1. Phylogeny  
   PEAK3, encoded by the gene C19orf35 and identified by UniProt ID Q6ZS72, is a member of the PEAK family of pseudokinases. The PEAK family comprises three related proteins—PEAK1, PEAK2, and PEAK3—with PEAK3 showing comparatively higher sequence divergence from its paralogues. Phylogenetic analyses based on human kinome studies place PEAK3 within the atypical or non‐catalytic kinase subgroup; although it retains a conserved kinase fold, it has evolved to function primarily as a scaffold rather than as an enzyme that transfers phosphate groups. Orthologs of PEAK3 are detectable in a broad range of vertebrate species, and its expression pattern appears to be restricted primarily to hematopoietic tissues such as lymphoid organs and granulocytes. This lineage‐specific expression, together with evidence of purifying selection in these tissues, indicates that despite its structural divergence from its paralogues, PEAK3 has maintained a conserved functional role in these cell types (OpenTargets Search: acute myeloid leukemia,leukemia,cancer-PEAK3,C19orf35,Q6ZS72, ounoughene2021sheddependentoncogenicsignaling pages 1-2). Comparative kinome analyses, as outlined in seminal works on the evolution of protein kinase signaling, suggest that PEAK3 and its family members originally arose from an ancestral kinase gene; subsequent gene duplication events and progressive loss of catalytic motifs resulted in the emergence of these scaffolding pseudokinases that now serve regulatory functions rather than catalytic ones (ounoughene2021sheddependentoncogenicsignaling pages 2-4).
2. Reaction Catalyzed  
   In contrast to conventional active kinases, PEAK3 does not catalyze the classical phosphorylation reaction that transfers a phosphate group from ATP to a serine, threonine, or tyrosine residue on a substrate protein. The typical reaction catalyzed by active kinases can be represented as:  
     ATP + [protein]‑(L‑serine/threonine) → ADP + [protein]‑(L‑serine/threonine)‑phosphate + H⁺.  
   However, PEAK3 is characterized as a probable catalytically inactive kinase because its ATP-binding site is sterically occluded by side chain orientations and key catalytic residues are either substituted or absent. As a result, PEAK3 does not support the transfer of a phosphate group and does not exhibit measurable phosphotransferase activity; its primary function is mediated through the assembly of signaling complexes and scaffolding interactions rather than through enzymatic catalysis (ounoughene2021sheddependentoncogenicsignaling pages 2-4, torosyan2023structuralinsightsinto pages 5-7).
3. Cofactor Requirements  
   Since PEAK3 is a pseudokinase and lacks conventional phosphotransferase activity, it does not require the typical cofactors—such as Mg²⁺ or Mn²⁺—that are essential for the catalytic functions of active kinases. Structural studies indicate that the ATP-binding cleft in PEAK3 is occluded, thereby precluding the necessity for divalent metal cofactors that would normally coordinate ATP binding and phosphotransfer. Consequently, the biochemical activity of PEAK3 does not depend on any cofactors in the same manner as catalytically active kinases (ounoughene2021sheddependentoncogenicsignaling pages 2-4).
4. Substrate Specificity  
   In active kinases, substrate specificity is defined by consensus motifs—for example, the preference for an RxRxxp[ST] motif in some serine/threonine kinases or specific phosphotyrosine recognition sequences in tyrosine kinases. In the case of PEAK3, however, its role as a pseudokinase implies that it does not phosphorylate substrates. Instead, substrate “specificity” is determined by its capacity to interact with select protein partners via conserved binding motifs. PEAK3 has been shown to interact with adaptor proteins such as CRK-II and GRB2, as well as other signaling molecules like ASAP1. These interactions are mediated through specific phosphotyrosine or proline-rich motifs that allow PEAK3 to assemble and modulate signaling complexes. For instance, PEAK3 antagonizes CRK-II-dependent signaling by interfering with the formation of membrane ruffles and lamellipodia-like extensions, and its substrate scope is thus defined in terms of the proteins it recruits rather than through direct catalytic phosphorylation (ounoughene2021sheddependentoncogenicsignaling pages 7-10).
5. Structure  
   The overall structure of PEAK3 reveals a modular organization that is consistent with its role as a scaffolding protein. The protein is organized into several distinct regions. At the extreme N-terminus, PEAK3 possesses a relatively short intrinsically disordered segment that contains a conserved 14-3-3 binding motif with the consensus sequence RTQSLP (approximately residues 66–71). This motif is crucial for mediating interactions with 14-3-3 proteins, which can further modulate the localization and stability of PEAK3 (torosyan2023structuralinsightsinto pages 2-3).  
   Immediately following the disordered region is the central pseudokinase domain. Despite its overall resemblance to conventional kinase domains—featuring an N-lobe composed predominantly of β-sheets and an αC helix, and a C-lobe that is largely α-helical—key catalytic features are modified. Notably, although PEAK3 retains an intact DFG motif in what has been termed a “DFG-in” conformation, the nucleotide-binding pocket is occluded. Substitutions in the αC helix disrupt the formation of the critical salt bridge between the catalytic lysine and the glutamate—which in active kinases is necessary for stabilizing the binding of ATP. As such, the architecture of the pseudokinase domain underscores the loss of catalytic activity while preserving the overall kinase fold (torosyan2023structuralinsightsinto pages 5-7, ounoughene2021sheddependentoncogenicsignaling pages 2-4).  
   Adjacent to the pseudokinase domain, the C-terminal region harbors the Split Helical Dimerization (SHED) domain. The SHED domain is a conserved helical module that mediates both homo-dimerization and hetero-dimerization among PEAK family members. This dimerization is a crucial structural determinant for PEAK3’s function as a scaffold, as it facilitates the formation of higher-order signaling complexes. Structural studies using cryo-electron microscopy have provided a view of full-length PEAK3 in complex with a 14-3-3 heterodimer, revealing an asymmetric interaction in which one monomer of 14-3-3 engages the phosphorylated N-terminal segment of PEAK3. In these complexes, the SHED domain contributes significantly to stabilizing the dimeric architecture, which in turn is essential for the recruitment of downstream signaling partners (torosyan2023structuralinsightsinto pages 14-15, ounoughene2021sheddependentoncogenicsignaling pages 2-4).  
   Additional features of the structure include a relatively rigid kinase-like core that is juxtaposed against flexible flanking regions. The overall three-dimensional organization, as determined by cryo-EM and supplemented by computational modeling, reflects a balance between structural rigidity—necessary for sustaining specific protein-protein interactions—and flexibility mediated by the intrinsically disordered N-terminus. This balance is central to the role of PEAK3 as an adaptable scaffold in diverse signaling contexts (torosyan2023structuralinsightsinto pages 1-2, 2-3).
6. Regulation  
   The regulatory mechanisms of PEAK3 center on its capacity to function as a molecular scaffold through regulated dimerization and phosphorylation-dependent interactions with adaptor proteins. One of the principal regulatory features is the SHED domain, which mediates dimerization that is essential for the formation of signaling complexes. Disruption of the dimerization interface has been shown to impair PEAK3’s ability to activate downstream signaling pathways (ounoughene2021sheddependentoncogenicsignaling pages 15-17).  
   In addition to the structural regulation conferred by dimerization, post-translational modifications further modulate PEAK3 activity. Phosphorylation events within the N-terminal region, particularly within the 14-3-3 binding motif, are critical for the interaction with 14-3-3 proteins. The binding of 14-3-3 not only stabilizes the overall structure of PEAK3 but also may regulate its subcellular localization, and thereby influence its function as a scaffold for other proteins (torosyan2023structuralinsightsinto pages 14-15).  
   Epigenetic regulation also plays a role in modulating the expression of PEAK3. Differential methylation of the C19orf35 gene has been observed in various gastrointestinal cancers, with studies demonstrating that the methylation status of this locus can vary according to tumor histologic subtype. These findings indicate that the regulation of PEAK3 may occur both at the protein–protein interaction level and at the transcriptional level via epigenetic modifications (chong2014dnamethylationstatus pages 7-9).  
   Thus, regulation of PEAK3 is achieved by a combination of mechanisms that include the control of its dimerization via the SHED domain, phosphorylation-dependent interactions with 14-3-3 proteins, and epigenetic modifications of its gene, all of which ultimately influence its capacity to assemble downstream signaling complexes (ounoughene2021sheddependentoncogenicsignaling pages 7-10, torosyan2023structuralinsightsinto pages 14-15).
7. Function  
   Functionally, PEAK3 operates as a scaffolding pseudokinase that orchestrates intracellular signaling without engaging in conventional catalytic activity. Its primary role is to interact with and modulate the function of key adaptor proteins. One established function is its antagonism of CRK-II-dependent signaling. By binding to CRK-II, PEAK3 interferes with the formation of CRK-II-mediated membrane ruffles and lamellipodia-like extensions, structures that are critical for cell motility. In doing so, PEAK3 serves as a negative regulator of CRK-II signaling and thus impacts cell movement (information section, ounoughene2021sheddependentoncogenicsignaling pages 1-2, 7-10).  
   In addition, PEAK3 engages in interactions with other regulatory molecules. It forms complexes with proteins such as GRB2 and ASAP1, and studies have shown that through these interactions it can facilitate the activation of downstream kinases like PYK2. This modulation leads to the activation of the AKT signaling pathway under conditions that may not require exogenous growth factors, thereby promoting cell survival and proliferation in a context often associated with oncogenic signaling (ounoughene2021sheddependentoncogenicsignaling pages 7-10, 15-17, 17-18).  
   Expression studies indicate that PEAK3 is predominantly expressed in hematopoietic tissues—including the spleen, lymph nodes, and granulocytes—which is consistent with reports of its upregulation in acute myeloid leukemia (AML). Elevated PEAK3 transcript and protein levels in AML patient samples support the concept that PEAK3 contributes to oncogenic processes in blood cancers (OpenTargets Search: acute myeloid leukemia,leukemia,cancer-PEAK3,C19orf35,Q6ZS72, ounoughene2021sheddependentoncogenicsignaling pages 1-2).  
   Overall, the biological role of PEAK3 is defined by its function as a regulatory scaffold that modulates cell migration, adhesion, and proliferation by controlling the assembly and spatial organization of multi-protein signaling complexes. Its interactions with CRK-II and other adaptor proteins provide a mechanistic basis for its involvement in cellular processes that are frequently deregulated in cancer, particularly in the context of leukemogenesis (ounoughene2021sheddependentoncogenicsignaling pages 7-10, 15-17).
8. Other Comments  
   Although PEAK3 lacks the intrinsic catalytic activity observed in conventional kinases, its role as a scaffolding protein renders it an attractive potential target for therapeutic intervention. Currently, no specific small-molecule inhibitors have been developed to target PEAK3 directly. Instead, therapeutic strategies may focus on its interaction surfaces—such as the dimerization interface mediated by the SHED domain or the binding interface for 14-3-3 adaptor proteins—to disrupt its assembly into oncogenic signaling complexes (ounoughene2021sheddependentoncogenicsignaling pages 15-17, torosyan2023structuralinsightsinto pages 14-15).  
   Epigenetic regulation has also been noted as an important factor affecting PEAK3 expression. Differential DNA methylation of the C19orf35 locus has been reported in gastrointestinal malignancies, including early gastric cancer, where distinct methylation patterns can serve as molecular markers for tumor subtypes. This epigenetic component suggests that, beyond protein–protein interactions, transcriptional regulation via DNA methylation may contribute to the aberrant expression of PEAK3 in cancer (chong2014dnamethylationstatus pages 7-9).  
   Additionally, the absence of prevalent disease-associated mutations in PEAK3 indicates that its oncogenic function is more closely tied to changes in expression level and alterations in its scaffolding interactions rather than to mutational activation or inactivation. As such, future research may emphasize the development of therapeutic agents aimed at modulating its protein interactions, a strategy that could potentially impair the downstream signaling pathways that drive tumor cell growth and dissemination.  
   Given its elevated expression in acute myeloid leukemia and its role in oncogenic signaling, PEAK3 represents a promising candidate for further investigation as a biomarker and therapeutic target in hematologic as well as possibly solid tumors (OpenTargets Search: acute myeloid leukemia,leukemia,cancer-PEAK3,C19orf35,Q6ZS72, ounoughene2021sheddependentoncogenicsignaling pages 1-2, 15-17).
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