Identification of the RASSF1A-Associated Mitotic and Apoptotic Complex (RAMAC) in Mammalian Cells

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RASSF1A (RAS association domain family protein 1A) is encoded by a putative tumor suppressor gene whose expression is frequently silenced in various cancers as a result of hypermethylation of a CpG island in its promoter. Previously, we demonstrated that tumor suppressor RASSF1A is recruited to spindle poles by RABP1 and regulates timing of mitotic progression by inhibiting APC-Cdc20 activity at prometaphase. Here, by using proteomics, we have now newly identified a RASSF1A-associated mitotic and apoptotic complex, here designated as 'RAMAC", which contains MST1, MST2, hWW45, EDD, LATS2. In addition, we confirm their interactions in vivo and particularly demonstrate that the tumor suppressor protein RASSF1A enhances Mst1 activation as well as Mst1-mediated apoptosis in response to death-receptor apoptotic signal. Coimmunoprecipitation and colocalization studies show that Mst1 associated with RASSF1A *in vivo* and they colocalized to microtubules throughout the cell cycle. In addition, overexpression of RASSF1A augmented the Mst1 kinase activity in mammalian cell. Both apoptosis and Mst1 activation after anti-Fas treatment were significantly suppressed in RASSF1A-depleted cells or increased in RASSF1A-complemented cells, respectively. Notably, further increase of Fas-induced apoptosis by RASSF1A overexpression was abrogated by Mst1 depletion with RNA interference. These findings indicate that RASSF1A contributes to the apoptotic function of Mst1 by activating Mst1 kinase *in vivo*. Since these Drosophila homologs have been shown to promote both restriction of cell proliferation and apoptosis, newly identified 'RAMAC" also may play an important role in cell proliferation and apoptosis.