1. Phylogeny – MST1 (STK4) is a member of the mammalian STE20‐like kinase family and falls specifically within the GCK‐II subfamily of serine/threonine kinases. MST1 is evolutionarily conserved and has orthologs in all mammalian species, sharing a high degree of sequence and functional homology with MST2, its closest paralog. Its evolutionary relationship extends to kinases such as the Drosophila Hippo, which is the invertebrate homolog and a central regulator of tissue size and apoptosis, and even further back to yeast STE20‐related kinases, underscoring its ancient origin within eukaryotes (ling2008biosignalingofmammalian pages 1-2, record2010structuralcomparisonof pages 1-3, gagnon2012molecularphysiologyof pages 1-2). The conserved nature of the kinase domain and the presence of regulatory modules such as the SARAH domain in MST1 indicate that it emerged from a common ancestral gene that gave rise to the core components of the Hippo pathway in higher eukaryotes (tang2014expandingthehippo pages 19-23).
2. Reaction Catalyzed – MST1 catalyzes a classical serine/threonine phosphorylation reaction. In this reaction, MST1 utilizes ATP as a phosphate donor and transfers the γ‐phosphate group to specific serine or threonine residues on substrate proteins, thereby producing ADP, a phosphorylated substrate, and a proton (H⁺). This reaction underlies its role as a signal transducer in multiple pathways, particularly in the regulation of apoptosis and cell proliferation (record2010structuralcomparisonof pages 1-3).
3. Cofactor Requirements – The catalytic activity of MST1 is dependent on the presence of divalent metal ions, with Mg²⁺ being the primary cofactor required for proper ATP binding and phosphoryl transfer. Although related kinases in the MST family have been reported to tolerate or even preferentially utilize alternative metal ions such as Mn²⁺ to some extent, available evidence specifically for MST1 indicates that Mg²⁺ is the essential cofactor for its enzymatic activity (record2010structuralcomparisonof pages 1-3, stegert2005functionalcharacterisationof pages 29-34).
4. Substrate Specificity – MST1 exhibits substrate specificity toward serine/threonine residues found in proteins involved in apoptotic and growth regulatory pathways. One well characterized substrate of MST1 is histone H2B, whose phosphorylation plays a key role in chromatin condensation during apoptosis. In addition, MST1 targets transcription factors such as FOXO3 by phosphorylating specific serine/threonine sites, thereby promoting their nuclear translocation and pro-apoptotic function. More broadly, MST1’s substrate recognition is consistent with motifs present in proteins that are central to the Hippo signaling pathway, though no single consensus substrate sequence has been rigorously defined for MST1 in the literature available from the provided sources (ling2008biosignalingofmammalian pages 4-6, record2010structuralcomparisonof pages 1-3).
5. Structure – MST1 is organized into a modular architecture that includes an approximately 300–350 amino acid N‐terminal catalytic (kinase) domain and a C‐terminal regulatory region containing a SARAH (Salvador/RASSF/Hippo) domain. The kinase domain comprises the conserved bilobal structure typical of serine/threonine kinases with an N‐lobe primarily responsible for ATP binding and a C‐lobe that contributes to substrate binding and catalytic activity. Key structural features include a conserved glycine-rich loop, a catalytic lysine that forms a salt bridge with a conserved glutamate in the αC-helix, and an activation loop whose phosphorylation is required for full kinase activation (record2010structuralcomparisonof pages 4-6, ling2008biosignalingofmammalian pages 3-4). The regulatory SARAH domain at the C-terminus mediates homodimerization as well as heterodimerization with other SARAH domain–containing proteins—most notably SAV1—which facilitates efficient signal transduction through the Hippo pathway (tang2014expandingthehippo pages 13-19, ling2008biosignalingofmammalian pages 3-4). Furthermore, MST1 contains caspase cleavage sites adjacent to its kinase domain; cleavage of the inhibitory C-terminal segment by caspases during apoptotic conditions results in a constitutively active kinase fragment that is capable of nuclear translocation (ling2008biosignalingofmammalian pages 3-4, stegert2005functionalcharacterisationof pages 103-107). Structural comparisons with related MST kinases have demonstrated that the conformation of the ATP-binding pocket and the arrangement of the activation loop are critical for inhibitor binding and regulatory control, insights that have been instrumental in guiding inhibitor design strategies targeting MST1 (record2010structuralcomparisonof pages 6-8, tang2014expandingthehippo pages 78-82).
6. Regulation – The regulation of MST1 occurs at multiple levels. Autophosphorylation of the activation loop is a critical step in establishing its catalytic competence, and this autophosphorylation is further enhanced by dimerization via the SARAH domains. During apoptotic signaling, caspase-mediated cleavage removes an inhibitory C-terminal domain from MST1, leading to its nuclear translocation and a marked increase in pro-apoptotic activity; this proteolytic processing is a key regulatory mechanism that converts MST1 to its active form (ling2008biosignalingofmammalian pages 3-4, stegert2005functionalcharacterisationof pages 103-107). MST1 interacts with several regulatory proteins such as SAV1 (Salvador homolog), which stabilizes the kinase and facilitates its phosphorylation of downstream targets; it also forms complexes with tumor suppressors like RASSF1A and adaptor proteins such as NORE1 that modulate its activity and substrate specificity. In addition, MST1 activity is modulated by upstream signaling pathways, with phosphorylation by Akt serving as an inhibitory mechanism that suppresses its pro-apoptotic function, thereby integrating signals from growth factor pathways (ling2008biosignalingofmammalian pages 4-6, tang2014expandingthehippo pages 92-97). Other regulatory inputs include allosteric interactions mediated by hMOB3 and possibly other MOB family proteins; hMOB3, for instance, has been shown to inhibit MST1 caspase cleavage under certain conditions and thereby modulate its apoptotic signaling output (tang2014expandingthehippo pages 78-82, ling2008biosignalingofmammalian pages 8-9).
7. Function – MST1 functions primarily as a stress-activated, pro-apoptotic kinase that plays a central role in the Hippo signaling pathway, which governs organ size, tissue homeostasis, and tumor suppression. Upon activation—either via autophosphorylation or caspase-mediated cleavage—MST1 translocates to the nucleus where it phosphorylates histone H2B, leading to chromatin condensation and internucleosomal DNA fragmentation that culminates in apoptosis (ling2008biosignalingofmammalian pages 4-6, stegert2005functionalcharacterisationof pages 103-107). In the canonical Hippo pathway, MST1 forms a functional complex with its regulatory partner SAV1 to phosphorylate and activate LATS1/2 kinases; activated LATS kinases, in turn, phosphorylate the transcriptional co-activators YAP1 and WWTR1/TAZ. Phosphorylation of YAP1 by LATS kinases results in its cytoplasmic retention through binding to 14-3-3 proteins, thereby inhibiting YAP1-dependent gene transcription that promotes cell proliferation and survival (tang2014expandingthehippo pages 13-19, record2010structuralcomparisonof pages 1-3). Beyond its role in controlling cell proliferation, MST1 is also implicated in immune regulation. It has been shown to regulate T cell adhesion, migration, and survival in lymphoid tissues, which constitutes a fundamental component of immune homeostasis (ling2008biosignalingofmammalian pages 7-8, tang2014expandingthehippo pages 8-13). Moreover, MST1 contributes to the regulation of organ size by repressing the proliferation of mature hepatocytes and preventing the activation of facultative liver stem cells, functions that are critical for tumor suppression and the maintenance of tissue architecture (record2010structuralcomparisonof pages 1-3, sczaniecka2015theroleand pages 152-155).
8. Other Comments – Structural studies of MST1 have enabled the identification of ATP-competitive inhibitors, including quinazoline-based compounds that have been co-crystallized with MST family kinases; these inhibitors target the conserved ATP-binding pocket and have provided a framework for the development of more selective kinase inhibitors (record2010structuralcomparisonof pages 1-3, record2010structuralcomparisonof pages 4-6). Although specific disease-associated mutations in MST1 are not extensively catalogued in the provided literature, dysregulation of MST1—whether through altered expression levels, impaired caspase-mediated cleavage, or inappropriate inhibitory phosphorylation—has been linked to diverse pathological conditions including cancer, cardiac dysfunction, and immunodeficiency (tang2014expandingthehippo pages 87-92, sczaniecka2015theroleand pages 152-155). In the context of cancer, MST1’s role in the Hippo pathway is particularly important because its proper function is essential for maintaining controlled cell proliferation; loss of MST1 activity can contribute to tumor progression and metastasis by failing to inactivate the oncogenic activity of YAP1 (ling2008biosignalingofmammalian pages 9-10, tang2014expandingthehippo pages 92-97). Furthermore, MST1’s involvement in triggering apoptosis through chromatin condensation and DNA fragmentation has positioned it as a potential therapeutic target for interventions aimed at reactivating apoptotic pathways in cancer cells. Future research into more selective MST1 inhibitors and a deeper understanding of its regulatory mechanisms may lead to novel strategies for treating cancers and other diseases linked to aberrant Hippo signaling (record2010structuralcomparisonof pages 6-8, tang2014expandingthehippo pages 23-29).
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