1. Phylogeny  
   Serine/threonine‐protein kinase DCLK1 (also known as DCAMKL1, DCDC3A, or KIAA0369) is a member of the calcium/calmodulin‐dependent kinase (CAMK) clade within the broader eukaryotic serine/threonine kinome. DCLK1 orthologs are found across metazoans and are particularly well conserved in vertebrates, where alternative splicing generates isoforms with distinct regulatory C-terminal tail regions. Phylogenetic analyses indicate that DCLK1, together with DCLK2 and DCLK3, emerged from an ancestral kinase gene early in metazoan evolution, and the vertebrate DCLK1 isoforms evolved distinct structural features, including tandem N‐terminal doublecortin (DCX) domains and a variable C-terminal regulatory tail, which are absent or significantly shorter in invertebrate homologs (venkat2023mechanisticandevolutionary pages 2-4, venkat2023mechanisticandevolutionary pages 4-5).
2. Reaction Catalyzed  
   DCLK1 catalyzes the phosphorylation of serine/threonine residues on substrate proteins by transferring the γ‐phosphate group from adenosine triphosphate (ATP) to the hydroxyl group of the target amino acid. The chemical reaction can be summarized as follows: ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺ (chen2023molecularmechanismof pages 3-5, johnson2023anatlasof pages 1-2).
3. Cofactor Requirements  
   The catalytic activity of DCLK1 requires the presence of divalent metal ions, particularly Mg²⁺, which serve as a cofactor necessary for proper ATP binding and phosphoryl transfer during catalysis (patel2016biochemicalandstructural pages 5-6).
4. Substrate Specificity  
   As a serine/threonine kinase, DCLK1 phosphorylates target proteins on serine or threonine residues. Although detailed consensus motifs for DCLK1 have not been fully established in isolation, its substrate specificity is generally consistent with that of other CAMK family members. Published kinome substrate profiling has revealed that many kinases of this class preferentially recognize specific sequence contexts flanking the phosphoacceptor site. In the case of DCLK1, phosphorylation has been demonstrated on peptide substrates derived from proteins involved in microtubule regulation, with the requirement for local amino acid configurations that facilitate access to the catalytic cleft. Accordingly, substrates are expected to contain a serine or threonine residue surrounded by residues that contribute to an optimal binding orientation in the catalytic pocket (chen2023molecularmechanismof pages 3-5, johnson2023anatlasof pages 7-7).
5. Structure  
   DCLK1 possesses a multidomain architecture that underlies its dual functionality in kinase signaling and microtubule regulation. The N-terminal region contains tandem doublecortin (DCX) domains that mediate microtubule binding and are critical for neuronal migration during brain development (patel2016biochemicalandstructural pages 1-3). Interposed between these domains and the kinase domain is a PEST linker, a sequence rich in proline, glutamic acid, serine, and threonine residues that is prone to proteolytic processing and may modulate the connectivity between microtubule-binding and catalytic functions. The C-terminal region harbors a serine/threonine kinase domain that adopts a bilobal structure characteristic of protein kinases, including a conserved ATP-binding lobe (N-terminal) and a substrate-binding lobe (C-terminal). Within this kinase domain, key structural features such as the activation loop, the DFG motif (including the aspartate crucial for magnesium coordination), the catalytic HRD motif, and the C-helix are present and arranged in a conformation that can be autoinhibited. In some isoforms, an autoinhibitory domain (AID) resides in the region adjacent to the kinase domain; it acts as a pseudo-substrate by occupying the ATP-binding pocket and hindering substrate access. This interaction is mediated via defined helices (termed R2 and R3 in structural studies) and involves conserved residues, including salt-bridge formation (e.g., between K692 and residues D511 and D533) that are critical for catalytic regulation (chen2023molecularmechanismof pages 1-3, patel2016biochemicalandstructural pages 3-4, venkat2023mechanisticandevolutionary pages 12-14).
6. Regulation  
   DCLK1 is subject to multiple regulatory mechanisms that modulate its kinase activity. A prominent mode of regulation is autoinhibition mediated by a built-in autoinhibitory domain (AID) that binds to and occludes the ATP-binding site, thereby preventing phosphorylation activity. Post-translational modifications, especially phosphorylation at specific serine and threonine residues within the kinase domain and the C-terminal tail, further modulate the activity of DCLK1. For instance, autophosphorylation events within the activation loop are critical for switching DCLK1 from an inactive to an active state. Additionally, mutations found in cancer, such as S660L, P675L, G681E, and A686T, disrupt the interaction between the AID and the kinase domain, releasing autoinhibition and resulting in increased kinase activity (chen2023molecularmechanismof pages 3-5, venkat2023mechanisticandevolutionary pages 15-17). The conformational dynamics of the C-terminal tail, including its docking into the ATP-binding pocket (mimicking ATP binding through residues such as Val682, Val684, and Thr687), further regulate autoinhibition. Phosphorylation of residues in this region, notably Thr688 in certain isoforms, stabilizes the autoinhibited conformation by forming hydrogen bonds with elements of the glycine-rich loop (venkat2023mechanisticandevolutionary pages 12-14, venkat2023mechanisticandevolutionary pages 17-18).
7. Function  
   DCLK1 is implicated in the regulation of several fundamental cellular processes, particularly within the nervous system. It is involved in calcium-dependent signaling pathways that control neuronal migration during brain development, a process mediated in part through its microtubule-binding DCX domains. In mature neurons, DCLK1 is thought to participate in the modulation of microtubule dynamics, which is essential for maintaining neuronal structure and function. Beyond its roles in normal neurodevelopment, aberrant expression and activation of DCLK1 have been associated with oncogenic processes. Elevated DCLK1 kinase activity has been linked to increased tumor cell proliferation, invasiveness, and metastasis in various cancers, including colorectal and pancreatic cancers. Expression levels and autoinhibition mechanisms appear to differ between isoforms, with some variants predominantly expressed during embryogenesis and others in the adult brain, suggesting isoform-specific roles in developmental and pathological contexts (chen2023molecularmechanismof pages 1-3, patel2016biochemicalandstructural pages 1-3, venkat2023mechanisticandevolutionary pages 2-4).
8. Other Comments  
   Several small-molecule inhibitors have been identified that target the kinase domain of DCLK1 by binding to key residues within the ATP-binding site, such as D533. Compounds like NVP-TAE684 and inhibitors identified through screening efforts have shown promising binding affinities, with some newer candidates demonstrating dissociation constants in the low micromolar range based on complementary in silico docking, molecular dynamics simulations, and surface plasmon resonance assays (chen2023molecularmechanismof pages 10-13, chen2023molecularmechanismof pages 13-15). Disease associations for DCLK1 are notably linked to its overexpression in tumors, where mutations that disrupt autoinhibition can contribute to tumorigenesis. In experimental models, upregulated DCLK1 activity correlates with markers of cancer stem cells and increased tumor cell invasiveness, underscoring the potential of DCLK1 as a therapeutic target in oncology. In addition, its established role in neuronal migration and microtubule regulation highlights its importance in nervous system development and function (chen2023molecularmechanismof pages 17-17, venkat2023mechanisticandevolutionary pages 1-2).
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