1. Phylogeny  
   DCLK2 (gene symbol DCLK2; also known as DCAMKL2, DCDC3B, or DCK2) belongs to the doublecortin (DCX) family of serine/threonine kinases. It is evolutionarily related to other members of this family, including DCLK1 and DCLK3, and shares a common ancestry with proteins harboring tandem DCX domains fused to a kinase domain. The DCX family is broadly conserved among vertebrates, and DCLK2 can be traced to common ancestors within the metazoan lineage, similar to other CaMK-like kinases that have undergone diversification in their regulatory and catalytic properties (reiner2006theevolvingdoublecortin pages 11-12, hu2024kinomewidesirnascreen pages 1-3).
2. Reaction Catalyzed  
   DCLK2 catalyzes the transfer of a phosphate group from ATP to the hydroxyl group of serine and/or threonine residues on substrate proteins. In chemical terms, the reaction is as follows:  
   ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺ (hu2024kinomewidesirnascreen pages 10-11).
3. Cofactor Requirements  
   Like many serine/threonine kinases, DCLK2 requires divalent metal ions—most notably Mg²⁺—as a cofactor for catalytic activity. Although members of the CaMK family typically demonstrate calcium/calmodulin responsiveness, DCLK2 is characterized by a significantly reduced Ca²⁺/calmodulin affinity and dependence compared to canonical CaMKs (hu2024kinomewidesirnascreen pages 10-11).
4. Substrate Specificity  
   DCLK2 exhibits a high degree of substrate specificity. In studies of clear cell renal cell carcinoma (ccRCC), DCLK2 was shown to selectively phosphorylate TANK-binding kinase 1 (TBK1) on serine 172—a modification that is essential for TBK1 activation in an oncogenic signaling cascade. Importantly, DCLK2 does not phosphorylate the closely related kinase IKKε at the corresponding site, underscoring its substrate selectivity (hu2024kinomewidesirnascreen pages 10-11). In addition, activation of TBK1 by DCLK2 initiates downstream phosphorylation events, such as the TBK1-dependent phosphorylation of p62 on serine 366, further delineating a distinct substrate specificity that appears restricted to components of this signaling pathway (hu2024kinomewidesirnascreen pages 10-11).
5. Structure  
   DCLK2 is a modular protein that comprises distinct structural domains. The N-terminal portion contains one or more doublecortin (DCX) domains, which serve to mediate microtubule binding and are characteristic of the DCX superfamily. The tandem DCX repeats are thought to facilitate microtubule stabilization and bundling, similar to other family members (reiner2006theevolvingdoublecortin pages 11-12). The C-terminal region of DCLK2 contains the serine/threonine kinase domain. Within this catalytic domain, a conserved lysine residue (K423 in functional studies) is critical for ATP binding and phosphotransfer, as mutation of this residue to alanine (K423A) abrogates kinase activity and the ability to phosphorylate TBK1 (hu2024kinomewidesirnascreen pages 10-11). Notably, DCLK2 isoforms show structural differences; for example, the predominant isoform in ccRCC, designated DCLK2203, lacks an auto-inhibitory C-terminal threonine residue that is present in alternate isoforms such as DCLK2201. The absence of this regulatory residue correlates with increased kinase activity, suggesting that the structural architecture of the C-terminal domain plays a critical role in modulating catalysis (hu2024kinomewidesirnascreen pages 10-11). Overall, although DCLK2 shares the canonical CaMK-like kinase fold, it is distinguished by a reduced reliance on calcium/calmodulin binding and by isoform-specific variations in regulatory motifs.
6. Regulation  
   DCLK2 is regulated at multiple levels. Alternative splicing generates different isoforms with distinct regulatory properties. For example, the DCLK2203 isoform, which predominates in clear cell renal cell carcinoma (ccRCC), lacks an auto-inhibitory C-terminal threonine residue that is present in other isoforms such as DCLK2201. This molecular difference results in markedly higher kinase activity for DCLK2203, as demonstrated by its ability to phosphorylate TBK1 efficiently (hu2024kinomewidesirnascreen pages 10-11). In addition, the catalytic activity of DCLK2 relies on an intact kinase domain, with point mutations (e.g., K423A) rendering the enzyme catalytically dead and unable to support oncogenic processes such as anchorage-independent growth or xenograft tumor formation (hu2024kinomewidesirnascreen pages 10-11). Regulation may also occur at the post-transcriptional level; for example, there is evidence suggesting that reduced expression of the nonsense-mediated mRNA decay (NMD) factor UPF1 in ccRCC can lead to increased expression of the hyperactive DCLK2203 isoform (hu2024kinomewidesirnascreen pages 10-11). While direct evidence of phosphorylation sites within DCLK2 (aside from its substrate TBK1 phosphorylation site) is not detailed in the available excerpts, autophosphorylation events and alternative splicing clearly contribute to its overall regulatory profile.
7. Function  
   DCLK2 functions as a serine/threonine kinase involved in oncogenic signaling. In studies focused on clear cell renal cell carcinoma, DCLK2 was identified as a critical regulator of TBK1 activity. It phosphorylates TBK1 on serine 172 within the activation loop, a modification that is essential for the activation of TBK1 and subsequent downstream signaling. Activated TBK1 is implicated in promoting the phosphorylation of p62 on serine 366, which supports an oncogenic cascade that drives tumor cell growth and metastasis (hu2024kinomewidesirnascreen pages 10-11). Functionally, depletion of DCLK2 reduces anchorage-independent colony formation in vitro and impairs tumor growth in xenograft models, underscoring its role as a tumor-promoting kinase (hu2024kinomewidesirnascreen pages 1-3). In addition, despite its structural similarity to CaMKs, DCLK2 is characterized by a significantly reduced Ca²⁺/calmodulin affinity; consequently, its function is largely independent of calcium regulation. By phosphorylating specific substrates such as TBK1—and, by similarity to other family members, potentially the CREB coactivator CRTC2/TORC2—DCLK2 is involved in the modulation of transcriptional responses related to cell proliferation (hu2024kinomewidesirnascreen pages 10-11). In ccRCC, the oncogenic role of DCLK2 is further supported by genomics and functional screening data, which highlight it as a candidate therapeutic target whose inhibition may selectively down-regulate TBK1 activity while sparing related innate immune pathways (hu2024kinomewidesirnascreen pages 1-3).
8. Other Comments  
   Recent efforts to develop selective inhibitors for the doublecortin-like kinase family have yielded compounds such as DCLK1-IN-1, which also inhibit DCLK2 with high selectivity, providing experimental chemical probes for modulating kinase activity in cancer cells (ferguson2020discoveryofa pages 1-2). Known mutations and structure–function studies indicate that alteration of key catalytic residues, such as mutation of K423, abrogates kinase activity, and such mutations have been instrumental in elucidating the functional importance of DCLK2 in oncogenic signaling (hu2024kinomewidesirnascreen pages 10-11). Furthermore, the differential regulation of DCLK2 isoforms via alternative splicing and mRNA decay mechanisms suggests that post-transcriptional controls contribute significantly to its activity and expression in tumor cells. In addition to clear cell renal cell carcinoma, although not detailed in the current excerpts, DCLK family members have been implicated in diverse biological processes including neuronal development and microtubule dynamics. However, the primary experimental evidence for DCLK2 points to its role as an oncogenic kinase involved in the activation of TBK1. These properties and the selective inhibition by emerging chemical probes underscore the potential of DCLK2 both as a therapeutic target in cancer and as a tool for further dissecting kinase-substrate relationships within the doublecortin family.
9. References  
   hu2024kinomewidesirnascreen pages 10-11, hu2024kinomewidesirnascreen pages 1-3, reiner2006theevolvingdoublecortin pages 11-12, burgess2002alternativesplicevariants pages 10-10, ferguson2020discoveryofa pages 1-2

References

1. (hu2024kinomewidesirnascreen pages 10-11): Lianxin Hu, Yanfeng Zhang, Lei Guo, Hua Zhong, Ling Xie, Jin Zhou, Chengheng Liao, Hongwei Yao, Jun Fang, Hongyi Liu, Cheng Zhang, Hui Zhang, Xiaoqiang Zhu, Maowu Luo, Alex von Kriegsheim, Bufan Li, Weibo Luo, Xuewu Zhang, Xian Chen, Joshua T. Mendell, Lin Xu, Payal Kapur, Albert S. Baldwin, James Brugarolas, and Qing Zhang. Kinome-wide sirna screen identifies a dclk2-tbk1 oncogenic signaling axis in clear cell renal cell carcinoma. Molecular Cell, 84:776-790.e5, Feb 2024. URL: https://doi.org/10.1016/j.molcel.2023.12.010, doi:10.1016/j.molcel.2023.12.010. This article has 5 citations and is from a highest quality peer-reviewed journal.
2. (burgess2002alternativesplicevariants pages 10-10): Harold A. Burgess and Orly Reiner. Alternative splice variants of doublecortin-like kinase are differentially expressed and have different kinase activities\*. The Journal of Biological Chemistry, 277:17696-17705, May 2002. URL: https://doi.org/10.1074/jbc.m111981200, doi:10.1074/jbc.m111981200. This article has 91 citations.
3. (hu2024kinomewidesirnascreen pages 1-3): Lianxin Hu, Yanfeng Zhang, Lei Guo, Hua Zhong, Ling Xie, Jin Zhou, Chengheng Liao, Hongwei Yao, Jun Fang, Hongyi Liu, Cheng Zhang, Hui Zhang, Xiaoqiang Zhu, Maowu Luo, Alex von Kriegsheim, Bufan Li, Weibo Luo, Xuewu Zhang, Xian Chen, Joshua T. Mendell, Lin Xu, Payal Kapur, Albert S. Baldwin, James Brugarolas, and Qing Zhang. Kinome-wide sirna screen identifies a dclk2-tbk1 oncogenic signaling axis in clear cell renal cell carcinoma. Molecular Cell, 84:776-790.e5, Feb 2024. URL: https://doi.org/10.1016/j.molcel.2023.12.010, doi:10.1016/j.molcel.2023.12.010. This article has 5 citations and is from a highest quality peer-reviewed journal.
4. (reiner2006theevolvingdoublecortin pages 11-12): Orly Reiner, Frédéric M Coquelle, Bastian Peter, Talia Levy, Anna Kaplan, Tamar Sapir, Irit Orr, Naama Barkai, Gregor Eichele, and Sven Bergmann. The evolving doublecortin (dcx) superfamily. BMC Genomics, 7:188-188, Jul 2006. URL: https://doi.org/10.1186/1471-2164-7-188, doi:10.1186/1471-2164-7-188. This article has 146 citations and is from a peer-reviewed journal.
5. (ferguson2020discoveryofa pages 1-2): F. Ferguson, Behnam Nabet, Srivatsan Raghavan, Srivatsan Raghavan, Yan Liu, Alan L. Leggett, Miljan Kuljanin, R. Kalekar, R. Kalekar, Annan Yang, Annan Yang, Shuning He, Jinhua Wang, Raymond W.S. Ng, Raymond W.S. Ng, Rita Sulahian, Lianbo Li, Emily J Poulin, Ling Huang, Jošt Vrabič Koren, Nora Diéguez-Martínez, Sergio Espinosa, Zhiyang Zeng, Cesear R. Corona, J. Vasta, R. Ohi, Taebo Sim, N. Kim, W. Harshbarger, W. Harshbarger, J. Lizcano, M. Robers, Senthil Muthaswamy, Charles Y. Lin, A. Look, K. Haigis, J. Mancias, B. Wolpin, Andrew J. Aguirre, Andrew J. Aguirre, William C. Hahn, William C. Hahn, K. Westover, and N. Gray. Discovery of a selective inhibitor of doublecortin like kinase 1. Nature Chemical Biology, 16:635-643, Apr 2020. URL: https://doi.org/10.1038/s41589-020-0506-0, doi:10.1038/s41589-020-0506-0. This article has 100 citations and is from a highest quality peer-reviewed journal.