



Review

RASSF tumor suppressor gene family: Biological functions and regulation

Natalia Volodko^a, Marilyn Gordon^a, Mohamed Salla^b, Haya Abu Ghazaleh^d, Shairaz Baksh^{a,b,c,*},1

^a Department of Pediatrics, Faculty of Medicine and Dentistry, 113 St. 87 Ave., University of Alberta, Edmonton, AB T6G 2E1, Canada

^b Department of Biochemistry, Faculty of Medicine and Dentistry, 113 St. 87 Ave., University of Alberta, Edmonton, AB T6G 2E1, Canada

^c Department of Oncology, Faculty of Medicine and Dentistry, 113 St. 87 Ave., University of Alberta, Edmonton, AB T6G 2E1, Canada

^d The American University of the Middle East, P.O. Box: 220 Dasman, 15453, Kuwait

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ABSTRACT

Genetic changes through allelic loss and nucleic acid or protein modifications are the main contributors to loss of function of tumor suppressor proteins. In particular, epigenetic silencing of genes by promoter hypermethylation is associated with increased tumor severity and poor survival. The RASSF (Ras association domain family) family of proteins consists of 10 members, many of which are tumor suppressor proteins that undergo loss of expression through promoter methylation in numerous types of cancers such as leukemia, melanoma, breast, prostate, neck, lung, brain, colorectal and kidney cancers. In addition to their tumor suppressor function, RASSF proteins act as scaffolding agents in microtubule stability, regulate mitotic cell division, modulate apoptosis, control cell migration and cell adhesion, and modulate NFκB activity and the duration of inflammation. The ubiquitous functions of these proteins highlight their importance in numerous physiological pathways. In this review, we will focus on the biological roles of the RASSF family members and their regulation.

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1. Introduction

The RASSF family of proteins is comprised of ten members each with multiple splice variants, with the exception of RASSF9 and 10 [1,2]. These proteins were named due to the presence of a Ras association (RA) domain in their N-terminus or C-terminus. The RA domain potentially interacts with the Ras GTPase family of proteins [2] that control a number of cellular processes including membrane trafficking, apoptosis, and proliferation [1–5]. Direct association with K-Ras has been only observed for RASSF2, 4, 5A, 6 and 9 [6–8]. In addition, RASSF proteins have several other functional domains that modulate associations with other proteins (see Table 1 for the list of RASSF interacting partners). These include a Salvador-RASSF-Hippo (SARAH) domain involved in several protein–protein interactions and for homo- and heterodimerization of RASSF isoforms. RASSFs can associate via the SARAH domain with downstream kinases such as mammalian sterile 20-like kinases (MST1 and MST2 [mammalian Hippo] also known as or STK4 and

STK3 respectively) and the mammalian orthologue of the tumor suppressor Salvador, SAV1 in order to promote apoptosis [9]. These various structural domains allow for associations with numerous molecules and determine RASSF proteins' involvement in several biological pathways in order to carry out tumor suppressor functions.

An ATM phosphorylation site is present on some RASSF proteins (RASSF1A and RASSF1C), and RASSF1A and RASSF1C also contain an N-terminal protein kinase C conserved region 1 (C1) domain that co-localizes with microtubules [1]. The C1 domain has also been demonstrated to allow for the association of RASSF1A with death receptor complexes, such as TNF-R1 and TRAIL [10]. The ATM phosphorylation site has been found to be phosphorylated in several RASSF proteins upon DNA damage/repair [11] (Reviewed in [12]).

2. The diverse nature and biological function of RASSF proteins

2.1. RASSF1A

In 2000 Dammann et al. cloned a gene that was mapped to the 3p21.3 region, whose genomic instability was frequently described in lung cancer. The gene was named RASSF1 as it contains Ras-association domain [13]. RASSF1 has eight transcripts (A–H),

* Corresponding author at: 3020E Katz Research Building, Department of Pediatrics, Faculty of Medicine and Dentistry, 113 St. 87 Ave., University of Alberta, Edmonton, AB T6G 2E1, Canada.

E-mail address: sbaksh@ualberta.ca (S. Baksh).

¹ Member, Alberta IBD Consortium.

Table 1
Summary of currently known biological information of the RASSF family of proteins.

RASSF isoform	Chromosome/MW	Biological functions	Interacting proteins	Mice knockout
1A	3p21 39.2 kDa	Regulates: <ul style="list-style-type: none"> • MST kinase-dependent apoptosis [42,44,109] • Death-receptor dependent apoptosis [40,41,175] • Microtubule formation and stabilization [14,176] • Cell cycle [35,165,176] • Mitosis [25] • Stability of mitotic cyclins and the timing of mitotic progression by inhibiting APC-Cdc20 [27,28,31] • Cardiac hypertrophy [49] Modulates NFκB activity [46,50] Inhibits β-catenin accumulation [5] Involved in DNA repair [12] Stabilizes p53 [36] and p73 [11,45] Protects from inflammation-induced injury [46]	MST1 [42–44,50,168,177] MST2 [45,177] MOAP1 [40,41,175] 14–3–3 [109] RABP1 [25] Cdc20 [28] Aurora A/B [29,165,169] MAP1B, MAP1S [14] MDM2 [36] Ran [176] Rap1A [178] RASS1A, RASSF5A [81] p120 E4F [33] Chk1 [170] DDB1 [172] H-Ras [179] ATM [11] Skp2 [166]	Yes [47,48]. Spontaneous tumorigenesis [47]; susceptible to DSS-induced colitis [46]
1C	3p21 31.2 kDa	Stimulates cell growth, may promote metastasis and survival of cancer cells [16] Promotes cell migration, attenuates apoptosis [17] Silencing resulted in a decrease in osteosarcoma and lung cancer cell proliferation [16,18] Inhibits β-catenin degradation [5] Activates SAPK/JNK signaling pathway [54] Down-regulated by CAS/CS1L [180]	MST1 [43,177] MST2 [177] IGFBP5 [18] TFP1-2 [56] βTrCP [5] DAXX [54,55]	No
2	20p13 37.8 kDa	Inhibits cell growth, arrests cell cycle [58,62] Involved in actin cytoskeleton organization [148] Induces apoptosis [58,62,145] Suppresses transcriptional activity of NFκB [181] Inhibits MST2 activity [93]	K-Ras [62] PAR4 [64] MST1 [66,177] MST2 [177]	Yes [67] growth retardation, systematic haematopoietic anomalies, defects in osteoclast and osteoblast differentiation
3	12q14.1 28.6 kDa	Regulates apoptosis and cell cycle via p53 stabilization, possibly involved in DNA repair [69,72]	MOAP1, Mdm2 [72] MST1, MST2 [72,177]	Not available
4	10q11.21 36.7 kDa	Required for apoptosis and growth inhibition [8] Inhibits MST2 activity [93] Modulates the MAP kinase signal downstream of the Ras signal [77]	K-Ras [8] ST1, MST2 [177] MST1 [75]	Not available
5A (Nore1A)	1q32 47.1 kDa	Suppresses tumour growth via apoptosis induction or cell cycle delay [7,82] Regulates microtubule formation [182] Induces degradation of HIPK1 oncoprotein [183] Required for the TNFα mediated apoptosis and full activation of MST1 [80]	Ras, Carma1 [182,184] MST1 [43,177] MST2 [177] tubulin, Aurora A [182] Mdm2 [183] Itch [185]	Yes [80] resistant to TNFα-induced apoptosis, fail to activate Mst1 in vivo
5C (Nore1B, RAPL)	1q32 30.4 kDa	Regulates lymphocyte adhesion, T cell migration, T cell receptor regulation [84,86,186] Controls the directional migration of vascular endothelial cells [6]	Ras, Carma1 [184] Rap1, Rap2, MST1 [6,84,86,186]	Yes [86] impaired lymphocyte trafficking and lymphoid organ abnormalities
6	4q13 43.4 kDa	Regulates apoptosis [87,93,94] Regulates cell cycle [94] Suppresses NFκB pathway [87,187] Stabilizes p53 [94] Potentially plays a role in the inflammatory response to respiratory syncytial virus-induced bronchiolitis [87,88] Possible obesity regulator [149]	MST2 [93] K-Ras, MOAP-1 [87] MST1, MST2 [177] MDM2 [94]	Not available
7	11p15 39.9 kDa	Regulates cell growth and mitosis [102,103] Has anti apoptotic activity [104]	N-Ras [104] MST1, MST2 [177] CHMP1B, DISC1 [3]	Not available
8	12p12 48.3 kDa	Inhibits cell growth, regulates Wnt and NFκB pathways, regulates cell–cell adhesion [4]	14–3–3γ, FRMD4A, PSMD4 [3]	Not available
9	12q21.31 50.0 kDa	Possible role in endosome recycling [110] Essential for epidermal homeostasis [112]	Peptidylglycine α-amidating monooxygenase [111] N-, K- and R-Ras [188] (contradicts to [177])	Yes [112] alopecia, shorter life expectancy and growth retardation
10	11p15 56.9 kDa	Suppress tumor cell growth [189,190] Induces apoptosis, inhibits Wnt/β-catenin pathway [116]	None described	Not available

The table summarizes current knowledge of the RASSFs with their chromosomal location, protein molecular weights (MW), biological functions, association partners and genetic knockouts.

arising from alternative splicing and differential promoter usage. Among the RASSF1 subtypes, 1A and 1C are the most extensively studied members with both localized to microtubules and involved in regulation of growth and migration [14,15]. Whereas RASSF1C may promote the proliferative capacity of cancer cells (especially breast and lung cancer cells) [16–18], RASSF1A is a bona fide tumor suppressor [1,2]. Thus, despite harboring 60% amino acid identity (mainly after amino acid 121 of RASSF1A), RASSF1A and RASSF1C display distinctive biological properties.

Several groups have demonstrated the importance of RASSF1A for microtubule stability and have mapped residues important for this function [10,14,19–21]. RASSF1A interacts with microtubules though interaction with microtubule associated proteins, such as MAP1B (microtubule-associated protein 1B) and MAP1S (microtubule-associated protein 1S) [14]. The loss of RASSF1A microtubule localization results in the inhibition of tumor suppressor properties, loss of tubulin stability, inhibition of death receptor-dependent cell death and promotion of genomic instability through the loss of centrosome and mitotic spindle body structures [14,19,20]. One of the possible mechanisms of microtubule stabilization by RASSF1A involves RAN GTPase [22], and another mechanism discovered recently includes the suppression of histone deacetylase 6 which functions as a tubulin deacetylase [23]. The ability of RASSF1A to regulate microtubule stability, spindle assembly and chromosome attachment determine RASSF1A tumor suppressor functions towards controlling cell growth, transformation, motility and invasiveness [15,19,24].

Further studies have shown that interaction of RASSF1A with RABP1 (RASSF1A binding protein 1/C19ORF5/MAP1S) leads to its recruitment to the spindle poles in pro-metaphase and its interaction with Cdc20 [25] (please note that here is a contradictory report on RASSF1A interaction with Cdc20 [26]), inhibition of APC (anaphase promoting complex), accumulation of mitotic cyclins A and B and eventually mitotic arrest [25,27,28]. Upon phosphorylation by Aurora A, RASSF1A fails to interact with Cdc20 thereby relieving APC inhibition, leading to degradation of cyclins and mitotic progression [29]. Interestingly, RABP1/MAP1S was recently shown to enhance autophagy by suppressing genomic instability and tumorigenesis [30]. Furthermore, it has also been suggested that RASSF1A stabilization of microtubules may have a role in autophagy regulation [30].

Along with mitotic progression, RASSF1A was shown to regulate other aspects of the cell cycle. RASSF1A inhibits accumulation of cyclin D1 (possibly through JNK kinase pathway [31,32], suppression of AP-1 activity [27], or both) and blocks the cell cycle at the G1/S-phase [31,32]. RASSF1A was also shown to associate with and increase the activity of p120^{E4F}, a transcription factor known to bind the cyclin A2 promoter [33,34] and p21^{Cip/Waf1} cyclin-dependent kinase inhibitor [35]. Another mechanism by which RASSF1A controls cell cycle and apoptosis is by the disruption of the MDM2–DAXX–HAUSP complex leading to MDM2 self-ubiquitination and stabilization of p53 [36].

The role of RASSF1A in β -catenin signaling pathway was shown using *Apc*^{+/^{Min} mice, a model of intestinal tumorigenesis. Mutations of the adenomatous polyposis coli (APC) gene are a frequent and early event in colorectal cancers. APC inactivation leads to reduced β -catenin degradation and its nuclear accumulation, thus causing aberrant Wnt pathway signaling and adenoma initiation [37,38]. It was shown that loss of RASSF1A in *Apc*^{+/^{Min} mice resulted in a significant increase in adenomas of the small intestine and accelerated intestinal tumorigenesis [39]. Immunohistochemical analysis revealed increased nuclear accumulation of β -catenin in *Rassf1a*^{-/-}; *Apc*^{+/^{Min} mice, supporting a mechanistic link via loss of the known interaction of RASSF1A with β -TrCP that mediates degradation of β -catenin [39].}}}

We have demonstrated the importance of RASSF1A in death receptor dependent cell death via associations with TNF-R1, TRAIL-R1 and modulator of apoptosis (MOAP-1) [40,41]. In addition, RASSF1A can also associate with the pro-apoptotic kinase, MST1/2 to modulate its kinase activity and promote cell death [9,42–45]. These associations function to prevent excessive growth and allow RASSF1A to function as a tumor suppressor. We also recently demonstrated that, under conditions of acute intestinal inflammation, RASSF1A restricts NF κ B activity by interfering with the ability of the membrane proximal TLR/MyD88/TRAF6/IRAK2/4 complex to promote downstream signaling to NF κ B [46]. The restriction of NF κ B activity also led to restriction of tyrosine (Y) 357 phosphorylation of Yes associated protein (YAP), a key Hippo-dependent transcription factor linked to proliferation [46]. We determined that Y357 phosphorylation of YAP may arise due to increased inflammation and DNA damage – both of which are prominent during intestinal inflammation injury stimulated by the colonic irritant, dextran sodium sulfate (DSS) [46]. We are currently exploring the molecular mechanisms modulating the Y357 phosphorylation of YAP and of its importance for the appearance and/or progression of inflammatory bowel disease.

Rassf1a^{-/-} mice (on the C57BL/6 background) are viable, fertile and retain expression of isoform 1C and other RASSF gene family members. They have an increased tumor incidence by 12–16 months of age (especially in the breast, lung, gastrointestinal (GI) and immune system [B-cell related lymphomas]) and develop tumors in response to chemical carcinogens [47,48]. When *Rassf1a*^{-/-} mice were subjected to cardiac stress by pressure overload they responded with an exaggerated hypertrophic response with the formation of enlarged cardiac myocytes and hearts. Forced expression of RASSF1A in rat neonatal cardiac myocytes suppressed Raf-1 and ERK1/2 activation and inhibition of phenylephrine-induced cardiac myocyte growth [49]. *Rassf1a*^{-/-} mice revealed surprising cell specificity of RASSF1A signaling in the heart with cardiomyocytes and fibroblasts showing different signaling responses. In cardiomyocytes RASSF1A primarily induced apoptosis and inhibited hypertrophic cell growth. In cardiac fibroblasts, however, RASSF1A repressed NF κ B activity and inhibited TNF α secretion, preventing paracrine hypertrophic signaling between fibroblasts and myocytes [50]. For more detailed discussion of RASSF1A regulatory mechanisms in the heart please see recent review by Duan et al. [51].

Recently, we showed that *Rassf1a*^{-/-} mice are susceptible to DSS-induced colitis [46]. *Rassf1a*^{-/-} mice displayed clinical symptoms of inflammatory bowel disease (IBD) including increased intestinal permeability, enhanced cytokine/chemokine production, elevated NF κ B activity and elevated colonic cell death and epithelial cell injury [46]. Aged *Rassf1a*-null mice have an enhanced susceptibility to spontaneous inflammation at >6 months of age (S. Baksh, unpublished observations) and megaesophagus [52]. Histological examination revealed chronic inflammatory infiltrate and subsequent fibrosis of the myenteric plexus and the muscle layers [52]. These studies support a role of RASSF1A in modulating NF κ B activity and in restricting uncontrolled inflammation leading to several disease states.

2.2. RASSF1C

As mentioned earlier, in contrast to RASSF1A, RASSF1C does not have tumor suppressor properties with increasing evidence suggesting that it functions as an oncogene. Overexpression of RASSF1C in breast cancer cells resulted in enhanced cell migration/invasion [17]. It was also reported that RASSF1C could activate osteoblast cell proliferation through interaction with IGFBP-5 [18]. It was shown recently that RASSF1C is a very unstable protein

regulated by polyubiquitylation by the Mule E3 ligase under normal conditions. Upon DNA damage Mule as well as SFC ^{β -TrCP} are involved in the RASSF1C degradation [53]. The association of RASSF1C with SFC ^{β -TrCP} ligase was also described previously in a study whereby the authors showed that β -catenin accumulation was promoted either by the overexpression of RASSF1C (which inhibited β -catenin degradation) or by the silencing of RASSF1A, implying that the balance between the two isoforms is crucial for the β TrCP-mediated degradation of β -catenin [5].

RASSF1C has been shown to form a complex with DAXX and localize to promyelocytic leukaemia-nuclear bodies in the nucleus. Upon DNA damage DAXX is degraded and RASSF1C is released into the cytoplasm where it activates the SAPK/JNK pathway [54]. Using yeast two-hybrid system, Chen et al. found that RASSF1C may also interact and co-localize in the nucleus with tissue factor pathway inhibitor-2 (TFPI-2), a serine-proteinase inhibitor implicated in inflammation, angiogenesis and tumor growth/metastasis, suggesting other potential roles for RASSF1C [56]. To date, no known *Rassf1c* knockout mouse has been established.

2.3. RASSF2

The RASSF2 gene is transcribed into two major isoforms (A and C) that lack the C1 and ATM domains present in RASSF1A (Reviewed in [2]). It is primarily a nuclear protein unlike RASSF1A and 1C [57]. Promoter methylation and loss of expression of RASSF2 has been reported in different cancers and cancer cell lines [58–61] implicating the correlation and significance of RASSF2 in tumorigenesis. In addition, transient expression of RASSF2 in 293T embryonic kidney cells showed prominent growth inhibition enhanced by activated K-Ras [62]. Furthermore, *in situ* staining and fluorescent microscopy illustrated that the attenuation of cell growth in RASSF2 expression systems was the resultant effect of apoptosis [62]. Regulation of cell growth by RASSF2 was reported to be mediated by the MAPK pathway. It was shown that MAPK/ERK-2 mediated phosphorylation promotes efficient export of RASSF2 from the nucleus via CRM-1 dependent nuclear export pathway. A RASSF2 mutant defective in nuclear import failed to arrest the cell cycle at G1/S phase and apoptosis suggesting that nuclear retention is critical for RASSF2 mediated cell growth regulation and therefore tumor suppression [63]. It also appears that RASSF2 plays an essential role in the nuclear localization of prostate apoptosis response protein 4 (PAR-4) [64] which must be translocated to the nucleus to induce apoptosis [65].

It has also been demonstrated that MST1 regulates RASSF2 protein stability. Knockdown of MST1 in cancer cells destabilized RASSF2, and *Mst1*-deficient mice revealed reduced RASSF2 protein levels. Conversely, RASSF2 complexes with MST1 and activates MST1 resulting in MST-FOXO3 signaling pathway inhibition. RASSF2 also engages the JNK pathway and induces apoptosis in an MST1-independent manner [66]. RASSF2-deficient mice have bone remodeling defects and it was proposed that RASSF2 regulates osteoblast and osteoclast differentiation by inhibiting NF κ B signaling via limiting IKK activity [67].

2.4. RASSF3

RASSF3 has a 60% amino acid homology to RASSF1A [68] and can be found in both normal and tumor cells [68]. It is the smallest member of the C-terminal RASSF family of proteins. It has a CpG island in the promoter but its hypermethylation was only recently detected in a pituitary somatotroph adenoma [69]. RASSF3 expression levels were downregulated in 125 of a total 140 non-small-cell lung carcinomas (NSCLCs), however, DNA hypermethylation was found not to be a cause of RASSF3 down regulation [70]. When RASSF3 was overexpressed in HER2/Neu positive human and

mouse breast cancer cell lines, cell proliferation was inhibited [71] suggesting that RASSF3 has a protective role in tumorigenesis. On the other hand, RASSF3-knockdown NSCLC cells increased the migration rate in motility assays compared to the control cells [70]. It was recently shown that RASSF3 expression induces p53 stabilization by facilitating the ubiquitination of MDM2, the E3 ligase for p53 [72]. Thus, RASSF3 can exert its tumor suppression properties via p53-dependent apoptosis and DNA damage control mechanisms. To date, no known *Rassf3* knockout has been generated.

2.5. RASSF4

RASSF4 has 25% and 60% identity with RASSF1A and RASSF2 respectively [8]. Current studies demonstrate that RASSF4 is broadly expressed in normal tissues [8,73,74] and the reduction of RASSF4 expression due to promoter specific hypermethylation was detected in tumor cell lines and primary tumors [8,73,74] suggesting that RASSF4 might act as a tumor suppressor. However, a recent paper revealed a pro-growth role for RASSF4 in alveolar rhabdomyosarcoma (aRMS) [75]. One of the subtypes of aRMS is characterized by paired box-3-forkhead box protein O1 (PAX3-FOXO1) – a chimeric protein, arising as a result of chromosomal translocation. The expression of PAX3-FOXO1 is associated with an 8% survival of patients past 4 year [76]. Using cell free models it was demonstrated that PAX3-FOXO1 transcriptionally upregulates RASSF4 which in turn inhibits cellular senescence and promotes cell proliferation [75]. It was also demonstrated that RASSF4 interacted with and inhibited the tumor suppressor MST1/Hippo, suggesting that RASSF4 may act as a Hippo pathway inhibitor. A further study revealed suppressed Hippo signaling in aRMS, that was reflected in high expression level of YAP (Yes-associated protein 1). Although attempts to find a clear cause and effect relationship between YAP and RASSF4 biology failed, YAP was shown clearly to be involved in senescence regulation [75]. Interestingly, earlier studies have shown that RASSF4 associated directly with activated K-Ras and induced cell death in 293T embryonic kidney and MCF-7 breast cancer cells [8], indicating a tumor suppressor role of RASSF4. However, the human lung tumor cell line H1299 was completely resistant to RASSF4-mediated growth inhibition, suggesting cell line dependence of RASSF4 functions [8]. It will be interesting to explore what determines the pro- or anti-tumor functions of RASSF4. One of the proposed regulators might be p53 [8], since H1299 cells (but not MCF-7 cells) are defective for p53. It was also suggested that RASSF4 may suppress the MAP kinase signal by suppression of ERK phosphorylation [77] thus providing another possible mechanism for RASSF4-mediated tumor suppression. To date, no known RASSF4 knockout has been generated.

2.6. RASSF5

RASSF5 (also called NORE1 or RAPL) was the first member of RASSF proteins to be cloned [78]. It is expressed as three transcripts (A–C) via differential promoter usage and alternative splicing. The longest form of RASSF5A has 40% amino acid similarity with RASSF1A. RASSF5A has been shown to associate with cytoskeletal proteins through its RA domain and promote growth suppression via the ERK pathway [79]. In addition, RASSF5A can complex with MST1 kinase upon TNF- α and TNF-related apoptosis-inducing ligand (TRAIL) stimulation in order to drive apoptosis [80]. RASSF5A can also heterodimerize with RASSF1A via N-terminal interactions and RASSF5 can associate with Ras-like GTPases to promote cell death [81]. It has also been demonstrated that RASSF5A can inhibit cell proliferation independently of MST1/2 kinases and Ras-GTPases through a delay in cell cycle progression [82]. The

diversity of effectors through which RASSF5 exerts its proapoptotic role suggests that apoptosis control is a very important function of RASSF5.

The C57BL/6-*Rassf5a*^{-/-} mice were just recently generated but do not have an overt phenotype nor evidence of tumor formation as they age [80]. However, cells from the *Rassf5a*^{-/-} mice were resistant to TNF α and TRAIL-dependent cell death and reduced activation of JNK kinases in response to TNFR stimulation failed to activate MST1 in vivo [80]. In line with the role of RASSF5 as a tumor suppressor, K-Ras transfected mouse embryonic fibroblasts developed a significant amount of tumors when injected into immune compromised mice to support the role of RASSF5 in restricting excessive growth [80]. Recently it was demonstrated that RASSF5A plays a role in developing the nervous system [83].

The shortest RASSF5 isoform RASSF5C/NORE1B/RAPL is expressed predominantly in lymphoid tissues [84] and was shown to act as a mediator of the Rap1 induction of integrin clustering and activation after T-cell receptor stimulation [84], hence it was named as RAPL (regulator for cell adhesion and polarization enriched in lymphoid tissues). Whereas most functional studies were focused on RASSF5A, there is direct evidence that RASSF5C can suppress growth and colony formation in cells to the same extent as RASSF1A (although not in all cell lines tested) [82].

It was shown that RAPL is important for p27 (a regulator of cell cycle progression) nuclear localization in lymphocytes upon antigen receptor stimulation. RAPL deficiency resulted in the cytoplasmic localization of p27 and hyperproliferation of both T and B cells. RAPL-deficient mice developed lupus-like autoimmunity and B cell lymphomas, suggesting that the regulation of p27 subcellular localization by RAPL serves as a checkpoint for S phase entry to prevent immunoproliferative disorders [85]. Mice deficient in RASSF5C exhibited impaired lymphocyte trafficking and lymphoid organ abnormalities [86].

2.7. RASSF6

A few years following the discovery of RASSF1, a new Ras effector protein, RASSF6 showing similar structural topography to that of other RASSF proteins, was characterized and mapped [87,88]. To date, three isoforms of RASSF6 (A–C) have been identified (reviewed by [89]). Similar to other RASSFs, RASSF6 behaves like a tumor suppressor protein and is epigenetically silenced in childhood leukaemias and neuroblastomas [90,91]. Overexpression of RASSF6 in HeLa cells induced apoptosis via a signaling mechanism that is associated with Bax activation and cytochrome c release [92]. Furthermore, RASSF6 and MST2 inhibited each other under basal conditions but dissociated upon stimulation resulting in apoptosis in a RASSF6-WW45-dependent manner. However, the authors also demonstrated that RASSF6 was able to induce apoptosis in a parallel WW45-independent manner. RASSF6 association with MST2 inhibits canonical Hippo pathway activation [93]. Interestingly, it was demonstrated that RASSF6 binds to MDM2 and facilitates its self ubiquitination and degradation, stabilizing p53 (like RASSF1A and RASSF3), thereby regulating apoptosis and cell cycle [94]. It also has been demonstrated that, similar to RASSF1A and RASSF2, RASSF6 can inhibit NF κ B activity and possibly inflammation by unknown mechanisms [87]. To date, no known *Rassf6* knockout mouse has been generated.

2.8. RASSF7

In 1994 Weitzel and Patel identified an aggregate of genes on chromosome 11p15, surrounding HRAS1 cluster 1 [95]. The upstream gene was originally named HRC1 (HRAS1-related cluster protein1) and was later renamed as RASSF7. Due to alternative splicing, RASSF7 is comprised of three transcripts (A–C) containing

an N-terminal RA domain but lacking the SARAH domain conserved among RASSF1–6 family members [89]. RASSF7 is ubiquitously expressed in many tissues, specifically in brain, lung, and human cell lines and is up regulated in several carcinomas such as islet cell tumors [96], pancreatic ductal carcinoma [97–99], endometrial cancer [100] and ovarian clear cell carcinomas [101]. Protein and mRNA expression of RASSF7 was shown to be enhanced upon hypoxic insults [102] which, given the hypoxic nature of solid tumours, might explain the increase in RASSF7 expression seen in tumor cells. No promoter methylation of the RASSF7 gene was found in 57 cancer cell lines in the study by Recino et al. [102]. Conversely, Djos et al. reported that CpG island of the RASSF7 gene was heavily methylated in neuroblastoma cell lines suggesting that RASSF7 may be involved in the regulation of tumor formation [90]. Functional analyses demonstrated that RASSF7 induces cell growth through the regulation of spindle formation subsequently promoting mitotic progression [102,103]. A recent study illustrated the role of RASSF7 in cell survival during periods of stress. During initial stress, RASSF7 associates with N-Ras, inhibiting MKK7/JNK signaling pathway and inducing cell growth [104]. However, the anti-apoptotic property of RASSF7 is soon lost following prolonged exposure of UV radiation, due to degradation of RASSF7 through ubiquitination [104]. Thus, the mounting evidence so far argues a tumor suppressor function for this RASSF protein. Currently, no known *Rassf7* knockout has been generated.

2.9. RASSF8

Seven transcripts of RASSF8 have been identified so far [89]. Similarly to RASSF7, RASSF8 isoforms contain an N-terminal RA domain and lacks the SARAH region [89]. The tumor suppressor role of RASSF8 was initially proposed due to reduced transcript levels of RASSF8 in lung adenocarcinomas [105]. Loss of RASSF8 does not appear to be due to promoter methylation, except possibly in childhood leukemia [91] and the mechanism for RASSF8 down-regulation in many cancers remains to be elucidated. Further studies demonstrated that transient expression of RASSF8 in lung carcinoma cells attenuated cell growth in soft agar [105]. Collectively, these findings support the notion that RASSF8 regulates tumor development. Additional functions of RASSF8 include cell–cell adhesion due to its association with adherens junction linked to β -catenin/E-cadherin function [4]. Wound healing assays exhibited increased cell migration in cells lacking RASSF8 expression, suggesting that loss of RASSF8 may contribute to tumor aggressiveness [4]. However, serum analysis indicated that mRNA levels of RASSF8 in blood plasma were elevated in breast cancer patients in comparison to healthy controls [106], suggesting a tumor promoter effect of RASSF8. Early studies demonstrated that RASSF8 interacts with the adapter protein 14-3-3 [107], an ubiquitously expressed scaffolding protein that regulates various molecular processes including apoptosis and cell cycle progression [108]. Interestingly, RASSF1A-mediated cell death can be regulated by 14-3-3 as well [109]. Thus, these adapter proteins appear to be pivotal in the functional regulation of RASSF proteins. To date, no known *Rassf8* knockout has been generated.

2.10. RASSF9

RASSF9 was previously known as P-CIP1, peptidylglycine α -amidating monooxygenase COOH-terminal interactor protein-1 and regulates the distribution and ultimate fate of peptidylglycine alpha-amidating monooxygenase in endosomal pathways [110,111]. However, following BLAST analysis, P-CIP1 revealed similar structural homology to RASSF7 and RASSF8 and was later named RASSF9 [3]. RASSF9 contains the conserved RA domain localized at the C-terminus similarly to RASSF7, 8 and 10 [3]. Little

is known about the expression and functional significance of RASSF9. In 2011, Chang's group reported the predominant expression of RASSF9 in epithelial tissue [112]. *Rassf9*-deficient mice exhibited signs of senescence including increased alopecia, shorter life expectancy and growth retardation, implicating its role in epidermal development [112].

2.11. RASSF10

RASSF10 shares similar structural features to that of RASSF7–9, whereby it comprises an N-terminal RA domain and lacks the SARAH region [91]. RASSF10 was shown to be expressed in bone marrow as well as thyroid, brain, prostate and kidney [91,113–115]. Concomitantly, hypermethylation of RASSF10 was associated with loss of gene and protein expression in cancer conditions such as childhood leukaemias, thyroid carcinomas and gliomas [91,113,114]. The transcription of RASSF10 was restored using a demethylating agent [116]. In vitro analyses in U87 glioma cells treated with RNAi to knockdown RASSF10 expression, showed increased cell proliferation and cell survival [113]. Re-expression of RASSF10 in these cells inhibited cell growth and colony formation of glioma cells highlighting the tumor suppressor function of RASSF10 [113]. More recently, the tumor suppressor activity of RASSF10 was assessed in gastric cancer cells, demonstrating that reintroduction of RASSF10 in gastric cell lines JRST and BGC823 reduced cell viability and promoted apoptosis [116]. The pro-apoptotic nature of RASSF10 was shown to involve the Wnt/ β -catenin signaling pathway [116]. Additionally, RASSF10 may play a potential role in the regulation of mitotic progression due to its localization to centrosomes and microtubule association [113]. In light of the existing information, it appears that RASSF10 is a strong candidate as a prognostic marker to screen certain carcinomas.

3. Epigenetic regulation of the RASSFs

Epigenetics has now emerged as a key driver of disease states such as cardiovascular diseases, cancer, and schizophrenia. It is a mechanism that results in the changes of gene expression without an underlying change in the nucleotide sequence or in the ability of the organism to transfer the change to the next generation. Although epigenetic changes are not directly transferred to the next generation, in most cases the susceptibility for change to occur in a specific region is transferred and thus can have familial links.

Epigenetic changes can be in the form of histone modification leading to chromatin remodeling or by the conversion of cytosine to 5-methylcytosine. The latter occurs on specific CG-rich DNA stretches termed “CpG islands” within the promoter region and interferes with normal transcription [117,118]. DNA methylation is maintained by DNA methyltransferase 1 (DNMT1), which predominantly methylates hemi-methylated DNA. DNMT3a and DNMT3b methyltransferases are responsible for *de novo* methylation of unmethylated CpG. Although methylation is a drastic change, it can be reversed with the use of 5-Aza-2'-deoxycytidine (a cytidine analog that inhibits DNA methylation) [119]. However, the use of 5-Aza-2'-deoxycytidine has its limitations and targeted delivery to the affected area has posed challenges for its use as a potential therapeutic agent.

As mentioned earlier, several RASSF family members are regulated by promoter specific epigenetic changes. RASSF1A was the first to be identified and is considered to be one of the most methylated genes in human cancer. Not only is it epigenetically silenced in numerous cancers but it is considered as one of the earliest detectable changes in cancer [120]. Reports show that epigenetic silencing of RASSF1A occurs at a prevalence of 10–30% in cervical

cancer, 60% in breast cancers to 80% in small cell lung cancer and up to 99% in prostate cancer (see Table 3 for the complete list and references). Epigenetic modifications occur on >60 CpG sites spanning the RASSF1A promoter and exon 1. Silencing can be detected in numerous cell lines and can be reversed using 5-aza-2'-deoxycytidine, machanine [76], curcumin [121] or the approved FDA drug for treating acute myeloid leukemia, decitabine.

The correlation of RASSF1A methylation with different tumor characteristics has been reported by several groups. RASSF1A promoter methylation correlated inversely with K-Ras and B-Raf mutation status in cervical adenocarcinomas [122] and thyroid cancers [123–125]. RASSF1A methylation was also shown recently to inversely correlate with TP53 mutations [120], suggesting that they likely have independent mechanisms in tumorigenesis. A positive correlation between the degree of RASSF1A methylation and higher tumor grade [51,120], the ability of deeper myometrial invasion and positive metastatic involvement of pelvic lymph nodes in endometrial cancer was reported [51]. Interestingly RASSF1A hypermethylation correlated with chromosome instability in Wilm's tumors [126] supporting a role for RASS1A in controlling genomic stability. Subtype-specific difference in methylation was described in lung cancer with lower methylation of RASSF1A described for NSCLC and higher for SCLC (Table 2). In breast cancer, it was shown that RASSF1A has a higher methylation level in HER2 positive compared to HER2-negative breast tumors [120].

Interestingly, in acute myeloid leukemia, RASSF1A methylation occurs very rarely if ever (please see Table 1 in [127]) and RASSF1A expression is not affected [128] suggesting that RASSF1A is not involved in the pathogenesis. It will be interesting to determine why RASSF1A, which is methylated in such an extent in other tumors, remains intact in myeloid malignancy. It might be possible that other RASSFs such as RASSF6, 7 or 10 (please see Table 2) epigenetic silencing is responsible for leukemia's development.

With the advantage of pyrosequencing we can now study promoter methylation with greater detail, gaining information about the degree of methylation of individual CpG sites. Pyrosequencing revealed high variability of a distribution and level of RASSF1A promoter methylation within and among pancreatic cancer samples [129]. The same variability was shown ten years earlier in breast cancer using an oligonucleotide-based microarray technique [130]. Based on the detailed data, Yan et al. concluded that RASSF1A promoter is characterized by progressive accumulation of methylation starting from the first exon to the promoter area of the RASSF1A gene [130].

Besides cancer, epigenetic silencing of RASSF1A has been observed in ulcerative colitis (UC) patients [131], a form of inflammatory bowel disease (IBD). UC and Crohn's disease (CD) are chronic intestinal idiopathic diseases characterized by inflammation of the GI tract resulting in abdominal pain, chronic diarrhea, and weight loss. Molecularly, inflammation is characterized by the hyperactivation of transcription factors such as NF κ B and elevated production of cytokines to amplify the inflammatory response [132–134]. The activation of NF κ B can originate from multiple surface receptors including Toll like receptors (TLR) or tumor necrosis factor α (TNF α) receptor (TNF-R1) that will ultimately result in cytokine production, such as IL-6 production. Interestingly, IL-6 can drive epigenetic loss of RASSF1A via DNA methyltransferase 1 (DNMT1) up-regulation [135,136] suggesting that inflammation can drive expression loss of RASSF1A and may explain why UC patients have epigenetic loss of RASSF1A. Importantly, IBD patients show an increased likelihood of developing colorectal cancer later in life and RASSF1A promoter methylation has been observed in colorectal cancers, suggesting that RASSF1A loss may be an early event in colorectal cancer development [131]. A similar epigenetic silencing of RASSF1A can be detected in 44% of pancreatitis patients [137] and it is known that inflammation of the pancreas is

Table 2

This is a summary of the articles describing RASSFs promoter methylation investigated in various cancer types. The percentage of patient samples methylated in the indicated cancers are shown.

Gene	Cancer type	Percentage (where possible) and References
RASSF1A	Adrenocortical carcinoma	60% [S1]
	Endometrial cancer	85% [S2]
	Ewing sarcoma	68% [S3], 53% [S4]
	Hepatoblastoma	44% [S5]
	Merkel cell carcinoma	51% [S6]
	Papillary thyroid carcinoma	[S7]
	Parathyroid tumors	52% [S8]
	Pituitary adenomas	20–50% [S9]
	Breast cancer	38% [S10]
	Colorectal cancer	42% [S11]
RASSF2	Ewing sarcoma	[S4]
	Gastric cancer	Up to 80% [S12]
	Lung cancer (NSCLC)	31% [S13], 44% [S10]
	Merkel cell carcinoma	7% [S14]
	Nasopharyngeal carcinoma	51% [S15]
	Oral squamous cell carcinoma	26% [S16]
	Prostate cancer	67% [S17]
	Squamous cervical cancer	61% [S18]
	Thyroid cancer	63% [S19]
	Somatotroph adenoma	[S20]
RASSF3	Breast cancer	27% [S21], 54% of breast cancer cell lines [S21]
	Hepatocellular carcinomas	5% [S22]
RASSF4	Kidney cancer	44% of renal cell carcinoma cell lines [S21]
	Lung cancer	21% in SCLC, 22% NSCLC [S21, 23]
RASSF5A	Nasopharyngeal cancer	5% [S24]
	Colorectal cancer	39% [S25]
RASSF5C	Pheochromocytoma and abdominal paraganglioma	[S25]
	Hepatocellular carcinomas	62% [S26]
RASSF6	Merkel cell carcinoma	8% [S14]
	Childhood leukemia	94% of B-ALL [S27], 41% of T-ALL [S27]
RASSF7	Neuroblastoma	[S28]
	Chronic lymphocytic leukemia	16% [S29]
RASSF8	Neuroblastoma	[S28]
	Childhood leukemia	9% of B-ALL [S27], 10% of T-ALL [S27]
RASSF10	Lung adenocarcinoma in mice	[S20]
	Childhood leukemia	16% of B-ALL [S27], 88% of T-ALL [S27]
RASSF10	Chronic lymphocytic leukemia	50% [S29]
	Glioblastoma	[S30]
	Melanoma	68% [S31]
	Merkel cell carcinoma	23% [S14]
	Prostate carcinoma	[S32]
	Thyroid cancer	66% [S33]

For additional data for RASSF1A and RASSF5A please see [127], only new data are presented here. Note that RASSF1C CpG island hypermethylation has never been described. Please note that due to references number restriction references to this table are placed into online [Supplementary materials](#).

This is a summary of the articles describing RASSFs promoter methylation investigated in various cancer types. The percentage of patient samples methylated in the indicated cancers are shown.

* For these studies, pyrosequencing was used to determine methylation status with no indication of % prevalence indicated in the reference. The study only detected a degree of RASSF1A methylation and it was greater in patients compared to controls utilized.

predisposing factor for the appearance of pancreatic cancer where 83% of patients have *RASSF1A* epigenetic silencing [137]. Thus, the epigenetic loss of *RASSF1A* during a pre-cancer state (inflammation) may represent a molecular mechanism whereby chronic inflammation drives epigenetic silencing of the *RASSF1A* in order to promote malignancy.

Because the epigenetic loss of *RASSF1A* has been detected in numerous disease settings, there are several patents to utilize epigenetic loss of *RASSF1A* as a diagnostic tool for disease appearance (please see <http://www.google.co.in/patents/US6596488>; <http://www.google.com.tr/patents/WO2013041731A1?cl=en>; <http://www.google.com/patents/US8546078>). Epigenetic loss of *RASSF1A* has been observed in urine [138–140], nipple aspirates [141,142], and sputum [143] to mention a few. Thus, there is potential in utilizing epigenetic loss of *RASSF1A* as a diagnostic/prognostic tool for treating cancer and potentially other diseases as well. For example, the significant increase in the hypermethylation of *RASSF1A* in plasma of pregnant women with intrahepatic cholestasis was suggested as a diagnostic marker of this disease state [144].

The 5' CpG island of *RASSF2* spans approximately 1.6 kb and *RASSF2* has been shown to undergo epigenetic silencing in 88% of thyroid cancer patients, with 63% of these patients also showing *RASSF5A* epigenetic silencing [145]. Epigenetic silencing was not observed in patients with goiter or follicular adenomas. In the same samples, *RASSF3* and 4 were not epigenetically silenced but *RASSF5A* was, suggesting selective roles for *RASSF2* and 5A in the function of the thyroid gland. Furthermore, 5-aza-2'-deoxycytidine treatment was able to restore the expression of *RASSF2* and 5A in a thyroid cancer cell line. *RASSF2* methylation was also detected in colorectal cancer (CRC), gastric cancer and oral squamous cell carcinoma (please see Table 2 for the complete list and references). In squamous cervical cancer *RASSF2* hypermethylation was associated with shorter survival time [146]. Interestingly, in CRC patients the histone modification epigenetic mechanism was probably also involved in silencing of *RASSF2*, because treating cells with a histone deacetylase inhibitor augmented *RASSF2* expression [58].

Promoter methylation of *RASSF3* is rarely detected in cancer and was only described just recently in somatotroph adenoma

Table 3
RASSF1A kinases.

Kinase	Amino acid residue	RASSF1A function affected	References
Aurora A	T202 and/or S203	Phosphorylation disrupts RASSF1A interactions with microtubules and abolishes RASSF1A ability to induce M-phase cell cycle arrest. Phosphorylation leads to dissociation of RASSF1A from Cdc20, which allows for APC interaction with Cdc20, Cyclin A reduction and pro-metaphase progression	[29,165]
Aurora B	S203	Phosphorylation leads to interaction with Syntaxin16 and localization of Syntaxin16 to the midzone and midbody in late mitosis, and thus the completion of cytokinesis	[169]
Cyclin D Cyclin dependent kinase 4 (Cyclin D-CDK4)	S203	Phosphorylation is required for Skp2 dependent degradation of RASSF1A	[166]
Chk1 (checkpoint kinase)	S184	Phosphorylation causes RASSF1A to dissociate from microtubules and alters RASSF1A distribution in the cell, with “punctate distribution pattern”, not “network and fiber-like”. RASSF1A phosphorylation mimicking mutant abolishes the ability of RASSF1A to induce M-phase arrest	[170]
ATM (Ataxia telangiectasia mutated)	S131	Phosphorylation is required for the activation of MST2 and LATS1, which leads to p73 stabilization	[11]
PKC (Protein kinase C)	S197 and S203	Phosphorylation is required for the ability of RASSF1A to reorganize the microtubule network	[167]
MST1 (Mammalian STE20-like kinase 1)	T202 and S203	Phosphorylation is required for the capacity of the RASSF1A to activate NDR1/2	[168]
GSK-3 β (Glycogen synthase kinase 3 β)	S175, S178, and S179	Phosphorylation is required for RASSF1A association with 14-3-3 to inhibit association with TNF-R1 or TRAIL-R1	[109]

This table summarizes what is known about identified kinases for RASSF1A and, when known, how it affects RASSF1A biology.

(a subtype of pituitary adenoma) [69], however another recent study has shown that some polymorphisms can predispose to squamous cell carcinoma of the head and neck by modifying RASSF3 expression levels [147], providing another way of RASSF3 regulation.

Methylation of RASSF4 promoter in head and neck squamous cell carcinomas (HNSCC) was rather infrequent (13%, $n = 54$) [73] and a low methylation frequency (5%) was also reported for nasopharyngeal cancer [74]. However, a trend for higher RASSF4 methylation was detected in recurrent HNSCC. This correlates with a recent finding that RASSF4 expression was suppressed in a subpopulation of oral cancer stem-like cells/cancer-initiating cells [77], suggesting that RASSF4 plays a role in cell migration and invasiveness control.

Early studies demonstrated loss of RASSF5A expression in lung tumor cell lines and primary lung tumors [7], Wilms' tumor and renal cell carcinoma, colon cancer, as an apparent result of promoter specific hypermethylation (please see Table 2 for the complete list and references). Alternative to epigenetic silencing, RASSF5A can be downregulated through proteolysis catalyzed by calpain proteins, offering a means for tumor cells to flourish and escape growth inhibition [148]. RASSF5C promoter methylation is a rare event, possibly because its expression is limited to lymphoid tissue. Interestingly, it only has been detected in hepatocellular carcinoma and 8% of Merkel Cell Carcinoma (please see Table 2 for the complete list and references).

Similar to RASSF5, RASSF6 has been found to be epigenetically silenced in neuroblastoma [90] and childhood leukemia [91]. RASSF6 was found to be down-regulated by interactions with macrophages in vitro [149] to suggest that the immune system plays a role in regulation of RASSF6.

RASSF10 is another member of the RASSF family of proteins that is also epigenetically silenced. To date, 68% of malignant melanoma patient samples tested harbor epigenetically silenced RASSF10 [150]. In addition, RASSF10 promoter methylation has been observed in prostate cancer patients as well as up to 88% of paediatric leukemias, 50% of chronic lymphocytic leukemia, 66% of thyroid cancers, and 68% of melanoma (please see Table 2 for the complete list and references).

Not many reports exist currently on methylation of RASSF7 and 8 (see Table 2) and RASSF9 gene does not contain CpG island.

Although it is clear that epigenetics plays a crucial role in the regulation of expression of the RASSF proteins, the underlying mechanisms just started to emerge. It was shown recently that RASSF1A promoter methylation is regulated by p53 and death-associated protein 6 (DAXX) [151]. The authors have found that p53 binds to the RASSF1A promoter, recruits DAXX and DNMT1, leading to DNA methylation and subsequently to inactivation of RASSF1A. Interestingly, fluctuation in p53 protein levels did not affect the rates of RASSF1A methylation. Conversely, methylation of the RASSF1A promoter was critically controlled by DAXX, as the enforced overexpression of DAXX led to enhanced RASSF1A promoter methylation, whereas inhibition of DAXX reduced RASSF1A methylation [151].

To better understand the mechanism of RASSF1A epigenetic silencing, Palakurthy et al. performed a genome-wide RNAi screen and found that homeobox protein HOXB3 is required for RASSF1A promoter hypermethylation [152], a finding which was supported by a miRNA study later [153]. It appears that HOXB3 binds to the DNMT3B gene to increase its expression. DNMT3B then is recruited to the RASSF1A locus through interactions with polycomb repressor complex 2 (PRC2) and MYC, where it methylates the RASSF1A promoter [152].

Another player involved in epigenetic regulation of RASSF1A was reported recently [154]. Beckerdorff et al. showed that non-spliced long non-coding RNA transcribed from the antisense strand of RASSF1A forms an RNA/DNA hybrid at the RASSF1A transcription site and recruits polycomb repressor complex 2 (PRC2) to the RASSF1A promoter. This results in increased methylation of histone H3K27 at the RASSF1A promoter and specific reduction of transcriptional activity. No DNA hypermethylation was detected in RASSF1A promoter in this study.

4. Other mechanisms of RASSFs regulation

In this section we will describe several other mechanisms of RASSF regulation with focus on microRNA and post-translational modifications of RASSF1A. Single nucleotide polymorphisms do exist for RASSF1A as another mechanism responsible for altering the biology of RASSF1A. This was recently reviewed in detail by Gordon et al. [155] and will not be discussed here.

4.1. microRNA regulation of RASSF1A

MicroRNAs (miRNAs) are short (~22 nucleotide) RNAs important for the regulation of numerous physiological processes. It appears that about 60% of human protein coding genes are regulated by miRNAs [156]. Many miRNAs are epigenetically regulated, associated with CpG islands and regulated by histone modifications. It is well established that miRNAs are often dysregulated in many types of cancer, and it has been proposed that miRNAs may function as either oncogenes or tumor suppressors depending on the target genes [157,158]. Currently 1872 human miRNAs are registered in the miRBase database (release 20, June 2013) with many miRNAs assigned to regulate specific genes [159].

Bioinformatic analysis (microrna.org) suggests that RASSF1A mRNA can be targeted by at least fifteen miRNAs (miR-326, -330, -149, -16, -497, -504, -410, -99a, -99b, -100, -124, -193, -193b, -182, -181a–d). However the only one so far experimentally validated is miR-602, which was not predicted. It has been shown recently that inhibition of miR-602 expression in hepatoma cells increased RASSF1A expression, promoted hepatoma cell apoptosis and inhibited cell proliferation suggesting that miR-602 had regulatory role in early stage of HBV-mediated hepatocarcinogenesis by inhibiting tumor suppressive functions of RASSF1A [160]. Since aberrant miR-602 expression was detected in early stages of hepatocarcinogenesis and correlated with RASSF1A protein loss the authors suggested that it may be a potential early marker for that condition (and possibly others) and may serve as a therapeutic target for HBV-positive hepatocellular carcinoma.

Persistent inflammation and increased IL-6 levels have been shown to up regulate DNMT activity to epigenetically silence genes such as RASSF1A. We can speculate that microRNA targeting DNMTs or other proteins involved in epigenetic regulation may also affect RASSF1A expression. One study revealed that overexpressed DNMT1 in colorectal cancer tissues and cell lines was associated with downregulated miR-342 [161]. Restoration of miR-342 resulted in a dramatic reduction of the expression of DNMT1 and this in turn reactivated RASSF1A along with ADAM23, Hint1 and RECKS genes via promoter demethylation. Li et al. showed decreased DNMT3b association with RASSF1a promoter after miR-7 and miR-218 silencing of HOXB3 transcription factor in breast cancer cells [153]. Additionally, they detected increased histone acetylation at the RASSF1A promoter. It was demonstrated that miR-373 is able to regulate RASSF1A expression via MBD2 (Methyl-CpG-Binding Domain Protein 2) in hilar cholangiocarcinoma. RASSF1A expression increased following suppression of MBD2 by miR-373 in QBC₉₃₉ cells, and decreased after activation of MBD2 by anti-miR-373 inhibitor in HIBE_{pic} cells [162]. Interestingly, in a parallel study the same group revealed that miR-373 gene promoter contained a CpG island and was regulated by methylation [163]. This phenomenon of miRNA-epigenetics regulatory networks was reviewed recently [164]. Therefore, these studies reveal an importance of microRNA regulation of RASSF1A. Theoretically the other RASSF members can also be regulated by miRNAs, but currently there is no experimental proof of that in the scientific literature.

4.2. Post-translational modifications of RASSF1A

Post-translational modifications of RASSF1A are described as the regulatory mechanism that may affect the biological properties and stability of RASSF1A. RASSF1A is known to be phosphorylated at several serine and threonine residues under physiological conditions. Rong et al. reported that the mitotic kinase Aurora-A directly interacts with and phosphorylates RASSF1 (on Thr202/Ser203) during mitosis and this regulates the ability of RASSF1A to interact with microtubules and modulate cell cycle progression [165]. Recently, several other kinases, including ATM (ataxia telangiectasia

Table 4

Summary of transcription factor (TF) binding sites for RASSF genes.

RASSF	Potential TF binding sites
<i>N-terminal RASSFs:</i>	
RASSF1	NFκB, p53, AP1, c-Jun, HoxA5
RASSF2	NFκB, p53, NFAT, IRF-1, SRY
RASSF3	p53, ITF-2, Tal-1β, c-Myc, Max1, CP1A, NF-Y, CBF(2)
RASSF4	SRF, Pax-5, SRY, HOAA3, E2F, STAT1, STAT2
RASSF5A	NFκB, p53, Elk-1
(Nore1A)	
RASSF5C	NFκB, p53, AP4, c-Myb
(Nore1B)	
RASSF 6	NFκB, p53, FoxC1, Hlf, FOXO3, RFX1, RelA
<i>C-terminal RASSFs:</i>	
RASSF7	NFκB, STAT3, c-Myc, c-Myb, Max-1, NF-1, HNF-1
RASSF8	RORα2, POU3F, POU2F, RSFC4, Cdc5, RFX1
RASSF9	Nkx2, RORα2, CHOP-10, c/EBPα, Lhx3, Pax-4, ER-α, Evi-1, Olf-1
RASSF10	Cart-1, NRSF, CREB, RFX1, ATF-2, CRE-BP1, FOXO1a, FOXO1, HNF-3β

The most relevant TF for RASSF genes were gleaned from <http://www.genecards.org/> and TFbind program [191].

mutated) [11], CDK4 (cyclin-dependent kinase-4) [166], PKC (protein kinase C) [167], MST1 [168], Aurora A [165], Aurora-B [169], and Chk1 [170] have also been shown to phosphorylate RASSF1A. Each of these kinases seems to regulate different aspects of RASSF1A function (see Table 3), suggesting that regulation of RASSF1A by phosphorylation is a complex process with multiple kinases involved that modulate RASSF1A function needed for various cellular processes.

As discussed above RASSF1A protein arrests cells in G1 phase and loss of RASSF1A leads to uncontrolled cell division in cancer. Since RASSF1A is expressed in normal cells, they have to have a mechanism to reduce RASSF1A and progress through cell cycle. It is well known that ubiquitination machinery plays a major role in regulation of factors important for cell cycle progression (see Fig. 2 in [171]). It appeared that ubiquitylating enzyme complexes regulate RASSF1A as well. The Skp1-Cul1-F-box (SCF) ubiquitin ligase complex was the first complex discovered to regulate RASSF1A [166]. The Skp2 subunit of this complex interacts with RASSF1A and promotes its degradation at the G1-S transition of the cell cycle. Interestingly, RASSF1A has to be phosphorylated on Ser202 by cyclin D-cyclin-dependent kinase 4 to be able to interact with Skp2 [166]. In mitosis, Cullin 4A (CULL4A) promotes RASSF1A degradation [172]. In this case DNA damage-binding protein 1 directly interacts with RASSF1A and brings it to the CUL4A E3 ligase complex during mitosis. Ubiquitination therefore plays an important role in RASSF1A regulation and defects of ubiquitination machinery along with lower levels of RASSF1A expression were shown to be associated with hepatocellular carcinoma prognosis, and this may potentially serve as a diagnostic marker [173].

It was recently shown that the RASSF1A promoter has a p53 binding site 2718 bp upstream from the ATG transcription start codon, and that p53 is able to bind to the RASSF1A promoter and inhibit RASSF1A expression [174]. A transcription factor (TF) search revealed that almost all C-terminal RASSF genes (except RASSF4) have p53 and NFκB (except RASSF3 and 4) binding sites in their promoters (Table 4). At the same time, N-terminal RASSF genes have a distinct TF set, suggesting that not only is there structural differences between classical and N-terminal RASSFs [3], but they also might be transcriptionally regulated in different ways.

5. Conclusion

As described in this review, the tumor suppressor function of RASSF proteins are primarily determined by their ability to inhibit

cell growth and proliferation and to promote cell death in addition to other distinct functions. RASSF proteins exert their diverse functions through interaction with various proteins and are regulated through complex mechanisms, including promoter hypermethylation, histone modifications, protein post-translational modifications, polymorphic changes resulting in functional differences and miRNA regulation. Epigenetic silencing of RASSF genes via promoter specific methylation is reported in many cancers and is believed to have a diagnostic and prognostic value. Recent data suggest that inflammation may potentially serve as a trigger for epigenetic silencing which may contribute to cancer development. RASSF1A promoter hypermethylation was detected in inflammatory bowel disease patients as well as in pancreatitis patients to suggest that pre-cancer inflammatory states involve loss of RASSF1 function. It will be interesting to see if this holds true in other inflammatory diseases such as arthritis and asthma. Despite of all the progress made in recent years, cancer epigenetics still has many unanswered questions and holds big potential for future research in diagnostics and therapeutics. More research is needed to provide a better understanding of how these changes arise and if these changes can be developed into a diagnostic and/or prognostic markers. The RASSF family of proteins has given substantial insight into tumor suppression mechanisms and have been firmly demonstrated to be an important gene family for the appearance and progression of cancer.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.febslet.2014.02.041>.

References

- Gordon, M. and Baksh, S. (2010) RASSF1A: not a prototypical Ras effector. *Small GTPases* 2, 148–157.
- Richter, A.M., Pfeifer, G.P. and Dammann, R.H. (2009) The RASSF proteins in cancer; from epigenetic silencing to functional characterization. *Biochim. Biophys. Acta* 1796 (2), 114–128. 15p.
- Sherwood, V., Recino, A., Jeffries, A., Ward, A. and Chalmers, A.D. (2009) The N-terminal RASSF family: a new group of Ras-association-domain-containing proteins, with emerging links to cancer formation. *Biochem. J.* 425, 303–311.
- Lock, F.E., Underhill-Day, N., Dunwell, T., Matallanas, D., Cooper, W., Hesson, L., Recino, A., Ward, A., Pavlova, T., Zabarovsky, E., Grant, M.M., Maher, E.R., Chalmers, A.D., Kolch, W. and Latif, F. (2010) The RASSF8 candidate tumor suppressor inhibits cell growth and regulates the Wnt and NF-kappaB signaling pathways. *Oncogene* 29, 4307–4316.
- Estrabaud, E., Lassot, I., Blot, G., Le Rouzic, E., Tanchou, V., Quemeneur, E., Daviet, L., Margottin-Goguet, F. and Benarous, R. (2007) RASSF1C, an isoform of the tumor suppressor RASSF1A, promotes the accumulation of [beta]-catenin by interacting with [beta]TrCP. *Cancer Res.* 67, 1054–1061.
- Fujita, H., Fukuhara, S., Sakurai, A., Yamagishi, A., Kamioka, Y., Nakaoka, Y., Masuda, M. and Mochizuki, N. (2005) Local activation of Rap1 contributes to directional vascular endothelial cell migration accompanied by extension of microtubules on which RAPL, a Rap1-associating molecule, localizes. *J. Biol. Chem.* 280, 5022–5031.
- Vos, M.D., Martinez, A., Ellis, C.A., Vallecorsa, T. and Clark, G.J. (2003) The proapoptotic Ras effector Nore1 may serve as a Ras-regulated tumor suppressor in the lung. *J. Biol. Chem.* 278, 21938–21943.
- Eckfeld, K., Hesson, L., Vos, M.D., Bieche, I., Latif, F. and Clark, G.J. (2004) RASSF4/AD037 is a potential ras effector/tumor suppressor of the RASSF family. *Cancer Res.* 64, 8688–8693.
- Avruch, J., Xavier, R., Bardeesy, N., Zhang, X.F., Praskova, M., Zhou, D. and Xia, F. (2009) RASSF family of tumor suppressor polypeptides. *J. Biol. Chem.* 284, 11001–11005.
- El-Kalla, M., Onyskiw, C. and Baksh, S. (2010) Functional importance of RASSF1A microtubule localization and polymorphisms. *Oncogene* 29, 5729–5740.
- Hamilton, G., Yee, K.S., Scrace, S. and O'Neill, E. (2009) ATM regulates a RASSF1A-dependent DNA damage response. *Curr. Biol.* 19, 2020–2025.
- Scrace, S.F. and O'Neill, E. (2012) RASSF signalling and DNA damage: monitoring the integrity of the genome? *Mol. Biol. Int.* 2012, 141732.
- Dammann, R., Li, C., Yoon, J.H., Chin, P.L., Bates, S. and Pfeifer, G.P. (2000) Epigenetic inactivation of a RAS association domain family protein from the lung tumour suppressor locus 3p21.3. *Nat. Genet.* 25, 315–319.
- Dallol, A., Agathangelou, A., Fenton, S.L., Ahmed-Choudhury, J., Hesson, L., Vos, M.D., Clark, G.J., Downward, J., Maher, E.R. and Latif, F. (2004) RASSF1A interacts with microtubule-associated proteins and modulates microtubule dynamics. *Cancer Res.* 64, 4112–4116.
- Dallol, A., Agathangelou, A., Tommasi, S., Pfeifer, G.P., Maher, E.R. and Latif, F. (2005) Involvement of the RASSF1A tumor suppressor gene in controlling cell migration. *Cancer Res.* 65, 7653–7659.
- Amaar, Y.G., Minera, M.G., Hatran, L.K., Strong, D.D., Mohan, S. and Reeves, M.E. (2006) Ras association domain family 1C protein stimulates human lung cancer cell proliferation. *Am. J. Physiol. Lung Cell Mol. Physiol.* 291, L1185–L1190.
- Reeves, M.E., Baldwin, S.W., Baldwin, M.L., Chen, S.T., Moretz, J.M., Aragon, R.J., Li, X., Strong, D.D., Mohan, S. and Amaar, Y.G. (2010) Ras-association domain family 1C protein promotes breast cancer cell migration and attenuates apoptosis. *BMC Cancer* 10, 562.
- Amaar, Y.G., Baylink, D.J. and Mohan, S. (2005) Ras-association domain family 1 protein, RASSF1C, is an IGFBP-5 binding partner and a potential regulator of osteoblast cell proliferation. *J. Bone Miner. Res.* 20, 1430–1439.
- Vos, M.D., Martinez, A., Elam, C., Dallol, A., Taylor, B.J., Latif, F. and Clark, G.J. (2004) A role for the RASSF1A tumor suppressor in the regulation of tubulin polymerization and genomic stability. *Cancer Res.* 64, 4244–4250.
- Liu, L., Tommasi, S., Lee, D.H., Dammann, R. and Pfeifer, G.P. (2003) Control of microtubule stability by the RASSF1A tumor suppressor. *Oncogene* 22, 8125–8136.
- Rong, R., Jin, W., Zhang, J., Sheikh, M.S. and Huang, Y. (2004) Tumor suppressor RASSF1A is a microtubule-binding protein that stabilizes microtubules and induces G2/M arrest. *Oncogene* 23, 8216–8230.
- Dallol, A., Hesson, L.B., Matallanas, D., Cooper, W.N., O'Neill, E., Maher, E.R., Kolch, W. and Latif, F. (2009) RAN GTPase is a RASSF1A effector involved in controlling microtubule organization. *Curr. Biol.* 19, 1227–1232.
- Jung, H.Y., Jung, J.S., Whang, Y.M. and Kim, Y.H. (2013) RASSF1A suppresses cell migration through inactivation of HDAC6 and increase of acetylated alpha-tubulin. *Cancer Res. Treat.* 45, 134–144.
- Korah, R., Healy, J.M., Kunstman, J.W., Fonseca, A.L., Ameri, A.H., Prasad, M.L. and Carling, T. (2013) Epigenetic silencing of RASSF1A deregulates cytoskeleton and promotes malignant behavior of adrenocortical carcinoma. *Mol. Cancer* 12, 87.
- Song, M.S., Chang, J.S., Song, S.J., Yang, T.H., Lee, H. and Lim, D.S. (2005) The centrosomal protein RAS association domain family protein 1A (RASSF1A)-binding protein 1 regulates mitotic progression by recruiting RASSF1A to spindle poles. *J. Biol. Chem.* 280, 3920–3927.
- Liu, L., Baier, K., Dammann, R. and Pfeifer, G.P. (2007) The tumor suppressor RASSF1A does not interact with Cdc20, an activator of the anaphase-promoting complex. *Cell Cycle* 6, 1663–1665.
- Song, M.S. and Lim, D.S. (2004) Control of APC-Cdc20 by the tumor suppressor RASSF1A. *Cell Cycle* 3, 574–576.
- Song, M.S., Song, S.J., Ayad, N.G., Chang, J.S., Lee, J.H., Hong, H.K., Lee, H., Choi, N., Kim, J., Kim, H., Kim, J.W., Choi, E.J., Kirschner, M.W. and Lim, D.S. (2004) The tumour suppressor RASSF1A regulates mitosis by inhibiting the APC-Cdc20 complex. *Nat. Cell Biol.* 6, 129–137.
- Song, S.J., Song, M.S., Kim, S.J., Kim, S.Y., Kwon, S.H., Kim, J.G., Calvisi, D.F., Kang, D. and Lim, D.S. (2009) Aurora A regulates prometaphase progression by inhibiting the ability of RASSF1A to suppress APC-Cdc20 activity. *Cancer Res.* 69, 2314–2323.
- Xie, R., Nguyen, S., McKeenan, K., Wang, F., McKeenan, W.L. and Liu, L. (2011) Microtubule-associated protein 1s (MAP1s) bridges autophagic components with microtubules and mitochondria to affect autophagosomal biogenesis and degradation. *J. Biol. Chem.* 286 (12), 10367–10377.
- Shivakumar, L., Minna, J., Sakamaki, T., Pestell, R. and White, M.A. (2002) The RASSF1A tumor suppressor blocks cell cycle progression and inhibits cyclin D1 accumulation. *Mol. Cell. Biol.* 22, 4309–4318.
- Whang, Y.M., Kim, Y.H., Kim, J.S. and Yoo, Y.D. (2005) RASSF1A suppresses the c-Jun-NH2-kinase pathway and inhibits cell cycle progression. *Cancer Res.* 65, 3682–3690.
- Fenton, S.L., Dallol, A., Agathangelou, A., Hesson, L., Ahmed-Choudhury, J., Baksh, S., Sardet, C., Dammann, R., Minna, J.D., Downward, J., Maher, E.R. and Latif, F. (2004) Identification of the E1A-regulated transcription factor p120

- E4F as an interacting partner of the RASSF1A candidate tumor suppressor gene. *Cancer Res.* 64, 102–107.
- [34] Ahmed-Choudhury, J., Agathangelou, A., Fenton, S.L., Ricketts, C., Clark, G.J., Maher, E.R. and Latif, F. (2005) Transcriptional regulation of cyclin A2 by RASSF1A through the enhanced binding of p120E4F to the cyclin A2 promoter. *Cancer Res.* 65, 2690–2697.
- [35] Thaler, S., Hahnel, P.S., Schad, A., Dammann, R. and Schuler, M. (2009) RASSF1A mediates p21Cip1/Waf1-dependent cell cycle arrest and senescence through modulation of the Raf-MEK-ERK pathway and inhibition of Akt. *Cancer Res.* 69, 1748–1757.
- [36] Song, M.S., Song, S.J., Kim, S.Y., Oh, H.J. and Lim, D.S. (2008) The tumor suppressor RASSF1A promotes MDM2 self-ubiquitination by disrupting the MDM2-DAXX-HAUSP complex. *EMBO J.* 27, 1863–1874.
- [37] Su, L.K., Kinzler, K.W., Vogelstein, B., Preisinger, A.C., Moser, A.R., Luongo, C., Gould, K.A. and Dove, W.F. (1992) Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. *Science* 256, 668–670.
- [38] Carothers, A.M., Melstrom Jr., K.A., Mueller, J.D., Weyant, M.J. and Bertagnoli, M.M. (2001) Progressive changes in adherens junction structure during intestinal adenoma formation in Apc mutant mice. *J. Biol. Chem.* 276, 39094–39102.
- [39] van der Weyden, L., Arends, M.J., Dovey, O.M., Harrison, H.L., Lefebvre, G., Conte, N., Gergely, F.V., Bradley, A. and Adams, D.J. (2008) Loss of RASSF1A cooperates with Apc(Min) to accelerate intestinal tumorigenesis. *Oncogene* 27, 4503–4508.
- [40] Baksh, S., Tommasi, S., Fenton, S., Yu, V.C., Martins, L.M., Pfeifer, G.P., Latif, F., Downward, J. and Neel, B.G. (2005) The tumor suppressor RASSF1A and MAP-1 link death receptor signaling to Bax conformational change and cell death. *Mol. Cell* 18, 637–650.
- [41] Foley, C.J., Freedman, H., Choo, S.L., Onyskiw, C., Fu, N.Y., Yu, V.C., Tuszyński, J., Pratt, J.C. and Baksh, S. (2008) Dynamics of RASSF1A/MOAP-1 association with death receptors. *Mol. Cell Biol.* 28, 4520–4535.
- [42] Oh, H.J., Lee, K.K., Song, S.J., Jin, M.S., Song, M.S., Lee, J.H., Im, C.R., Lee, J.O., Yonehara, S. and Lim, D.S. (2006) Role of the tumor suppressor RASSF1A in Mst1-mediated apoptosis. *Cancer Res.* 66, 2562–2569.
- [43] Khokhlatchev, A., Rabizadeh, S., Xavier, R., Nedwidek, M., Chen, T., Zhang, X.F., Seed, B. and Avruch, J. (2002) Identification of a novel Ras-regulated proapoptotic pathway. *Curr. Biol.* 12, 253–265.
- [44] Praskova, M., Khokhlatchev, A., Ortiz-Vega, S. and Avruch, J. (2004) Regulation of the MST1 kinase by autophosphorylation, by the growth inhibitory proteins, RASSF1 and NRE1, and by Ras. *Biochem. J.* 381, 453–462.
- [45] Matallanas, D., Romano, D., Yee, K., Meissl, K., Kucerova, L., Piazzolla, D., Baccarini, M., Vass, J.K., Kolch, W. and O'Neill, E. (2007) RASSF1A elicits apoptosis through an MST2 pathway directing proapoptotic transcription by the p73 tumor suppressor protein. *Mol. Cell* 27, 962–975.
- [46] Gordon, M., El-Kalla, M., Zhao, Y., Fiteih, Y., Law, J., Volodko, N., Mohamed, A., El-Kadi, A.O., Liu, L., Odenbach, J., Thiesen, A., Onyskiw, C., Ghazaleh, H.A., Park, J., Lee, S.B., Yu, V.C., Fernandez-Patron, C., Alexander, R.T., Wine, E. and Baksh, S. (2013) The tumor suppressor gene, RASSF1A, is essential for protection against inflammation-induced injury. *PLoS One* 8, e75483.
- [47] Tommasi, S., Dammann, R., Zhang, Z., Wang, Y., Liu, L., Tsark, W.M., Wilczynski, S.P., Li, J., You, M. and Pfeifer, G.P. (2005) Tumor susceptibility of RASSF1A knockout mice. *Cancer Res.* 65, 92–98.
- [48] van der Weyden, L., Tachibana, K.K., Gonzalez, M.A., Adams, D.J., Ng, B.L., Petty, R., Venkitaraman, A.R., Arends, M.J. and Bradley, A. (2005) The RASSF1A isoform of RASSF1 promotes microtubule stability and suppresses tumorigenesis. *Mol. Cell Biol.* 25, 8356–8367.
- [49] Oceandy, D., Pickard, A., Prehar, S., Zi, M., Mohamed, T.M., Stanley, P.J., Baudoin-Stanley, F., Nadif, R., Tommasi, S., Pfeifer, G.P., Armesilla, A.L., Cartwright, E.J. and Neyses, L. (2009) Tumor suppressor Ras-association domain family 1 isoform A is a novel regulator of cardiac hypertrophy. *Circulation* 120, 607–616.
- [50] Del Re, D.P., Matsuda, T., Zhai, P., Gao, S., Clark, G.J., Van Der Weyden, L. and Sadoshima, J. (2010) Proapoptotic RASSF1A/Mst1 signaling in cardiac fibroblasts is protective against pressure overload in mice. *J. Clin. Invest.* 120, 3555–3567.
- [51] Duan, C., Liu, M., Zhang, J. and Ma, R. (2013) RASSF1A: a potential novel therapeutic target against cardiac hypertrophy. *Prog. Biophys. Mol. Biol.* 113, 284–288.
- [52] van der Weyden, L., Happerfield, L., Arends, M.J. and Adams, D.J. (2009) Megaesophagus in RASSF1A-null mice. *Int. J. Exp. Pathol.* 90, 101–108.
- [53] Zhou, X., Li, T.T., Feng, X., Hsiang, E., Xiong, Y., Guan, K.L. and Lei, Q.Y. (2010) Targeted polyubiquitylation of RASSF1C by the Mule and SCFbeta-TrCP ligases in response to DNA damage. *Biochem. J.* 441, 227–236.
- [54] Kitagawa, D., Kajihira, H., Negishi, T., Ura, S., Watanabe, T., Wada, T., Ichijo, H., Katada, T. and Nishina, H. (2006) Release of RASSF1C from the nucleus by Daxx degradation links DNA damage and SAPK/JNK activation. *EMBO J.* 25, 3286–3297.
- [55] Escobar-Cabrera, E., Lau, D.K., Giovinazzi, S., Ishov, A.M. and McIntosh, L.P. (2010) Structural characterization of the DAXX N-terminal helical bundle domain and its complex with RASSF1C. *Structure* 18, 1642–1653.
- [56] Chen, X., Li, Z., Zhang, J., Mao, Z., Ma, D. and Wang, H. (2012) Tissue factor pathway inhibitor-2 may interact with nuclear protein RASSF1C. *Acta Biochim. Biophys. Sin.* 44, 183–185.
- [57] Cooper, W.N., Dickinson, R.E., Dallol, A., Grigorieva, E.V., Pavlova, T.V., Hesson, L.B., Bieche, I., Broggin, M., Maher, E.R., Zabarovsky, E.R., Clark, G.J. and Latif, F. (2008) Epigenetic regulation of the ras effector/tumour suppressor RASSF2 in breast and lung cancer. *Oncogene* 27, 1805–1811.
- [58] Akino, K., Toyota, M., Suzuki, H., Mita, H., Sasaki, Y., Ohe-Toyota, M., Issa, J.P., Hinoda, Y., Imai, K. and Tokino, T. (2005) The Ras effector RASSF2 is a novel tumor-suppressor gene in human colorectal cancer. *Gastroenterology* 129, 156–169.
- [59] Hesson, L.B., Wilson, R., Morton, D., Adams, C., Walker, M., Maher, E.R. and Latif, F. (2005) CpG island promoter hypermethylation of a novel Ras-effector gene RASSF2A is an early event in colon carcinogenesis and correlates inversely with K-ras mutations. *Oncogene* 24, 3987–3994.
- [60] Zhang, Z., Sun, D., Van do, N., Tang, A., Hu, L. and Huang, G. (2007) Inactivation of RASSF2A by promoter methylation correlates with lymph node metastasis in nasopharyngeal carcinoma. *Int. J. Cancer* 120, 32–38.
- [61] Park, H.W., Kang, H.C., Kim, I.J., Jang, S.G., Kim, K., Yoon, H.J., Jeong, S.Y. and Park, J.G. (2007) Correlation between hypermethylation of the RASSF2A promoter and K-ras/BRAF mutations in microsatellite-stable colorectal cancers. *Int. J. Cancer* 120, 7–12.
- [62] Vos, M.D., Ellis, C.A., Elam, C., Ulku, A.S., Taylor, B.J. and Clark, G.J. (2003) RASSF2 is a novel K-Ras-specific effector and potential tumor suppressor. *J. Biol. Chem.* 278, 28045–28051.
- [63] Kumari, G. and Mahalingam, S. (2009) Extracellular signal-regulated kinase 2 (ERK-2) mediated phosphorylation regulates nucleocytoplasmic shuttling and cell growth control of Ras-associated tumor suppressor protein, RASSF2. *Exp. Cell Res.* 315, 2775–2790.
- [64] Donninger, H., Hesson, L., Vos, M., Beebe, K., Gordon, L., Sidransky, D., Liu, J.W., Schlegel, T., Payne, S., Hartmann, A., Latif, F. and Clark, G.J. (2010) The Ras effector RASSF2 controls the PAR-4 tumor suppressor. *Mol. Cell Biol.* 30, 2608–2620.
- [65] El-Guendy, N., Zhao, Y., Gurumurthy, S., Burikhanov, R. and Rangnekar, V.M. (2003) Identification of a unique core domain of par-4 sufficient for selective apoptosis induction in cancer cells. *Mol. Cell Biol.* 23, 5516–5525.
- [66] Song, H., Oh, S., Oh, H.J. and Lim, D.S. (2010) Role of the tumor suppressor RASSF2 in regulation of MST1 kinase activity. *Biochem. Biophys. Res. Commun.* 391, 969–973.
- [67] Song, H., Kim, H., Lee, K., Lee, D.H., Kim, T.S., Song, J.Y., Lee, D., Choi, D., Ko, C.Y., Kim, H.S., Shin, H.I., Choi, J., Park, H., Park, C., Jeong, D. and Lim, D.S. (2012) Ablation of RASSF2 induces bone defects and subsequent haematopoietic anomalies in mice. *EMBO J.* 31, 1147–1159.
- [68] Tommasi, S., Dammann, R., Jin, S.G., Zhang, X.F., Avruch, J. and Pfeifer, G.P. (2002) RASSF3 and NRE1: identification and cloning of two human homologues of the putative tumor suppressor gene RASSF1. *Oncogene* 21, 2713–2720.
- [69] Peng, H., Liu, H., Zhao, S., Wu, J., Fan, J. and Liao, J. (2013) Silencing of RASSF3 by DNA hypermethylation is associated with tumorigenesis in somatotroph adenomas. *PLoS One* 8, e59024.
- [70] Fukatsu, A., Ishiguro, F., Tanaka, I., Kudo, T., Nakagawa, K., Shinjo, K., Kondo, Y., Fujii, M., Hasegawa, Y., Tomizawa, K., Mitsudomi, T., Osada, H., Hata, Y. and Sekido, Y. (2014) RASSF3 downregulation increases malignant phenotypes of non-small cell lung cancer. *Lung Cancer* 83 (1), 23–39.
- [71] Jacquemart, I.C., Springs, A.E. and Chen, W.Y. (2009) RASSF3 is responsible in part for resistance to mammary tumor development in neu transgenic mice. *Int. J. Oncol.* 34, 517–528.
- [72] Kudo, T., Ikeda, M., Nishikawa, M., Yang, Z., Ohno, K., Nakagawa, K. and Hata, Y. (2012) The RASSF3 candidate tumor suppressor induces apoptosis and G1-S cell-cycle arrest via p53. *Cancer Res.* 72, 2901–2911.
- [73] Steinmann, K., Sandner, A., Schagdarsurengin, U. and Dammann, R.H. (2009) Frequent promoter hypermethylation of tumor-related genes in head and neck squamous cell carcinoma. *Oncol. Rep.* 22, 1519–1526.
- [74] Chow, L.S., Lo, K.W., Kwong, J., Wong, A.Y. and Huang, D.P. (2004) Aberrant methylation of RASSF4/AD037 in nasopharyngeal carcinoma. *Oncol. Rep.* 12, 781–787.
- [75] Crose, L.E., Galindo, K.A., Kephart, J.G., Chen, C., Fitamant, J., Bardeesy, N., Bentley, R.C., Galindo, R.L., Ashley Chi, J.T. and Linardic, C.M. (2014) Alveolar rhabdomyosarcoma-associated PAX3-FOXO1 promotes tumorigenesis via Hippo pathway suppression. *J. Clin. Invest.* 124, 285–296.
- [76] Sorensen, P.H., Lynch, J.C., Qualman, S.J., Tirabosco, R., Lim, J.F., Maurer, H.M., Bridge, J.A., Crist, W.M., Triche, T.J. and Barr, F.G. (2002) PAX3-FKHR and PAX7-FKHR gene fusions are prognostic indicators in alveolar rhabdomyosarcoma: a report from the children's oncology group. *J. Clin. Oncol.* 20, 2672–2679.
- [77] Michifuri, Y., Hirohashi, Y., Torigoe, T., Miyazaki, A., Fujino, J., Tamura, Y., Tsukahara, T., Kanaseki, T., Kobayashi, J., Sasaki, T., Takahashi, A., Nakamori, K., Yamaguchi, A., Hiratsuka, H. and Sato, N. (2013) Small proline-rich protein-1B is overexpressed in human oral squamous cell cancer stem-like cells and is related to their growth through activation of MAP kinase signal. *Biochem. Biophys. Res. Commun.* 439, 96–102.
- [78] Vavvas, D., Li, X., Avruch, J. and Zhang, X.F. (1998) Identification of Nore1 as a potential Ras effector. *J. Biol. Chem.* 273, 5439–5442.
- [79] Moshnikova, A., Frye, J., Shay, J.W., Minna, J.D. and Khokhlatchev, A.V. (2006) The growth and tumor suppressor NRE1A is a cytoskeletal protein that suppresses growth by inhibition of the ERK pathway. *The Journal of biological chemistry.* 281, 8143–8152.
- [80] Park, J., Kang, S.I., Lee, S.Y., Zhang, X.F., Kim, M.S., Beers, L.F., Lim, D.S., Avruch, J., Kim, H.S. and Lee, S.B. (2010) Tumor suppressor Ras-association domain family 5 (RASSF5/NRE1) mediates death receptor ligand-induced apoptosis. *The Journal of biological chemistry.* 285, 35029–35038.

- [81] Ortiz-Vega, S., Khokhlatchev, A., Nedwidek, M., Zhang, X.F., Dammann, R., Pfeifer, G.P. and Avruch, J. (2002) The putative tumor suppressor RASSF1A homodimerizes and heterodimerizes with the Ras-GTP binding protein Nore1. *Oncogene* 21, 1381–1390.
- [82] Aoyama, Y., Avruch, J. and Zhang, X.F. (2004) Nore1 inhibits tumor cell growth independent of Ras or the MST1/2 kinases. *Oncogene* 23, 3426–3433.
- [83] Nakamura, T., Yasuda, S., Nagai, H., Koinuma, S., Morishita, S., Goto, A., Kinashi, T. and Wada, N. (2013) Longest neurite-specific activation of Rap1B in hippocampal neurons contributes to polarity formation through RalA and Nore1A in addition to PI3-kinase. *Genes to cells: devoted to molecular & cellular mechanisms* 18, 1020–1031.
- [84] Katagiri, K., Maeda, A., Shimonaka, M. and Kinashi, T. (2003) RAPL, a Rap1-binding molecule that mediates Rap1-induced adhesion through spatial regulation of LFA-1. *Nat. Immunol.* 4, 741–748.
- [85] Katagiri, K., Ueda, Y., Tomiyama, T., Yasuda, K., Toda, Y., Ikehara, S., Nakayama, K.I. and Kinashi, T. (2011) Deficiency of Rap1-binding protein RAPL causes lymphoproliferative disorders through mislocalization of p27kip1. *Immunology* 34, 24–38.
- [86] Katagiri, K., Ohnishi, N., Kabashima, K., Iyoda, T., Takeda, N., Shinkai, Y., Inaba, K. and Kinashi, T. (2004) Crucial functions of the Rap1 effector molecule RAPL in lymphocyte and dendritic cell trafficking. *Nat. Immunol.* 5, 1045–1051.
- [87] Allen, N.P., Donninger, H., Vos, M.D., Eckfeld, K., Hesson, L., Gordon, L., Birrer, M.J., Latif, F. and Clark, G.J. (2007) RASSF6 is a novel member of the RASSF family of tumor suppressors. *Oncogene* 26, 6203–6211.
- [88] Hull, J., Rowlands, K., Lockhart, E., Sharland, M., Moore, C., Hanchard, N. and Kwiatkowski, D.P. (2004) Haplotype mapping of the bronchiolitis susceptibility locus near IL8. *Hum. Genet.* 114, 272–279.
- [89] van der Weyden, L. and Adams, D.J. (2007) The Ras-association domain family (RASSF) members and their role in human tumorigenesis. *Biochim. Biophys. Acta* 1776, 58–85.
- [90] Djos, A., Martinsson, T., Kogner, P. and Caren, H. (2012) The RASSF gene family members RASSF5, RASSF6 and RASSF7 show frequent DNA methylation in neuroblastoma. *Mol. Cancer* 11, 40.
- [91] Hesson, L.B., Dunwell, T.L., Cooper, W.N., Catchpole, D., Brini, A.T., Chiaromonte, R., Griffiths, M., Chalmers, A.D., Maher, E.R. and Latif, F. (2009) The novel RASSF6 and RASSF10 candidate tumour suppressor genes are frequently epigenetically inactivated in childhood leukaemias. *Mol. Cancer* 8, 42.
- [92] Ikeda, M., Hirabayashi, S., Fujiwara, N., Mori, H., Kawata, A., Iida, J., Bao, Y., Sato, Y., Iida, T., Sugimura, H. and Hata, Y. (2007) Ras-association domain family protein 6 induces apoptosis via both caspase-dependent and caspase-independent pathways. *Exp. Cell Res.* 313 (7), 1484–1495. 12p.
- [93] Ikeda, M., Kawata, A., Nishikawa, M., Tateishi, Y., Yamaguchi, M., Nakagawa, K., Hirabayashi, S., Bao, Y., Hidaka, S., Hirata, Y. and Hata, Y. (2009) Hippo pathway-dependent and -independent roles of RASSF6. *Sci. Signal.* 2, ra59.
- [94] Iwasa, H., Kudo, T., Maimaiti, S., Ikeda, M., Maruyama, J., Nakagawa, K. and Hata, Y. (2013) The RASSF6 tumor suppressor protein regulates apoptosis and the cell cycle via MDM2 protein and p53 protein. *J. Biol. Chem.* 288, 30320–30329.
- [95] Weitzel, J.N. and Patel, J. (1994) A single P1 clone bearing three genes from human chromosome 11p15.5: HRC1, HRAS1, and RNH. *Genet. Anal. Tech. Appl.* 11, 165–170.
- [96] Lowe, A.W., Olsen, M., Hao, Y., Lee, S.P., Taek Lee, K., Chen, X., van de Rijn, M. and Brown, P.O. (2007) Gene expression patterns in pancreatic tumors, cells and tissues. *PLoS One* 2, e323.
- [97] Brandt, R., Grutzmann, R., Bauer, A., Jesnowski, R., Ringel, J., Lohr, M., Pilarsky, C. and Hoheisel, J.D. (2004) DNA microarray analysis of pancreatic malignancies. *Pancreatology: official journal of the International Association of. Pancreatology* 4, 587–597.
- [98] Friess, H., Ding, J., Kleeff, J., Fenkell, L., Rosinski, J.A., Guweidhi, A., Reidhaar-Olson, J.F., Korc, M., Hammer, J. and Buchler, M.W. (2003) Microarray-based identification of differentially expressed growth- and metastasis-associated genes in pancreatic cancer. *Cell. Mol. Life Sci.* 60, 1180–1199.
- [99] Logsdon, C.D., Simeone, D.M., Binkley, C., Arumugam, T., Gresson, J.K., Giordano, T.J., Misek, D.E., Kuick, R. and Hanash, S. (2003) Molecular profiling of pancreatic adenocarcinoma and chronic pancreatitis identifies multiple genes differentially regulated in pancreatic cancer. *Cancer Res.* 63, 2649–2657.
- [100] Mutter, G.L., Baak, J.P., Fitzgerald, J.T., Gray, R., Neuberger, D., Kust, G.A., Gentleman, R., Gullans, S.R., Wei, L.J. and Wilcox, M. (2001) Global expression changes of constitutive and hormonally regulated genes during endometrial neoplastic transformation. *Gynecol. Oncol.* 83, 177–185.
- [101] Tan, D.S., Lambros, M.B., Rayter, S., Natrajan, R., Vatcheva, R., Gao, Q., Marchio, C., Geyer, F.C., Savage, K., Parry, S., Fenwick, K., Tamber, N., Mackay, A., Dexter, T., Jameson, C., McCluggage, W.G., Williams, A., Graham, A., Faratian, D., El-Bahrawy, M., Paige, A.J., Gabra, H., Gore, M.E., Zvebil, M., Lord, C.J., Kaye, S.B., Ashworth, A. and Reis-Filho, J.S. (2009) PPM1D is a potential therapeutic target in ovarian clear cell carcinomas. *Clin. Cancer Res.* 15, 2269–2280.
- [102] Recino, A., Sherwood, V., Flaxman, A., Cooper, W.N., Latif, F., Ward, A. and Chalmers, A.D. (2010) Human RASSF7 regulates the microtubule cytoskeleton and is required for spindle formation, Aurora B activation and chromosomal congression during mitosis. *Biochem. J.* 430, 207–213.
- [103] Sherwood, V., Manboddh, R., Sheppard, C. and Chalmers, A.D. (2008) RASSF7 is a member of a new family of RAS association domain-containing proteins and is required for completing mitosis. *Mol. Biol. Cell* 19 (4), 1772–1782.
- [104] Takahashi, S., Ebihara, A., Kajihio, H., Kontani, K., Nishina, H. and Katada, T. (2011) RASSF7 negatively regulates pro-apoptotic JNK signaling by inhibiting the activity of phosphorylated-MKK7. *Cell Death Differ.* 18, 645–655.
- [105] Falvella, F.S., Manenti, G., Spinola, M., Pignatiello, C., Conti, B., Pastorino, U. and Dragani, T.A. (2006) Identification of RASSF8 as a candidate lung tumor suppressor gene. *Oncogene* 25, 3934–3938.
- [106] Rykova, E.I., Skvortsova, T.E., Hoffmann, A.L., Tamkovich, S.N., Starikov, A.V., Bryzgunova, O.E., Permiakova, V.I., Warnecke, J.M., Sczakiel, G., Vlasov, V.V. and Laktionov, P.P. (2008) Breast cancer diagnostics based on extracellular DNA and RNA circulating in blood. *Biomed. Khim.* 54, 94–103.
- [107] Jin, J., Smith, F.D., Stark, C., Wells, C.D., Fawcett, J.P., Kulkarni, S., Metalnikov, P., O'Donnell, P., Taylor, P., Taylor, L., Zougman, A., Woodgett, J.R., Langeberg, L.K., Scott, J.D. and Pawson, T. (2004) Proteomic, functional, and domain-based analysis of in vivo 14-3-3 binding proteins involved in cytoskeletal regulation and cellular organization. *Curr. Biol.* 14, 1436–1450.
- [108] van Hemert, M.J., Steensma, H.Y. and van Heusden, G.P. (2001) 14-3-3 Proteins: key regulators of cell division, signalling and apoptosis. *Bioessays* 23, 936–946.
- [109] Ghazaleh, H.A., Chow, R.S., Choo, S.L., Pham, D., Olesen, J.D., Wong, R.X., Onyskiw, C. and Baksh, S. (2010) 14-3-3 Mediated regulation of the tumor suppressor protein, RASSF1A. *Apoptosis* 15, 117–127.
- [110] Chen, L., Johnson, R.C. and Milgram, S.L. (1998) P-CIP1, a novel protein that interacts with the cytosolic domain of peptidylglycine alpha-amidating monooxygenase, is associated with endosomes. *J. Biol. Chem.* 273, 33524–33532.
- [111] Alam, M.R., Caldwell, B.D., Johnson, R.C., Darlington, D.N., Mains, R.E. and Eipper, B.A. (1996) Novel proteins that interact with the COOH-terminal cytosolic routing determinants of an integral membrane peptide-processing enzyme. *J. Biol. Chem.* 271, 28636–28640.
- [112] Lee, C.M., Yang, P., Chen, L.C., Chen, C.C., Wu, S.C., Cheng, H.Y. and Chang, Y.S. (2011) A novel role of RASSF9 in maintaining epidermal homeostasis. *PLoS One* 6, e17867.
- [113] Hill, V.K., Underhill-Day, N., Krex, D., Robel, K., Sangan, C.B., Summersgill, H.R., Morris, M., Gentle, D., Chalmers, A.D., Maher, E.R. and Latif, F. (2011) Epigenetic inactivation of the RASSF10 candidate tumor suppressor gene is a frequent and an early event in gliomagenesis. *Oncogene* 30, 978–989.
- [114] Schagdarsurengin, U., Richter, A.M., Wohler, C. and Dammann, R.H. (2009) Frequent epigenetic inactivation of RASSF10 in thyroid cancer. *Epigenetics* 4, 571–576.
- [115] Dansranjav, T., Wagenlehner, F., Gattenloehner, S., Steger, K., Weidner, W., Dammann, R. and Schagdarsurengin, U. (2012) Epigenetic down regulation of RASSF10 and its possible clinical implication in prostate carcinoma. *Prostate* 72, 1550–1558.
- [116] Wei, Z., Chen, X., Chen, J., Wang, W., Xu, X. and Cai, Q. (2013) RASSF10 is epigenetically silenced and functions as a tumor suppressor in gastric cancer. *Biochem. Biophys. Res. Commun.* 432, 632–637.
- [117] Hu, S., Wan, J., Su, Y., Song, Q., Zeng, Y., Nguyen, H.N., Shin, J., Cox, E., Rho, H.S., Woodard, C., Xia, S., Liu, S., Lyu, H., Ming, G.L., Wade, H., Song, H., Qian, J. and Zhu, H. (2013) DNA methylation presents distinct binding sites for human transcription factors. *eLife* 2, e00726.
- [118] Harikrishnan, K.N., Chow, M.Z., Baker, E.K., Pal, S., Bassal, S., Brasacchio, D., Wang, L., Craig, J.M., Jones, P.L., Sif, S. and El-Osta, A. (2005) Brahma links the SWI/SNF chromatin-remodeling complex with MeCP2-dependent transcriptional silencing. *Nat. Genet.* 37, 254–264.
- [119] Christman, J.K. (2002) 5-Azacytidine and 5-aza-2'-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy. *Oncogene* 21, 5483–5495.
- [120] Klajic, J., Fleischer, T., Dejeux, E., Edvardsen, H., Warnberg, F., Bukholm, I., Lonning, P.E., Solvang, H., Borresen-Dale, A.L., Tost, J. and Kristensen, V.N. (2013) Quantitative DNA methylation analyses reveal stage dependent DNA methylation and association to clinico-pathological factors in breast tumors. *BMC Cancer* 13, 456.
- [121] Du, L., Xie, Z., Wu, L.C., Chiu, M., Lin, J., Chan, K.K., Liu, S. and Liu, Z. (2012) Reactivation of RASSF1A in breast cancer cells by curcumin. *Nutr. Cancer* 64, 1228–1235.
- [122] Kang, S., Kim, H.S., Seo, S.S., Park, S.Y., Sidransky, D. and Dong, S.M. (2007) Inverse correlation between RASSF1A hypermethylation, KRAS and BRAF mutations in cervical adenocarcinoma. *Gynecol. Oncol.* 105, 662–666.
- [123] Brait, M., Loyo, M., Rosenbaum, E., Ostrow, K.L., Markova, A., Papagerakis, S., Zahurak, M., Goodman, S.M., Zeiger, M., Sidransky, D., Umbrecht, C.B. and Hoque, M.O. (2012) Correlation between BRAF mutation and promoter methylation of TIMP3, RARbeta2 and RASSF1A in thyroid cancer. *Epigenetics* 7, 710–719.
- [124] Nakamura, N., Carney, J.A., Jin, L., Kajita, S., Pallares, J., Zhang, H., Qian, X., Sebo, T.J., Erickson, L.A. and Lloyd, R.V. (2005) RASSF1A and NRE1A methylation and BRAFV600E mutations in thyroid tumors. *Lab. Invest.* 85, 1065–1075.
- [125] Xing, M., Cohen, Y., Mambo, E., Tallini, G., Udelsman, R., Ladenson, P.W. and Sidransky, D. (2004) Early occurrence of RASSF1A hypermethylation and its mutual exclusion with BRAF mutation in thyroid tumorigenesis. *Cancer Res.* 64, 1664–1668.
- [126] Haruta, M., Matsumoto, Y., Izumi, H., Watanabe, N., Fukuzawa, M., Matsuura, S. and Kaneko, Y. (2008) Combined BubR1 protein down-regulation and RASSF1A hypermethylation in Wilms tumors with diverse cytogenetic changes. *Mol. Carcinog.* 47, 660–666.

- [127] Donninger, H., Vos, M.D. and Clark, G.J. (2007) The RASSF1A tumor suppressor. *J. Cell Sci.* 120, 3163–3172.
- [128] Zare-Abdollahi, D., Safari, S., Movafagh, A., Ghadiani, M., Riazi-Isfahani, S. and Omrani, M.D. (2014) Intact expression status of RASSF1A in acute myeloid leukemia. *Med. Oncol.* 31, 770.
- [129] Malpeli, G., Amato, E., Dandrea, M., Fumagalli, C., Debattisti, V., Boninsegna, L., Pelosi, G., Falconi, M. and Scarpa, A. (2010) Methylation-associated down-regulation of RASSF1A and up-regulation of RASSF1C in pancreatic endocrine tumors. *BMC Cancer* 11, 351.
- [130] Yan, P.S., Shi, H., Rahmatpanah, F., Hsiao, T.H., Hsiao, A.H., Leu, Y.W., Liu, J.C. and Huang, T.H. (2003) Differential distribution of DNA methylation within the RASSF1A CpG island in breast cancer. *Cancer Res.* 63, 6178–6186.
- [131] Abouzeid, H.E., Kassem, A.M., Abdel Wahab, A.H., El-mezayen, H.A., Sharad, H. and Abdel Rahman, S. (2010) Promoter hypermethylation of RASSF1A, MGMT, and HIC-1 genes in benign and malignant colorectal tumors. *Tumour Biol.* 32, 845–852.
- [132] de Ridder, L., Benninga, M.A., Taminiau, J.A., Hommes, D.W. and van Deventer, S.J. (2007) Infliximab use in children and adolescents with inflammatory bowel disease. *J. Pediatr. Gastroenterol. Nutr.* 45, 3–14.
- [133] Baumgart, D.C. and Carding, S.R. (2007) Inflammatory bowel disease: cause and immunobiology. *Lancet* 369, 1627–1640.
- [134] Xiao, C. and Ghosh, S. (2005) NF-kappaB, an evolutionarily conserved mediator of immune and inflammatory responses. *Adv. Exp. Med. Biol.* 560, 41–45.
- [135] Braconi, C., Huang, N. and Patel, T. (2010) MicroRNA-dependent regulation of DNA methyltransferase-1 and tumor suppressor gene expression by interleukin-6 in human malignant cholangiocytes. *Hepatology* 51, 881–890.
- [136] Foran, E., Garrity-Park, M.M., Mureau, C., Newell, J., Smyrk, T.C., Limburg, P.J. and Egan, L.J. (2010) Upregulation of DNA methyltransferase-mediated gene silencing, anchorage-independent growth, and migration of colon cancer cells by interleukin-6. *Mol. Cancer Res.* 8, 471–481.
- [137] Dammann, R., Schagdarsurengin, U., Liu, L., Otto, N., Gimm, O., Dralle, H., Boehm, B.O., Pfeifer, G.P. and Hoang-Vu, C. (2003) Frequent RASSF1A promoter hypermethylation and K-ras mutations in pancreatic carcinoma. *Oncogene* 22, 3806–3812.
- [138] Dimitriadis, E., Kalogeropoulos, T., Velaeti, S., Sotiriou, S., Vassiliou, E., Fasoulis, L., Klapsas, V., Synesiou, M., Apostolaki, A., Trangas, T. and Pandis, N. (2013) Study of genetic and epigenetic alterations in urine samples as diagnostic markers for prostate cancer. *Anticancer Res.* 33, 191–197.
- [139] Ahmed, H. (2010) Promoter methylation in prostate cancer and its application for the early detection of prostate cancer using serum and urine samples. *Biomarkers Cancer* 2010, 17–33.
- [140] Yu, J., Zhu, T., Wang, Z., Zhang, H., Qian, Z., Xu, H., Gao, B., Wang, W., Gu, L., Meng, J., Wang, J., Feng, X., Li, Y., Yao, X. and Zhu, J. (2007) A novel set of DNA methylation markers in urine sediments for sensitive/specific detection of bladder cancer. *Clin. Cancer Res.* 13, 7296–7304.
- [141] Krassenstein, R., Sauter, E., Dulaimi, E., Battagli, C., Ehya, H., Klein-Szanto, A. and Cairns, P. (2004) Detection of breast cancer in nipple aspirate fluid by CpG island hypermethylation. *Clin. Cancer Res.* 10, 28–32.
- [142] Martins, A.T., Monteiro, P., Ramalho-Carvalho, J., Costa, V.L., Dinis-Ribeiro, M., Leal, C., Henrique, R. and Jeronimo, C. (2011) High RASSF1A promoter methylation levels are predictive of poor prognosis in fine-needle aspirate washings of breast cancer lesions. *Breast Cancer Res. Treat.* 129, 1–9.
- [143] Honorio, S., Agathangelou, A., Schuermann, M., Pankow, W., Viacava, P., Maher, E.R. and Latif, F. (2003) Detection of RASSF1A aberrant promoter hypermethylation in sputum from chronic smokers and ductal carcinoma in situ from breast cancer patients. *Oncogene* 22, 147–150.
- [144] Yi, P., Yin, N., Zheng, Y., Jiang, H., Yu, X., Yan, Y., Liu, Q., Xiao, F. and Li, L. (2013) Elevated plasma levels of hypermethylated RASSF1A Gene sequences in pregnant women with intrahepatic cholestasis. *Cell Biochem. Biophys.* 67, 977–981.
- [145] Schagdarsurengin, U., Richter, A.M., Hornung, J., Lange, C., Steinmann, K. and Dammann, R.H. (2010) Frequent epigenetic inactivation of RASSF2 in thyroid cancer and functional consequences. *Mol. Cancer* 9, 264.
- [146] Guerrero-Setas, D., Perez-Janices, N., Blanco-Fernandez, L., Ojer, A., Cambra, K., Berdasco, M., Esteller, M., Maria-Ruiz, S., Torrea, N. and Guarch, R. (2013) RASSF2 hypermethylation is present and related to shorter survival in squamous cervical cancer. *Mod. Pathol.* 26, 1111–1122.
- [147] Guo, H., Liu, H., Wei, J., Li, Y., Yu, H., Guan, X., Li, E.W., Li, G., Sturgis, E.M., Wei, Q. and Liu, Z. (2013) Functional single nucleotide polymorphisms of the RASSF3 gene and susceptibility to squamous cell carcinoma of the head and neck. *Eur. J. Cancer* 3, e3997.
- [148] Kuznetsov, S. and Khokhlatchev, A.V. (2008) The growth and tumor suppressors NORE1A and RASSF1A are targets for calpain-mediated proteolysis. *PLoS One* 3, e3997.
- [149] Sanada, Y., Kumoto, T., Suehiro, H., Nishimura, F., Kato, N., Hata, Y., Sorisky, A. and Yanaka, N. (2013) RASSF6 expression in adipocytes is down-regulated by interaction with macrophages. *PLoS One* 8, e61931.
- [150] Helmbold, P., Richter, A.M., Walesch, S., Skorokhod, A., Marsch, W., Enk, A. and Dammann, R.H. (2012) RASSF10 promoter hypermethylation is frequent in malignant melanoma of the skin but uncommon in nevus cell nevi. *J. Invest. Dermatol.* 132, 687–694.
- [151] Zhang, H., He, J., Li, J., Tian, D., Gu, L. and Zhou, M. (2012) Methylation of RASSF1A gene promoter is regulated by p53 and DAXX. *FASEB J.* 27, 232–242.
- [152] Palakurthy, R.K., Wajapeyee, N., Santra, M.K., Gazin, C., Lin, L., Gobeil, S. and Green, M.R. (2009) Epigenetic silencing of the RASSF1A tumor suppressor gene through HOXB3-mediated induction of DNMT3B expression. *Mol. Cell* 36, 219–230.
- [153] Li, Q., Zhu, F. and Chen, P. (2012) MiR-7 and miR-218 epigenetically control tumor suppressor genes RASSF1A and Claudin-6 by targeting HoxB3 in breast cancer. *Biochem. Biophys. Res. Commun.* 424, 28–33.
- [154] Beckedorff, F.C., Ayupe, A.C., Crocci-Souza, R., Amaral, M.S., Nakaya, H.I., Soltys, D.T., Menck, C.F., Reis, E.M. and Verjovski-Almeida, S. (2013) The intronic long noncoding RNA ANRASSF1 recruits PRC2 to the RASSF1A promoter, reducing the expression of RASSF1A and increasing cell proliferation. *PLoS Genet.* 9, e1003705.
- [155] Gordon, M., El-Kalla, M. and Baksh, S. (2012) RASSF1 polymorphisms in cancer. *Mol. Biol. Int.* 2012, 365213.
- [156] Friedman, R.C., Farh, K.K., Burge, C.B. and Bartel, D.P. (2009) Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res.* 19, 92–105.
- [157] Esquela-Kerscher, A. and Slack, F.J. (2006) Oncomirs – microRNAs with a role in cancer. *Nat. Rev. Cancer* 6, 259–269.
- [158] Osada, H. and Takahashi, T. (2007) MicroRNAs in biological processes and carcinogenesis. *Carcinogenesis* 28, 2–12.
- [159] Kozomara, A. and Griffiths-Jones, S. (2011) MiRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Res.* 39, D152–D157.
- [160] Yang, L., Ma, Z., Wang, D., Zhao, W., Chen, L. and Wang, G. (2010) MicroRNA-602 regulating tumor suppressive gene RASSF1A is overexpressed in hepatitis B virus-infected liver and hepatocellular carcinoma. *Cancer Biol. Ther.* 9, 803–808.
- [161] Wang, H., Wu, J., Meng, X., Ying, X., Zuo, Y., Liu, R., Pan, Z., Kang, T. and Huang, W. (2010) MicroRNA-342 inhibits colorectal cancer cell proliferation and invasion by directly targeting DNA methyltransferase 1. *Carcinogenesis* 32, 1033–1042.
- [162] Chen, Y., Luo, J., Tian, R., Sun, H. and Zou, S. (2011) MiR-373 negatively regulates methyl-CpG-binding domain protein 2 (MBD2) in hilar cholangiocarcinoma. *Dig. Dis. Sci.* 56, 1693–1701.
- [163] Chen, Y., Gao, W., Luo, J., Tian, R., Sun, H. and Zou, S. (2011) Methyl-CpG binding protein MBD2 is implicated in methylation-mediated suppression of miR-373 in hilar cholangiocarcinoma. *Oncol. Rep.* 25, 443–451.
- [164] Sato, F., Tsuchiya, S., Meltzer, S.J. and Shimizu, K. (2011) MicroRNAs and epigenetics. *FEBS J.* 278, 1598–1609.
- [165] Rong, R., Jiang, L.Y., Sheikh, M.S. and Huang, Y. (2007) Mitotic kinase Aurora-A phosphorylates RASSF1A and modulates RASSF1A-mediated microtubule interaction and M-phase cell cycle regulation. *Oncogene* 26, 7700–7708.
- [166] Song, M.S., Song, S.J., Kim, S.J., Nakayama, K., Nakayama, K.I. and Lim, D.S. (2008) Skp2 regulates the antiproliferative function of the tumor suppressor RASSF1A via ubiquitin-mediated degradation at the G1-S transition. *Oncogene* 27, 3176–3185.
- [167] Verma, S.K., Ganesan, T.S. and Parker, P.J. (2008) The tumour suppressor RASSF1A is a novel substrate of PKC. *FEBS Lett.* 582, 2270–2276.
- [168] Vichalkovski, A., Gresko, E., Cornils, H., Hergovich, A., Schmitz, D. and Hemmings, B.A. (2008) NDR kinase is activated by RASSF1A/MST1 in response to Fas receptor stimulation and promotes apoptosis. *Curr. Biol.* 18, 1889–1895.
- [169] Song, S.J., Kim, S.J., Song, M.S. and Lim, D.S. (2009) Aurora B-mediated phosphorylation of RASSF1A maintains proper cytokinesis by recruiting Syntaxin16 to the midzone and midbody. *Cancer Res.* 69, 8540–8544.
- [170] Jiang, L., Rong, R., Sheikh, M.S. and Huang, Y. (2014) Mitotic Arrest by Tumor Suppressor RASSF1A is Regulated via Chk1 Phosphorylation. *Mol. Cancer Res.* 12 (1), 119–129.
- [171] Teixeira, L.K. and Reed, S.I. (2013) Ubiquitin ligases and cell cycle control. *Annu. Rev. Biochem.* 82, 387–414.
- [172] Jiang, L., Rong, R., Sheikh, M.S. and Huang, Y. (2010) Cullin-4A-DNA damage-binding protein 1 E3 ligase complex targets tumor suppressor RASSF1A for degradation during mitosis. *J. Biol. Chem.* 286, 6971–6978.
- [173] Calvisi, D.F., Ladu, S., Pinna, F., Frau, M., Tomasi, M.L., Sini, M., Simile, M.M., Bonelli, P., Mironi, M.R., Seddaiu, M.A., Lim, D.S., Feo, F. and Pascale, R.M. (2009) SKP2 and CKS1 promote degradation of cell cycle regulators and are associated with hepatocellular carcinoma prognosis. *Gastroenterology* 137 (18), 26 e1–10.
- [174] Tian, Y., Hou, Y., Zhou, X., Cheng, H. and Zhou, R. (2011) Tumor suppressor RASSF1A promoter: p53 binding and methylation. *PLoS One* 6, e17017.
- [175] Vos, M.D., Dallol, A., Eckfeld, K., Allen, N.P., Donninger, H., Hesson, L.B., Calvisi, D., Latif, F. and Clark, G.J. (2006) The RASSF1A tumor suppressor activates Bax via MOAP-1. *J. Biol. Chem.* 281 (8), 4557–4563.
- [176] Dallol, A., Kolch, W. and Latif, F. (2009) When RASSF1A RAN into tumor suppression: Ran GTPase is a RASSF1A effector involved in controlling microtubule organization. *Cell Cycle* 8, 3796–3797.
- [177] Chan, J.J., Flatters, D., Rodrigues-Lima, F., Yan, J., Thalassinou, K. and Katan, M. (2013) Comparative analysis of interactions of RASSF1–10. *Adv. Biol. Regul.* 53, 190–201.
- [178] Verma, S.K., Ganesan, T.S., Kishore, U. and Parker, P.J. (2011) The tumor suppressor RASSF1A is a novel effector of small G protein Rap1A. *Protein Cell* 2, 237–249.
- [179] Calvisi, D.F., Ladu, S., Gordon, A., Farina, M., Conner, E.A., Lee, J.S., Factor, V.M. and Thorgeirsson, S.S. (2006) Ubiquitous activation of Ras and Jak/Stat pathways in human HCC. *Gastroenterology* 130, 1117–1128.

- [180] Lorenzato, A., Martino, C., Dani, N., Oligschläger, Y., Ferrero, A.M., Biglia, N., Calogero, R., Olivero, M. and Di Renzo, M.F. (2012) The cellular apoptosis susceptibility CAS/CSE1L gene protects ovarian cancer cells from death by suppressing RASSF1C. *FASEB J.* 26, 2446–2456.
- [181] Clark, J., Freeman, J. and Donninger, H. (2012) Loss of RASSF2 Enhances Tumorigenicity of Lung Cancer Cells and Confers Resistance to Chemotherapy. *Mol. Biol. Int.* 2012, 705948.
- [182] Bee, C., Moshnikova, A., Mellor, C.D., Molloy, J.E., Koryakina, Y., Stieglitz, B., Khokhlatchev, A. and Herrmann, C. (2010) Growth and tumor suppressor NORE1A is a regulatory node between Ras signaling and microtubule nucleation. *J. Biol. Chem.* 285, 16258–16266.
- [183] Lee, D., Park, S.J., Sung, K.S., Park, J., Lee, S.B., Park, S.Y., Lee, H.J., Ahn, J.W., Choi, S.J., Lee, S.G., Kim, S.H., Kim, D.H., Kim, J., Kim, Y. and Choi, C.Y. (2012) Mdm2 associates with Ras effector NORE1 to induce the degradation of oncoprotein HIPK1. *EMBO Rep.* 13, 163–169.
- [184] Ishiguro, K., Avruch, J., Landry, A., Qin, S., Ando, T., Goto, H. and Xavier, R. (2006) Nore1B regulates TCR signaling via Ras and Carma1. *Cell. Signal.* 18, 1647–1654.
- [185] Suryaraja, R., Anitha, M., Anbarasu, K., Kumari, G. and Mahalingam, S. (2013) The E3 ubiquitin ligase Itch regulates tumor suppressor protein RASSF5/NORE1 stability in an acetylation-dependent manner. *Cell Death Dis.* 4, e565.
- [186] Miertzschke, M., Stanley, P., Bunney, T.D., Rodrigues-Lima, F., Hogg, N. and Katan, M. (2007) Characterization of interactions of adapter protein RAPL/Nore1B with RAP GTPases and their role in T cell migration. *J. Biol. Chem.* 282, 30629–30642.
- [187] Kim, J.H., Kim, W.S. and Park, C. (2013) Epstein-Barr virus latent membrane protein 1 increases genomic instability through Egr-1-mediated up-regulation of activation-induced cytidine deaminase in B-cell lymphoma. *Leukemia Lymphoma* 54, 2035–2040.
- [188] Rodriguez-Viciana, P., Sabatier, C. and McCormick, F. (2004) Signaling specificity by Ras family GTPases is determined by the full spectrum of effectors they regulate. *Mol. Cell. Biol.* 24, 4943–4954.
- [189] Hill, V. K., Underhill-Day, N., Krex, D., Robel, K., Sangan, C. B., Summersgill, H. R., Morris, M., Gentle, D., Chalmers, A. D., Maher, E. R. & Latif, F. (2010) Epigenetic inactivation of the RASSF10 candidate tumor suppressor gene is a frequent and an early event in gliomagenesis. *Oncogene*.
- [190] Richter, A.M., Walesch, S.K., Wurl, P., Taubert, H. and Dammann, R.H. (2012) The tumor suppressor RASSF10 is upregulated upon contact inhibition and frequently epigenetically silenced in cancer. *Oncogenesis* 1, e18.
- [191] Tsunoda, T. and Takagi, T. (1999) Estimating transcription factor bindability on DNA. *Bioinformatics* 15, 622–630.