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| Citation       | Kagey, Michael H., and Xi He. 2017. “Rationale for targeting the Wnt signalling modulator Dickkopf-1 for oncology.” British Journal of Pharmacology 174 (24): 4637-4650. doi:10.1111/bph.13894. http://dx.doi.org/10.1111/bph.13894. |
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| Published Version | doi:10.1111/bph.13894                                                                                                                                                                                                                                           |
| Accessed       | July 23, 2018 3:33:00 PM EDT                                                                                                                                                                                                                                      |
| Citable Link   | http://nrs.harvard.edu/urn-3:HUL.InstRepos:34651747                                                                                                                                                                                                              |
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Rationale for targeting the Wnt signalling modulator Dickkopf-1 for oncology

Michael H Kagey and Xi He

1Leap Therapeutics, Cambridge, MA, USA, and 2The F. M. Kirby Neurobiology Center Boston Children’s Hospital, Department of Neurology, Harvard Medical School, Boston, MA, USA

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
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; McConéchy 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 understood.
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 down-stream 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; Wang 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; Sedwick and D’Souza-Schorey, 2016). For example, Wnt-5α 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 (Borchering 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., 2002; 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
has been associated with lymph node metastasis in 4640 British Journal of Pharmacology (2017) 174 Liu et al. and cholangiocarcinoma tumours and this correlated with has been detected in a high percentage of oesophageal sion and metastasis (Table 1). For example, DKK1 staining advanced stage, decreased overall survival, vascular inva- was frequently associated with a poor prognosis, such as tion and metastasis (Smadja et al., 2011; Gurluler et al., 2014; Aguilera et al., 2015)

| 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)        |
| Endometriai                   | (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)                                                       |

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

devlopment 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  
• Cancer cells with metastatic potential avoided immune clearance by expressing DKK1                                                                                              | (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-DDK1 antibody had efficacy in a syngeneic mouse model  
• Cancer cells with metastatic potential avoided immune clearance by expressing DKK1  
• An anti-DDK1 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-DDK1 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-DDK1 antibodies had efficacy in mouse models  
• Anti-DDK1 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-DDK1 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)                                                                     |
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; Fang 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

Table 2 (Continued)

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

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

Acknowledgements

The authors wish to thank Walter Newman, David Shapiro, Cyndi Sirad and Melissa Geddie for critical reading of this manuscript and Tom DiCesare for graphic support. X.H. acknowledges support by NIH (RO1-GM057603 and RO1-AR060359) and by Boston Children's Hospital Intellectual and Developmental Disabilities Research Center (P30 HD-18655). X.H. is an American Cancer Society Research Professor.

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.
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