Mammalian Sterile 20–Like Kinases in Tumor Suppression: An Emerging Pathway

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Abstract

Emerging evidence suggests that the proapoptotic kinase mammalian sterile 20–like kinase 2 (MST2) acts in a novel tumor suppression pathway. Recently, we showed that Raf-1 kinase sequesters and inhibits MST2 and that this event is critical for Raf-mediated cell survival. In this review, we summarize Raf control of MST2 and we outline a novel pathway involving the downstream effector proteins Salvador and Warts/Lats that may act to limit the positive effects of Raf/mitogen-activated protein kinase signaling in cancer cells. (Cancer Res 2005; 65(13): 5485-7)

The Mammalian Sterile 20–Like Kinases

The kinase mammalian sterile 20 (Ste20)–like kinase 2 (MST2, Krs1) and its close homologue MST1 (Krs2) are members of the germinal center kinase group II (GCK II) family of mitogen-activated protein kinase (MAPK)–related kinases that includes the more distantly related kinases MST3, MST4, LOK, SOK, and SLK (1). This GCK II family also shares homology to the yeast Ste20 kinase in the kinase domain. GCK II kinases seem to be mainly involved in apoptotic signaling in response to stress signals, including Ste20 itself (2). Both MST1 and MST2 have been described as MAPK kinase kinases that lead to c-Jun-NH2-kinase (JNK) activation in response to stress and Fas signaling, apparently through a MEKK1/MKK7 route (3). Activation of JNK and subsequent activation of caspases seems to explain the original description of MST1 and MST2 as proapoptotic kinases. Once activated, caspase-3 removes the carboxyl-terminal portion of MST2; this also occurs on MST1 together with a distinct caspase-6/7–mediated cleavage event (4). The carboxyl-terminal region contains an inhibitory region together with the Salvador and RASSF homology (SARAH) protein-protein interaction domain, dimerization domain, and a nuclear export signal (4). Caspase cleavage results in a constitutively active kinase domain residing in the nucleus that phosphorlates histone 2B and potentiates the DNA condensation characteristic of apoptosis (5). The yeast homologue, Ste20, has been shown recently to function in a similar manner to promote an apoptotic-like response in yeast, which do not have caspase-like activity or Ste20 cleavage (2). Caspase regulation of MST1/2 may have arisen later in evolution as the full-length kinases seem to be able to be activated in cells by caspase-independent mechanisms. However, activation of the full-length kinase is not readily observed, in agreement with the idea that only a small pool of MST1/2 is active at one time (4). Activation of full-length MST1/2 occurs in response to stress conditions or treatment with staurosporine or okadaic acid (6), requiring a functional dimerization domain that is thought to mediate a trans-autophosphorylation event that is essential for kinase activity (4). At later times, usually several hours after initial activation, ensuing caspase activity severs the carboxyl-terminal regulatory domain from the kinase domain, further enhancing MST1/2 catalytic activity.

Raf Regulates MST2 Signaling

Raf-MAPK signaling mediates diverse cellular fates, including proliferation, differentiation, and survival. Activation of Raf occurs as a result of GTP loading of Ras in response to mitogens, which allows a cascade of activation through Raf and MEK kinases to the MAP kinases, also known as the extracellular signal-regulated kinases (ERK; ref. 7). The MAPK pathway does provide mechanisms to generate specific biological responses that can be imparted through the overall strength and duration of the ERK signal, allowing sustained versus transient activation of ERK substrates. The existence of multiple isofoms at each step in the pathway allows further specificity through distinct binding partners and, hence, integration of additional signals that could modulate the overall response (7). The kinase cascade consists of three Raf isofoms, Raf-1, B-Raf, and A-Raf, which are able to phosphorylate both isofoms of MEK (MEK1/2), which in turn can activate either ERK1 or ERK2 (7). Ras activates a group of pathways, but is the only known signal that activates the Raf kinases (7) and the importance of this step in cellular events has been established through the identification of mutation in Ras and the B-Raf kinase, which lead to increased ERK signaling in human cancers (8). Genetic ablation of the Raf isofoms in mice allowed us to separate some unique aspects of individual Raf isoform signaling. Most notably, mice in which the raf1 gene was deleted died in utero due to an overwhelming level of apoptosis, which occurred despite normal expression of the alternate Raf isoforms and undisturbed ERK activation (9).

The fact that ERK activation was unaffected in cells from Raf-1−/− mice pointed to a survival signal, other than Ras-induced ERK signaling, that is defective and that led to the apoptotic phenotype of these mice (10, 11). A survival function for Raf-1 that is independent of ERK-promoting activity was confirmed by phenotypic rescue of the Raf-1 null mice with a Raf-1-YY340/341FF derivative that is not activated by mitogens and that has low basal kinase activity (10). Mouse embryo fibroblasts derived from the Raf-1−/− mice also exhibited a hyperapoptotic phenotype; furthermore, the sensitivity was specific to certain stimuli (i.e., serum starvation and engagement of the death receptor Fas). Using this system, we identified the pathway that leads to the proapoptotic phenotype, defining MST1 and MST2 in proteomic screens for potential pathways through which Raf-1 could provide a survival signal (12).

The interaction of Raf-1 and MST2 in cells occurred in the absence of serum growth factors and was dissociated by signaling by mutant Ras, growth factors, or proapoptotic stimuli, only the latter of which led to MST2 activation, however (12, 13). Ras and growth factors also contribute to cell survival by collateral signaling.
pathways involving ERK and Akt (13); it is currently unknown whether these pathways may also impinge on MST2 activation. Significantly, the interaction with Raf-1 suppressed MST2 phosphorylation and kinase activity, and a Raf-1 mutant devoid of kinase activity was equally active as observed in reconstitution experiments of Raf-1/C0/C0 cells. Thus, the kinase activity of Raf-1 is dispensable for MST2 control, strongly suggesting a different structural mechanism. Indeed, Raf-1 binding to MST2 sequesters MST2 monomers and prevents the dimerization required for trans-autophosphorylation and activation. Additionally, Raf-1 recruits a phosphatase that dephosphorylates MST2 preserving the inactive state (12). This phosphatase is presumably protein phosphatase 2A (PP2A), which has been previously shown to associate with Raf-1 and remove an inhibitory phosphorylation from Ser259 during Raf-1 activation (7). Thus, PP2A could coordinate the activation of Raf-1 with the concomitant deactivation of MST2, possibly explaining why growth factor stimulation can dissociate the Raf-1/MST2 complex without inducing MST2 activation. This double control of MST2 by Raf-1 seems to ensure that the release of MST2 only results in MST2 activation and proapoptotic activity if MST2 phosphorylation is permitted at the same time.

The apoptotic sensitivity of the Raf-1/C0/C0 mouse embryo fibroblasts in response to Fas signaling and serum starvation indeed correlates with an enhancement of MST2 kinase activity. Ablation of MST2 in the knockout cells reversed their elevated apoptotic sensitivity, whereas overexpression of exogenous MST2 increased this sensitivity. Similar experiments in Raf-1/C0/C0 control fibroblasts indicated that MST2 had to be overexpressed to an extent where its levels exceeded the levels of Raf-1 before an apoptotic sensitivity manifested, suggesting that MST2 is quantitatively sequestered by Raf-1 (12). In human cell lines, down-regulation of Raf-1 and MST2 by small interfering RNA confirmed that MST2 had to be overexpressed to an extent where its levels exceeded the levels of Raf-1 before an apoptotic sensitivity manifested, suggesting that MST2 is quantitatively sequestered by Raf-1 (12). Notably, B-Raf does not associate with MST1 or MST2 and hence has no direct control over MST induced apoptosis like Raf-1 (12).

**A Novel Tumor Suppressor Pathway that Includes MST2, Salvador, and Warts/Lats**

Genetic analyses conducted in *Drosophila* suggest that the MST2 homologue Hippo functions in a conserved tumor suppressor pathway that includes the Salvador adapter protein and the Warts/Lats kinase(s). As mentioned above, MST2 kinase activity functions to promote apoptosis via JNK and caspase activation (3). However, the recently described MST2/Hippo pathway has been implicated in both apoptosis and cell cycle inhibition in *Drosophila* (14). Intriguingly, this pathway seems to be conserved in mammals. Hippo (dMST) has been proposed to phosphorylate and activate the AGC-like kinase Warts, facilitated by the scaffolding protein Salvador,
which presents separate interaction domains for Hippo and Warts (14). Warts signaling reduces the transcription and stability of the inhibitor of apoptosis protein diAP, thereby priming cells for apoptosis. It further blocks cell cycle progression by diminishing the levels of cyclin E (14). Whereas there is conjecture over the tumor suppressor status of human Salvador homologue, hSav1, the human homologues of warts, Lats1 and Lats2, seem to be bona fide tumor suppressors and regulators of cell cycle progression and apoptosis. Lats1 ablation promotes tumorigenesis in mice, highlighting its status as a tumor suppressor (15). Recently, biochemical evidence has been provided for the activation of human Lats1 by MST2 (16). However, no binding between MST2 and Lats1 was observed, suggesting that in humans the activation mechanism may be different than in Drosophila. Nevertheless, the skeleton of the pathway seems to be conserved, and it will be interesting to map the further elements that connect the MST activation of Lats to the regulation of cyclin E and IAP levels. In human cells, Lats1/2 have been shown to affect cyclin E and cyclins A/B, inducing cell cycle arrest at G1-S or G2-M accordingly (17, 18). Failure to maintain correct cyclin expression patterns benefits tumor growth, whereas overexpression of IAP proteins is commonly observed to aid tumor survival (19).

The upstream regulation of MST2 is just beginning to be elucidated. The removal of the negative regulation by Raf-1 is not the sole means of control over MST2 activation. Interestingly, MST2/1 each have been shown to be associated with members of the RASSF tumor suppressor family through the SARAH protein-protein interaction domain (20). The RASSF family consists of six members that each have numerous splice variants. Methyltransferase silencing of a RASSF1-specific splice form promoter was observed in the 3p tumor suppressor locus of lung, breast, and ovarian tumors (21, 22). Further studies showed a much wider penetration of epigenetic silencing in the majority of primary tumors analyzed. This silencing—mainly of the RASSF1a splice isoform—is linked to severity of disease and/or poor prognosis. Whereas variation between splice forms arises from independent promoters, producing exclusive amino-terminal regions, commonality exists in a conserved Ras association (RA) domain that defines the family and a SARAH domain that is responsible for the interaction with MST.

As the RASSF proteins do not have any enzymatic activity themselves, it has been suggested that these proteins provide a scaffold function for the MST kinases (4). This may explain the limited activation of MST kinase activity that is observed via physiologic stimulation, a scaffold may simply target the restricted activity to a specific region of the cell when required. Through RA domain association, RASSF family members have been shown to variably associate with a variety of GTP-binding proteins, including Ki-Ras, Ha-Ras, R-Ras, M-Ras, and Rap1/2 (Fig. 1; ref. 4). Rap1B binding to RASSF5b/Nore/RapL has been shown to play a role in lymphocyte migration and adhesion along endothelial vessel walls, indicating additional functions of RASSF proteins in addition to apoptosis and cell cycle control (23). The different associations through the RA domain show a potential for spatial targeting within the cell of the different isoforms. Further support for this idea comes from the presence of a diacylglycerol binding domain that is specific to the amino terminal of the RASSF1a isoform and is absent from the 1c isoform that remains expressed in tumors (4).

Spatial localization of MST kinases may also allow access to activation signals that would be required for full activity. When Raf-1 control of MST2 is removed by serum or active Ras, MST2 still requires an activation step that could potentially be through a RASSF interaction. Some evidence for this has been provided by the potentiation of MST1-induced apoptosis via CKN1 binding (24). Once Raf-1 control is relieved and MST is activated, the RASSF proteins may also serve to deliver the kinase activity to potential substrates, such as apoptotic targets or components of the cell cycle, e.g., the anaphase promoting complex (APC/C; ref. 25). Elucidation of how the RASSF-MST interaction is regulated by Raf-1 and the RA domain of RASSF will help explain the activation and/or targeting of the MST kinase activities. Moreover, exactly how MST activity is affected by variation in RASSF splice forms is likely to provide an important insight into the tumor relevance of the RASSF1a silencing.

Acknowledgments

References


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5487 Cancer Res 2005; 65: (13). July 1, 2005

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