1. Phylogeny  
   STK31 (also known as SgK396 or NYD‐SPK) is a member of the serine/threonine kinase family that falls within the evolutionary framework of Hanks‐type kinases. Its kinase domain shares overall sequence homology with canonical serine/threonine kinases; however, a key alteration—a substitution of the conserved catalytic aspartate located in the HRD motif with serine (D854S)—leads to its classification as a pseudokinase. This modification distinguishes STK31 from catalytically active family members, while its multi‐domain architecture remains evolutionarily conserved. The protein displays a modular organization that includes an N‐terminal Tudor domain, a central coiled‐coil domain, and a C‐terminal serine/threonine kinase domain. In normal physiology, STK31 expression is largely restricted to developing spermatocytes in the testis, a feature that suggests a conserved role in germ cell biology among mammals. Furthermore, its close evolutionary relationship with other pseudokinases—proteins that have retained structural similarity to active kinases despite minimal or alternative catalytic activity—places it within an ancient core set of signaling proteins that can be traced back to the common ancestry of eukaryotes as observed in other members of the serine/threonine kinase superfamily (moore2021theeffectsof pages 74-85, moore2021theeffectsofa pages 74-85).
2. Reaction Catalyzed  
   STK31 is expected to catalyze a phosphate‐transfer reaction that follows the well‐established mechanism for serine/threonine kinases. The canonical reaction involves the binding of ATP and transfer of the gamma (γ) phosphate to the hydroxyl group of a serine or threonine residue on a substrate protein. In chemical terms, the reaction can be summarized as follows:  
     ATP + [protein]–(L‑serine or L‑threonine) → ADP + [protein]–(L‑serine/threonine‑phosphate) + H⁺.  
   Despite its pseudokinase classification—which is a direct consequence of the D854S substitution—the expected reaction, were an alternative catalytic mechanism operative, would remain consistent with this classical serine/threonine phosphorylation reaction (moore2021theeffectsof pages 74-85).
3. Cofactor Requirements  
   Similar to canonical serine/threonine kinases, the catalytic activity of STK31 is predicted to require divalent metal ions as cofactors. In most cases, Mg²⁺ is necessary to coordinate ATP binding and support the phosphotransfer reaction. Although STK31 has an altered catalytic core that questions its conventional kinase activity, the cofactor requirement remains consistent with those of other enzymes in this family (moore2021theeffectsof pages 74-85).
4. Substrate Specificity  
   Experimental investigations into the substrate specificity of STK31 have not yet yielded a definitive consensus phosphorylation motif. Analysis of functionally critical residues within the kinase domain—such as the mutated catalytic residue—and subsequent site‐directed mutagenesis (targeting residues including D872, N859, K758, and the D872/D874 double mutant) did not abolish its pro‐proliferative effects in vivo. These observations imply that the biological functions of STK31 may be mediated less by a traditional catalytic substrate recognition and more by alternative mechanisms such as scaffolding or by recruiting unidentified substrates that contribute to oncogenic signaling pathways. Consequently, although STK31 is structurally equipped with a serine/threonine kinase domain, its precise substrate preference remains to be fully characterized experimentally (moore2021theeffectsof pages 85-91, foulkes2018biochemicalanalysisof pages 234-236).
5. Structure  
   STK31 exhibits a distinct multi‐domain architecture consisting of an N‐terminal Tudor domain, a central coiled‐coil domain, and a C‐terminal serine/threonine kinase domain. The Tudor domain is typically involved in recognizing methylated protein or RNA partners and is implicated in RNA‐related processes such as RNA silencing, particularly through its interaction with MIWI proteins in developing spermatocytes. The central coiled‐coil domain likely mediates homotypic or heterotypic protein–protein interactions, contributing to the assembly of multi‐protein signaling complexes. The C‐terminal kinase domain, although organized similarly to other Hanks‐type serine/threonine kinases, contains a critical substitution—the replacement of the catalytic aspartate by serine (D854S) in the HRD motif—that categorizes the domain as pseudokinase. Structural studies using mass spectrometry of purified STK31 proteins have detected an anomalous 313 Da adenosyl‐like mass shift, which may indicate a novel post‐translational modification that could influence the conformation and function of the kinase domain. Moreover, the functional sensitivity observed upon epitope tagging (with V5 or FLAG tags) suggests that precise structural integrity is required for its biological activity and that even minor alterations can disrupt its function (moore2021theeffectsof pages 74-85, moore2021theeffectsof pages 85-91).
6. Regulation  
   The regulation of STK31 occurs both at the transcriptional level and through potential post‐translational modifications. Under normal physiological conditions, STK31 expression is largely restricted to the testis, where it is present in developing spermatocytes and is thought to interact with MIWI, thereby playing a role in RNA silencing pathways. In contrast, aberrant overexpression of STK31 has been documented in a variety of human cancers—including colon, gastric, lung, pancreatic, and cervical cancer—and this overexpression is frequently associated with promoter hypomethylation. Such epigenetic dysregulation leads to increased protein levels in non‐testicular tissues. In experimental models, CRISPR knockout screens in hepatocytes have identified STK31 as a strong positive regulator of cell proliferation, and its overexpression in both in vitro and in vivo systems has been shown to enhance tumor development. Importantly, mutagenesis studies targeting several conserved residues within the pseudokinase domain (for example, D872A, N859A, and K758A substitutions) did not significantly modify its tumor‐promoting activity, indicating that the regulatory mechanism of STK31 may not rely solely on conventional catalytic activity but rather on scaffolding or alternative non‐canonical functions. In addition, mass spectrometry analysis revealing a 313 Da mass shift suggests that STK31 may be subject to a unique post‐translational modification, the precise role of which in regulating kinase function is yet to be elucidated. Finally, the functional impairment observed when epitope tags are added underscores the highly sensitive nature of its structural conformation for proper regulatory control (moore2021theeffectsof pages 74-85, moore2021theeffectsof pages 85-91, foulkes2018biochemicalanalysisof pages 234-236).
7. Function  
   STK31 is functionally recognized as a pro‐proliferative factor with significant implications in oncogenesis. Under normal conditions, its transcript and protein are predominantly restricted to the testis, where its interaction with MIWI proteins is thought to modulate RNA silencing during spermatocyte development. Despite the apparent functional redundancy observed in knockout mouse models in the reproductive context, the aberrant overexpression of STK31 in several human cancers points to a distinct pathological role. In a number of tumor types—including colon, gastric, lung, pancreatic, and cervical cancers—STK31 is overexpressed, a phenomenon that has been linked to epigenetic changes such as promoter hypomethylation. Notably, in liver cancer models, an in vivo CRISPR knockout screen targeting pseudokinases in hepatocytes identified STK31 as a critical positive regulator of cell proliferation; subsequent overexpression studies in HEK293T cells and mouse models of hepatocellular carcinoma demonstrated that enhanced levels of STK31 correspond with increased tumor burden. In these contexts, the kinase domain of STK31, despite its classification as a pseudokinase, is integral for its pro‐proliferative function. Although conventional catalytic activity may be compromised because of the D854S mutation, STK31 appears to exert its biological effects either via residual non‐canonical catalytic activity or by serving as a scaffolding protein that organizes and stabilizes oncogenic signaling complexes—potentially engaging with components of the c‐Myc/β‐catenin pathway. This functional divergence between its physiological role in germ cell development and its pathological role in cancer underscores the adaptability and context‐dependency of STK31’s signaling functions (moore2021theeffectsof pages 74-85, moore2021theeffectsof pages 85-91, foulkes2018biochemicalanalysisof pages 234-236).
8. Other Comments  
   STK31 is of considerable interest due to its classification as a cancer‐associated pseudokinase. Genetic associations reported by the Open Targets Platform link STK31 to various traits such as metabolite measurement, chronotype, telomere length, and body height; however, these associations stem primarily from genetic–epidemiological studies and do not yet provide a direct mechanistic insight into its cellular function. The atypical structure of STK31’s kinase domain, particularly the D854S substitution, suggests that its functional contributions may not involve conventional phosphorylation but could include alternative catalytic or scaffolding roles. Experimental evidence indicates that even when key conserved residues in the pseudokinase domain are mutated, the oncogenic potential of STK31 remains largely intact. This observation has led to the hypothesis that STK31 may mediate its pro‐proliferative effects by assembling and modulating other signaling components rather than through direct phosphorylation of canonical substrates. In this regard, STK31 represents a promising target for therapeutic intervention in cancers where its expression is pathologically elevated. Finally, the sensitivity of its functional activity to structural perturbations—as observed by the differential behavior of epitope‐tagged constructs—emphasizes the necessity of preserving its native conformation in both experimental and potential drug development contexts (moore2021theeffectsof pages 85-91, OpenTargets Search: -STK31).
9. References
10. moore2021theeffectsof pages 74-85.
11. moore2021theeffectsof pages 85-91.
12. moore2021theeffectsofa pages 74-85.
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