

DCLK1 and its interaction partners: An effective therapeutic target for colorectal cancer (Review)

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Abstract. Doublecortin-like kinase protein 1 (DCLK1) is a microtubule-associated protein with a C-terminal serine/threonine kinase domain. Its expression was first reported in radial glial cells, where it serves an essential role in early neurogenesis, and since then, other functions of the DCLK1 protein have also been identified. Initially considered to be a marker of quiescent gastrointestinal and pancreatic stem cells, DCLK1 has recently been identified in the gastrointestinal tract as a marker of tuft cells. It has also been implicated in different types of cancer, where it regulates several vital pathways, such as Kras signaling. However, its underlying molecular mechanisms remain unclear. The present review discusses the different roles of DCLK1 and its interactions with other proteins that are homologically similar to DCLK1 to develop a novel therapeutic strategy to target cancer cells more accurately.

Contents

1. Introduction
2. Functions of DCLK1 and its role in cancer
3. DCLK1 and its interacting partners
4. Genomic evidence
5. DCLK1 as a potential small-molecule target in CRC
6. Conclusion

1. Introduction

Doublecortin-like kinase protein 1 (DCLK1), also known as DCAMLK1 and CLICK1, was first identified in the developing

rodent brain as a brain-specific protein (1,2). The *DCLK1* gene is present on the 13q13.3 loci of the human chromosome 13, and codes for a member from the protein kinase superfamily and the doublecortin family (2). The DCLK1 protein contains two N-terminal doublecortin domains, which bind to microtubules and regulate their polymerization, while its C-terminal serine/threonine-protein kinase domain, with substantial homology to Ca²⁺/calmodulin-dependent protein kinase (3-6), is in between the doublecortin and the protein kinase domains, mediating multiple protein-protein interactions (7). Previous studies have reported that this protein is highly expressed in the radial glial cells and neuronal precursors, suggesting a potential role in early neurogenesis (3,4,7).

DCLK1 is expressed in tuft cells of the gastrointestinal tract, and is also expressed at low levels in normal gastrointestinal cells and upregulated in several gastrointestinal malignancies (8). It is also considered a marker in cancer stem cells and cancer-initiating cells (8-11). The *DCLK1* gene is speculated to serve a role in the epithelial-to-mesenchymal transition (EMT) of tumor cells by regulating the NOTCH, WNT and NFκB signaling pathways (12,13). Fig. 1 illustrates the important findings of DCLK1 throughout the years. Proteins that share similar functional homology and co-expression patterns have been demonstrated to interact with each other (14). The present review discusses proteins that exhibit homology, co-expression or interaction with DCLK1. Previous studies have reported the role of DCLK1 in cancer cell self-renewal (14,15). Thus, it is important to identify a novel inhibitor for DCLK1.

2. Functions of DCLK1 and its role in cancer

Normal function of DCLK1. DCLK1 is highly expressed in tuft cells of the gastrointestinal tract. These tuft cells are similar to taste cells and serve a chemosensory role in the small intestine and colon (16). However, their primary function is to initiate a T helper cell immune response against parasites (17). Tuft cells exhibit self-renewal proliferative abilities due to the expression of DCLK1 (18). The microtubule-associated protein coded by *DCLK1* has three major splice variants (DCLK, DCLK DCX-like and CPG16), with altered kinase activities. In addition, DCLK1 exhibits differential splicing in embryonic tissues compared with adult tissues (5). The embryonic forms of DCLK1 and DCLK-like proteins exhibit considerably higher expression in post-mitotic neurons and

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neuronal progenitor cells (radial glial cells) compared with other neuronal cells (5). The post-mitotic neurons also express DCX (3,5). Furthermore, DCLK1 is highly expressed in developing mammalian brains, particularly in the neocortex and cerebellum regions, where active neurogenesis occurs. However, this expression is diminished in adults (19). DCLK1 stimulates the polymerization of tubulins while interacting with microtubulins through the tandem DCX domains, and is highly expressed in the brain (5).

Given that the temporal and spatial expression pattern of doublecortin is very similar to that of DCLK1, DCLK1 is proposed to have functions similar to that of doublecortin (20,21). Thus, it may serve a role in neuronal migration, axon transport, synapse maturation and brain development (21-24). Furthermore, DCLK1 has two isoforms of varying lengths because of the epithelial changes that occur with different functions, where the shorter isoform DCLK1-S induces tumorigenesis in colorectal cancer (CRC) (7,25,26). DCLK1 labels a subset of dendritic microtubules and is required for trafficking KIF1-dependent dense-core vesicles into dendrites and dendritic development (23). Although these tuft cells have significant self-renewal properties, they are not stem cells. Lineage tracing studies have demonstrated that while these cells can be a part of the reserve progenitor cells that originate from rapidly cycling or quiescent stem cells, they do not exhibit lineage tracing similar to that of the stem cells while resting or under duress (27-30). Thus, they cannot be considered quiescent or active intestinal stem cells (30). DCLK1 helps in maintaining intestinal homeostasis, along with assisting tissue regeneration (8,31-32). Thus, knocking down DCLK1 in mouse models of DDS-induced colitis followed by completely irradiating it results in the loss of DCLK1, exacerbating tissue injury and halting the tissue regeneration (33,34). However, whether this is due to a deficiency in the critical tuft cell-derived niche factors or a contribution to the regenerative program by DCLK1-expressing epithelial cells remains to be investigated (35,36).

DCLK1 expression in cancer. DCLK1 performs a variety of functions associated with tumorigenesis (37). The malignancy of every cancer is dependent on the metastasizing ability of a tumor; for example, the ability to move to a different organ. Angiogenesis and EMT are essential for the metastasis of cancer cells. DCLK1 regulates metastasis by controlling signaling pathways, such as the NOTCH, WNT, RTK, TGF- β and Hedgehog pathways (37). DCLK1 is also associated with the enhancement of angiogenesis in pancreatic tumors (38). Upregulation of the *DCLK1* gene downregulates microRNA (miRNA/miR)-200a expression, which in turn upregulates the expression of EMT-related transcription factors, such as ZEB1, ZEB2, SNAIL and SLUG (25,39), resulting in increased angiogenesis and metastasis. Similarly, upregulation of DCLK1 also downregulates miR-143/145 expression in CRC and pancreatic cancer, which increases the expression of maintenance factors, such as NANOG, OCT4, KLF4, SOX2, RREB1 and KRAS, eventually increasing the pluripotency tumorigenicity of these types of cancer (40,41). DCLK1 serves a role in various cancer functions, such as drug resistance, metastasis, secondary tumor formation and cancer recurrence (12,42-46). In addition, it helps regulate cell proliferation and invasion in Hodgkin's lymphoma (47). Overexpression of DCLK1 in

primary human hepatocytes has been demonstrated to form spheroids in suspension cultures. Furthermore, these cells express high levels of β -catenin, α -fetoprotein and SOX9, suggesting that DCLK1 can induce clonogenicity in hepatoma cells (48). Table I illustrates the role of DCLK1 in three of the most prevalent types of cancer. It also lists information on different proteins and pathways regulated by DCLK1.

DCLK1 as a cancer stem cell marker. Analyzing pre-invasive pancreatic cancer cells in mouse models has demonstrated that a subpopulation of these cancer cells are morphologically similar to the gastrointestinal tuft cells, with similar stem cell-like properties (49). It has also been reported that DCLK1 is highly expressed in these cells, serving as a potential biomarker for cancer stem cells (50). Following this discovery, it has been demonstrated that DCLK1 has similar biomarker properties in CRC and osteosarcoma (50). Immunoassay methods were performed to identify circulating cellular protein DCLK1 in CRC stem cells, proving it to be the most promising cancer stem cell marker (51-54). Thus, DCLK1 is not just a target for diagnostic purposes but is also useful in therapeutic settings (12,42,55-59).

DCLK1 as a therapeutic target for cancer. Targeting DCLK1 with antibodies has helped accurately screen for CRC (60). Inhibition of DCLK1 using miR-137 and other alternate splicing methods is effective in decreasing tumorigenesis in CRC and kidney cancer (10). miR-195 targets DCLK1 and successfully reduces the pluripotency and EMT in pancreatic cancer cells (61). It is also targeted in non-small cell lung carcinoma to increase chemosensitivity of the cancer cells (62). In CRC, the sensitivity of cancer cells against chemotherapy and radiation therapy is enhanced by targeting DCLK-KRAS, using miR-15b (63). DCLK-KRAS is associated with increased tumor cell invasion in 95% of pancreatic ductal adenocarcinomas (PDAC), and is also considered an undruggable target (63). DCLK1 is associated with increased KRAS expression via the PI3K/AKT/mTOR-pathway (40). Thus, DCLK1 can potentially be targeted to decrease KRAS expression and control tumor cell invasion in PDAC.

The B cell-specific moloney murine leukemia virus insertion site 1 (Bmi-1) is a crucial regulator for the self-renewal, malignant transformation and EMT of cancer stem cells, and is upregulated in pancreatic cancer (64). DCLK1 knockdown suppresses cell proliferation, both *in vitro* and *in vivo*, and inhibits the migration and invasion capacities of pancreatic cancer cells by decreasing Bmi-1 expression; thus, suggesting a potential novel strategy to treat pancreatic cancer (64). It has been reported that DCLK1 can be selectively silenced using let-7a miRNA, which arrests tumor growth in human CRC cells (65). Recently, it was demonstrated that the co-localization of DCLK1 with autophagy-related protein p62 happens due to accumulation of DCLK1 in colon cancer cells (65). In addition, crocetin acid targets DCLK1 in cancer cells by inhibiting the hedgehog signaling pathway in pancreatic cancer cells (65).

3. DCLK1 and its interacting partners

DCLK1 and DCX. DCX or doublecortin is present on the X chromosome at Xq23 and codes for the doublecortin family

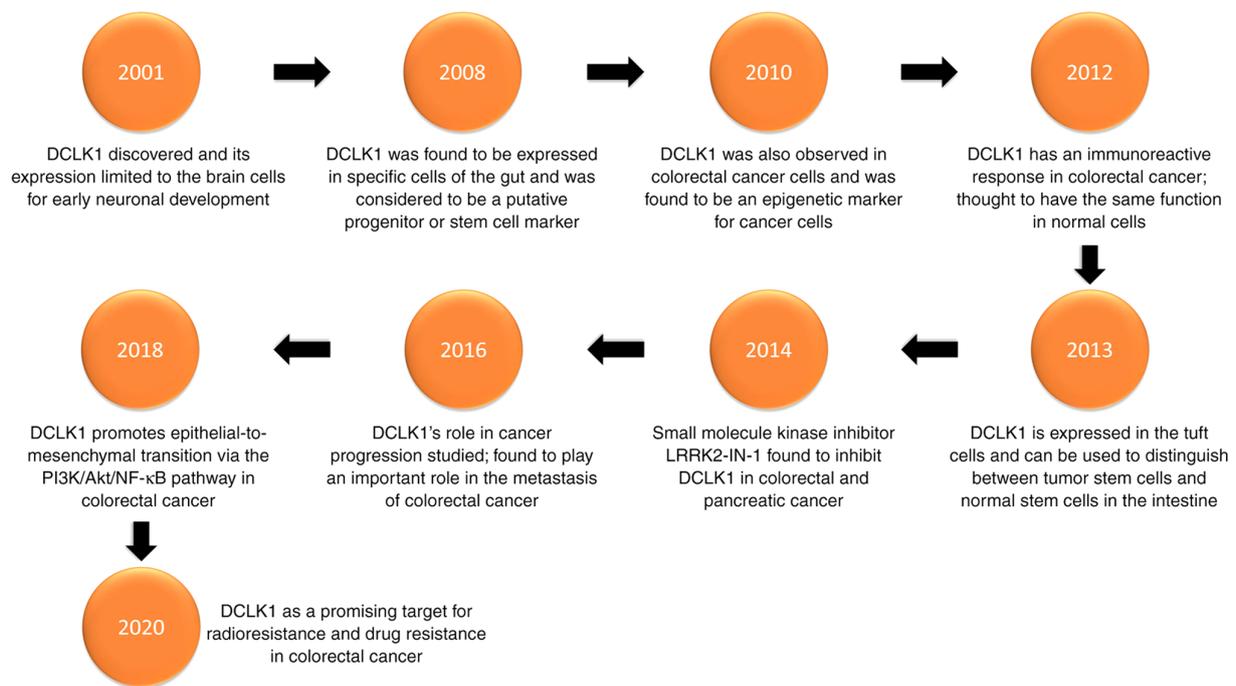


Figure 1. A timeline illustrating current breakthroughs and findings surrounding DCLK1 in colorectal cancer. DCLK1, doublecortin-like kinase protein 1; LRRK2-IN-1, leucine-rich repeat kinase 2 inhibitor 1.

of proteins (66,67). DCX is a cytoplasmic microtubule-associated protein that helps stabilize microtubules (68-70). DCX and DCLK1 have protein profiles with very high homology and similar functions (71), suggesting a functional redundancy or functional equivalence. Their functional association was assessed by knocking down the *DCLK1* gene via the RNAi strategy in rats. The results demonstrated that a dose-dependent interaction exists between DCX and DCLK1 in commissural fiber tract formation (72), which is also associated with humans, although not proven by experimental procedures (73,74). In humans, DCX and DCLK1 are hypothesized to compete with each other to bind to the target proteins. This competitive binding may be a way for either protein to participate in a signaling pathway crucial for neuronal interaction before and during migration, which may be part of a calcium ion-dependent signal transduction pathway (75). Fig. 2 presents the co-expression of DCLK1 and DCX, and demonstrates the interaction between them.

DCLK1 and ANK2. The *ANK2* gene codes for a family of proteins that play key roles in cellular functions, such as cell motility, activation, proliferation, cell-cell contact signaling and the maintenance of specialized membrane domains (76). In humans, the *ANK2* and *DCLK1* genes are implicated in co-expression networks, with an RNA co-expression score of 0.305 (Fig. 2) (71). *ANK2*, similarly to DCLK1, serves a role in the progression of gastric cancer. While DCLK1 plays a role in promoting the EMT process of gastric cancer and helps in lymphovascular invasion (77,78), *ANK2* serves a role in promoting the proliferation of the cancer cells. Targeting and silencing *ANK2* using miR-647 inhibits the proliferation of gastric cancer cells (79,80). Notably, while trying to identify the genes regulating normal hearing, an audiometric pattern has been observed in the expression of the genes. Differences

between different mice genotypes have also been observed, supporting *ANK2*'s role in hearing function (81-83).

DCLK1 and MSII. The *MSII* gene, also known as Musashi RNA binding protein 1, encodes a protein containing two conserved tandem RNA recognition motifs (83). Similar proteins in other species function as RNA-binding proteins, playing central roles in post-transcriptional gene regulation (84). The *MSII* protein is associated with various factors, including the grade of the malignancy, proliferative activity in glioma melanomas, esophageal cancer and colon cancer (85-87). The *MSII* gene was initially identified as a neuronal stem cell marker, which can affect cell cycle regulation, proliferation and apoptosis by suppressing the expression of certain mRNAs and other genes (88,89). The *MSII* gene, similar to the *DCLK1* gene, is also an intestinal stem cell marker, which is expressed alongside DCLK1 in gastric cancer cells (90). Targeting and silencing of the *MSII* gene activates certain tumor-suppressing mRNAs, inhibiting the growth of cancers (88,91-93). The Musashi RNA binding protein activates the WNT and NOTCH signaling pathways to regulate proliferation (91). In humans, the *MSII* and *DCLK1* genes are a part of multiple co-expression networks (71), and it has been reported that DCLK1 is often expressed in cells that also express the *MSII* gene, namely the long-lived tuft cells (94). This suggests that *MSII* may interact with DCLK1 to help tumor progression.

TNIK and CALM1 proteins. According to GeneMANIA (<https://genemania.org>), DCLK1 physically interacts with TRAF2 and NCK interacting Kinase (TNIK) and calmodulin (CALM1) proteins (95). The TNIK protein is an activator of the WNT signaling pathway, which is regulated in several types of cancer, including CRC (96). Thus, DCLK1 can directly or

Table I. Comparing three of the most prevalent types of cancer and the functions of DCLK1.

Characteristic	Colorectal cancer	Pancreatic cancer	Gastric cancer
Role of DCLK1	Promotes EMT	Promotes stem cell pluripotency, angiogenesis and EMT	Cancer initiation, progression and EMT
Proteins regulated by DCLK1	P65 subunit of the NF- κ B transcription factor; Sp1 transcription factor	Pluripotency factors, such as OCT4, SOX2, c-MYC, LIN28, NANOG and KLF4	P65 subunit of the NF- κ B transcription factor; Sp1 transcription factor
Pathways regulated by DCLK1	PI3K/Akt pathway	NOTCH signalling pathway	NOTCH, NF- κ B, KRAS, and WNT molecular signalling pathways
Therapeutic treatment	Targeting DCLK1 with inhibitors, such as LRRk2-IN-1 small molecule	Downregulation of DCLK1 upregulates tumor suppressor mRNAs	Targeting DCLK1 with inhibitors, such as LRRk2-IN-1 small molecule

DCLK1, doublecortin-like kinase protein 1; EMT, epithelial-to-mesenchymal transition; NF- κ B, nuclear factor κ light chain enhancer of activated B cells; OCT4, octamer-binding transcription factor 4; SOX2, SRY (sex determining region Y)-box 2; KLF4, Kruppel-like factor 4; LRRk2-In-1, Leucine-rich repeat kinase 2 Inhibitor 1.

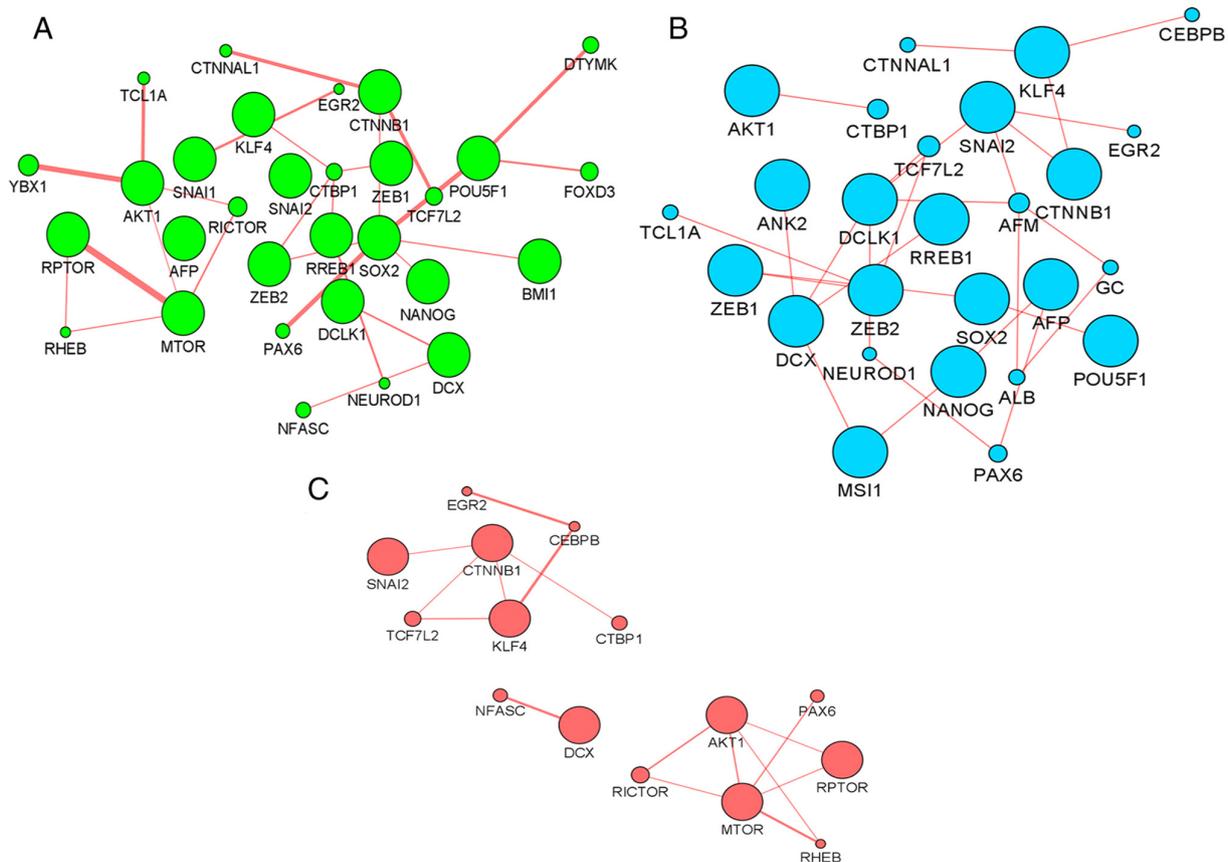


Figure 2. Networks generated using Cytoscape software (v.3.8.2; on Java 11.0.6 by AdoptOpenJDK) to elucidate the interaction between DCLK1 and different proteins. (A) Co-expression patterns, (B) consolidated pathways and (C) physical interactions. Circles represent proteins; DCLK1, doublecortin-like kinase protein 1.

indirectly interact with TNIK to regulate the WNT signaling pathway. The CALM1 protein mediates the control of various enzymes, ion channels, aquaporins and other proteins via calcium-binding (97). The C-terminal of the DCLK1 protein

has a serine/threonine-protein kinase domain, which exhibits substantial homology to Ca²⁺/calmodulin-dependent protein kinase, making it possible for the CALM1 protein to regulate DCLK1 through its domain (Fig. 2).

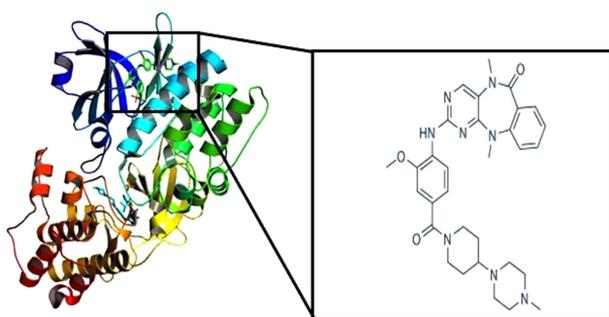


Figure 3. Doublecortin-like kinase protein 1 protein bound to the kinase domain of small molecule LRRk2-IN-1 (left). Chemical structure of LRRk2-IN-1 (right). LRRk2-IN-1, leucine-rich repeat kinase 2 inhibitor 1.

4. Genomic evidence

In 2015, scientists performed a series of genomic experiments to identify the protein interactions, where high-throughput affinity purification mass spectroscopy was performed to identify the interacting partners of 2,594 proteins in HEK293T cells (75). The resulting network (BioPlex) revealed 23,744 interactions among 7,668 proteins, with 86% interactions previously undocumented (75). In 2017, the same scientists released Bioplex 2.0, which contained interacting partners of 25% of protein-coding genes, with >29,000 previously unknown co-associations (76). According to the Bioplex 2.0 network, the highest-scoring interacting proteins with DCLK1 were cell division cycle associated 8 (CDCA8), deoxyguanosine kinase (DGUOK), filamin binding LIM protein 1 (FBLIM1), HCLS1-associated protein X1 (HAX1) and nuclear FMR1 interacting protein (NUFIP1). All these five proteins were found to be molecular targets of DCLK1. CDCA8 plays roles in mitosis and cell division, and also helps in chromatin-induced microtubule stabilization (98). Being a microtubule-associated protein, DCLK1 may be associated with CDCA8. Overexpression of CDCA8 promotes proliferation of bladder cancer cells (98). DGUOK has a similar kinase function to DCLK1 and can regulate cancer cell stemness (99). FBLIM1 helps in cell adhesion and motility (100), and when overexpressed in glioma, it increases cancer cell migration, which can be induced by DCLK1 as it plays a role in angiogenesis and metastasis (100). HAX1 is a potential oncogene for hypopharyngeal carcinoma, which increases cancer cell proliferation and migration (101). The NUFIP1 protein interacts with the tumor suppressor protein, BRCA (102). Notably, proteins with high scores have an implicated role in different types of cancer.

5. DCLK1 as a potential small-molecule target in CRC

The aforementioned profile insights of the *DCLK1* gene prove that they are key components of CRC pathogenesis (13). Targeting the DCLK1 kinase domain to inhibit its function may be an effective strategy for treating CRC (52). The interaction profile of DCLK1, along with its co-expressing genes, seem to share a strong homology (52,103); thus, there is a requirement for a specific small molecule to inhibit DCLK1. It has been demonstrated that the inhibitor of Leucine-rich repeat kinase 2

(LRRk) protein, LRRk2-IN-1, a key regulator in Alzheimer's disease, inhibits DCLK1 *in vitro* (103). Recently, an analog of LRRk2-IN-1 was designed and successfully tested as a DCLK1 inhibitor (Fig. 3) (103). Although these small molecules are effective, there is ambiguity regarding their non-specificity to DCLK1, since DCLK1 also has strong homology to other interaction partners (103,104). Thus, 3D pharmacophore-based strategy for high throughput virtual screening (HTVS) can be applied against a natural compound databases (105). These databases have millions of compounds that are commercially available. Thus, HTVS combined with molecular docking and molecular dynamics studies may provide insights into secondary structure changes induced by small-molecule binding. Such computational methods combined with *in vitro* validation can help identify effective inhibitors for DCLK1 (105). Furthermore, building a pharmacophore model from molecular dynamics simulations of LRRk2-IN-1, which is a recent approach in identifying potential small molecule inhibitors, can be an effective strategy in designing a novel small molecule inhibitor for DCLK1 (106). A computational quantum mechanical modeling method, such as density functional theory, can be used to study the electronegativity of these small molecules interacting with DCLK1 (107-109).

6. Conclusion

DCLK1 was initially identified for its function in neuronal development, but has recently been implicated as an epigenetic marker in tuft cells of the intestine, and also in tumorigenesis. DCLK1 interacts with several proteins and regulates various pathways to perform functions, ranging from cancer initiation to metastasis. Understanding the pathways and their interactions with DCLK1 is important to develop effective treatment strategies for different types of cancer. Currently, targeting and silencing DCLK1 using small molecule kinase inhibitors is a good therapeutic strategy for treating colorectal cancer. However, this strategy is non-specific and thus is inefficient. In addition, the underlying molecular mechanisms of DCLK1 remain unclear. Proteins with a similar homology and co-expression pattern to DCLK1 can be used to determine the exact molecular mechanism of the protein. With the development of techniques, such as next-generation 3D pharmacophore modeling, the profiles of DCLK1 and its interaction partners can be extensively investigated (110-114). Understanding the underlying molecular mechanisms of DCLK1 is important in identifying small molecules that can effectively bind and inhibit DCLK1. In addition, targeting the interacting partners of DCLK1 may be an effective treatment strategy.

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Authors' contributions

MV drafted the initial manuscript. MB generated and interpreted the images and contributed to a concept that deals with the interaction of DCLK1 protein. SR critically reviewed the manuscript for important intellectual content. AT conceptualized the hypothesis and performed a critical review of the paper. Data authentication is not applicable. All authors have read and approved the final manuscript.

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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