Role of DCLK1 in oncogenic signaling (Review)

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Abstract. Doublecortin-like kinase 1 (DCLK1) has been identified as a novel biomarker of cancer stem cells among several different cancer types, including colon, breast, pancreas, kidney, liver, stomach and esophageal cancers. Studies have demonstrated that DCLK1 regulates tumorigenesis and epithelial-mesenchymal transformation via several important pathways, such as Notch, Wnt/β-catenin, RAS and multiple microRNAs. The function and biological mechanisms, including their association with the molecular structure and isoforms of DCLK1, are gradually being elucidated. However, the currently available knowledge regarding DCLK1 in terms of developing effective anti-cancer drugs remains incomplete. In the present review, the molecular characteristics, biomarker function and biological mechanisms of DCLK1 are summarized and DCLK1 is proposed as a potential anti-tumor target via the glucose metabolism pathway.

Contents

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Abbreviations: DCLK1, doublecortin-like kinase 1; CSCs, cancer stem cells; DCX, doublecortin; AcTub, acetylated tubulin; EMT, epithelial-mesenchymal transition

Key words: doublecortin-like kinase 1, cancer stem cells, molecular structure and function, signaling pathway, metabolism

1. Introduction

In 2008, the Houchen group proposed that serine-threonine kinase, doublecortin-like kinase 1 (DCLK1, then known as DCAMKL-1), was a specific marker protein for intestinal adenoma stem cells (1), which was the first of a series of research reports providing evidence that it may be an effective target for oncology drug development. To date, DCLK1 has been reported to be a selective marker of several types of cancer stem cells (CSCs), including those in colon, breast, pancreas, kidney and esophageal cancers (2,3). The first high-level evidence for the CSC marker status of DCLK1 came in 2012, when Nakanishi *et al* (4) reported that DCLK1 does not mark normal stem cells, but specifically marks CSCs in the adenomatous polyposis coli (APC) loss-driven $APC^{Min/+}$ model of intestinal tumorigenesis. Furthermore, in normal gastrointestinal epithelia, DCLK1 has been indicated to mark fully differentiated epithelial tuft cells among several other cell types in the gastric antrum, bile duct and pancreas (5,6). Epithelial tuft cells are characterized by microtubule bundles located at the cell apex and express DCLK1 and acetylated α-tubulin to take part in regulating the microenvironment (7). DCLK1+ tuft cells have a key role in tumorigenesis by regulating inflammation of the microenvironment via expression of proteins such as Cox1, Cox2 and hematopoietic prostaglandin-D synthase in intestinal cancer (8).

After 20 years of research, DCLK1 is accepted as a specific marker of tuft cells and several types of CSCs and evidence of its ability to regulate tumor growth and metastasis has been provided (9). DCLK1 is expressed in lung, liver, heart, spleen, thymus, prostate and intestine and strongly marks specific cell types (Fig. 1) (10-12). In the present review, the molecular structure and biological mechanisms of DCLK1 in tumorigenesis and metastasis were summarized and its role

in metabolism was highlighted as a potentially novel area for further exploration.

2. Molecular structure and function of DCLK1

The human DCLK1 gene is located on the long arm 13q12.3-q13 of the 13th chromosome, which contains two different promoter sequences to form splicing variants with different protein functional domains (13). Structural domains include two N-terminal doublecortin (DCX) domains and one C-terminal serine/threonine protein kinase domain, homologous to the protein kinase superfamily and DCX (14,15). The structural characteristics of DCX1 are similar to those of DCX, which is able to specifically bind to microtubules, and DCX2 mainly interacts with microtubules and their dimers. These two DCX domains allow DCLK1 to bind microtubules and regulate microtubule aggregation to affect neuronal migration. The C-terminal domain is similar to calmodulin dependent kinase II, but lacks a typical calmodulin binding site (16,17). At present, there are several splicing variants in the DCLK1 gene that have been identified, including a full-length type with all domains and a poly-arginine region, a DCX-like type containing only the microtubule-binding domains, and a smaller molecular-weight type containing a phosphoserine-rich region and kinase domain. These variations in protein domains resulting from alternative splicing and multiple transcriptional promoter regions are hypothesized to result in completely different molecular functions (18). Human DCLK1 includes 82- and 52-kDa isoforms, which are transcribed from an upstream (A, CpG-regulated) or downstream promoter (B, TATA-box) with differing C-terminal domains (Fig. 2). The A isoforms contain N-terminal doublecortin domains to bind to microtubules and a protein kinase domain, while the B isoforms lack N-terminal doublecortin domains. Later, DCX-like was identified and only includes N-terminal DCX domains produced from the A promoter and Camk-related peptide, a 56 amino acid B-promoter-derived peptide with unknown function, was also identified (19,20).

The biological activity of DCLK1 in cancer is different between β-promoter (alternatively termed as DCLK1-S or DCLK3/4, 45-52 kDa, isoform 3/4) and α-promoter (termed as DCLK1-L or DCLK1/2, ~82 kDa, isoform 1/2) isoforms. One study determined that hypermethylation of the α-promoter directly led to the absence of expression of DCLK1-L in 15 human colon cancer cell lines, and that the α-promoter was activated by β-catenin and T-cell factor-4/lymphoid enhancer factor (LEF), while the β-promoter was activated by NF-κB p65 in cancer cells. In this study, the majority of human CRCs were reported to express DCLK1-S, which developed an invasive phenotype and this was associated with unfavorable overall survival (19). Park *et al* (21) identified DCLK1-B transcription as directly activated by Wnt/β-catenin signaling and that LEF1 mediates Wnt-induced CSC properties. Sarkar *et al* (22) reported that DCLK1-L and DCLK1-S are in nuclear and mitochondrial fractions, as well as plasma membrane and cytosolic fractions, but DCLK1-S is in the nuclei and mitochondria in colon cancer. DCLK1 α-promoter demonstrated hypermethylation in cholangiocarcinoma, but hypomethylation in α- and β-promoter regions in renal cell carcinoma (RCC) (23). Of note, two mouse models using DCLK1 A-promoter isoforms to drive Cre-recombinase (DCLK1-CreERT and DCLK1-CreERT2) demonstrated lineage tracing of CRC tumors in the presence of APC mutation (24,25). Interestingly, Ge *et al* (26) reported that both DCLK1-AS (isoform 1, 82 kD) and DCLK1-BL (isoform 4) isoforms are able to efficiently activate epithelial-to-mesenchymal transition (EMT) in pancreatic ductal adenocarcinoma (PDAC) cell lines. Overall, there is a significant shortcoming in the literature regarding the function of DCLK1 isoforms in cancer. The combined evidence suggests that all major DCLK1 isoforms are oncogenic, but there may be a variation among different tumor types or even among tumor subtypes.

3. Biological mechanism of DCLK1 in different types of cancer

Colorectal cancer (CRC). The biological function of DCLK1 in tumorigenesis and metastasis as a marker of tuft cells and CSCs is most thoroughly studied in CRC. In normal human intestinal tissue, stem cells are located at the base of the intestinal crypt epithelium, where they are marked by leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) without co-expressing gut endocrine markers chromogranin A and somatostatin (Fig. 3) (27). DCLK1 marks fully differentiated intestinal tuft cells located in the crypt and villus (28). Long-lived DCLK1+ tuft cells with characteristic microvilli feature self-renewal ability and potential quiescent stem-like functionality (29). Knockdown of the Wnt regulator APC does not alter this quiescence, but subsequent activation through inflammation induced by dextran sodium sulfate is sufficient to initiate colon cancer in Dclk1-Cre/Apc$^{flox/flox}$ (Dclk1-Cre/Apc$^{flox/flox}$ transgenic mice featuring knocking out APC gene in DCLK1^{+} cells) transgenic mice (30). These findings are supported in DCLK1-knockout mice where deficiency results in increased epithelial barrier permeability, higher levels of pro-inflammatory cytokines and chemokines, decreased levels of LGR5 and dysregulated Wnt/β-Catenin pathway genes in Villin-Cre/Dclk1$^{flox/flox}$ (Villin-Cre/Dclk1$^{flox/flox}$ mice featuring deletion of DCLK1 expression in villin-positive cells) mice (31). In 2009, Gerbe *et al* (32) provided conclusive evidence that DCLK1 was in fact a tuft cell rather than stem cell marker, as indicated by the position and marker co-expression (cyclooxygenase enzymes 1/2, advillin and tubulin) of DCLK1-expressing cells. Lineage tracing studies demonstrated that DCLK1-positive cells also express colorectal CSC markers, such as CD133 and CD44 (3,4,33). Of note, DCLK1-positive normal intestinal epithelial cells isolated by fluorescence-assisted cell sorting form spheroids that may assemble into glandular epithelial structures and express multiple markers of gut epithelial lineages when implanted subcutaneously in athymic nude mice (27). Self-renewal and differentiation characteristics of DCLK1-positive cells and low expression in normal tissue both led researchers to speculate that they may mark a type of stem cell (34,35), but these findings are no longer supported in the literature and previous results are likely artifacts resulting from the existence of rare DCLK1/LGR5 double-positive cells.

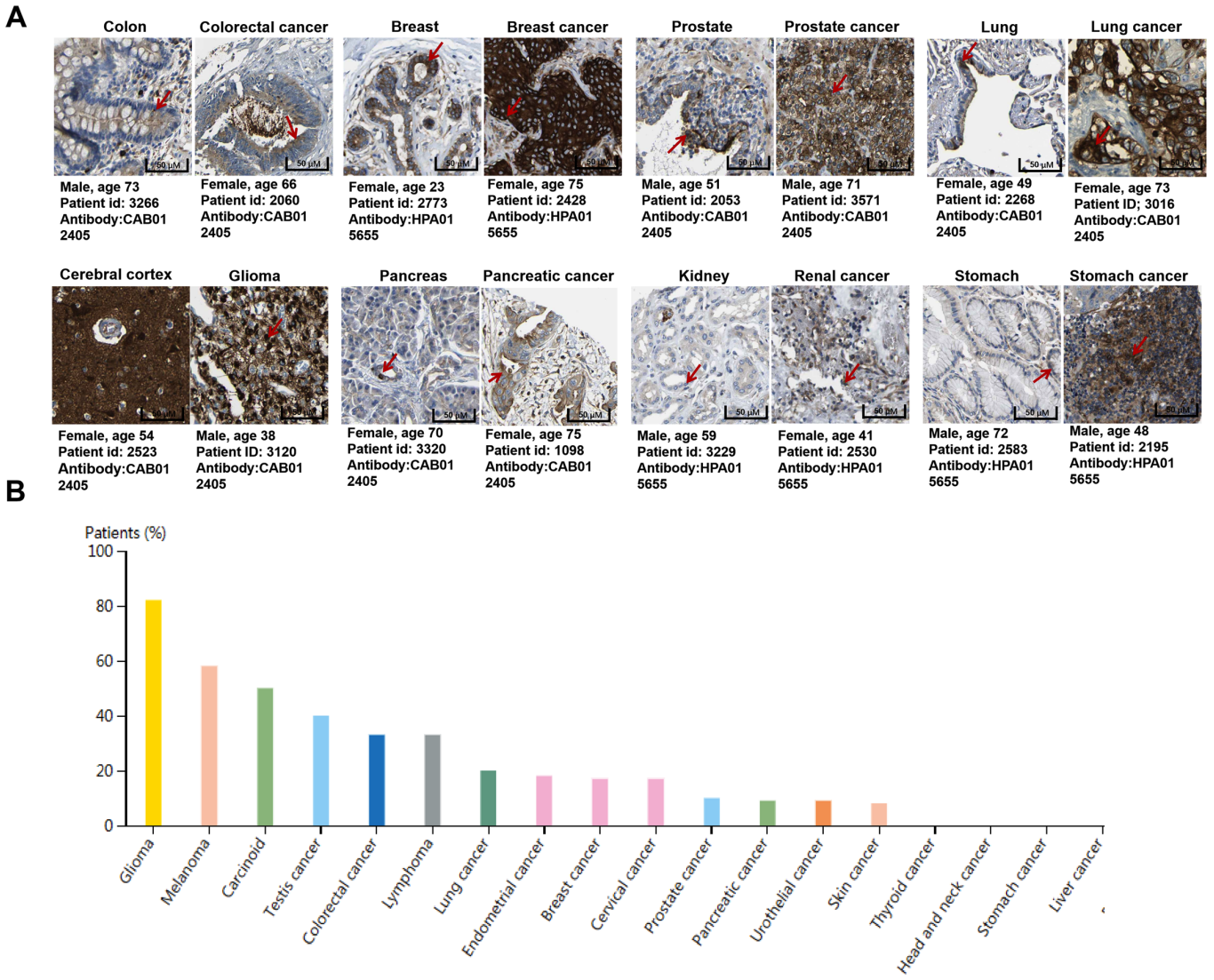

Figure 1. DCLK1 protein expression in different human normal and cancerous tissues. (A) Representative immunohistochemistry of DCLK1 staining in different human organs. The red arrows point at DCLK1-positive cells. Representative immunohistochemistry of DCLK1 staining in different types of human cancer (scale bars, 50 μm). (B) DCLK1 protein expression levels in different types of human cancer. All data were obtained from the human protein atlas (https://www.proteinatlas.org/search/dclk1). The Y-axis indicates the percentage of patients (maximum 12 patients) with high and medium DCLK1 protein expression. DCLK1, doublecortin-like kinase 1.

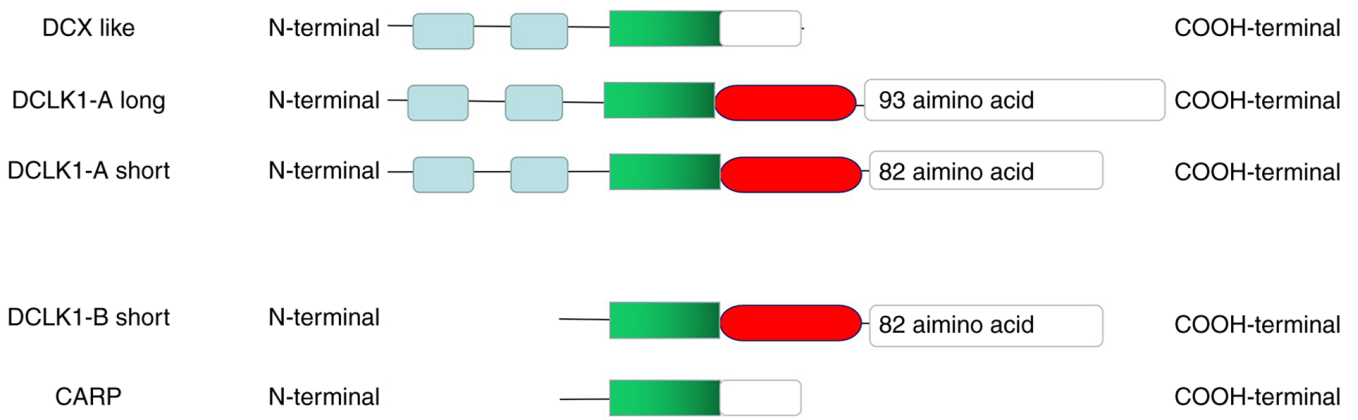

Figure 2. Schematic representation of the isoforms of n DCLK1 in humans. The light blue box represents DCX-like domains, the green box represents ser/pro-rich domains, the red box represents serine/threonine protein kinase domains and the white box represents different C-terminal regions. DCLK1-A long is 82.2 KD, including 740 AA, DCLK1-A short is 81.1 KD, including 729 AA, DCLK1-B long is 47.7 KD, including 433 AA, and DCLK1-B short is 46.5 KD, including 422 AA. CARP is ~56 AA. DCLK1, doublecortin-like kinase 1; DCX, doublecortin; AA, amino acids.

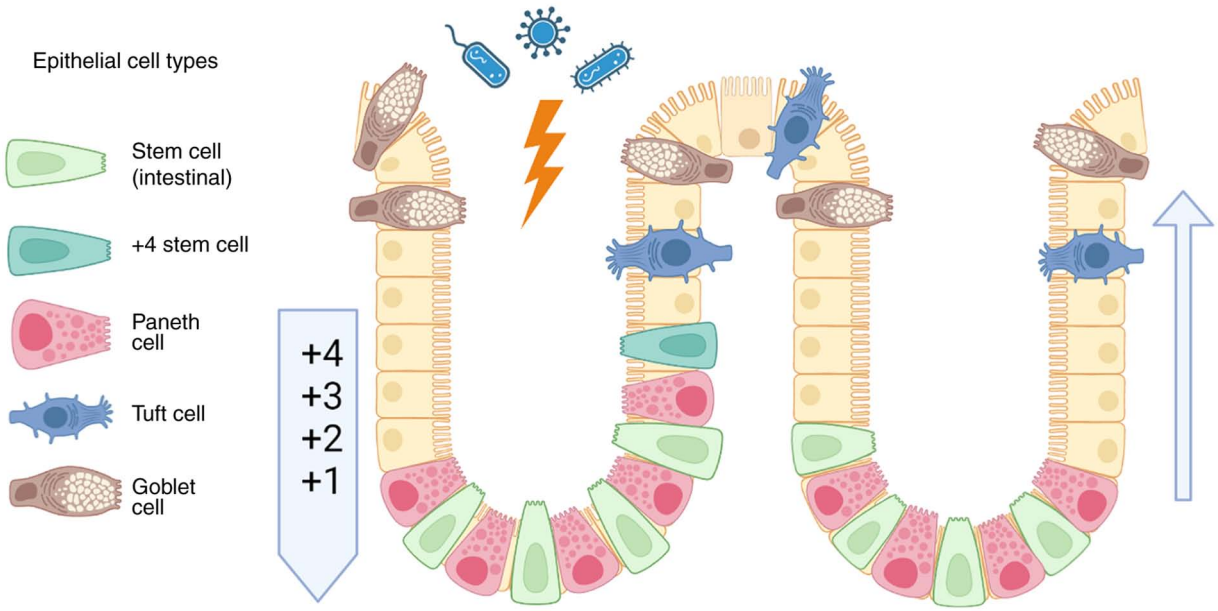

Figure 3. Self-renewal of the intestinal epithelium. Intestinal epithelial cells include ISCs, Paneth cells, enterocytes, goblet cells, tuft cells and enteroendocrine cells. At the base of the crypt, ISCs reside in a quiescent state (+4 position) and active state (+1 to +4 positions) interspersed with Paneth cells. To maintain homeostasis, and particularly in response to stimulation by injury from bacteria or pathogens and hypoxia, crypt base ISCs proliferate and then differentiate along the crypt-villus axis and move up, fully replacing the intestinal epithelium in short order. ICSs, intestinal stem cells.

Certain studies reported that DCLK1 expression is significantly higher in CRC tissue and adenomas compared to normal tissue. In addition, increased levels are seen in distant metastases and it is closely associated with CRC recurrence (36,37). Key evidence demonstrated that DCLK1 specifically marked CSCs in the intestine, which continuously produce tumor progeny to prompt polyp growth, but there is no apparent effect on normal tissue after deletion of these cells (38). Furthermore, overexpression of DCLK1 was observed to enhance the percentage of stem-like human CRC cells *in vitro* (39,40). It has become clear that DCLK1, while not a *bona fide* normal stem cell marker, is instead a key marker of differentiated intestinal epithelial tuft cells (6,32), which, in the context of mutation and tumorigenesis, may identify specific CRC stem cells.

A series of reports evidence that DCLK1 regulates EMT, proliferation and CSC maintenance through the Notch, Ras and Wnt pathways via interaction with different microRNAs (miRNAs/miRs). MiR-1291 was observed to directly bind to the 3'-untranslated region sequence of DCLK1 and then inhibited the stemness and cell cycle through the cyclin-dependent kinase inhibitors $p21^{WAF1/CIP1}$ and $p27^{KIP1}$ (41). The levels of miR-137 and miR-15a were inversely correlated with high levels of DCLK1 detected in CRC with larger tumor size, poor differentiation and lymph node involvement (42). Knockdown of DCLK1 expression led to downregulation of miR-200a, miR-144 and miR-let7a along with downregulation of EMT-associated transcription factors [zinc finger E-box binding homeobox 1 (ZEB1), ZEB2, Snail, Slug and Twist], c-Myc, KRAS and Notch-1 in human colon cancer cells (43). In a non-tumorigenic context, a recent study indicated that miR-195 is able to directly interact with DCLK1 mRNA, resulting in suppressed function for tuft and paneth cells in the small intestinal epithelium by inhibiting DCLK1 translation (44).

In CRC cell lines, Notch pathway-regulated markers of CRC CSCs [DCLK1, LGR5, aldehyde dehydrogenase 1 family member A1 (ALDH1) and CD44] by JAK2, STAT3 and ERK1/2 phosphorylation and increased expression of Jagged 1 to promote stemness (45). On the basis of the above findings, researchers have proposed potential therapeutics to suppress proliferation, colony formation and reduce the number of DCLK1+ cells via the Notch pathway (46,47). The Wnt pathway promotes elongator acetyltransferase complex subunit 3 expression and SOX9 translation, which in turn support LGR5(+)/DCLK1(+) intestinal cancer stem cells in response to intestinal regeneration after radiation-induced injury (28). Furthermore, β-catenin nuclear translocation is increased by overexpression of RNA binding motif protein 3, which induces stemness in DCLK1(+)/LGR5(+)/CD44(+) CRC cells (48). Wnt/β-catenin pathway and pluripotency transcription factors c-Myc, KLF transcription factor 4, OCT4 and SOX2 are activated by commensal-polarized macrophages through a microbiome-induced bystander effect in *E. faecalis*-colonized IL10 knockout mice, leading to increases in the number of DCLK1(+)/CD44(+) cells through gene mutation, chromosomal instability and endogenous transformation to promote tumorigenicity (35). Basic research indicated that compounds, such as γ-mangostin present in the mangosteen (*Garcinia mangostana*) fruit, WNT5A agonist FOXY5 and niclosamide, are able to regulate chemotherapy resistance and cancer stemness by decreasing the number of DCLK1-positive cells (21,49,50). KRAS mutation was observed to upregulate DCLK1 protein levels, which was reversed by inhibiting KRAS expression (51).

Pancreatic cancer. DCLK1 marks a small subpopulation of morphologically and functionally distinct pancreatic cancer cells, which promote tumorigenesis in multiple mouse

models (52,53). In normal adult pancreas, DCLK1 is expressed in ductal epithelial cells and islet cells (54) and it is upregulated in murine and human pancreatic intraepithelial neoplasia (55). It is co-expressed with neurogenin-3 and somatostatin, and pancreatic stem cell markers, but not with insulin and glucagon, which mark pancreatic α cells (24). DCLK1-positive cells isolated by flow cytometry injected into nude mice give rise to nodules with a hyperplastic appearance (56). Acetylated tubulin (AcTub), a marker of differentiation of specific pancreatic intraepithelial neoplasia, is frequently co-expressed with DCLK1 and regulates epithelial-mesenchymal transformation of pancreatic cancer cells. AcTub and DCLK1-marked cells demonstrate a typical tuft cell morphology with prominent microvilli at the apical surface of the cell and lead to increased size and number of spheroids in cancer self-renewal assays (53,57). Furthermore, these cells express high levels of ABL proto-oncogene 1, non-receptor tyrosine kinase and insulin-like growth factor 1 receptor, which are drug targets in clinical cancer therapy (58,59). DCLK1 was also reported to be a marker of a population of pancreatic cancer-initiating cells with morphological and molecular features of gastrointestinal tuft cells (53), which drive pancreatic tumor growth by immune cell-derived IL17, which in turn regulates POU class 2 homeobox 3, ALDH1A1 and IL17 receptor C (60). In the pancreas, DCLK1 marks pancreatic tuft and acinar, but rarely islet cells. DCLK1+ tuft cells expand in response to chronic injury or chronic inflammation, and DCLK1+ epithelial cells are a source of acinar-ductal metaplasia after Kras-G12D mutation. These findings indicated that DCLK1+ pancreatic cells may act as pancreatic intraepithelial neoplasia stem cells, but whether or not these arise from pancreatic DCLK1+ tuft cells or DCLK1+ acinar cells is a matter of debate (5,61).

In zebrafish and mouse models, it has been confirmed that $DCLK1^{+}$ cells are enriched in pancreatic intraepithelial neoplasia and their expansion is an early event in KRAS-induced pancreatic tumorigenesis (52,62). In an established KRAS transgenic mouse model of pancreatic cancer, DCLK1-positive CSC-like cells increased, while at the same time, knockdown of DCLK1 expression in human pancreatic cells reduced c-Myc and KRAS through a let-7a miRNA-dependent mechanism (43). In clinical tissue samples of PDAC, KRAS and TP53 mutations were indicated to be associated with DCLK1 gene overexpression, which may contribute to the migration, proliferation and colony formation abilities of pancreatic cancer cells (63). Mutation of KRAS in DCLK1+ pancreatic cells does not affect cell quiescence or longevity but contributes functionally to the pathogenesis of pancreatic cancer (24). In 2019, Qu *et al* (64) reported that DCLK1-α-long increases invasion and drug resistance by activating the PI3K/AKT/MTOR signaling pathway through increasing KRAS activity in pancreatic and duodenal homeobox $Pdx1^{Cre}KRAS^{G12D}$ transformation-related protein 53^{R172H} mouse models and in the human pancreatic cancer cell lines AsPC-1 and MiaPaCa-2. The hepatocyte growth factor/c-MET axis is necessary for the expression of DCLK1 in tumor cells and the recent two papers indicated that it is strongly associated with tumor immune escape, including the promotion of M2-macrophages and decrease of CD4+ and CD8+ T cells in PDAC (26,65). It facilitates pancreatic cancer progression by mediating the interaction between PCSCs and stromal pancreatic stellate cells (66). Overall, despite these complex and emerging findings, overwhelming evidence has indicated that DCLK1 is a vital pancreatic CSC-like marker, which is upregulated and closely associated with precancerous lesions, tumorigenesis and invasion (67-70).

Gastric cancer and esophageal cancer. In human normal stomach tissue, stem cells are located in the isthmus of gastric glands and DCLK1-positive cells were originally located in the gastric stem cell zone. These DCLK1-positive cells were not able to be labeled by bromodeoxyuridine, which was consistent with static stem cells lacking typical cell proliferation ability, suggesting that DCLK1 may be a marker of quiescent stem cells (71). The expression of DCLK1 in gastric cancer tissues was significantly higher than that in adjacent normal tissue and significantly correlated with lymph node metastasis and prognosis (72-75). A recent study suggested that long non-coding RNA small nucleolar RNA host gene 1 promoted the effects of DCLK1/Notch1 on the EMT process through regulating miR-15b expression (76). Small extracellular vesicle (exosome) isolated from a DCLK1-overexpressing human gastric cancer cell line promoted the migration of non-transfected gastric cancer cells in a kinase-dependent manner (77). DCLK1 is also a potential biomarker to predict the survival of patients with gastric cancer (78).

DCLK1 expression progressively increases from Barrett's esophagus to dysplasia and then to esophageal adenocarcinoma (2,79). In human esophageal squamous cell carcinoma (ESCC) cells, DCLK1-S induced MMP2 expression via MAPK/ERK signaling to activate the EMT (80). Knockdown of DCLK1 inhibited the progression of ESCC by regulating proliferation, migration and invasion by suppressing the β-catenin/c-Myc pathway (81). These results indicated that DCLK1 levels are associated with the occurrence and development of esophageal cancer. In Barrett's esophageal adenocarcinoma, the expression of DCLK1 and LGR5 are significantly increased in squamous epithelial cells located at the gastric spout, which indicates that Barrett's esophageal adenocarcinoma probably comes from gastric cancer (82,83).

Breast cancer. Serum estradiol levels are an important factor of increased risk of postmenopausal breast cancer. Haakensen *et al* (84) detected differentially expressed genes by analysis of gene microarrays and indicated that DCLK1 was one of six influenced by serum estradiol. DCLK1 gene expression was downregulated in breast carcinoma samples compared with normal tissue samples but did not exhibit any significantly differential expression between invasive breast cancer and ductal carcinoma *in situ*. DCLK1 was not significant as an independent factor associated with serum estradiol in a linear regression model. A series of subsequent studies on DCLK1 expression in breast carcinomas were developed and the clinical results indicated DCLK1 was associated with clinicopathological features, estrogen receptor status and neuroendocrine markers (85). A cohort study including 1,132 cases reported that DCLK1 levels varied in several molecular subtypes. Luminal cancers had higher DCLK1 expression than HER2-overexpression and triple negative breast cancers (TNBCs). Elevated DCLK1 was associated with a lower histologic grade, absence of lymphovascular invasion, fibrotic foci, necrosis and lower pN stage. DCLK1 did not correlate with other breast CSC markers and stem cell features, but significant correlations were found with

estrogen receptor and neuroendocrine markers. Zhao *et al* (86) used DCLK1 to devise a clinically practical method based on immunohistochemistry for the molecular subtyping of the mesenchymal subtype TNBC. Specifically, DCLK1 marked a mesenchymal subtype enriched in stem cell-related gene signatures and activated JAK/STAT3 pathway, which is highly correlated with CSC-like breast cancer cells (86). In support of these findings, Ramamoorthy *et al* (87) reported that DCLK1 is downregulated with ALDH and CD133 downstream of the Notch signaling pathway, which results in inhibition of TNBC stemness. In breast cancer cell lines, silencing of DCLK1 decreased the levels of Wnt/β-catenin pathway proteins such as β-catenin, c-Myc and cyclin D1 to decrease cell migration and invasion (88). Further basic studies indicated that DCLK1 is a molecular regulator of breast cancer proliferation, migration, invasion and a degradome-related metastatic stem-like profile (88-90). Furthermore, miR-424-5p was indicated to act as a tumor suppressive miRNA regulating breast cancer cell proliferation, migration and invasion via binding DCLK1 *in vitro* (90). In combination, these findings suggest that DCLK1 is a potential therapeutic target in breast cancer, but further mechanistic studies are required.

Renal cancer. Only a small number of known markers of CSCs in kidney cancers is available. Among these are the commonly reported broad CSC markers ALDH, CD44 and CD133. Ge *et al* (91) reported that DCLK1 stimulated essential molecular and functional characteristics of renal CSCs, including expression of ALDH, self-renewal and resistance to approved tyrosine kinase inhibitors sunitinib, sorafenib, everolimus and temsirolimus, suggesting that DCLK1 is a potential renal CSC marker. Furthermore, they indicated that overexpression of DCLK1 was a direct regulatory factor in renal clear carcinoma progression, supporting the notion that DCLK1 is a potential CSC target to inhibit RCC metastasis in early stages (3). Of note, treatment with a DCLK1-targeted monoclonal antibody was able to inhibit tumorigenesis in ACHN renal cancer xenografts, suggesting a potential therapeutic strategy for this highly chemoresistant cancer (91). In addition, a small-molecule kinase inhibitor of DCLK1, DCLK1-IN-1, demonstrated obvious inhibition of immune checkpoint ligand programmed death ligand 1 and an apparent increase in immune-mediated cytotoxicity alone or in combination with anti-programmed death 1 therapy by suppressing DCLK1 phosphorylation and downregulating pluripotency factors and CSC- or EMT-associated markers, including c-MET, c-MYC and N-cadherin in RCC cell lines. These experimental results were consistent with the analysis of clinical populations in which DCLK1 predicted RCC survival. In addition, its expression was correlated with reduced CD8+ cytotoxic T-cell infiltration and increased in M2 immunosuppressive macrophage populations (92).

Liver cancer. To date, DCLK1 has not been identified to be a hepatocellular CSC marker. However, the expression of DCLK1 in chronic hepatitis, cirrhosis and hepatocellular carcinoma was significantly increased (93). DCLK1 is mainly expressed in epithelial and stromal cells, lymphocytes and bile duct cells of liver tissue of patients with chronic hepatitis C virus infection. Furthermore, the level of DCLK1 is related to the expression of S100A9 protein (94). S100A9 is a key protein of pro-inflammatory signaling by binding to advanced glycation end product receptor and toll-like receptor 4 to activate the NFκB pathway (95). Upregulation of DCLK1 may promote the expression of S100A9 protein, while downregulation of DCLK1 directly reduces the expression of S100A9 protein and reduces signal cell infiltration of inflammatory cells (96). Ali *et al* (94) reported that DCLK1 was overexpressed in liver cells infected with hepatitis C virus and further results indicated that DCLK1 was involved in the replication of hepatitis C virus. According to recent findings, tuft cells express CD300lf (a murine norovirus receptor) and are virally induced to proliferate through this receptor to improve murine norovirus infection. Although research in this area is limited, it is worth considering if tuft cells in the intestine may similarly take part in the replication of hepatitis C and other viruses (97). Liver tissues from patients with cirrhosis and HCC exhibited overexpression of DCLK1, β-catenin and cleaved E-cadherin. DCLK1-overexpressing hepatoma cells induced high levels of β-catenin, α-fetoprotein and SOX9, which led to clonogenicity and dedifferentiated phenotypes (98). In HCC tumors, DCLK1-positive cells have characteristics of CSCs and co-express marker proteins CD133, LGR5, Lin28, AFP and c-Myc (99,100). DCLK1 may be a new target for the treatment of hepatitis C virus-induced tumorigenesis. However, the stem cell characteristics of DCLK1 in hepatocellular carcinoma require confirmation by further research.

All related signaling pathways of DCLK1 in different types of cancer are illustrated in Fig. 4.

4. DCLK1, a promising anti-tumor target

DCLK1 is one of the most important CSC markers due to its role in promoting tumorigenesis, metastasis, invasion and drug resistance by supporting self-renewal, stemness properties and quiescence, with activating signaling pathways including Wnt, Ras and Notch (101,102). DCLK1 represents a more specific CSC marker, compared with previously studied markers for colorectal, pancreatic and possibly other cancer types, such as gastric cancer, esophageal cancer, breast cancer and renal carcinoma. Development of drugs targeting DCLK1 has been reported, including kinase inhibitors LRRK2-IN-1, XMD8-92 and DCLK1-IN-1; monoclonal antibody CBT-15 (targeting DCLK1's extracellular C-terminus); and chimeric antigen receptor T-cell therapy (CAR-T_ CBT-511) (91,103-105). These drugs exhibited anti-tumor effects via regulating EMT, angiogenesis, proliferation, migration, invasion, apoptosis, cell cycle, DNA damage and stemness in several different cancer types (106) (Table I).

Notch, Wnt/β-catenin and RAS pathways are closely related to DCLK1 in regulating stemness, tumorigenesis, metastasis and drug resistance of several different cancer types. At present, increasing attention is paid to the energy metabolism of CSCs, as the common antitumor treatments aiming to decrease tumor size or reduce proliferating tumor cells may fail to target CSCs, which accounts for this therapeutic treatment resistance (107). Furthermore, the metabolic type for CSCs is primarily dominated by oxidative phosphorylation but not glycolysis, as CSCs consume more oxygen, produce higher levels of ATP and increase mitochondrial mass and membrane potential compared with the bulk of differentiated cancer cells, which rely on glycolysis. The

Table I. Studies reporting inhibition of DCLK1.

Cancer type	Cancer subtype	Intervention	Level of evidence	Pathways affected	Functional effect	(Refs.)
CRC	None	Honokiol with ionizing radiation	*In vivo* and *in vitro*	Notch pathway	Inhibition of tumor growth and enhancement of the sensitivity to radiation	(115)
CRC	None	AG490 and PD98059	*In vivo* and *in vitro*	Notch pathway	Prolactin activates the Notch pathway, then regulating the CSC population	(45)
Pancreatic cancer	None	Quinomycin	*In vivo* and *in vitro*	Notch pathway	Targeting of CSCs and inhibition of tumor growth	(47)
CRC	None	*Alcea rosea* seed extract	*In vivo* and *in vitro*	Notch pathway and Wnt/β-catenin pathway	Inhibition of tumor growth	(46)
CRC	None	XAV939, dibenzazepine, siRNA to DCLK1	*In vivo* and *in vitro*	miR-153-3p/Wnt/β-catenin pathway	Infection-induced signals regulate the Wnt signaling pathway	(116)
CRC	None	Cucurbitacin B and cucurbitacin I	*In vivo* and *in vitro*	Notch pathway	Inhibition of tumor growth and proliferation, induction of apoptosis	(117)
Pancreatic cancer	None	siRNA-mediated knockdown of DCAMKL-1	*In vivo* and *in vitro*	miR-200a/ZEB1, miR-144/notch, miR-let-7a/Kras	Inhibition of stemness, invasion and EMT	(68)
Breast cancer	Triple negative	Celastrol and triptolide	*In vitro*	Notch pathway	Inhibition of proliferation and stemness	(87)
CRC	None	(2'Z,3'E)-6-bromoindirubin-3'-oxime	*In vitro*	Wnt/β-catenin pathway	RBM3 induces stemness	(48)
CRC	None	Niclosamide	*In vivo* and *in vitro*	Wnt/β-catenin pathway	Suppression of tumor growth and cancer stem-like cell populations	(21)
CRC	None	Foxy5	*In vivo*	Wnt/β-catenin pathway and PGE2 signaling pathway	Modulation of CSCs	(50)
CRC	None	Silibinin	*In vitro*	Wnt/β-catenin pathway	Induction of apoptosis, suppression of migration, elimination of CSCs and attenuation of EMT	(118)
Pancreatic cancer	None	XMD8-92	*In vivo* and *in vitro*	pluripotency, angiogenesis and anti-apoptotic pathway	Inhibition of cell proliferation, EMT and tumor xenograft growth	(119)
Pancreatic cancer	None	Gemcitabine, everolimus, LY-294002 and ABT-199	*In vivo* and *in vitro*	PI3K/AKT/mTOR	Inhibition of tumorigenesis, metastasis and drug resistance	(64)
Pancreatic cancer	None	UNC0638	*In vivo* and *in vitro*	MAPK pathway	Initiation of oncogenic Kras-driven pancreatic carcinogenesis	(120)

AG490 is a JAK inhibitor, PD98059 is an ERK inhibitor, XAV939 is a Wnt/β-catenin inhibitor, dibenzazepine is a γ-secretase inhibitor, LRRK2-IN-1 is a DCLK1 inhibitor, (2'Z,3'E)-6-bromoindirubin-3'-oxime is a GSK3β inhibitor, XMD8-92 is a kinase inhibitor, everolimus is an mTOR inhibitor, LY-294002 is a PI3K inhibitor, ABT-199 is a BCL-2 inhibitor, UNC0638 is a G9a inhibitor and Foxy5 is a WNT5A agonist. LEF1, lymphoid enhancer-binding factor 1; RBM3, RNA binding protein RBM3; PGE, prostaglandin E; ZEB1, zinc finger E-box binding homeobox 1; CRC, colorectal cancer; CSC, cancer stem cell; EMT, epithelial-to-mesenchymal transition; siRNA, small inhibitory RNA; miR, microRNA; DCLK1, doublecortin-like kinase 1.

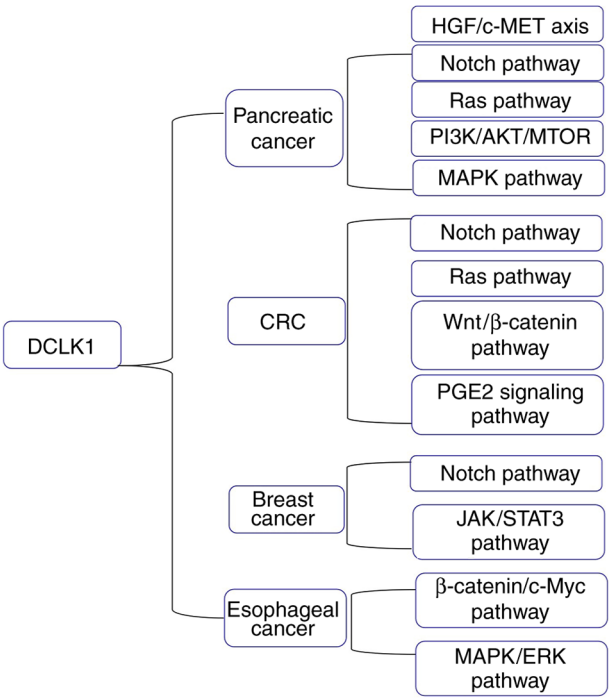

Figure 4. DCLK1-related signaling pathways in different cancer types. It has been indicated that DCLK1 has a key role in the Notch, Ras and PI3K/AKT/mTOR pathways in pancreatic cancer; Notch, Ras, Wnt/β-catenin and PGE2 signaling pathways in CRC; and Notch and JAK/STAT3 pathways in breast cancer. CRC, colorectal cancer; PGE, prostaglandin E; HGF, hepatocyte growth factor; DCLK1, doublecortin-like kinase 1.

limited but emerging data in this field suggest the importance of further investigation of the relationship between DCLK1 and DCLK1+ CSCs and metabolism (108).

Despite promising findings regarding DCLK1-targeted agents, successfully targeting DCLK1 and avoiding toxicity and other concerns will require a thorough exploration of the roles of DCLK1 in other biological aspects. In 2013, Verissimo *et al* (109) first reported that knockdown of DCL, a splice variant of DCLK1, is related to reduced mitochondrial activity, which significantly decreased tumor growth in neuroblastoma xenografts. In this study, DCL affected oxidative phosphorylation by interacting with the mitochondrial outer membrane protein outer membrane protein 25/synaptojanin 2 binding protein. However, DCL lacks the kinase domain and kinase catalytic and autoinhibitory activity present in other prominent DCLK1 isoforms (110). However, new evidence suggests that DCLK1 may also be important in conditions of altered metabolism. First, MCF-7 breast cancer cells deregulated the metabolism by triggering transcriptomic reprogramming closely related to DCLK1 levels (111). These findings suggest accelerated dedifferentiation towards a more stem-like state and that DCLK1 may be a key part of this process. Coincidentally, in a non-cancer context, an isoform of DCLK1, candidate plasticity gene 16 (CPG16; also known as DCLK1-BL or DCLK1-Short), was identified as a negative regulator of insulin gene expression, which was increased by long-term exposure of pancreatic β-cells to a high-glucose medium (112). In addition, CPG16 suppressed the jun dimerization protein 2-mediated upregulation of insulin promoter activity in a kinase activity-dependent manner under glucotoxic conditions (113). Of note, Zhao *et al* (114) reported that glycolysis promotes the expression of DCLK1 and maintains the CSC and EMT phenotypes via maintenance of low reactive oxygen species levels in gemcitabine-resistant Patu8988 pancreatic cancer cells. Together, these findings suggest that DCLK1 may be a key target of glucose metabolism inhibiting drugs such as metformin, which may be helpful in decreasing the incidence of cancer. The limited but emerging data in this field suggest the importance of further investigation of the relationship between DCLK1 function and metabolism.

5. Conclusion and future directions

DCLK1 as a marker of tuft cells and CSCs is closely related to tumorigenesis and metastasis in various cancer types, including gastrointestinal, breast, renal and other cancers. The DCLK1 isoforms have different functions in the development and progression of the above cancers. Furthermore, the evidence for the emergence of tumors related to various signaling pathways has been linked to DCLK1 in the literature (e.g. Notch, WNT and RAS signaling pathways). Several drugs have been developed by targeting the genetic or kinase activity of DCLK1, and in the future, metabolic regulation via glycolysis and regulation of insulin expression by targeting DCLK1 is worthy of further study.

It is well known that DCLK1 expression is obviously significant in melanoma, testicular cancer, lymphoma and endometrial cancer (9), besides the above ones, but only a small number of studies have been performed on them until now. Thus, by including these data, it is esteemed that other groups in these specific subfields of oncology may become aware of and consider researching DCLK1 in their respective projects.

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Availability of data and materials

Data sharing is not applicable.

Authors' contributions

Study conception and design: ZC, ZY and QL; Searching and selection the literature: QL, HF, HC and JD; writing the original manuscript: QL, HF, HC; revision of the manuscript: ZC, ZY and NW. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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