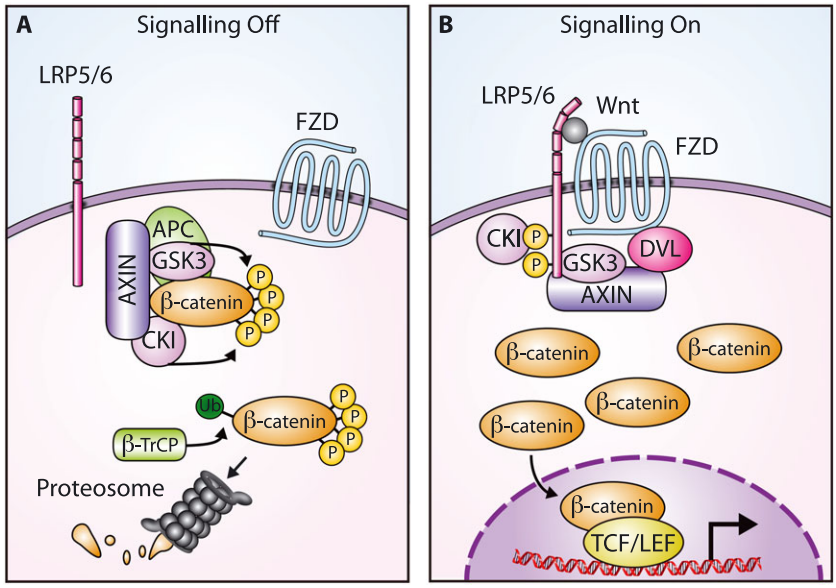

Figure 1

Overview of β-catenin-dependent Wnt signalling. (A) In the absence of Wnt, β-catenin is bound by the 'destruction' complex and phosphorylated by GSK3 and casein kinase I (CKI). Phosphorylation results in targeting for ubiquitin-mediated degradation. (B) Wnt binding to a FZD receptor and the LRP5/6 co-receptor disrupts the 'destruction' complex and stabilizes β-catenin. The β-catenin protein translocates to the nucleus, interacts with T-cell factor/lymphoid enhancer factor (TCF/LEF) family transcription factors and activates a Wnt-responsive transcriptional programme. β-TrCP, β-transducin repeat containing protein; DVL, Dishevelled.

understood and others, such as a Wnt/Ca^{2+} pathway, have been proposed (Semenov *et al.*, 2007; Liu *et al.*, 2016a). β-catenin-independent Wnt signalling does not require LRP5/6 and is instead initiated through Wnt interaction with FZD or additional receptors, such as **receptor tyrosine kinase-like orphan receptor (ROR)** and **receptor-like tyrosine kinase (RYK)** (Green *et al.*, 2014). Many downstream mediators are utilized adding further complexity (Sugimura and Li, 2010; van Amerongen, 2012). For example, heterotrimeric G-proteins, Rho-family small GTPases, JNK and calcium/calmodulin-dependent protein kinase II have all been implicated as downstream components. Some investigators have suggested that, similar to β-catenin-dependent Wnt signalling, these β-catenin-independent pathways may directly tie into gene regulation through nuclear factor of activated T-cells (NFAT), ATF2 and c-Jun transcription factors, although this requires further substantiation (Saneyoshi *et al.*, 2002; Schambony and Wedlich, 2007; Rao and Kuhl, 2010; Bengoa-Vergniory *et al.*, 2014). Not surprisingly, given that β-catenin-independent Wnt signalling regulates cell motility, the pathway has been implicated in promoting cancer (Katoh, 2005; Wang, 2009; Sedgwick and D'Souza-Schorey, 2016). For example, **Wnt-5a** activates β-catenin-independent Wnt pathways, leading to invasion, metastasis and proliferation of some cancers (Asem *et al.*, 2016; Kumawat and Gosens, 2016). **ROR1**, a receptor for Wnt-5a, is also involved in cancer progression and is over-expressed in both haematological and solid malignancies (Borcherding *et al.*, 2014). The therapeutic intervention of β-catenin-independent Wnt signalling has promise; however, a better understanding of this pathway will be necessary to fully exploit targeting it for oncology.

Discovery and characterization of DKK1

Dickkopf-1 (DKK1) is a member of the Dickkopf family and has most extensively been characterized as a secreted protein that is an inhibitor of β-catenin-dependent Wnt signalling. The Dickkopf family consists of four members (DKK1–4), which contain two conserved cysteine-rich (Cys) domains involved in protein–protein interactions (Niehrs, 2006). The Cys domains define the family and there is not a high degree of sequence similarity outside of these regions. DKK1 is essential for development, and homozygous null mice die at birth with severe head defects and limb dysmorphogenesis (Mukhopadhyay *et al.*, 2001). Mice with reduced DKK1 expression levels are viable but have increased bone mass, indicating a role for DKK1 in bone development and homeostasis (MacDonald *et al.*, 2007; Pinzone *et al.*, 2009). DKK1 expression in adult tissues does not appear to be as ubiquitous; however, DKK1 has been detected in various tissues including bone, placenta, intestine, colon and prostate (Glinka *et al.*, 1998; Fedi *et al.*, 1999; Monaghan *et al.*, 1999; Zhang *et al.*, 2004; Forget *et al.*, 2007; Aguilera *et al.*, 2015). Of the Dickkopf family members, DKK1 is the best understood.

DKK1 was originally identified in *Xenopus* as an inhibitor of β-catenin-dependent Wnt signalling and an inducer of head formation during embryogenesis, a phenotype that coined the Dickkopf (German for 'big head, stubborn') nomenclature (Glinka *et al.*, 1998). Its human homologue was also characterized as a potent Wnt inhibitor (Fedi *et al.*, 1999). Thereafter, multiple labs demonstrated that DKK1 impeded β-catenin-dependent Wnt signalling by binding to the LRP6 co-receptor with high affinity and blocking signalling (Bafico *et al.*, 2001; Mao *et al.*, 2001; Semenov *et al.*, 2001). More recent structural studies have supported this model and expanded our understanding of DKK1-mediated inhibition of β-catenin-dependent Wnt signalling. The crystal structures of DKK1 and LRP6 along with binding data suggest that DKK1 occupies multiple Wnt domains on LRP6 and that this presumably prevents virtually all Wnt binding to the co-receptor (Ahn *et al.*, 2011; Bourhis *et al.*, 2011; Chen *et al.*, 2011; Cheng *et al.*, 2011; Bao *et al.*, 2012). Furthermore, DKK1 binding to LRP6 can induce a conformational change that may allosterically impede Wnt binding (Matoba *et al.*, 2017). DKK1 can also form a ternary complex with the **kremen 1** co-receptor and LRP6, possibly leading to depletion of LRP6 from the cell surface and decreased signalling; however, this model remains controversial (Mao *et al.*, 2002; Semenov *et al.*, 2008; Wang *et al.*, 2008; Zebisch *et al.*, 2016). Although DKK1 clearly regulates Wnt signalling through inhibition of the β-catenin-dependent pathway, this may be an oversimplification because DKK1 has also been linked to the activation of β-catenin-independent Wnt signalling. For example, DKK1 has been implicated in promoting β-catenin-independent Wnt signalling during *Xenopus* and zebrafish development, neurite outgrowth, in Alzheimer's disease pathogenesis, as well as in oncology models (Pandur *et al.*, 2002; Caneparo *et al.*, 2007; Endo *et al.*, 2008; Thudi *et al.*, 2011; Wang and Zhang, 2011; Tao *et al.*, 2013; Killick *et al.*, 2014; Krause *et al.*, 2014; Marzo *et al.*, 2016). DKK1 activation of β-catenin-independent Wnt signalling is not well understood but is probably indirect and involves DKK1 shifting the Wnt signalling balance from the β-catenin-dependent pathway to β-catenin-independent pathways (discussed in a later section). Thus, the effect of DKK1 on cellular function presumably involves the interrogation of outputs from both β-catenin-dependent and independent Wnt pathways, adding further complexity to its regulation of Wnt signalling.

Based on the ability of DKK1 to inhibit β-catenin-dependent Wnt signalling, a pathway that is frequently overactivated in cancer, it is not surprising that DKK1 was initially characterized as a tumour suppressor. Early studies in gastrointestinal cancer showed that DKK1 expression was decreased in tumours and that the gene was frequently methylated and silenced (Gonzalez-Sancho *et al.*, 2005; Aguilera *et al.*, 2006; Sato *et al.*, 2007). Additional studies indicated DKK1 could suppress tumours by inducing apoptosis and inhibiting tumour growth, proliferation, invasion and angiogenesis (Lee *et al.*, 2004; Maehata *et al.*, 2008; Mikheev *et al.*, 2008; Qiao *et al.*, 2008; Hirata *et al.*, 2011; Kim *et al.*, 2012; Menezes *et al.*, 2012; Qi *et al.*, 2012). However, paradoxically, many correlative and functional studies have linked DKK1 to the promotion of cancer (Tables 1 and 2) (Mazon *et al.*, 2016). The ability of DKK1 to function as a tumour suppressor or promoter is probably dependent on numerous contextual factors such as the type of cancer, heterogeneity within the tumour, Wnt signalling pathway wiring and the tumour micro-environment. Deciphering this will advance the

Table 1

Cancers with tumours that express DKK1 or induce elevated patient serum levels

Cancer	Reference
Bladder	(Sun *et al.*, 2015)
Breast	(Forget *et al.*, 2007; Voorzanger-Rousselot *et al.*, 2007; Bu *et al.*, 2008; Sato *et al.*, 2010; Smadja *et al.*, 2010; Xu *et al.*, 2012; Zhou *et al.*, 2014; Rachner *et al.*, 2014a)
Chondrosarcoma	(Chen *et al.*, 2014; Zarea *et al.*, 2016)
Cholangiocarcinoma	(Sato *et al.*, 2010; Shi *et al.*, 2013; Shi *et al.*, 2016)
Cervical	(Jiang *et al.*, 2009; Sato *et al.*, 2010; Jiang *et al.*, 2013)
Colon/rectal	(Kemik *et al.*, 2011; Gurluler *et al.*, 2014; Aguilera *et al.*, 2015)
Endometrial	(Jiang *et al.*, 2009)
Oesophageal	(Yamabuki *et al.*, 2007; Darlavoix *et al.*, 2009; Makino *et al.*, 2009; Li *et al.*, 2011; Begenik *et al.*, 2014; Lyros *et al.*, 2015)
Gastric	(Sato *et al.*, 2010; Gao *et al.*, 2012; Gomceli *et al.*, 2012; Lee *et al.*, 2012; Liu *et al.*, 2016b)
Glioblastoma	(Zhou *et al.*, 2010)
Kidney	(Wirths *et al.*, 2003; Forget *et al.*, 2007)
Liver	(Wirths *et al.*, 2003; Patil *et al.*, 2005; Yu *et al.*, 2009; Sato *et al.*, 2010; Tung *et al.*, 2011; Shen *et al.*, 2012; Chen *et al.*, 2013; Tao *et al.*, 2013; Yang *et al.*, 2013; Huang *et al.*, 2014; Zhang *et al.*, 2014; Kim *et al.*, 2015; Desert *et al.*, 2016)
Laryngeal	(Shi *et al.*, 2014)
Lung	(Forget *et al.*, 2007; Yamabuki *et al.*, 2007; Sheng *et al.*, 2009; Sato *et al.*, 2010; Li *et al.*, 2013; Chu *et al.*, 2014; Dong *et al.*, 2014; Xiang *et al.*, 2015; Kimura *et al.*, 2016; Yao *et al.*, 2016)
Malignant fibrous histiocytoma	(Matushansky *et al.*, 2007)
Multiple myeloma	(Tian *et al.*, 2003; Politou *et al.*, 2006; Qian *et al.*, 2007)
Osteosarcoma	(Lee *et al.*, 2007)
Ovarian	(Chamorro *et al.*, 2005; Shizhuo *et al.*, 2009; Wang and Zhang, 2011)
Pancreatic	(Sato *et al.*, 2010; Takahashi *et al.*, 2010; Han *et al.*, 2015; Kimura *et al.*, 2016)
Prostate	(Hall *et al.*, 2008; Rachner *et al.*, 2014b)
Solid tumours, meta-analysis	(Liu *et al.*, 2014)
Urothelial	(Shen *et al.*, 2010)

development of DKK1-targeted therapies for oncology currently undergoing clinical development (Lu *et al.*, 2016).

DKK1 overexpression in cancer

Clinical studies in a range of cancers have detected elevated levels of DKK1 in patient serum or tumours and this was frequently associated with a poor prognosis, such as advanced stage, decreased overall survival, vascular invasion and metastasis (Table 1). For example, DKK1 staining has been detected in a high percentage of oesophageal and cholangiocarcinoma tumours and this correlated with a decrease in overall survival (Yamabuki *et al.*, 2007; Shi *et al.*, 2013). In tumours from breast, cholangiocarcinoma, laryngeal squamous cell carcinoma, liver, rectal and gastric cancers, elevated levels of DKK1 have been observed with vascular invasion, lymphatic invasion or VEGF-C expression, implicating DKK1 in promoting cancer cell migration and metastasis (Smadja *et al.*, 2010; Kemik *et al.*, 2011; Tung *et al.*, 2011; Shi *et al.*, 2013, 2014; Tao *et al.*, 2013; Liu *et al.*, 2016b). In support of this, DKK1 positivity has been associated with lymph node metastasis in cancers (Kemik *et al.*, 2011; Li *et al.*, 2013; Shi *et al.*, 2013; Shi *et al.*, 2014; 2016). DKK1 staining in tumours has also been co-detected with β-catenin, and in some instances, patients with dual staining had a worse prognosis, including decreased overall survival (Yu *et al.*, 2009; Xu *et al.*, 2012; Chen *et al.*, 2013, 2014; Shi *et al.*, 2014, 2016). It is puzzling that DKK1, an inhibitor of β-catenin-dependent Wnt signalling, is detected in tumours with β-catenin, an indicator of activated Wnt/β-catenin signalling. However, activated β-catenin-dependent Wnt signalling can result in the up-regulation of DKK1, potentially as a negative feedback mechanism under physiological conditions (Niida *et al.*, 2004; Chamorro *et al.*, 2005; Gonzalez-Sancho *et al.*, 2005; Bu *et al.*, 2008; Chen *et al.*, 2016). In tumours that stain for β-catenin and DKK1, the negative feedback may have been disrupted by, for example, stabilizing mutations in β-catenin that would render the inhibitory activity of DKK1 inoperative. It is interesting to speculate that in the context of constitutively activated β-catenin-dependent Wnt signalling, the increased expression of DKK1 is contributing to tumour growth and poor prognosis. Further research is required to address this issue.

Table 2

Preclinical evidence for DKK1 promoting cancer pathogenesis

Cancer	Selected evidence for DKK1 cancer-promoting activity	Reference
Breast	• DKK1 increased tumour growth and neovascularization in a xenograft model • Cancer cells with metastatic potential avoided immune clearance by expressing DKK1	(Voorzanger-Rousselot *et al.*, 2007; Smadja *et al.*, 2010; Malladi *et al.*, 2016)
Cholangiocarcinoma	• DKK1 knockdown decreased migration, invasion, proliferation, tumour growth and expression of VEGF-C and MMP9	(Shi *et al.*, 2013; Shi *et al.*, 2016)
Colorectal	• DKK1 regulated the expression of cancer-related genes	(Aguilera *et al.*, 2015)
Oesophageal	• Overexpression of DKK1 increased proliferation and invasion	(Li *et al.*, 2011)
Liver	• DKK1 promoted migration, invasion, tumour growth, metastasis and angiogenesis • Cancer stem cell-like liver cells had increased expression of DKK1	(Yu *et al.*, 2009; Tung *et al.*, 2011; Chen *et al.*, 2013; Tao *et al.*, 2013; Huang *et al.*, 2014; Kim *et al.*, 2015; Chen *et al.*, 2016)
Laryngeal	• DKK1 knockdown reduced migration, invasion and proliferation	(Shi *et al.*, 2014)
Lung	• An anti-DKK1 antibody had efficacy in a syngeneic mouse model • Cancer cells with metastatic potential avoided immune clearance by expressing DKK1 • An anti-DKK1 antibody induced apoptosis, reduced invasion, decreased proliferation and had efficacy in a xenograft model • DKK1 overexpression promoted invasion, migration and proliferation • DKK1 activated PI3K/Akt signalling through a novel receptor (CKAP4)	(Sato *et al.*, 2010; Li *et al.*, 2013; Salim *et al.*, 2015; D'Amico *et al.*, 2016; Kimura *et al.*, 2016; Malladi *et al.*, 2016; Yao *et al.*, 2016; Pang *et al.*, 2017)
Melanoma	• An anti-DKK1 antibody had efficacy in a syngeneic mouse model	(D'Amico *et al.*, 2016)
MFH	• DKK1 promoted transformation of hMSCs to MFH cells	(Matushansky *et al.*, 2007)
Multiple myeloma	• Anti-DKK1 antibodies had efficacy in mouse models • Anti-DKK1 antibodies improved bone health in mouse models	(Yaccoby *et al.*, 2007; Fulciniti *et al.*, 2009; Heath *et al.*, 2009; Pozzi *et al.*, 2013)
Osteosarcoma	• An anti-DKK1 antibody had efficacy in PDX models • DKK1 overexpression increased proliferation and tumour growth	(Gregory *et al.*, 2003; Krause *et al.*, 2014; Goldstein *et al.*, 2016)
Ovarian	• DKK1 knockdown had efficacy in a xenograft model	(Wang and Zhang, 2011)

continues

Table 2 (Continued)

Cancer	Selected evidence for DKK1 cancer-promoting activity	Reference
Pancreatic	• DKK1 knockdown decreased migration, invasion and proliferation • DKK1 activated PI3K/Akt signalling through a novel receptor (CKAP4)	(Takahashi *et al.*, 2010; Kimura *et al.*, 2016)
Prostate	• DKK1 overexpression increased tumour growth and metastasis • An anti-DKK1 antibody reduced tumour growth in a xenograft model	(Hall *et al.*, 2010; Thudi *et al.*, 2011)

MFH, malignant fibrous histiocytoma; hMSCs, human mesenchymal stem cells; PDX, patient-derived xenograft.

DKK1 promotes proliferation, invasion and tumour growth in preclinical models

In addition to clinical data, direct evidence for DKK1 cancer-promoting activity exists for preclinical cancer models (Table 2). For example, DKK1 promoted migration and/or invasion in cholangiocarcinoma, oesophageal, liver, laryngeal, lung and pancreatic cancer cell lines (Table 2). For some of these cancer cell lines, this may have occurred through DKK1 regulation of **MMP** expression, a family of proteases with well characterized roles in cancer cell migration (Kessenbrock *et al.*, 2010; Chen *et al.*, 2013; Shi *et al.*, 2013; Shi *et al.*, 2016). Furthermore, DKK1 knockdown reduced the expression of VEGF-C, a protein associated with promoting metastasis to lymph nodes, in cholangiocarcinoma (Park *et al.*, 2006; Shi *et al.*, 2013). Along with migration and invasion, DKK1 also stimulated proliferation in cell culture experiments (Table 2). However, this was not a universal feature, and for certain cancers, DKK1 promoted migration and/or invasion without having a detectable effect on proliferation (Tung *et al.*, 2011; Wang and Zhang, 2011; Chen *et al.*, 2013; Li *et al.*, 2013). It is currently not well understood why there was this difference, but it may be due to variations in Wnt signalling pathways across cancer cell lines or different cell culture conditions. Elevated DKK1 expression also occurred in liver, breast and lung cancer cells that had stem cell-like characteristics, suggesting that it may contribute to the development of an undifferentiated phenotype (Chen *et al.*, 2016; Malladi *et al.*, 2016). Supporting this, DKK1 overexpression prevented the differentiation of osteosarcoma cells and increased the level of the cancer stem cell marker aldehyde dehydrogenase 1 in these cells (Krause *et al.*, 2014). Taken together, there is ample evidence from cell culture model systems that DKK1 may contribute to cancer progression by promoting migration, invasion, proliferation and cancer stem cell-like properties.

DKK1 has been documented to affect tumour growth in *in vivo* models representing a range of cancers. For example, tumour models for breast cancer, cholangiocarcinoma, liver cancer, lung cancer, melanoma, multiple myeloma, osteosarcoma, ovarian cancer and prostate cancer all responded to changes in DKK1 levels (Table 2). In multiple myeloma, treatment with anti-DKK1 antibodies reduced disease burden and improved bone health in mouse models (Yaccoby *et al.*, 2007; Fulciniti *et al.*, 2009; Pozzi *et al.*, 2013). Lung cancer, melanoma, osteosarcoma and prostate cancer also responded to anti-DKK1 antibody treatment *in vivo* (Hall *et al.*, 2010; Sato *et al.*, 2010; D'Amico *et al.*, 2016; Goldstein *et al.*, 2016). In both breast cancer and hepatocellular carcinoma xenograft models, DKK1 increased tumour growth and promoted angiogenesis, suggesting that DKK1 has pro-angiogenic activity (Smadja *et al.*, 2010; Tung *et al.*, 2011). In support of this, knockdown of DKK1 decreased tumour growth and angiogenesis in hepatocellular carcinoma (Huang *et al.*, 2014). In addition to affecting primary tumour growth, DKK1 has also been linked to the development of metastasis in bone, breast, liver, lung and prostate cancer models, possibly related to the prominent role of DKK1 in stimulating migration and invasion observed *in vitro* (Thudi *et al.*, 2011; Tao *et al.*, 2013; Huang *et al.*, 2014; Goldstein *et al.*, 2016; Malladi *et al.*, 2016; Pang *et al.*, 2017). Taken together, these data demonstrate that DKK1 has tumour-promoting activity in animal models via effects on tumour growth, metastasis and angiogenesis.

DKK1 modulation of signalling pathways in cancer cells

Emerging evidence is improving our understanding of how DKK1 can promote tumour growth and metastasis through the modulation of signalling pathways in cancer cells. For example, an elegant study has demonstrated that DKK1 contributes to metastasis in an *in vivo* model through inhibition of β-catenin-dependent Wnt signalling (Figure 2A) (Malladi *et al.*, 2016). The authors initially identified and characterized latency competent cancer (LCC) cells that had a stem cell-like phenotype and tumour-initiating capability by avoiding immune clearance. Impeding DKK1 expression re-sensitized these LCC cells to β-catenin-dependent Wnt signalling and up-regulated the expression of activating ligands for natural killer (NK) cells, leading to NK cell-mediated clearance of the LCC cells and reduced metastasis. These results suggest

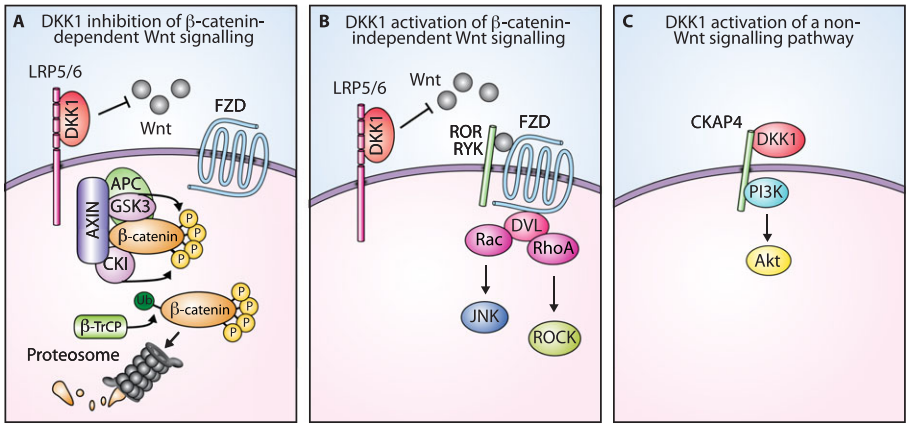

Figure 2

DKK1 regulation of signalling pathways. (A) DKK1 inhibition of β-catenin-dependent Wnt signalling. DKK1 inhibits β-catenin-dependent Wnt signalling by binding to the LRP5/6 co-receptor and blocking Wnt binding, which results in β-catenin degradation. (B) Model of DKK1 activation of β-catenin-independent Wnt signalling. DKK1 binding to the LRP5/6 co-receptor shifts Wnt and the FZD receptor to β-catenin-independent signalling pathways. A simplified version of the β-catenin-independent Wnt/PCP pathway is shown as an example. (C) DKK1 activation of a non-Wnt signalling pathway. DKK1 binds to the CKAP4 receptor and activates PI3K/Akt signalling. GSK3, glycogen synthase kinase 3; CKI, casein kinase I; β-TrCP, β-transducin repeat containing protein; RYK, receptor-like tyrosine kinase; DVL, Dishevelled; ROCK, Rho-associated protein kinase.

the intriguing possibility that the reactivation of β-catenin-dependent Wnt signalling could be an effective way to eliminate tumour-initiating cells with metastatic potential through immune surveillance. However, caution and further studies are warranted, since the reactivation of β-catenin-dependent Wnt signalling may also induce proliferation of LCC cells and in principle increase tumour growth. An important issue to address is how to enhance tumour immune surveillance without promoting tumour growth.

For certain cancer cells, inhibition of β-catenin-dependent Wnt signalling by DKK1 can favour the formation of an undifferentiated phenotype, which in general is more malignant. For example, DKK1 has been implicated in having a role in limiting the ability of malignant fibrous histiocytoma cells to differentiate by blocking β-catenin-dependent Wnt signalling (Matushansky *et al.*, 2007). In osteosarcoma, DKK1 inhibited β-catenin-dependent Wnt signalling and impeded differentiation (Goldstein *et al.*, 2016). Treatment with an anti-DKK1 antibody reduced tumour growth in patient-derived xenograft models, increased nuclear β-catenin staining and increased the expression of osteopontin, a bone differentiation marker. Taken together, these results indicate that for some cancers DKK1 can contribute to tumour growth by impeding β-catenin-dependent Wnt signalling.

DKK1 can also promote cancer pathogenesis by activating β-catenin-independent Wnt signalling. This finding is not unexpected, given the role of β-catenin-independent Wnt signalling in cell migration and polarity during development (Sedgwick and D'Souza-Schorey, 2016). In liver cancer cells, knockdown of DKK1 decreased metastasis and reduced the levels of phosphorylated **JNK**, a downstream mediator of the Wnt/PCP pathway, suggesting that signalling was occurring through β-catenin-independent Wnt pathways (Tao *et al.*, 2013). The overexpression of DKK1 in prostate cancer cells increased metastatic potential and resulted in JNK activation without affecting β-catenin levels, suggesting that DKK1 was acting primarily through a β-catenin-independent Wnt pathway (Thudi *et al.*, 2011). A similar result has been observed in ovarian cancer cells where DKK1 promoted cell invasion and increased phosphorylated JNK without affecting β-catenin levels (Wang and Zhang, 2011). Furthermore, DKK1 staining in ovarian tumours correlated with that of phosphorylated JNK. In osteosarcoma, DKK1 overexpression increased tumour growth, RhoA expression and JNK phosphorylation, further supporting its role in activating β-catenin-independent Wnt signalling (Krause *et al.*, 2014). Taken together, these results implicate DKK1 activation of β-catenin-independent Wnt signalling in cancer cells as a potential driver of tumour growth and metastasis. Even though evidence exists for DKK1 activation of β-catenin-independent Wnt signalling in developmental and cancer models, the mechanistic details have not been elucidated. It has been hypothesized that DKK1 shifts the Wnt signalling balance from the β-catenin-dependent pathway to β-catenin-independent pathways (Figure 2B) (Endo *et al.*, 2005, 2008; Caneparo *et al.*, 2007; Wang and Zhang, 2011; Krause *et al.*, 2014). This potentially occurs through DKK1 binding to the LRP5/6 co-receptor blocking Wnt and increasing the availability of a Wnt pool to activate β-catenin-independent signalling pathways. Likewise, DKK1 could shift FZD receptors to β-catenin-independent pathways since there would be fewer Wnt bound LRP5/6 co-receptors to interact with. Going forward, it will be important to increase our understanding of how DKK1 promotes the activation of β-catenin-independent Wnt signalling and the extent that this contributes to tumour growth and metastasis.

DKK1 modulates β-catenin-dependent and -independent Wnt signalling in cancer cells, but is it limited to these pathways? For instance, even though DKK1 showed a clear phenotypic response in lung, pancreatic and prostate cancer cell lines in culture, attempts to detect measurable changes in

either β-catenin-dependent or independent Wnt signalling pathways were largely unsuccessful, leading the authors to speculate that the effects of DKK1 occurred through an undefined signalling pathway (Hall *et al.*, 2010; Sato *et al.*, 2010; Takahashi *et al.*, 2010). This question has begun to be addressed by a screening approach that has identified the cytoskeleton-associated protein 4 (CKAP4) as a novel DKK1 receptor (Kimura *et al.*, 2016). Further characterization indicated that CKAP4 interacted with **PI3K** and that DKK1 binding resulted in the activation of **Akt** signalling and increased the proliferation of the cancer cells (Figure 2C). Disrupting DKK1–CKAP4 signalling with either a shRNA-targeting DKK1 or a CKAP4 antibody impeded tumour growth in pancreatic and lung xenograft models. These data suggest a scenario in which DKK1 can signal through a Wnt receptor-independent pathway to promote tumour growth.

DKK1 modulation of signalling pathways in immune cells

Immune modulation has revolutionized the treatment paradigm for cancer and delivered significant clinical benefit (Topalian *et al.*, 2015). With the approval of immune checkpoint inhibitors, such as therapeutic antibodies targeting cytotoxic t-lymphocyte-associated protein 4 (CTLA-4), programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1), it has become possible to harness the immune system for an anti-tumour response. However, only a subset of patients responds to these therapies, and many novel immune mediated strategies are being pursued to overcome this limitation. For example, the presence of myeloid-derived suppressor cells (MDSCs) in the tumour micro-environment is associated with a poor prognosis, and blocking the function of these cells may have therapeutic benefits (Draghiciu *et al.*, 2015). Recently, DKK1 was shown to signal to MDSCs through the inhibition of β-catenin-dependent Wnt signalling and thereby promote tumour growth in murine syngeneic models (D'Amico *et al.*, 2016). An anti-DKK1 antibody resulted in tumour regression and a shift in the tumour micro-environment from anti-inflammatory to pro-inflammatory, suggesting that the anti-DKK1 antibody was having immune modulatory activity. This was supported by linking DKK1 to MDSC immunosuppressive activities, such as the production of ROS and suppression of T-cell proliferation. Interestingly, the primary source of DKK1 was not from the tumour, but rather from bone, suggesting that high levels of DKK1 in tumours may not necessarily be a prerequisite for efficacy from a DKK1-targeted therapy. Mechanistically, this study gives a clear example of DKK1 signalling to immune cells and the benefit of blocking DKK1 in order to promote an anti-tumour immune response.

DKK1 may also contribute to an immunosuppressive tumour micro-environment by modulating signalling in additional immune cells besides MDSCs. DKK1 from activated platelets signalled to $CD4^+$ T-cells and promoted a pathogenic $CD4^+$ T-helper 2 response as a result of environmental challenges (Chae *et al.*, 2016). Intriguingly, this may not occur through β-catenin-dependent Wnt signalling. Even though this study was not conducted in a cancer model, DKK1 antagonized T-helper 1 polarization and suppressed the secretion of the pro-inflammatory cytokine interferon γ, which are both cellular events that are usually not favourable for an anti-tumour immune response (Fridman *et al.*, 2012). Given these clinical implications, it will be important to elucidate whether DKK1 signals to $CD4^+$ T-cells and additional immune cells in the tumour micro-environment and the extent this contributes to immunosuppression.

A model for DKK1 cancer-promoting activity and clinical implications

DKK1 has diverse functional consequences on cancer and immune cells that contribute to cancer progression. Here, we propose a model highlighting the multitude of potential mechanisms involving DKK1 (Figure 3). DKK1 from the tumour or a host tissue source, such as bone, signals to both tumour cells and immune cells to promote tumour growth. The modulation of Wnt signalling in immune cells by DKK1 results in an immunosuppressive tumour micro-environment. In addition, DKK1 regulation of Wnt signalling and PI3K/Akt signalling in cancer cells contributes to tumour growth and immune evasion and may favour a cancer stem cell phenotype.

Even though DKK1 can clearly promote tumour growth, it has also been hypothesized to function as a tumour suppressor by inhibiting β-catenin-dependent Wnt signalling in cancer cells. This apparent paradox can be explained in part by the diverse functional outcomes of Wnt signalling and thus DKK1, which can vary depending on cancer types. Here, we

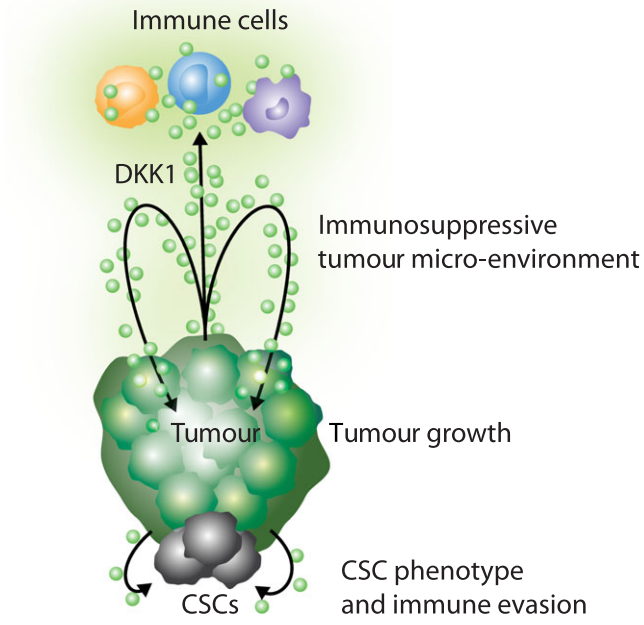

Figure 3
Model for DKK1 cancer-promoting activity. DKK1 signals to tumour cells and immune cells, resulting in an immunosuppressive tumour micro-environment, tumour growth, metastasis, a cancer stem cell (CSC) phenotype and immune evasion.

propose three non-mutually exclusive mechanistic models to reconcile how DKK1, an inhibitor of β-catenin-dependent Wnt signalling, can have tumour promoting activity. (i) Depending on the Wnt signalling wiring in a cancer cell, inhibition of β-catenin-dependent Wnt signalling is not necessarily tumour suppressive. As discussed earlier, DKK1 and its inhibition of β-catenin-dependent Wnt signalling favoured an undifferentiated phenotype in osteosarcoma and the tumour-initiating ability of LCC cells. (ii) The characterization of DKK1 as only an inhibitor of β-catenin-dependent Wnt signalling in cancer cells is an oversimplification. It is important to consider other potential regulatory outcomes of DKK1. For example, DKK1 activation of β-catenin-independent Wnt signalling and/or PI3K/Akt signalling in cancer cells or DKK1 signalling to immune cells may outweigh any tumour-suppressive activity of DKK1 inhibition of β-catenin-dependent Wnt signalling in cancer cells. (iii) In some tumours, β-catenin-dependent Wnt signalling is constitutively activated downstream of DKK1. In this context, it can be hypothesized that DKK1 is unable to inhibit β-catenin-dependent Wnt signalling, thereby eradicating its potential tumour suppressor activity. Further mechanistic insights are crucial for understanding the cancer-promoting activities of DKK1 and deciding which indications are most likely to respond to DKK1-targeted therapies.

Two anti-DKK1 neutralizing antibodies have been or are currently being evaluated clinically for potential use in oncology (Lu *et al.*, 2016). BHQ880, an antibody developed by Novartis Pharmaceuticals, has completed phase 1B trials in multiple myeloma (NCT00741377, NCT01337752 and NCT01302886). Published data indicate that BHQ880 is well tolerated, and some clinical benefit was observed when it was given in combination with zoledronic acid and anti-myeloma therapies (Iyer *et al.*, 2014). Leap Therapeutics is developing DKN-01, a humanized anti-DKK1 monoclonal antibody. An initial dose finding study (NCT01457417) in patients with advanced malignancies demonstrated that DKN-01 monotherapy was well tolerated, and clinical activity in patients with refractory non-small cell lung cancer was observed (Edenfield *et al.*, 2014). Currently, DKN-01 is being evaluated in phase 1B trials for advanced cholangiocarcinoma and relapsed/refractory oesophageal/gastro-oesophageal junction and gastric cancer in combination with standard of care chemotherapy (NCT02375880 and NCT02013154). Preliminary data indicate promising clinical activity in both diseases, and DKN-01 continues to be well tolerated (Eads *et al.*, 2016; Ryan *et al.*, 2016). Based on these results and the increasing understanding of DKK1 tumour promoting activity, further clinical development is warranted.

Wnt signalling is exceedingly complex, and the role of DKK1 in modulating this pathway and additional signalling pathways in both cancer and immune cells to promote tumour growth and metastasis has not been fully elucidated. Even given the complexity, we believe that DKK1 is a promising oncology target for the following reasons. (i) Preliminary clinical data with anti-DKK1 therapeutic antibodies are encouraging, demonstrating a good safety profile and potential benefit when used as a monotherapy or in combination. (ii) For many cancers, elevated tumour levels of DKK1 correlated with a poor prognosis. (iii) Emerging evidence suggests that DKK1 signals to both tumour and immune cells to promote cancer. Therefore, a DKK1-neutralizing therapy could have the benefit of both a direct anti-tumour effect and the stimulation of a pro-inflammatory anti-tumour response. (iv) DKK1 does not appear to be widely expressed in adult tissues, suggesting that on target toxicity from a DKK1-directed therapy would be limited, possibly avoiding or ameliorating the immune-mediated adverse events observed with approved checkpoint inhibitors.

Concluding remarks

Wnt signalling is a fundamental pathway involved in development and adult tissue homeostasis. It is frequently dysregulated in oncology, and multiple therapeutics are currently in preclinical and clinical development. DKK1 is an attractive therapeutic target for oncology given its potential for broad clinical applicability. Elevated levels of DKK1 are detected in the serum and tumours of many cancer patients, spanning a wide range of malignancies and frequently correlate with a poor prognosis. Preclinically blocking DKK1 activity decreases proliferation, migration and invasion in cancer cell lines and has efficacy in multiple mouse tumour models. Clinically, anti-DKK1 neutralizing antibodies have shown promise and are well tolerated. Although the mechanisms of action of DKK1 are not fully characterized, it is becoming increasingly apparent that it can modulate signalling pathways on both cancer and immune cells to promote tumour growth and metastasis. Current and future clinical trials will address the promise of therapeutically targeting DKK1 for oncology.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.*, 2015a,b,c).

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Conflict of interest

M.H.K. is an employee and shareholder of Leap Therapeutics. X.H. is a member of the scientific advisory board of Leap Therapeutics.

References

Aguilera O, Fraga MF, Ballestar E, Paz MF, Herranz M, Espada J *et al.* (2006). Epigenetic inactivation of the Wnt antagonist DICKKOPF-1 (DKK-1) gene in human colorectal cancer. Oncogene 25: 4116–4121.

Aguilera O, Gonzalez-Sancho JM, Zazo S, Rincon R, Fernandez AF, Tapia O *et al.* (2015). Nuclear DICKKOPF-1 as a biomarker of chemoresistance and poor clinical outcome in colorectal cancer. Oncotarget 6: 5903–5917.

Ahn VE, Chu ML, Choi HJ, Tran D, Abo A, Weis WI (2011). Structural basis of Wnt signaling inhibition by Dickkopf binding to LRP5/6. Dev Cell 21: 862–873.

Alexander SPH, Davenport AP, Kelly E, Marrion N, Peters JA, Benson HE *et al.* (2015a). The Concise Guide to PHARMACOLOGY 2015/16: G protein-coupled receptors. Br J Pharmacol 172: 5744–5869.

Alexander SPH, Fabbro D, Kelly E, Marrion N, Peters JA, Benson HE *et al.* (2015b). The Concise Guide to PHARMACOLOGY 2015/16: Catalytic receptors. Br J Pharmacol 172: 5979–6023.

Alexander SPH, Fabbro D, Kelly E, Marrion N, Peters JA, Benson HE *et al.* (2015c). The Concise Guide to PHARMACOLOGY 2015/16: Enzymes. Br J Pharmacol 172: 6024–6109.

Anastas JN, Moon RT (2013). WNT signalling pathways as therapeutic targets in cancer. Nat Rev Cancer 13: 11–26.

Asem MS, Buechler S, Wates RB, Miller DL, Stack MS (2016). Wnt5a signaling in cancer. Cancers (Basel) 8: 1–18.

Assie G, Letouze E, Fassnacht M, Jouinot A, Luscap W, Barreau O *et al.* (2014). Integrated genomic characterization of adrenocortical carcinoma. Nat Genet 46: 607–612.

Bafico A, Liu G, Yaniv A, Gazit A, Aaronson SA (2001). Novel mechanism of Wnt signalling inhibition mediated by Dickkopf-1 interaction with LRP6/Arrow. Nat Cell Biol 3: 683–686.

Bao J, Zheng JJ, Wu D (2012). The structural basis of DKK-mediated inhibition of Wnt/LRP signaling. Sci Signal 5: pe22.

Begenik H, Kemik AS, Emre H, Dulger AC, Demirkiran D, Ebinc S *et al.* (2014). The association between serum Dickkopf-1 levels and esophageal squamous cell carcinoma. Hum Exp Toxicol 33: 785–788.

Bengoa-Vergniory N, Gorrono-Etxebarria I, Gonzalez-Salazar I, Kypta RM (2014). A switch from canonical to noncanonical Wnt signaling mediates early differentiation of human neural stem cells. Stem Cells 32: 3196–3208.

Borcherding N, Kusner D, Liu GH, Zhang W (2014). ROR1, an embryonic protein with an emerging role in cancer biology. Protein Cell 5: 496–502.

Bourhis E, Wang W, Tam C, Hwang J, Zhang Y, Spittler D *et al.* (2011). Wnt antagonists bind through a short peptide to the first beta-propeller domain of LRP5/6. Structure 19: 1433–1442.

Bu G, Lu W, Liu CC, Selander K, Yoneda T, Hall C *et al.* (2008). Breast cancer-derived Dickkopf1 inhibits osteoblast differentiation and osteoprotegerin expression: implication for breast cancer osteolytic bone metastases. Int J Cancer 123: 1034–1042.

Caneparo L, Huang YL, Staudt N, Tada M, Ahrendt R, Kazanskaya O *et al.* (2007). Dickkopf-1 regulates gastrulation movements by coordinated modulation of Wnt/beta catenin and Wnt/PCP activities, through interaction with the Dally-like homolog Knypek. Genes Dev 21: 465–480.

Chae WJ, Ehrlich AK, Chan PY, Teixeira AM, Henegariu O, Hao L *et al.* (2016). The Wnt antagonist Dickkopf-1 promotes pathological type 2 cell-mediated inflammation. Immunity 44: 246–258.

Chamorro MN, Schwartz DR, Vonica A, Brivanlou AH, Cho KR, Varmus HE (2005). FGF-20 and DKK1 are transcriptional targets of beta-catenin and FGF-20 is implicated in cancer and development. EMBO J 24: 73–84.

Chen C, Zhou H, Zhang X, Ma X, Liu Z, Liu X (2014). Elevated levels of Dickkopf-1 are associated with beta-catenin accumulation and poor prognosis in patients with chondrosarcoma. PLoS One 9: e105414.

Chen L, Li M, Li Q, Wang CJ, Xie SQ (2013). DKK1 promotes hepatocellular carcinoma cell migration and invasion through beta-catenin/MMP7 signaling pathway. Mol Cancer 12: 157.

Chen S, Bubeck D, MacDonald BT, Liang WX, Mao JH, Malinauskas T *et al.* (2011). Structural and functional studies of LRP6 ectodomain reveal a platform for Wnt signaling. Dev Cell 21: 848–861.

Chen W, Zhang YW, Li Y, Zhang JW, Zhang T, Fu BS *et al.* (2016). Constitutive expression of Wnt/betacatenin target genes promotes proliferation and invasion of liver cancer stem cells. Mol Med Rep 13: 3466–3474.

Cheng Z, Biechele T, Wei Z, Morrone S, Moon RT, Wang L *et al.* (2011). Crystal structures of the extracellular domain of LRP6 and its complex with DKK1. Nat Struct Mol Biol 18: 1204–1210.

Chu T, Teng J, Jiang L, Zhong H, Han B (2014). Lung cancer-derived Dickkopf1 is associated with bone metastasis and the mechanism involves the inhibition of osteoblast differentiation. Biochem Biophys Res Commun 443: 962–968.

Clevers H, Loh KM, Nusse R (2014). Stem cell signaling. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. Science 346 1248012.

Clevers H, Nusse R (2012). Wnt/beta-catenin signaling and disease. Cell 149: 1192–1205.

D'Amico L, Mahajan S, Capietto AH, Yang Z, Zamani A, Ricci B *et al.* (2016). Dickkopf-related protein 1 (Dkk1) regulates the accumulation and function of myeloid derived suppressor cells in cancer. J Exp Med 213: 827–840.

Darlavoix T, Seelentag W, Yan P, Bachmann A, Bosman FT (2009). Altered expression of CD44 and DKK1 in the progression of Barrett's esophagus to esophageal adenocarcinoma. Virchows Arch 454: 629–637.

Desert R, Mebarki S, Desille M, Sicard M, Lavergne E, Renaud S *et al.* (2016). "Fibrous nests" in human hepatocellular carcinoma express a Wnt-induced gene signature associated with poor clinical outcome. Int J Biochem Cell Biol 81: 195–207.

Dong LL, Qu LY, Chu LY, Zhang XH, Liu YH (2014). Serum level of DKK-1 and its prognostic potential in non-small cell lung cancer. Diagn Pathol 9: 52.

Draghiciu O, Lubbers J, Nijman HW, Daemen T (2015). Myeloid derived suppressor cells–an overview of combat strategies to increase immunotherapy efficacy. Oncoimmunology 4: e954829.

Eads J, El-Khoueiry A, Manji G, Abrams T, Khorana AA, Miksad R *et al.* (2016). Phase I study of DKN-01 (D), an anti-DKK1 monoclonal antibody, in combination with gemcitabine (G) and cisplatin (C) in patients (pts) with advanced biliary cancer (ABC). Ann Oncol 27 (suppl_6): 698P.

Edenfield WJ, Richards DA, Vukelja SJ, Weiss GJ, Sirard CA, Landau SB *et al.* (2014). A phase 1 study evaluating the safety and efficacy of DKN-01, an investigational monoclonal antibody (Mab) in patients

(pts) with advanced non-small cell lung cancer. J Clin Oncol 32 5s (suppl; abstr 8068).

Endo Y, Beauchamp E, Woods D, Taylor WG, Toretsky JA, Uren A *et al.* (2008). Wnt-3a and Dickkopf-1 stimulate neurite outgrowth in Ewing tumor cells via a Frizzled3- and c-Jun N-terminal kinase-dependent mechanism. Mol Cell Biol 28: 2368–2379.

Endo Y, Wolf V, Muraiso K, Kamijo K, Soon L, Uren A *et al.* (2005). Wnt-3a-dependent cell motility involves RhoA activation and is specifically regulated by dishevelled-2. J Biol Chem 280: 777–786.

Fedi P, Bafico A, Nieto Soria A, Burgess WH, Miki T, Bottaro DP *et al.* (1999). Isolation and biochemical characterization of the human Dkk-1 homologue, a novel inhibitor of mammalian Wnt signaling. J Biol Chem 274: 19465–19472.

Forget MA, Turcotte S, Beauseigle D, Godin-Ethier J, Pelletier S, Martin J *et al.* (2007). The Wnt pathway regulator DKK1 is preferentially expressed in hormone-resistant breast tumours and in some common cancer types. Br J Cancer 96: 646–653.

Fridman WH, Pages F, Sautes-Fridman C, Galon J (2012). The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 12: 298–306.

Fulciniti M, Tassone P, Hideshima T, Vallet S, Nanjappa P, Ettenberg SA *et al.* (2009). Anti-DKK1 mAb (BHQ880) as a potential therapeutic agent for multiple myeloma. Blood 114: 371–379.

Gao C, Xie R, Ren C, Yang X (2012). Dickkopf-1 expression is a novel prognostic marker for gastric cancer. J Biomed Biotechnol 2012: 804592.

Giannakis M, Hodis E, Jasmine Mu X, Yamauchi M, Rosenbluh J, Cibulskis K *et al.* (2014). RNF43 is frequently mutated in colorectal and endometrial cancers. Nat Genet 46: 1264–1266.

Glinka A, Wu W, Delius H, Monaghan AP, Blumenstock C, Niehrs C (1998). Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. Nature 391: 357–362.

Goldstein SD, Trucco M, Bautista Guzman W, Hayashi M, Loeb DM (2016). A monoclonal antibody against the Wnt signaling inhibitor dickkopf-1 inhibits osteosarcoma metastasis in a preclinical model. Oncotarget 7: 21114–21123.

Gomceli I, Bostanci EB, Ozer I, Kemik AS, Turhan N, Tez M *et al.* (2012). A novel screening biomarker in gastric cancer: serum Dickkopf-1. Hepatogastroenterology 59: 1661–1664.

Gonzalez-Sancho JM, Aguilera O, Garcia JM, Pendas-Franco N, Pena C, Cal S *et al.* (2005). The Wnt antagonist DICKKOPF-1 gene is a downstream target of beta-catenin/TCF and is downregulated in human colon cancer. Oncogene 24: 1098–1103.

Green J, Nusse R, van Amerongen R (2014). The role of Ryk and Ror receptor tyrosine kinases in Wnt signal transduction. Cold Spring Harb Perspect Biol 6: 1–12.

Gregory CA, Singh H, Perry AS, Prockop DJ (2003). The Wnt signaling inhibitor dickkopf-1 is required for reentry into the cell cycle of human adult stem cells from bone marrow. J Biol Chem 278: 28067–28078.

Gurluler E, Tumay LV, Guner OS, Kucukmetin NT, Hizli B, Zorluoglu A (2014). The role of preoperative serum levels for Dickkopf-related protein 1 as a potential marker of tumor invasion in patients with stage II and III colon cancer. Eur Rev Med Pharmacol Sci 18: 1742–1747.

Hall CL, Daignault SD, Shah RB, Pienta KJ, Keller ET (2008). Dickkopf-1 expression increases early in prostate cancer development and decreases during progression from primary tumor to metastasis. Prostate 68: 1396–1404.

Hall CL, Zhang H, Baile S, Ljungman M, Kuhstoss S, Keller ET (2010). p21CIP-1/WAF-1 induction is required to inhibit prostate cancer growth elicited by deficient expression of the Wnt inhibitor Dickkopf-1. Cancer Res 70: 9916–9926.

Han SX, Zhou X, Sui X, He CC, Cai MJ, Ma JL *et al.* (2015). Serum Dickkopf-1 is a novel serological biomarker for the diagnosis and prognosis of pancreatic cancer. Oncotarget 6: 19907–19917.

Hao HX, Jiang X, Cong F (2016). Control of Wnt receptor turnover by R-spondin-ZNRF3/RNF43 signaling Module and its dysregulation in cancer. Cancers (Basel) 8: 1–12.

Heath DJ, Chantry AD, Buckle CH, Coulton L, Shaughnessy JD Jr, Evans HR *et al.* (2009). Inhibiting Dickkopf-1 (Dkk1) removes suppression of bone formation and prevents the development of osteolytic bone disease in multiple myeloma. J Bone Miner Res 24: 425–436.

Hirata H, Hinoda Y, Nakajima K, Kawamoto K, Kikuno N, Ueno K *et al.* (2011). Wnt antagonist DKK1 acts as a tumor suppressor gene that induces apoptosis and inhibits proliferation in human renal cell carcinoma. Int J Cancer 128: 1793–1803.

Huang Y, Yang X, Zhao F, Shen Q, Wang Z, Lv X *et al.* (2014). Overexpression of Dickkopf-1 predicts poor prognosis for patients with hepatocellular carcinoma after orthotopic liver transplantation by promoting cancer metastasis and recurrence. Med Oncol 31: 966.

Iyer SP, Beck JT, Stewart AK, Shah J, Kelly KR, Isaacs R *et al.* (2014). A Phase IB multicentre dose-determination study of BHQ880 in combination with anti-myeloma therapy and zoledronic acid in patients with relapsed or refractory multiple myeloma and prior skeletal-related events. Br J Haematol 167: 366–375.

Jiang T, Huang L, Zhang S (2013). DKK-1 in serum as a clinical and prognostic factor in patients with cervical cancer. Int J Biol Markers 28: 221–225.

Jiang T, Wang S, Huang L, Zhang S (2009). Clinical significance of serum DKK-1 in patients with gynecological cancer. Int J Gynecol Cancer 19: 1177–1181.

Kahn M (2014). Can we safely target the WNT pathway? Nat Rev Drug Discov 13: 513–532.

Katoh M (2005). WNT/PCP signaling pathway and human cancer (review). Oncol Rep 14: 1583–1588.

Kemik O, Kemik AS, Sumer A, Begenik H, Purisa S, Tuzun S *et al.* (2011). Relationship between clinicopathologic variables and serum and tissue levels of dickkopf-1 in patients with rectal cancer. J Invest Med 59: 947–950.

Kessenbrock K, Plaks V, Werb Z (2010). Matrix metalloproteinases: regulators of the tumor microenvironment. Cell 141: 52–67.

Killick R, Ribe EM, Al-Shawi R, Malik B, Hooper C, Fernandes C *et al.* (2014). Clusterin regulates beta-amyloid toxicity via Dickkopf-1-driven induction of the WNT–PCP–JNK pathway. Mol Psychiatry 19: 88–98.

Kim BR, Shin HJ, Kim JY, Byun HJ, Lee JH, Sung YK *et al.* (2012). Dickkopf-1 (DKK-1) interrupts FAK/PI3K/mTOR pathway by interaction of carbonic anhydrase IX (CA9) in tumorigenesis. Cell Signal 24: 1406–1413.

Kim SU, Park JH, Kim HS, Lee JM, Lee HG, Kim H *et al.* (2015). Serum Dickkopf-1 as a biomarker for the diagnosis of hepatocellular carcinoma. Yonsei Med J 56: 1296–1306.

Kimura H, Fumoto K, Shojima K, Nojima S, Osugi Y, Tomihara H *et al.* (2016). CKAP4 is a Dickkopf1 receptor and is involved in tumor progression. J Clin Invest 126: 2689–2705.

Krause U, Ryan DM, Clough BH, Gregory CA (2014). An unexpected role for a Wnt-inhibitor: Dickkopf-1 triggers a novel cancer survival mechanism through modulation of aldehyde-dehydrogenase-1 activity. Cell Death Dis 5: e1093.

Kumawat K, Gosens R (2016). WNT-5A: signaling and functions in health and disease. Cell Mol Life Sci 73: 567–587.

Kwong LN, Dove WF (2009). APC and its modifiers in colon cancer. Adv Exp Med Biol 656: 85–106.

Lee AY, He B, You L, Xu Z, Mazieres J, Reguart N *et al.* (2004). Dickkopf-1 antagonizes Wnt signaling independent of beta-catenin in human mesothelioma. Biochem Biophys Res Commun 323: 1246–1250.

Lee HS, Lee HE, Park DJ, Kim HH, Kim WH, Park KU (2012). Clinical significance of serum and tissue Dickkopf-1 levels in patients with gastric cancer. Clin Chim Acta 413: 1753–1760.

Lee N, Smolarz AJ, Olson S, David O, Reiser J, Kutner R *et al.* (2007). A potential role for Dkk-1 in the pathogenesis of osteosarcoma predicts novel diagnostic and treatment strategies. Br J Cancer 97: 1552–1559.

Li S, Qin X, Guo X, Cui A, He Y, Wei S *et al.* (2013). Dickkopf-1 is oncogenic and involved in invasive growth in non small cell lung cancer. PLoS One 8: e84944.

Li S, Qin X, Liu B, Sun L, Zhang X, Li Z *et al.* (2011). Dickkopf-1 is involved in invasive growth of esophageal cancer cells. J Mol Histol 42: 491–498.

Liu LJ, Xie SX, Chen YT, Xue JL, Zhang CJ, Zhu F (2016a). Aberrant regulation of Wnt signaling in hepatocellular carcinoma. World J Gastroenterol 22: 7486–7499.

Liu QR, Li YF, Deng ZQ, Cao JQ (2016b). Prognostic significance of Dickkopf-1 in gastric cancer survival: a meta-analysis. Genet Test Mol Biomarkers 20: 170–175.

Liu Y, Tang W, Xie L, Wang J, Deng Y, Peng Q *et al.* (2014). Prognostic significance of Dickkopf-1 overexpression in solid tumors: a meta-analysis. Tumour Biol 35: 3145–3154.

Logan CY, Nusse R (2004). The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 20: 781–810.

Lu B, Green BA, Farr JM, Lopes FC, Van Raay TJ (2016). Wnt drug discovery: weaving through the screens, patents and clinical trials. Cancers (Basel) 8: 1–15.

Lyros O, Rafiee P, Nie L, Medda R, Jovanovic N, Otterson MF *et al.* (2015). Wnt/beta-catenin signaling activation beyond robust nuclear beta-catenin accumulation in nondysplastic Barrett's esophagus: regulation via Dickkopf-1. Neoplasia 17: 598–611.

MacDonald BT, Joiner DM, Oyserman SM, Sharma P, Goldstein SA, He X *et al.* (2007). Bone mass is inversely proportional to Dkk1 levels in mice. Bone 41: 331–339.

MacDonald BT, Tamai K, He X (2009). Wnt/beta-catenin signaling: components, mechanisms, and diseases. Dev Cell 17: 9–26.

Maehata T, Taniguchi H, Yamamoto H, Nosho K, Adachi Y, Miyamoto N *et al.* (2008). Transcriptional silencing of Dickkopf gene family by CpG island hypermethylation in human gastrointestinal cancer. World J Gastroenterol 14: 2702–2714.

Makino T, Yamasaki M, Takemasa I, Takeno A, Nakamura Y, Miyata H *et al.* (2009). Dickkopf-1 expression as a marker for predicting clinical outcome in esophageal squamous cell carcinoma. Ann Surg Oncol 16: 2058–2064.

Malladi S, Macalinao DG, Jin X, He L, Basnet H, Zou Y *et al.* (2016). Metastatic latency and immune evasion through autocrine inhibition of WNT. Cell 165: 45–60.

Mao B, Wu W, Davidson G, Marhold J, Li M, Mechler BM *et al.* (2002). Kremen proteins are Dickkopf receptors that regulate Wnt/beta-catenin signalling. Nature 417: 664–667.

Mao B, Wu W, Li Y, Hoppe D, Stannek P, Glinka A *et al.* (2001). LDL-receptor-related protein 6 is a receptor for Dickkopf proteins. Nature 411: 321–325.

Marzo A, Galli S, Lopes D, McLeod F, Podpolny M, Segovia-Roldan M *et al.* (2016). Reversal of synapse degeneration by restoring Wnt signaling in the adult hippocampus. Curr Biol 26: 2551–2561.

Matoba K, Mihara E, Tamura-Kawakami K, Miyazaki N, Maeda S, Hirai H *et al.* (2017). Conformational freedom of the LRP6 ectodomain Is regulated by N-glycosylation and the binding of the Wnt antagonist Dkk1. Cell Rep 18: 32–40.

Matushansky I, Hernando E, Socci ND, Mills JE, Matos TA, Edgar MA *et al.* (2007). Derivation of sarcomas from mesenchymal stem cells via inactivation of the Wnt pathway. J Clin Invest 117: 3248–3257.

Mazon M, Masi D, Carreau M (2016). Modulating Dickkopf-1: a strategy to monitor or treat cancer? Cancers (Basel) 8: 1–9.

McConechy MK, Ding J, Senz J, Yang W, Melnyk N, Tone AA *et al.* (2014). Ovarian and endometrial endometrioid carcinomas have distinct CTNNB1 and PTEN mutation profiles. Mod Pathol 27: 128–134.

Menezes ME, Devine DJ, Shevde LA, Samant RS (2012). Dickkopf1: a tumor suppressor or metastasis promoter? Int J Cancer 130: 1477–1483.

Mikheev AM, Mikheeva SA, Maxwell JP, Rivo JV, Rostomily R, Swisshelm K *et al.* (2008). Dickkopf-1 mediated tumor suppression in human breast carcinoma cells. Breast Cancer Res Treat 112: 263–273.

Monaghan AP, Kioschis P, Wu W, Zuniga A, Bock D, Poustka A *et al.* (1999). Dickkopf genes are co-ordinately expressed in mesodermal lineages. Mech Dev 87: 45–56.

Mukhopadhyay M, Shtrom S, Rodriguez-Esteban C, Chen L, Tsukui T, Gomer L *et al.* (2001). Dickkopf1 is required for embryonic head induction and limb morphogenesis in the mouse. Dev Cell 1: 423–434.

Niehrs C (2006). Function and biological roles of the Dickkopf family of Wnt modulators. Oncogene 25: 7469–7481.

Niida A, Hiroko T, Kasai M, Furukawa Y, Nakamura Y, Suzuki Y *et al.* (2004). DKK1, a negative regulator of Wnt signaling, is a target of the beta-catenin/TCF pathway. Oncogene 23: 8520–8526.

Pandur P, Lasche M, Eisenberg LM, Kuhl M (2002). Wnt-11 activation of a non-canonical Wnt signalling pathway is required for cardiogenesis. Nature 418: 636–641.

Pang H, Ma N, Jiao M, Shen W, Xin B, Wang T *et al.* (2017). The biological effects of dickkopf1 on small cell lung cancer cells and bone metastasis. Oncol Res 25: 35–42.

Park BK, Paik YH, Park JY, Park KH, Bang S, Park SW *et al.* (2006). The clinicopathologic significance of the expression of vascular endothelial growth factor-C in intrahepatic cholangiocarcinoma. Am J Clin Oncol 29: 138–142.

Patil MA, Chua MS, Pan KH, Lin R, Lih CJ, Cheung ST *et al.* (2005). An integrated data analysis approach to characterize genes highly expressed in hepatocellular carcinoma. Oncogene 24: 3737–3747.

Pinzone JJ, Hall BM, Thudi NK, Vonau M, Qiang YW, Rosol TJ *et al.* (2009). The role of Dickkopf-1 in bone development, homeostasis, and disease. Blood 113: 517–525.

Polakis P (2012). Wnt signaling in cancer. Cold Spring Harb Perspect Biol 4: 1–13.

Politou MC, Heath DJ, Rahemtulla A, Szydlo R, Anagnostopoulos A, Dimopoulos MA *et al.* (2006). Serum concentrations of Dickkopf-1 protein are increased in patients with multiple myeloma and reduced after autologous stem cell transplantation. Int J Cancer 119: 1728–1731.

Pozzi S, Fulciniti M, Yan H, Vallet S, Eda H, Patel K *et al.* (2013). In vivo and in vitro effects of a novel anti-Dkk1 neutralizing antibody in multiple myeloma. Bone 53: 487–496.

Qi L, Sun B, Liu Z, Li H, Gao J, Leng X (2012). Dickkopf-1 inhibits epithelial-mesenchymal transition of colon cancer cells and contributes to colon cancer suppression. Cancer Sci 103: 828–835.

Qian J, Xie J, Hong S, Yang J, Zhang L, Han X *et al.* (2007). Dickkopf-1 (DKK1) is a widely expressed and potent tumor-associated antigen in multiple myeloma. Blood 110: 1587–1594.

Qiao L, Xu ZL, Zhao TJ, Ye LH, Zhang XD (2008). Dkk-1 secreted by mesenchymal stem cells inhibits growth of breast cancer cells via depression of Wnt signalling. Cancer Lett 269: 67–77.

Rachner TD, Gobel A, Thiele S, Rauner M, Benad-Mehner P, Hadji P *et al.* (2014a). Dickkopf-1 is regulated by the mevalonate pathway in breast cancer. Breast Cancer Res 16: R20.

Rachner TD, Thiele S, Gobel A, Browne A, Fuessel S, Erdmann K *et al.* (2014b). High serum levels of Dickkopf-1 are associated with a poor prognosis in prostate cancer patients. BMC Cancer 14: 649.

Rao TP, Kuhl M (2010). An updated overview on Wnt signaling pathways: a prelude for more. Circ Res 106: 1798–1806.

Ryan DP, Murphy J, Mahalingam D, Strickler J, Stein S, Sirard C *et al.* (2016). Current results of a phase I study of DKN-01 in combination with paclitaxel (P) in patients (pts) with advanced DKK1+ esophageal cancer (EC) or gastro-esophageal junction tumors (GEJ). Ann Oncol 27 (suppl_2) ii108.

Salim H, Zong D, Haag P, Novak M, Mork B, Lewensohn R *et al.* (2015). DKK1 is a potential novel mediator of cisplatin-refractoriness in non-small cell lung cancer cell lines. BMC Cancer 15: 628.

Saneyoshi T, Kume S, Amasaki Y, Mikoshiba K (2002). The Wnt/calcium pathway activates NF-AT and promotes ventral cell fate in *Xenopus* embryos. Nature 417: 295–299.

Sato H, Suzuki H, Toyota M, Nojima M, Maruyama R, Sasaki S *et al.* (2007). Frequent epigenetic inactivation of DICKKOPF family genes in human gastrointestinal tumors. Carcinogenesis 28: 2459–2466.

Sato N, Yamabuki T, Takano A, Koinuma J, Aragaki M, Masuda K *et al.* (2010). Wnt inhibitor Dickkopf-1 as a target for passive cancer immunotherapy. Cancer Res 70: 5326–5336.

Schambony A, Wedlich D (2007). Wnt-5A/Ror2 regulate expression of XPAPC through an alternative noncanonical signaling pathway. Dev Cell 12: 779–792.

Sedgwick AE, D'Souza-Schorey C (2016). Wnt signaling in cell motility and invasion: drawing parallels between development and cancer. Cancers (Basel) 8: 1–15.

Semenov MV, Habas R, Macdonald BT, He X (2007). SnapShot: noncanonical Wnt signaling pathways. Cell 131: 1378.

Semenov MV, Tamai K, Brott BK, Kuhl M, Sokol S, He X (2001). Head inducer Dickkopf-1 is a ligand for Wnt coreceptor LRP6. Curr Biol 11: 951–961.

Semenov MV, Zhang X, He X (2008). DKK1 antagonizes Wnt signaling without promotion of LRP6 internalization and degradation. J Biol Chem 283: 21427–21432.

Seshagiri S, Stawiski EW, Durinck S, Modrusan Z, Storm EE, Conboy CB *et al.* (2012). Recurrent R-spondin fusions in colon cancer. Nature 488: 660–664.

Shen CH, Hsieh HY, Wang YH, Chen SY, Tung CL, Wu JD *et al.* (2010). High Dickkopf-1 expression is associated with poor prognosis in patients with advanced urothelial carcinoma. Exp Ther Med 1: 893–898.

Shen Q, Fan J, Yang XR, Tan Y, Zhao W, Xu Y *et al.* (2012). Serum DKK1 as a protein biomarker for the diagnosis of hepatocellular carcinoma: a large-scale, multicentre study. Lancet Oncol 13: 817–826.

Sheng SL, Huang G, Yu B, Qin WX (2009). Clinical significance and prognostic value of serum Dickkopf-1 concentrations in patients with lung cancer. Clin Chem 55: 1656–1664.

Shi RY, Yang XR, Shen QJ, Yang LX, Xu Y, Qiu SJ *et al.* (2013). High expression of Dickkopf-related protein 1 is related to lymphatic metastasis and indicates poor prognosis in intrahepatic cholangiocarcinoma patients after surgery. Cancer 119: 993–1003.

Shi XD, Yu XH, Wu WR, Xu XL, Wang JY, Xu LB *et al.* (2016). Dickkopf-1 expression is associated with tumorigenity and lymphatic metastasis in human hilar cholangiocarcinoma. Oncotarget 7: 70378–70387.

Shi Y, Gong HL, Zhou L, Tian J, Wang Y (2014). Dickkopf-1 is a novel prognostic biomarker for laryngeal squamous cell carcinoma. Acta Otolaryngol 134: 753–759.

Shizhuo W, Tao J, Shulan Z, Bing Z (2009). The expression and significance of Dickkopf-1 in epithelial ovarian carcinoma. Int J Biol Markers 24: 165–170.

Smadja DM, d'Audigier C, Weiswald LB, Badoual C, Dangles-Marie V, Mauge L *et al.* (2010). The Wnt antagonist Dickkopf-1 increases endothelial progenitor cell angiogenic potential. Arterioscler Thromb Vasc Biol 30: 2544–2552.

Southan C, Sharman JL, Benson HE, Faccenda E, Pawson AJ, Alexander SP *et al.* (2016). The IUPHAR/BPS Guide to PHARMACOLOGY in 2016: towards curated quantitative interactions between 1300 protein targets and 6000 ligands. Nucleic Acids Res 44: D1054–D1068.

Sugimura R, Li L (2010). Noncanonical Wnt signaling in vertebrate development, stem cells, and diseases. Birth Defects Res C Embryo Today 90: 243–256.

Sun DK, Wang L, Wang JM, Zhang P (2015). Serum Dickkopf-1 levels as a clinical and prognostic factor in patients with bladder cancer. Genet Mol Res 14: 18181–18187.

Takahashi N, Fukushima T, Yorita K, Tanaka H, Chijiiwa K, Kataoka H (2010). Dickkopf-1 is overexpressed in human pancreatic ductal adenocarcinoma cells and is involved in invasive growth. Int J Cancer 126: 1611–1620.

Tao YM, Liu Z, Liu HL (2013). Dickkopf-1 (DKK1) promotes invasion and metastasis of hepatocellular carcinoma. Dig Liver Dis 45: 251–257.

Thudi NK, Martin CK, Murahari S, Shu ST, Lanigan LG, Werbeck JL *et al.* (2011). Dickkopf-1 (DKK-1) stimulated prostate cancer growth and metastasis and inhibited bone formation in osteoblastic bone metastases. Prostate 71: 615–625.

Tian E, Zhan F, Walker R, Rasmussen E, Ma Y, Barlogie B *et al.* (2003). The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. N Engl J Med 349: 2483–2494.

Topalian SL, Drake CG, Pardoll DM (2015). Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell 27: 450–461.

Tung EK, Mak CK, Fatima S, Lo RC, Zhao H, Zhang C *et al.* (2011). Clinicopathological and prognostic significance of serum and tissue Dickkopf-1 levels in human hepatocellular carcinoma. Liver Int 31: 1494–1504.

van Amerongen R (2012). Alternative Wnt pathways and receptors. Cold Spring Harb Perspect Biol 4: 1–18.

Voorzanger-Rousselot N, Goehrig D, Journe F, Doriath V, Body JJ, Clezardin P *et al.* (2007). Increased Dickkopf-1 expression in breast cancer bone metastases. Br J Cancer 97: 964–970.

Wang K, Zhang Y, Li X, Chen L, Wang H, Wu J *et al.* (2008). Characterization of the Kremen-binding site on Dkk1 and elucidation of the role of Kremen in Dkk-mediated Wnt antagonism. J Biol Chem 283: 23371–23375.

Wang S, Zhang S (2011). Dickkopf-1 is frequently overexpressed in ovarian serous carcinoma and involved in tumor invasion. Clin Exp Metastasis 28: 581–591.

Wang Y (2009). Wnt/planar cell polarity signaling: a new paradigm for cancer therapy. Mol Cancer Ther 8: 2103–2109.

Wirths O, Waha A, Weggen S, Schirmacher P, Kuhne T, Goodyer CG *et al.* (2003). Overexpression of human Dickkopf-1, an antagonist of wingless/WNT signaling, in human hepatoblastomas and Wilms' tumors. Lab Invest 83: 429–434.

Xiang XJ, Liu YW, Chen DD, Yu S (2015). Differential expression of Dickkopf-1 among non-small cell lung cancer cells. Mol Med Rep 12: 1935–1940.

Xu WH, Liu ZB, Yang C, Qin W, Shao ZM (2012). Expression of Dickkopf-1 and beta-catenin related to the prognosis of breast cancer patients with triple negative phenotype. PLoS One 7: e37624.

Yaccoby S, Ling W, Zhan F, Walker R, Barlogie B, Shaughnessy JD Jr (2007). Antibody-based inhibition of DKK1 suppresses tumor-induced bone resorption and multiple myeloma growth in vivo. Blood 109: 2106–2111.

Yamabuki T, Takano A, Hayama S, Ishikawa N, Kato T, Miyamoto M *et al.* (2007). Dikkopf-1 as a novel serologic and prognostic biomarker for lung and esophageal carcinomas. Cancer Res 67: 2517–2525.

Yang H, Chen GD, Fang F, Liu Z, Lau SH, Zhang JF *et al.* (2013). Dickkopf-1: as a diagnostic and prognostic serum marker for early hepatocellular carcinoma. Int J Biol Markers 28: 286–297.

Yao L, Zhang D, Zhao X, Sun B, Liu Y, Gu Q *et al.* (2016). Dickkopf-1-promoted vasculogenic mimicry in non-small cell lung cancer is associated with EMT and development of a cancer stem-like cell phenotype. J Cell Mol Med 20: 1673–1685.

Yu B, Yang X, Xu Y, Yao G, Shu H, Lin B *et al.* (2009). Elevated expression of DKK1 is associated with cytoplasmic/nuclear beta-catenin accumulation and poor prognosis in hepatocellular carcinomas. J Hepatol 50: 948–957.

Zarea M, Mohammadian Bajgiran A, Sedaghati F, Hatami N, Taheriazam A, Yahaghi E *et al.* (2016). Diagnostic investigations of DKK-1 and PDCD5 expression levels as independent prognostic markers of human chondrosarcoma. IUBMB Life 68: 597–601.

Zebisch M, Jackson VA, Zhao Y, Jones EY (2016). Structure of the dual-mode Wnt regulator Kremen1 and insight into ternary complex formation with LRP6 and Dickkopf. Structure 24: 1599–1605.

Zhan T, Rindtorff N, Boutros M (2017). Wnt signaling in cancer. Oncogene 36: 1461–1473.

Zhang J, Zhao Y, Yang Q (2014). Sensitivity and specificity of Dickkopf-1 protein in serum for diagnosing hepatocellular carcinoma: a meta-analysis. Int J Biol Markers 29: e403–e410.

Zhang Y, Wang Y, Li X, Zhang J, Mao J, Li Z *et al.* (2004). The LRP5 high-bone-mass G171V mutation disrupts LRP5 interaction with Mesd. Mol Cell Biol 24: 4677–4684.

Zhou SJ, Zhuo SR, Yang XQ, Qin CX, Wang ZL (2014). Serum Dickkopf-1 expression level positively correlates with a poor prognosis in breast cancer. Diagn Pathol 9: 161.

Zhou Y, Liu F, Xu Q, Wang X (2010). Analysis of the expression profile of Dickkopf-1 gene in human glioma and the association with tumor malignancy. J Exp Clin Cancer Res 29: 138.

Zucman-Rossi J, Villanueva A, Nault JC, Llovet JM (2015). Genetic landscape and biomarkers of hepatocellular carcinoma. Gastroenterology 149: 1226–1239 e1224.