**1. Phylogeny:**  
SCYL3, also known as protein-associating with the carboxyl-terminal domain of ezrin or PACE-1, is a member of the SCY1‐like protein family and is classified as a pseudokinase. It is evolutionarily related to its family members SCYL1 and SCYL2, which are present in animal systems and known to be involved in intracellular trafficking and other regulatory roles. Studies in hepatocellular carcinoma models and in cellular systems have confirmed that SCYL3 is a conserved protein expressed in mammalian species, and sequence analyses indicate that its kinase-like domain, despite lacking some canonical catalytic motifs, is preserved across these organisms (lei2023scyl3asa pages 1-3, jung2017scyl2genesare pages 11-15). In contrast to SCYL1 and SCYL2, which have roles in Golgi dynamics and clathrin-mediated vesicle trafficking respectively, SCYL3 shows a unique pattern of expression that is primarily linked to cell adhesion and migratory functions. The evolutionary conservation of the SCY1-like pseudokinase family implies that the divergence from full catalytic activity in SCYL3 may represent an adaptation for specialized regulatory functions in metazoans. Orthologous relationships suggest that while SCYL3 is widespread among vertebrates, there are no identified homologs in plants, highlighting its specialized function in animal cells (lei2023scyl3asa pages 1-3, jung2017scyl2genesare pages 11-15).

**2. Reaction Catalyzed:**  
The classical reaction catalyzed by typical kinases involves the transfer of a phosphate group from adenosine triphosphate (ATP) to specific serine, threonine, or tyrosine residues on substrate proteins, resulting in the formation of adenosine diphosphate (ADP) and a phosphorylated protein. SCYL3, although harboring a kinase domain, is categorized as a pseudokinase because it lacks several critical catalytic motifs, including the canonical HRD motif that is normally essential for efficient phosphotransfer. In vitro studies have demonstrated that SCYL3 is capable of exhibiting measurable kinase activity; however, the reaction following the standard chemical equation—ATP + [protein] → ADP + [protein]-phosphate—has not been unequivocally documented for SCYL3, and a definitive phosphoryl transfer reaction remains to be clearly established (lei2023scyl3asa pages 3-4).

**3. Cofactor Requirements:**  
Standard kinase catalysis typically relies on divalent metal ions, most notably magnesium (Mg²⁺), to facilitate efficient ATP binding and to stabilize the transition state during phosphotransfer. For SCYL3, direct experimental evidence regarding cofactor requirements is not explicitly provided in the current literature. It is conceivable, based on biochemical precedents for other kinase enzymes, that any residual enzymatic function present in SCYL3 would require Mg²⁺; however, no dedicated studies have confirmed a specific cofactor dependency for SCYL3 (lei2023scyl3asa pages 3-4).

**4. Substrate Specificity:**  
Unlike many classical kinases that are defined by a conserved consensus motif for substrate phosphorylation, SCYL3 does not exhibit a clearly delineated substrate motif in available studies. Instead, its biological activity is largely attributed to its ability to associate with regulatory proteins, particularly by binding to and stabilizing other kinases such as ROCK2. SCYL3 physically interacts with ROCK2 through its C-terminal domain, and this interaction is essential for enhancing ROCK2’s stability and transactivating activity. Furthermore, SCYL3 has been shown to bind to ezrin, a protein that links membrane proteins to the actin cytoskeleton, suggesting that the substrate recognition of SCYL3 is mediated by selective protein–protein interactions rather than by a defined amino acid sequence motif in substrates (lei2023scyl3asa pages 5-6, jung2017scyl2genesare pages 15-18).

**5. Structure:**  
SCYL3 is a 110 kDa protein encoded on chromosome 1q24.2, and its primary structure is characterized by a central kinase-like domain along with four HEAT repeats. The kinase-like domain, while adopting the typical bilobal structure seen in protein kinases, lacks several conserved catalytic residues, such as the HRD motif, and is therefore classified as a pseudokinase domain. This feature suggests that SCYL3’s central domain is more likely involved in binding to active kinases or regulatory partners rather than in catalysis of phosphate transfer. In addition to the kinase-like region, the presence of four HEAT repeats points toward a role in scaffolding and mediating protein–protein interactions. These repeats are often involved in flexible binding and are thought to facilitate the assembly of multi-protein complexes. Moreover, the C-terminal region includes an ezrin-binding domain that is critical for SCYL3’s ability to interact with and stabilize ROCK2. Deletion mutants lacking the C-terminal domain have been shown to lose their capacity to bind ROCK2 and to promote cell migration and tumor growth, underscoring the functional importance of this structural segment. Although explicit three-dimensional structures from crystallographic studies or AlphaFold predictions are not detailed in the available literature, the described domain organization provides significant insights into the modular function of SCYL3 in mediating cytoskeletal dynamics and signal transduction (lei2023scyl3asa pages 1-3, lei2023scyl3asa pages 8-9, jung2017scyl2genesare pages 11-15).

**6. Regulation:**  
The regulation of SCYL3 occurs predominantly at the level of protein expression and through its interaction with key signaling proteins within oncogenic pathways. Overexpression of SCYL3 has been consistently reported in hepatocellular carcinoma as well as in certain breast and colon cancers, where elevated levels correlate with aggressive tumor behavior and poor patient prognosis. Functionally, SCYL3 acts by binding to ROCK2 and enhancing its stability, which in turn promotes the formation of actin stress fibers and focal adhesions—structural components critical for cell migration and invasion. Experimental evidence obtained through shRNA-mediated knockdown of SCYL3 in hepatocellular carcinoma cell lines has demonstrated that a reduction in SCYL3 expression results in decreased cell proliferation, migration, and invasive potential. This downregulation is accompanied by a marked reduction in ROCK2 protein levels and a subsequent decrease in the phosphorylation of downstream targets such as myosin light chain 2 (MLC2), indicating that SCYL3 positively regulates ROCK2-mediated signaling cascades. Although the precise post-translational modifications of SCYL3 itself, including any potential phosphorylation events, have not been fully characterized, its functional effects are clearly mediated via the stabilization and modulation of partner proteins rather than through classical catalytic regulation. In summary, SCYL3 regulation is largely driven by transcriptional upregulation in cancer contexts and by its ability to exert control over cytoskeletal reorganization through protein–protein interactions (lei2023scyl3asa pages 6-7, lei2023scyl3asa pages 7-8, lei2023scyl3asa pages 9-10, jung2017scyl2genesare pages 15-18).

**7. Function:**  
SCYL3 plays an important role in modulating cell adhesion and migration by serving as a regulatory scaffold within the cell. Its interaction with ezrin and ROCK2 places SCYL3 at a critical junction in the control of cytoskeletal dynamics, which are essential for processes such as cell motility, invasion, and metastasis. Overexpression studies in hepatocellular carcinoma have demonstrated that increased levels of SCYL3 correlate with enhanced tumor cell proliferation, greater migratory capacity, and a higher incidence of metastasis to distant organs such as the lungs. These findings are supported by both in vitro assays, where SCYL3 overexpression in hepatocellular carcinoma cell lines results in increased cell migration and invasion, and by in vivo orthotopic mouse models, in which heightened SCYL3 expression leads to more irregular tumor growth fronts and augmented dissemination of tumor nodules. Furthermore, clinical analyses using quantitative PCR and publicly available datasets have revealed that elevated SCYL3 mRNA and protein levels are associated with shorter overall survival, disease-free survival, and progression-free survival in patients with hepatocellular carcinoma. This pattern of expression is also observed in other cancer types, including colon and breast cancers, suggesting that SCYL3 may play a broader role in oncogenesis beyond liver cancer. The biological functions of SCYL3 are mediated primarily through its capacity to stabilize ROCK2, thus enhancing ROCK2-dependent signaling pathways that regulate the rearrangement of the actin cytoskeleton and the formation of focal adhesions, culminating in increased cell motility and invasive behavior. By mediating these processes, SCYL3 contributes to both the local invasion of tumor cells and to distant metastatic spread in cancer (lei2023scyl3asa pages 1-3, lei2023scyl3asa pages 5-6, lei2023scyl3asa pages 9-10, jung2017scyl2genesare pages 15-18, OpenTargets Search: cancer,hepatocellular carcinoma-SCYL3).

**8. Other Comments:**  
Although specific inhibitors targeting SCYL3 have not yet been developed or reported in the literature, its strong association with aggressive cancer phenotypes indicates that it could represent a promising target for future therapeutic intervention. High levels of SCYL3 expression have been implicated in mediating resistance to sorafenib treatment in hepatocellular carcinoma patients, as evidenced by reduced disease-free and progression-free survival rates in those with elevated SCYL3 expression. In addition to its role in cancer, SCYL3 is located on chromosome 1q24.2—a region that is frequently amplified in tumors—while it is also subject to deletion in the context of 1q24 microdeletion syndrome when co-deleted with neighboring genomic loci. Such genomic alterations suggest that both overexpression and loss-of-function events affecting SCYL3 may have pathological consequences. Despite its in vitro kinase-like activity, the predominant biological function of SCYL3 appears to be regulatory, operating through the stabilization and enhancement of ROCK2 activity and the subsequent modulation of cytoskeletal and adhesion dynamics. The current body of evidence underscores the need for further research to comprehensively characterize SCYL3’s post-translational modifications, detailed mutational landscape, and to explore potential small-molecule modulators that could target its interaction interfaces (lei2023scyl3asa pages 9-10, OpenTargets Search: cancer,hepatocellular carcinoma-SCYL3).

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