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RASSF5: An MST activator and tumor suppressor *in vivo* but opposite *in vitro*

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Is RASSF5 a tumor suppressor or activator? RASSF5 links K-Ras and the Hippo pathway. Hippo's signaling promotes YAP1 phosphorylation and degradation. YAP1 overexpression promotes cancer. Most reports point to RASSF5 suppressing cancer; however, some point to its promoting cancer. Our mechanistic view explains how RASSF5 can activate MST1/2 and suppress cancer *in vivo*; but inhibits MST1/2 *in vitro*. We propose that both activation and inhibition of MST1/2 can take place via SARAH heterodimerization. Our thesis *in vivo*, membrane-anchored Ras dimers (or nanoclusters) can promote SARAH domain heterodimerization, Raf-like MST1/2 kinase domain homodimerization and trans-autophosphorylation. In contrast, *in vitro*, K-Ras binding also releases the RASSF5 SARAH stimulating MST1/2's SARAH heterodimerization; however, without membrane, no MST1/2 kinase domain homodimerization/trans-autophosphorylation.

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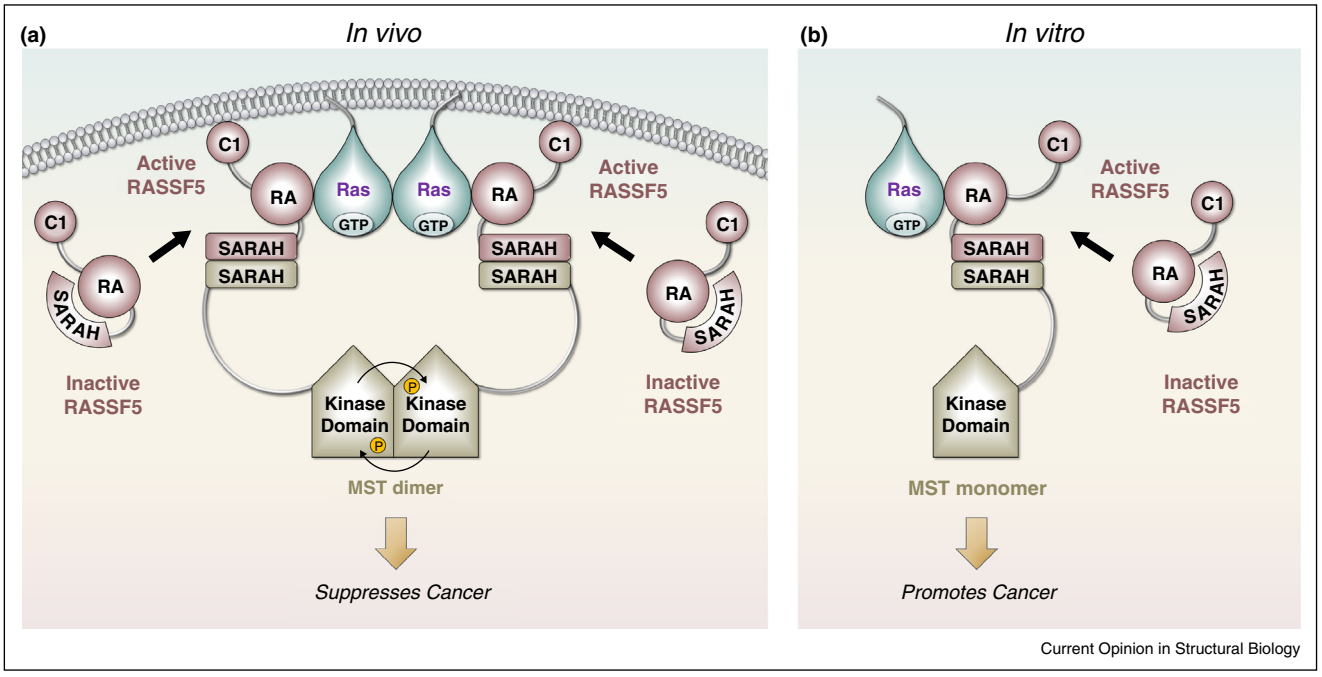
Introduction

Ras association domain family member 5 (RASSF5, also known as NORE1A) is an effector of the Ras protein [1•]. It also binds to mammalian sterile 20-like serine/threonine 1/2 (MST1/2) in the Hippo pathway. Hippo's signaling promotes phosphorylation of Yes-associated protein 1 (YAP1) [2,3•,4]. Phosphorylation is an essential signal that tags YAP1 for degradation. Overexpression of YAP1 is often observed in cancer, including those cancers where the mitogen-activated protein kinases (MAPK) pathway (Ras/Raf/MEK/ERK) is also mutated [5–7,8•,9]. Thus, RASSF5 is a key network node linking active, GTP-bound Ras, including the highly oncogenic K-Ras4B, to the Hippo pathway and YAP1 abundance in cancer. *KRAS4B* is the most abundant mutated oncogene in cancer, particularly of the pancreas (over 95%), lung and colorectal [10,11•,12]. The clear importance of RASSF5 as a potential drug target has recently led to increasing interest in the community. Signaling through the Hippo pathway, a conserved kinase cascade that controls organ size by regulating cell proliferation, apoptosis, and stem cell self-renewal, is modulated not only by the RASSF5, but also by cell contact inhibition [13]. Dysregulation of the Hippo pathway can result in higher levels of YAP1 and cancer development.

Here we focus on the linkage between K-Ras and Hippo's signaling through RASSF5. From the mechanistic standpoint, RASSF5 has raised a number of fundamental and perplexing questions. Among these is first, how Ras activates RASSF5; second, can RASSF5 act as both a suppressor and activator of cancer which is what some experimental reports suggest and if so how; third, MST1/2 trans-autophosphorylation requires that the kinase domain homodimerizes. Is the kinase domain homodimerization promoted by MST1/2 SARAH (Sav-RASSF-Hippo) domain homodimerization or heterodimerization of SARAH domains involving RASSF5 and MST1/2? Understanding how RASSF5 links Ras to MST1/2, and via Hippo signaling to YAP1, will provide a good grasp of a key linkage in cancer cell biology, and thus in drug discovery. Here we will comment on these questions from the structural standpoint, as well as through the lens of our views of pathway-driven tumor proliferation.

Below we provide a mechanistic perspective of RASSF5 in *KRAS4B*-driven cancer. We describe how K-Ras4B activates RASSF5, which in turn can promote — or as some experimental data suggest — inhibit MST1/2 activation. We argue that *in vivo* activated RASSF5 can function to activate MST1/2 and suppress cancer (Figure 1a) whereas *in vitro*, activated RASSF5 can function to inhibit MST1/2 which in a cell scenario would activate cancer (Figure 1b). We also overview K-Ras pathway-driven tumor initiation and the RASSF5 linkage

Figure 1



As an adaptor, RASSF5 binds to Ras and MST through its RA domain and SARAH domain, respectively. **(a)** *In vivo*, active Ras is anchored into the membrane, forming dimers, and nanoclusters. The C1 domain of RASSF5 attaches to the membrane as well. The interaction with Ras leads to a conformational change in RASSF5, allosterically activating it, shifting the landscape toward an open SARAH domain, which makes it available for interaction with the SARAH domain of MST. The RASSF5/MST SARAH heterodimer has a higher affinity than RASSF5/RASSF5 or MST/MST SARAH homodimers. Membrane-anchored Ras-bound RASSF5 has increased effective local concentration and is preoriented for productive MST homodimerization, with trans-autophosphorylation with RASSF5 acting as an adaptor linking active GTP-bound Ras and MST. The active MST then activates the Hippo pathway which results in phosphorylation of YAP1, leading to its degradation, thus suppressing cancer. By contrast **(b)**, *in vitro*, without the benefits of the membrane, active Ras lacks anchoring and its effective local concentration is low. Due to the stronger interaction of the RASSF5/MST SARAH heterodimer than the MST/MST SARAH homodimer, the MST/RASSF5 complex is still preferred, which prevents MST kinase domain homodimerization and MST trans-autophosphorylation. The inactive MST inactivates the Hippo pathway. YAP1 is overexpressed, which promotes cell proliferation.

to the Hippo pathway in this light. The Hippo and MAPK signaling have similar roles in tumor cell proliferation, and the Hippo pathway, as well as YAP1, are frequently mutated or overexpressed in Ras-driven cancers and drug resistance. Hippo and MAPK are independent *core* pathways fulfilling similar roles in the cell cycle [8•].

Our thesis: RASSF5 is an adaptor protein

How can RASSF5 act as both suppressor and activator of cancer as some literature reports suggest? Figure 1 provides a schematic diagram of our thesis. RASSF5 is a Ras effector. It interacts with Ras via its Ras association (RA) domain. The interaction is at the same effector site where other Ras effectors, such as the serine-threonine protein kinase Raf, the lipid kinase phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) and Ral guanine nucleotide dissociation stimulator (RalGDS), the exchange factor for other GTPases, also interact [14–16,17•]. The interfaces of Ras/Raf, Ras/PI3K, and Ras/RalGDS overlap that of Ras/RASSF5, and the Ras binding domains (RBDs) of Raf, PI3K and the RA domains of RalGDS and RASSF5 are all similar to each other (Figure 2) [11•]. GTP-bound Ras activates all its effectors. Among Ras effectors, Raf's activation involves the homodimerization of its catalytic kinase domain and trans-autophosphorylation, where the catalytic domains cross-phosphorylate each other [18•,19]. A long, ~165-residue linker, characteristic to all Raf proteins, connects Raf's RBD with Raf's catalytic domain. Allosteric effects elicited by the binding of the RBD to Ras, and 14-3-3 recognition of a phosphorylated motif in the highly flexible hinge region (Ser259 in human c-Raf), result in the homodimerization of the catalytic kinase domain, Raf's activation, and MAPK — also a phosphorylation cascade — signaling. MAPK signaling acts in the early phase of the G1 (Gap 1) into the S (Synthesis) cell cycle stage [14]. Ras dimers (and nanoclusters) increase the effective local concentration of Raf and may restrain its orientation, preconditioning it for productive binding [20,21,22•].

MST1/2 does not have a Ras binding domain; RASSF5 does, and like MST1/2 it has a SARAH domain [23]. The