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Review

The RASSF proteins in cancer; from epigenetic silencing to functional characterization

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ABSTRACT

The Ras-Association Domain Family (RASSF) comprises ten members, termed RASSF1 to RASSF10. RASSF1 to RASSF6 harbor a C-terminal Ras-association (RA) domain and RASSF7 to RASSF10 contain an N-terminal RA domain. Interestingly, it was observed that in various tumor types distinct RASSFs transcripts (e.g. *RASSF1A* and *RASSF2A*) are missing due to hypermethylation of their CpG island promoter. Since methylation of the *RASSF1A* promoter is described as an early and frequent event in tumorigenesis, *RASSF1A* could serve as a useful diagnostic marker in cancer screens. RASSFs are implicated in various cellular mechanisms including apoptosis, cell cycle control and microtubule stabilization, though little is known about the underlying mechanisms. Tumor suppressing functions were reported for several members. Here we review the current literature on RASSF members focusing on structural, functional and epigenetic aspects. Characterizing the cellular mechanisms that regulate the signaling pathways RASSFs are involved in, could lead to a deeper understanding of tumor development and, furthermore, to new strategies in cancer treatment.

Contents

Abbreviations: AP-1, Activator protein-1; APC/C-CDC20, complex anaphase-promoting complex/cyclosom-cell division cycle 20 homolog; ATM, ataxia telangiectasia mutated; C19ORF5, chromosome 19 open reading frames 5; CDC20, cell division cycle protein 20; CDH1, Cdc20 homolog 1; CDK, cyclin dependent kinase; CNK1, connector enhancer of KSR; DAXX, death-domain associated protein; DNMTs, DNA-methyltransferases; Hpo, Hippo; Id-1, Inhibitor of DNA binding/differential-1; LATS, large tumor suppressor; MOAP-1/MAP1, modulator of apoptosis-1; MAP1B, microtubule-associated protein 1B; MST, mammalian sterile 20-like kinase; MTOC, microtubule organizing center; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; NORE1, Novel Ras Effector 1; PKC, protein kinase C; RA, Ras-association (RalGDS/AF-6) domain; SARAH, Salvador, RASSF and Hippo; Sav, Salvador; SKP2, S-phase kinase-associated protein 2; TNFα, tumor necrosis factor alpha; Wts, Warts; WW45, human Salvador homolog; YAP1, Yes-associated protein 1

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1. Introduction

This review will give an insight into the broad spectrum of investigations regarding the Ras-Association Domain Family (RASSF), which has been studied with ever-growing interest since the first description and characterization of RASSF1 in the year 2000 [1].

Ras-Association Domain Family 1 (RASSF1) was first identified using a (presumably false-positive) yeast-two-hybrid screen through its interaction with XPA, a protein known to function in DNA excision repair [1]. The C-terminus of RASSF1 displayed a high homology to mouse Nore1 (later termed Rassf5), a known Ras effector [2]. Dammann et al. further identified three different RASSF1 transcripts: *RASSF1A*, *B* and *C*. These variants were shown to share four common exons, encoding a Ras-association *(RalGDS/AF-6)* domain, from which the name of the family was derived. The Ras-Association Domain Family 1 (*RASSF1*) gene is located on the small arm of chromosome 3 (3p21.3) [1]. Earlier loss of heterozygosity (LOH) studies were investigating the very same chromosomal region in various tumor entities and had already proposed the existence of tumor suppressor genes at 3p21.3 [3,4].

Different cellular mechanisms can contribute to tumorigenesis. Loss of function of tumor suppressor genes and gain of function of proto-oncogenes can be observed [5]. Molecular changes lead to the activation of oncogenes by mutations, amplifications, increased promoter activity and/or translation of fusion-proteins. Regarding tumor suppressor genes, loss of one allele can be compensated by the intact remaining one, therefore, the crucial inactivation is the silencing of the second allele [6]. Loss of function of tumor suppressors can occur through mutations, deletions, mitotic recombination events or by epigenetic inactivation, through methylation of CpG islands in the promoter region [7]. The term Epigenetics comprises all hereditary changes of gene regulation that are not based on the DNA sequence itself [8]. So-called CpG islands are DNA regions in which dinucleotides of cytosine–guanine are statistically overrepresented in comparison to the whole genome. CpG islands are often associated with promoters; genes, whose promoters are especially rich in CpG sequences, tend to be expressed in most tissues. Cytosines in CpG islands can be methylated at the 5-position by DNA-methyltransferases (DNMTs) and the corresponding gene (e.g. tumor suppressor gene) can, therefore, be silenced. Methylated CpGs are recognized by methyl-CpG-binding proteins that can form histone deacetylase silencing complexes that regulate gene expression at the chromatin level [9–11]. Epigenetic inactivation of the tumor suppressor RASSF1A was frequently reported in different tumor entities as reviewed previously [12,13].

2. The Ras-Association Domain Family

The Ras-Association Domain Family comprises ten members from RASSF1 to RASSF10 as well as various isoforms, which are listed in Fig. 1. One characteristic features of this family is the Ras-association domain (RA), which can be found either C-terminally (RASSF1 to RASSF6) or N-terminally (RASSF7 to RASSF10). The other characteristic feature is the Sav–RASSF–Hpo (SARAH) domain, encoding a protein–protein interaction domain, which however is only found in RASSF1 to 6. Prominent and most intensely studied family members are RASSF1A (an isoform of RASSF1) and RASSF5, also called NORE1. Whereas RASSF7 to RASSF10 joined the family only recently and therefore little data exist to date.

3. The RASSF domains

The Ras-Association Domain Family proteins contain several distinct domains that are depicted for each member in Fig. 1. The **RA domain** is a Ras-association (RalGDS/AF-6) domain and a characteristic feature of Ras-effectors and Ras-related-GTPases [14], that gave its name to the whole Ras-Association Domain Family [1]. The **C1 domain** was named after its high homology with a cysteine-rich diacylglycerol/phorbol ester (DAG)-binding domain also called protein kinase C conserved region 1 (C1). Its central C1 zinc finger [15] is characteristic for the domain and RASSF1A was shown to associate with the TNF-R1/MOAP-1 or TRAIL-R1/MOAP-1 complex via its C1 domain [16]. The **ATM domain** corresponds, by its sequence, to a putative ATM-kinase phosphorylation motive. It was shown, that this peptide becomes phosphorylated by ATM at least *in vitro* [17]. The ATM kinase (ataxia telangiectasia mutated) is of central importance for the regulation of cell cycle checkpoints that lead to DNA-repair and apoptosis [18]. However, a functional relevance of this ATM domain has not been confirmed *in vivo* yet. The **SARAH domain** is a protein–protein interaction domain, named after the tumor suppressors *Sa*lvador (in *D. melanogaster*; orthologue of human WW45), *RA*SSF and *H*ippo (in *D. melanogaster*; orthologue of the human proapoptotic kinase MST1). This domain is characterized by its length of 50 amino acids and its distal C-terminal position. SARAH domains are found in WW45, MST1 and different RASSFs and heteromeric and as well as homomeric interactions can be conducted via SARAH domains [19], e.g. MST and WW45 or RASSF1A and RASSF5 interaction [20–22]. Ortiz-Vega et al. reported that recombinant RASSF1C exhibits a much weaker ability to homodimerize or heterodimerize with RASSF5 in comparison to RASSF1A [20]. Regarding RASSF2 it was shown to associate with RASSF3 and RASSF5 [23] and RASSF5 with RASSF1A through their nonhomologous amino-terminal segments [20]. The functional consequence of these RASSF interactions need to be addressed in further studies, as it is known that SARAH domains play a central role in the newly discovered Hippo signaling pathway in *D. melanogaster*, which regulates cell proliferation and apoptosis [19,24].

4. The RASSF members

4.1. RASSF1

The *RASSF1* gene, which is located on the small arm of chromosome 3 (locus 3p21.3) codes for eight exons (1α, 1β, 2αβ, 2γ, 3, 4, 5 and 6) (Fig. 2). There are seven different RASSF1 isoforms (RASSF1A to RASSF1G) that are generated by differential usage of two promoters (distance 3.5 kb) and through alternative splicing [25]. So far however, the biological relevance of only two isoforms, RASSF1A and RASSF1C, was demonstrated. Regarding the transcripts *RASSF1B* and *RASSF1E* there is currently not enough evidence to support a biological role, as well as for the candidates *RASSF1F* and *RASSF1G* that possibly enter