Non-coding RNA Research 7 (2022) 123–131

Contents lists available at ScienceDirect

Non-coding RNA Research

journal homepage: www.keaipublishing.com/en/journals/non-coding-rna-research

Review Article

Regulation of RASSF by non-coding RNAs in different cancers: RASSFs as masterminds of their own destiny as tumor suppressors and oncogenes

Ammad Ahmad Farooqi [a,*], Gulnara Kapanova [b,c], Abay Z. Kussainov [d], Zaure Datkhayeva [e], Karlygash Raganina [f], Bolat N. Sadykov [g]

[a] Institute of Biomedical and Genetic Engineering (IBGE), Islamabad, Pakistan
[b] Al-Farabi Kazakh National University, Almaty, 71 al-Farabi Ave, Almaty, 050040, Kazakhstan
[c] Scientific Center of Anti-infectious Drugs, Kazakhstan, Almaty, 75 a al-Faraby Ave, 050040, Kazakhstan
[d] Department of Pediatric Surgery, Kazakh National Medical University, Kazakhstan
[e] Department of Obstetrics and Gynecology, Kazakh National Medical University, Kazakhstan
[f] Department of Pharmaceutical Technology, Asfendiyarov Kazakh National Medical University, Kazakhstan
[g] Department of General Surgery, Kazakhstan Medical University KSPH, Almaty, 050004, Kazakhstan

A B S T R A C T

Ras-association domain family (RASSF) proteins are tumor suppressors and have gained phenomenal limelight because of their mechanistic role in the prevention/inhibition of carcinogenesis and metastasis. Decades of research have demystified wide ranging activities of RASSF molecules in multiple stages of cancers. Although major fraction of RASSF molecules has tumor suppressive roles, yet there is parallel existence of proof-of-concept about moonlighting activities of RASSF proteins as oncogenes. RASSF proteins tactfully rewire signaling cascades for prevention of cancer and metastasis but circumstantial evidence also illuminates oncogenic role of different RASSF proteins in different cancers. In this review we have attempted to provide readers an overview of the complex interplay between non-coding RNAs and RASSF proteins and how these versatile regulators shape the landscape of carcinogenesis and metastasis.

1. Introduction

Increasingly it is being realized that RASSF proteins connect to a surprisingly broader range of signaling cascades that regulate carcinogenesis and metastasis. RASSF molecules are non-enzymatic adaptors structurally characterized by the identification of Ras-association (RA) domain as well as a C-terminal Salvador-Rassf-Hippo (SARAH) domain. Intriguingly, RASSF1A, a comprehensively studied family member particularly works as a ubiquitous tumor suppressor [1,2]. Different other RASSF proteins have also been structurally and functionally analyzed in wide variety of cancers. Intricate crosstalks of RASSF proteins with different signaling cascades have fundamental role in multiple stages of cancer and metastasis.

Cutting-edge research works have shown that transcription of the mammalian genome is enormously complex. In this context, discovery of microRNA was ground-breaking and revolutionized our understanding about "central dogma" of molecular biology [3–6]. High-throughput RNA sequencing studies have shown that transcription of the human genome generates different types of long noncoding RNAs that have no apparent protein-coding functions [7–12]. Circular RNAs (circRNAs) are single-stranded covalently closed RNA molecules. Molecular biologists have begun to demystify the central physiological roles of circRNAs in normal development and pathogenesis of different diseases [13–16].

A glimpse into the molecular biology of multiple emerging non-coding RNA systems reveals comprehensive landscape of their functions and roles in regulation of RASSF-driven signaling. In recent years, compelling evidence has emerged indicating that RASSF members have been linked to cell death and inhibition of carcinogenesis.

In this review we have summarized most recent and exciting experimental evidence about the interplay between non-coding RNAs and RASSF proteins. We have exclusively discussed RASSF1A, RASSF2, RASSF3, RASSF4, RASSF5, RASSF6, RASSF7, RASSF8, RASSF9 and RASSF10 in different cancers.

2. RASSF1

Seminal research works have shown that K-Ras activation and RASSF1A-defective background promoted carcinogenesis in transgenic mouse model (17). Transgenic mice having K-Ras4b^{G12D} under the control of doxycycline were crossed with RASSF1A knockout mice to scientifically analyze how K-Ras activity promoted cancer in the absence of RASSF1A [17]. The results highlighted that RASSF1A loss

* Corresponding author.
E-mail address: Farooqiammadahmad@gmail.com (A.A. Farooqi).

https://doi.org/10.1016/j.ncrna.2022.04.001
Received 10 April 2022; Received in revised form 26 April 2022; Accepted 30 April 2022
Available online 13 May 2022

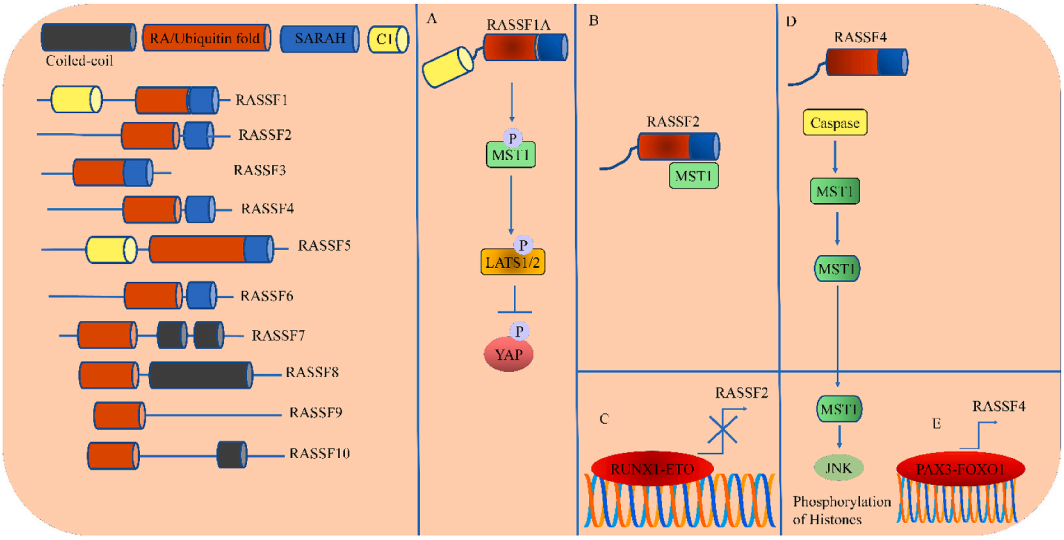

Fig. 1. Diagrammatic representation of RASSF proteins. (A) RASSF1A inhibited nuclear accumulation of YAP. (B) Assembly of RASSF2 and MST1 as a multi-protein complex is essential in the maintenance of RASSF2 stability. (C) RUNX1-ETO transcriptionally repressed RASSF2. (D) RASSF4 promoted caspase-mediated cleavage of MST1. Proteolytically processed MST1 moved into the nucleus of JNK and phosphorylation of histones. (E) PAX3-FOXO1 fusion oncoprotein transcriptionally upregulated RASSF4.

consequently resulted in carcinogenesis.

Series of cutting-edge studies had earlier shown that phosphorylation of YAP and TAZ by RASSF1A/MST/LATS cascade resulted in cytoplasmic retention and subsequent proteasomal degradation (Fig. 1). Sphere forming ability of RASSF1A-overexpressing CNE-2 cells was found to be reduced [18]. RASSF1A restoration caused significant reduction in the proliferative and sphere forming properties of RASSF1A-knockdown CNE-1 cells. Nuclear accumulation of YAP1 was profoundly abolished in RASSF1A-overexpressing CNE-2 cells. Transient knockdown of YAP1 reduced secretion of PDGF-BB and sphere forming properties of cancer cells. Additionally, human recombinant PDGF-BB induced an increase in the sphere formation and invasive abilities of YAP1-silenced RASSF1A-depleted CNE-1 cells [18]. Collectively, scientific evidence provided proof-of-concept that RASSF1A mediated the inhibition of PDGFB *via* YAP1 inactivation in NPC cells.

Ursolic acid, a medicinally important bioactive molecule effectively induced YAP phosphorylation [19]. RASSF1, MST1, MST2 and p-YAP were noted to be increased in the UA-treated xenograft tumor tissues. Importantly, E-cadherin level was enhanced, whereas MMP9, Twist and Vimentin levels were noted to be reduced in Ursolic acid-treated tissues of tumor xenografts in nude mice [19]. Overall, these experimentally verified evidence indicated that RASSF1 interfered with nuclear accumulation of YAP and prevented carcinogenesis.

3. RASSF2

RUNX1-ETO transcriptionally repressed RASSF2 in leukemic cells (Fig. 1) [20]. Co-expression of RASSF2 and RUNX1-ETO resulted in a significant delay in RE9a leukemia onset. The delay in the onset of leukemia was linked with notable reduction in leukemic burden in the peripheral blood and less severe anemia. MST1/2 was indispensable for RASSF2-mediated inhibitory effects on RUNX1-ETO-induced leukemic transformation. RASSF2 deletion mutant lacking the SARAH domain completely lost its capability to impair RUNX1-ETO-driven leukemic transformation and simultaneous induction of apoptotic death in RUNX1-ETO-expressing cells [20].

RASSF2 level was found to be reduced in MST1 depleted cells [21]. Assembly of RASSF2 and MST1 as a multi-protein complex is essential in the maintenance of RASSF2 stability (Fig. 1). RASSF2 level in MST1$^{-/-}$ mice was reduced to varying levels in various organs. RASSF2 is a substrate for MST1 kinase and also greatly potentiates MST1 activity. RASSF2 overexpression activated JNK pathway in association with PARP activation and cleavage of caspase-3 [21].

Excitingly, series of experiments had shown that mutations in the nuclear localization signal severely hampered the nuclear accumulation of RASSF2A [22]. RASSF2A expressing MCF7 cancer cells were inoculated in SCID mice and over the course of 140 days, palpable tumors were not observed [22].

4. RASSF3

MMTV/neu transgenic mouse line has been shown to be suitable for studies related to HER2/neu-related breast cancer [23]. Upregulation of RASSF3 has been reported in mammary glands of tumor-resistant MMTV/neu rodent model in comparison to tumor-susceptible littermates. RASSF3 is upregulated in neu-specific mammary tumors in comparison to adjacently located normal tissues. Importantly, RASSF3 upregulation hampered the proliferation rate of HER2/neu positive breast cancer cells (human and rodent), possibly through apoptotic death. Incidence of development of mammary tumors in RASSF3-overexpressing bi-transgenic mice model was considerably delayed in comparison to MMTV/neu$^{+/-}$ littermates [23].

Downregulation of RASSF3 was found to be correlated strongly with promoter hypermethylation in somatotroph adenomas [24]. Trichostatin A and 5-Aza stimulated RASSF3 expression in somatotroph adenoma cells. p53 silencing suppressed apoptotic death and restored cell growth in RASSF3-overexpressing GH3 cells. Collectively, these results revealed that p53 knockdown robustly blocked the antitumor effects of RASSF3 [24].

5. RASSF4

Treatment of human myeloma cell lines with decitabine (DNMT inhibitors) and quisinostat (HDAC inhibitors) significantly enhanced RASSF4 levels [25]. RASSF4 overexpression significantly increased bortezomib sensitivity in JJN3 and XG-7 cells. Furthermore, multiple myeloma patients having high expression of RASSF4 show superior overall survival upon bortezomib treatment. Likewise, animal models inoculated with RASSF4-overexpressing cells demonstrated a considerable improvement in the overall survival rates. There was a strong increase in the phosphorylated levels of SAPK/JNK, p53, c-Jun, p38-MAPK in RASSF4-overexpressing cells. Activation of MST1 is

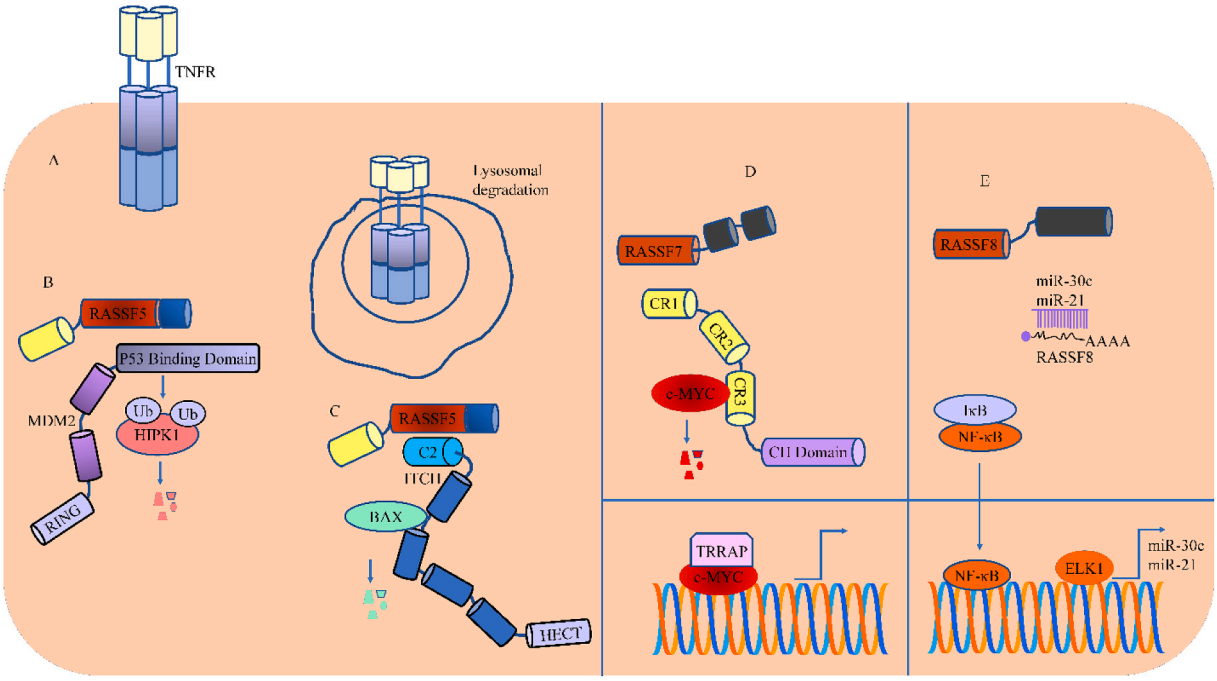

Fig. 2. Diagrammatic representation of RASSF-mediated signaling. (A) RASSF5 promoted endocytosis-driven lysosomal degradation of TNFRI. (B) RASSF5 induced HIPK1 degradation by the E3 ubiquitin ligase activities of MDM2. (C). RASSF5 protected BAX from ITCH-mediated ubiquitylation to promote TNF-induced apoptotic death. (D) RASSF7 promoted E3 ligase Cullin-4B-mediated ubiquitylation and proteasomal degradation of c-Myc. RASSF7 abolished c-Myc- driven binding of the co-activator TRRAP to the promoter regions of the target genes. (E) ELK1 transcriptionally upregulates miR-30c and miR-21. These miRNAs target RASSF8 and promote cancer progression. RASSF8 impaired oncogenic signaling by increasing IκB-mediated inactivation of NF-κB.

controlled by caspase-dependent cleavage, resulting in approximately 35 kDa cleavage fragments (Fig. 1). These hyperactive MST1 fragments shuttle to the nucleus from cytoplasm and activate JNK cascade, resulting in the phosphorylation of histones (H2B, H2AX), condensed chromatin and apoptotic death. Enforced expression of RASSF4 although activated MST1 but the level of hyperactive MST1 was profoundly increased in the cells co-treated with bortezomib [25].

Oncogenic role: PAX3-FOXO1 fusion oncoprotein transcriptionally upregulated RASSF4 (Fig. 1) [26]. RASSF4-silenced Rh28 aRMS xenografts exhibited a significant delay in gaining maximum tumor burden. RASSF4-silenced tumors contained larger cells with prominent nucleoli, increased cytoplasm and an abundance of cells with large, multinucleated giant cells and irregularly shaped nuclei [26].

6. RASSF5

RASSF5 induced poly-ubiquitination and proteasomal degradation of HIPK1. RASSF5 induced HIPK1 degradation by the E3 ubiquitin ligase activities of MDM2 [27]. RASSF5 acted as a scaffold protein and enhanced physical interaction between MDM2 and HIPK1. Subcutaneous xenografts derived from HIPK1-silenced-A549 cells were smaller in size [27].

Depletion of RASSF5 substantially suppressed TNFα-mediated apoptotic death mainly through its interactions with pro-apoptotic kinase MST1 [28]. MST1 knockdown also induced resistance development against TNFα-induced apoptosis. For a detailed analysis of the central roles of RASSF5 in experimental models, RASSF5-deficient rodent models were generated. RASSF5 inactivation in mouse embryonic fibroblasts (MEFs) resulted in resistance to TNFα- and TRAIL-mediated apoptotic death. Essentially, RASSF5-null mice were found to be greatly resistant to TNFα-induced apoptotic death and did not activate MST1. Importantly, RASSF5 loss also caused spontaneous immortalization of MEFs during earlier passages and RASSF5-null immortalized MEFs were transformed fully by K-RasG12V [28]. These findings clearly indicated that loss of RASSF5 increased the susceptibility of tumorigenesis.

TNF/TNFR pathway transduced pro-survival signals by activation of NF-κB [29]. RASSF5 promoted endocytosis-driven lysosomal degradation of TNFRI (Fig. 2). RASSF5 increased K48-linked ubiquitylation of TNFRI and enhanced its lysosomal degradation. RASSF5 facilitated the assembly of ITCH-induced destruction complexes. RASSF5 protected BAX from ITCH-mediated ubiquitylation to promote TNF-induced apoptotic death (Fig. 2). In response to TNF injections, $RASSF5^{+/+}$ tumors exhibited a substantially higher regression rates as compared to $RASSF5^{-/-}$ tumors. TNF injections triggered activation of BAX, PARP cleavage, and apoptotic death in RASSF5-overexpressing tumors but not in RASSF5-silenced tumors [29]. Overall, these interesting findings indicated that RASSF5 switched TNF-driven signals from pro-survival to pro-apoptotic by inactivation of NF-κB and enhancing the stability of BAX.

Treatment of rhabdomyosarcoma with DNMT inhibitors led to upregulation of RASSF1 and RASSF5 by demethylation of promoter regions [30]. Both these molecules activated classical Hippo pathway and increased YAP1 inactivation by phosphorylation. However, surprisingly, YAP1 is activated non-canonically by YES1 protein. YAP1 and YES1 interacted in the nucleus of the Rh30 alveolar rhabdomyosarcoma cells. Moreover, YES1 knockdown promoted cytoplasmic accumulation of YAP1 and caused transcriptional repression of YAP1-target genes [30]. YAP1 is bi-directionally regulated by Hippo-mediated and Hippo-independent (via YES1) in rhabdomyosarcoma.

7. RASSF6

Levels of MMP2 and MMP9 were found to be reduced in RASSF6-overexpressing-HepG2 cells. Shrinkage of the tumor xenografts was noticed in mice inoculated with RASSF6-overexpressing-HepG2 cells [31].

RASSF6-stably expressing cells demonstrated shrinkage of tumor weight and size, but RASSF6-silenced-HT-29 cells caused expansion of the tumor mass in rodent models [32].

786-O cells reconstituted with RASSF6 led to evident reduction in sorafenib resistance. Sorafenib induced apoptotic death in RASSF6-overexpressing ACHN/R cells. Functionally, RASSF6 overexpression steadily suppressed MCL-1 levels in ACHN/R cells. Treatment of ACHN/R-RASSF6-expressing cells with JNK inhibitors restored MCL-1 levels and sorafenib resistance. Tumor mass derived from ACHN cells was more sensitive to treatments as compared to tumors derived from RASSF6-knockdown cells [33].

Oncogenic roles: Ubiquitylation levels of TRIM16 were greatly enhanced by RASSF6, which tagged TRIM16 to undergo proteasomal degradation. Pulmonary metastatic nodules were reported to be considerably reduced in the mice injected with RASSF6-silenced cells [34].

8. RASSF7

RASSF7 promoted E3 ligase Cullin-4B-mediated ubiquitylation and proteasomal degradation of c-Myc [35]. CUL4B protein levels were noted to be reduced in RASSF7-depleted cancer cells. Additionally, RASSF7 interfered with c-Myc-mediated transcriptional regulation of target gene networks. Previous studies had shown that c-Myc-induced recruitment of co-activator TRRAP caused transcriptional regulation of target genes (Fig. 2). However, RASSF7 abolished c-Myc- driven binding of the co-activator TRRAP to the promoter regions of the target genes. Furthermore, RASSF7 also impaired formation of heterodimeric complexes between c-Myc and Max [35].

Oncogenic role: RASSF7 overexpression caused reduction in the phosphorylated levels of MST1/2, LATS1 and YAP. Loss of phosphorylation of YAP potently enhanced its nuclear accumulation. Tumor volume and weights, as well as the number of pulmonary metastatic nodules were increased in mice inoculated with RASSF7-overexpressing cancer cells [36].

9. RASSF8

RASSF8 inhibition resulted in an increase in the levels of VEGF-C and promoted VEGF-C-driven downstream signaling. Importantly, lymphatic vessel densities in the tumors developed from RASSF8-overexpressing cells were low. On the contrary, lymphatic vessel densities were found to be profoundly increased in tumors developed from RASSF8 knockdown cells [37].

ELK1 has been shown to transcriptionally upregulate miR-30c and miR-21 in cancer cells [38]. MiR-30c and miR-21 targeted RASSF8 and promoted cancer progression. RASSF8 impaired oncogenic signaling by increasing IκB-mediated inactivation of NF-κB (Fig. 2). Whereas, miR-30c and miR-21 promoted nuclear transportation of NF-κB. K-Ras$^{LSL-G12D}$ mice were systemically administered with LNA-anti-miR-21 and intraperitoneally injected with cisplatin for analysis of lung tumorigenesis. miR-21 silencing caused complete eradication of the appearance of hyperplasia and adenomas [38].

RASSF8 co-localized with β-catenin at the cell peripheral regions in H1792 and A549 lung cancer cells. However, β-catenin is re-localized from cell-cell contacts and undergoes trafficking from peripheral regions to the cytoplasm and nucleus. p65 is localized to the cell periphery and co-localizes with β-catenin at Adherens junctions. Likewise, after the depletion of RASSF8, p65 undergoes re-location from cell-cell contacts and accumulates in the cytoplasm and nuclear compartments. β-Catenin and p65 move into the nucleus and control the target transcriptional networks to promote carcinogenesis [39].

10. RASSF9

TAK1 phosphorylated RASSF9 at Serine-284th which resulted in notable reduction in dimerization of RAS proteins, that caused inhibition of the RAF/MEK/ERK signal transduction. RASSF9 knockdown delayed growth and proliferative potential of cancer cells [40].

RASSF9 positively regulated RAS pathway by promoting the dimerization of RAS proteins which activated downstream transduction cascades including MEK and ERK to drive proliferation of tumor cells. There was an evident increase in p-MEK and p-ERK levels in tumor tissues of mice injected subcutaneously with RASSF9-overexpressing-A549 cells [41].

11. RASSF10

RASSF10 is a haplo-insufficient tumor suppressor and the loss of one allele is sufficient for its loss of functions and contributory role in carcinogenesis [42].

RASSF10 expression is regulated by TGFβ in cancer cells. There is an increase in RASSF10 levels in TGFβ-treated A549 cancer cells. SNAI2 is a

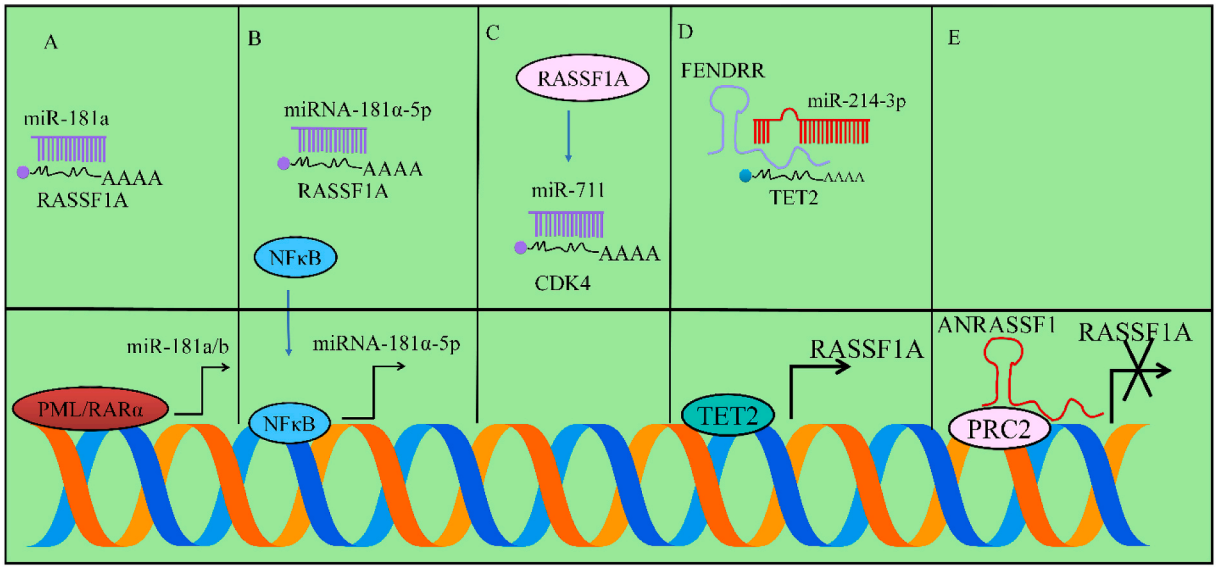

Fig. 3. Shows (A–B) Different proteins like PML/RARα and NFκB have been shown to transcriptionally upregulate miR-181. miR-181 has the ability to target RASSF1A and promote carcinogenesis. (C) RASSF1A increased the levels of miR-711 and promoted miR-711-induced targeting of CDK4. (D) FENDRR blocked miR-214-3p mediated targeting of TET2. TET2 stimulated the expression of RASSF1A. (E) ANRASSF1 recruited PRC2 to the promoter region of RASSF1A thus reducing the expression of RASSF1A.

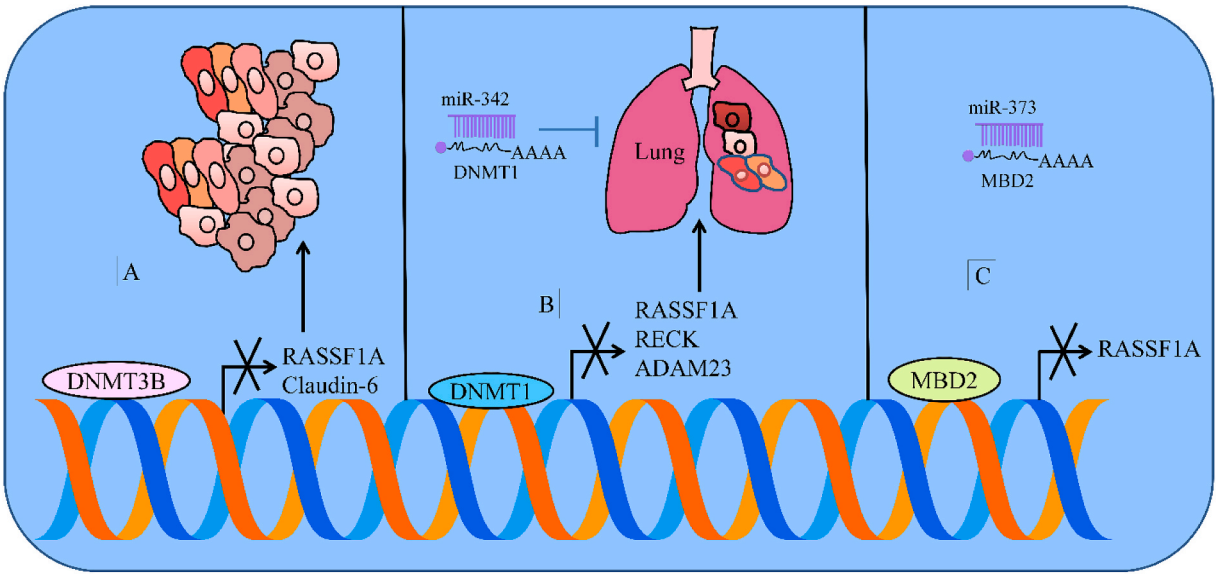

Fig. 4. Shows (A) DNMT3B enhanced proliferation of cancer cells through epigenetic inactivation of RASSF1A and Claudin-6. (B) miR-342 targeted DNMT1 and blocked pulmonary metastasis. DNMT1 epigenetically inactivated RASSF1A, RECK and ADAM23 and promoted pulmonary metastasis. (C) miR-373-mediated targeting of MBD2 induced upregulation of tumor suppressive RASSF1A.

transcriptional repressor of E-cadherin and CDH1 is downregulated in TGFβ-treated cancer cells. Interestingly, E-cadherin levels were also found to be reduced upon the knockdown of RASSF10. Structurally, RASSF10 interacted with ASPP2 and potently enhanced its stability. Knockdown of RASSF10 or ASPP2 triggered SMAD2 phosphorylation. Loss of RASSF10 or ASPP2 caused constitutive activation of TGFβ pathway and epithelial-to-mesenchymal transition [43].

Binding of RASSF10 through its coiled-coil domains to LRP6 reduced p-LRP6 levels, eventually prohibiting nuclear transportation of β-catenin. RASSF10 inhibited proliferative and invasive potential of NSCLC cells [44].

RASSF10 demonstrated rapid ubiquitination followed by proteasomal degradation in Nucleophosmin-knockdown cells. Lysine residues at 183rd and 476th positions are potential ubiquitination sites in RASSF10. Nucleophosmin abolished ubiquitination and proteasomal degradation of RASSF10 by downregulation of the expression of E3 ligase RNF2. RASSF10 failed to promote nuclear accumulation of GADD45a in Nucleophosmin-depleted cells. RASSF10-induced GADD45a upregulation was abrogated in p53 knockdown cells [45].

RASSF10 overexpression led to an increase in the levels of Bax and Bad but decreased BCL-2 and BCL-XL in MHCC97H and Huh7 cells. RASS10 knockdown suppressed E-cadherin levels but increased N-cadherin and Vimentin in Hep3B cells. Tumor growth rates were found to be reduced in mice implanted with RASSF10-overexpressing MHCC97H cells [46].

After providing a schematic overview of RASSF proteins for our readers, we will now analyze how non-coding RNAs regulate different RASSF proteins during various steps of cancer progression.

Regulation of RASSF by non-coding RNAs: Mounting evidence about the critical role of non-coding RNAs in the regulation of RASSF proteins is intriguing and systematically unveiling the fundamental role of these interactions in shaping multiple steps of carcinogenesis and metastasis.

12. RASSF1A

RASSF1A is the best-characterized example of the RASSF family and major fraction of our knowledge regarding the molecular functions of these proteins emanates from studies related to RASSF1A.

RASSF1A inhibited the growth of cancer cells by reducing the phosphorylation of JNK [47]. Tumors derived from RASSF1A-overexpressing-SMMC7721 cells were smaller in size. Levels of p-JNK and JNK were found to be reduced in RASSF1A-overexpressing tumors. miR-602 promoted the recurrence of liver cancer by inhibition of RASSF1A [47].

RASSF1A has also been shown to be directly targeted by miR-181α-5p in osteosarcoma cells [48]. Migratory and invasive capacities were suppressed significantly upon the knockdown of miR-181α-5p in 143B and MG63 cells. IL-1β secreted by M2-TAMs exerted the effects through NFκB signaling pathway. Consequently, IL-1β secreted by M2-TAMs potently promoted osteosarcoma metastases by NFκB/miRNA-181α-5-p/RASSF1A cascade (Fig. 3) [48].

RASSF1A knockdown attenuated the effects of miR-181a downregulation on the apoptosis of SGC-7901 cells. miR-181a targeted RASSF1A and inhibited apoptosis in cancer cells [49]. Collectively, these findings indicated that miR-181a acted as an oncogenic miRNA and interfered with apoptotic death of cancer cells.

miRNA-181a/b is triggered by PML/RARα (oncogenic fusion protein) (Fig. 3) [50]. There was a significant increase in the expression of miRNA-181a/b in PML/RARα knock-in mouse samples. Moreover, there was a significant reduction in tumor suppressor RASSF1A levels in PML/RARα knock-in mice [50].

Traditional Chinese medicine formula Jianpi-Huayu (JPHY) was found to be effective against hepatocellular carcinoma [51]. Tumors were smaller in size in mice treated with JPHY. JPHY reduced the expression of miR-602 and simultaneously increased the levels of RASSF1A [51].

RASSF1A increased the expression of miR-711 in SGC-7901 cancer cells [52]. CDK4 levels were noted to be reduced in miR-711-mimics-transfected SGC-7901 cells but high in the miR-711-inhibitors-transfected SGC-7901 cancer cells. RASSF1A inhibited proliferation of gastric cancer cells by miR-711- mediated targeting of CDK4 [52].

miR-602 has been shown to negatively regulate RASSF1A to promote carcinogenesis. There was a notable increase in apoptosis in HepG2 and HepG2-HBX cells after miR-602 inhibition [53].

However, surprisingly, RASSF1C has oncogenic role. It has been convincingly revealed that overexpression of RASSF1C downregulated tumor suppressor miR-33a but RASSF1C knockdown upregulated miR-33a expression in lung cancer cells. Furthermore, RASSF1C

overexpression led to a rapid increase in the levels of β-catenin, SNAIL and vimentin [54].

It is important to consider the delicate balance between RASSF1A and RASSF1C in the experiments in order to gain relatively deeper mechanistic insights to their distinct and/or overlapping contributory role in carcinogenesis and metastasis. More efforts are expected from basic and clinical oncologists to elucidate the actual functions of RASSF1A and RASSF1C isoforms in wide variety of cancers and how non-coding RNAs can regulate different isoforms to inhibit/prevent cancer progression.

Epigenetic regulation of RASSF1A: DNMT3B is involved in hypermethylation of promoter regions of RASSF1A and Claudin-6 (CLDN6) (shown in Fig. 4) [55]. Reconstitution of miR-7 and miR-218 caused marked suppression in the interaction of DNMT3B with promoters of RASSF1A and Claudin-6. Acetylation of H3 at promoter regions of RASSF1A and CLDN6 was also reported to be increased by miR-218 and miR-7 in MDA-MB-231 cancer cells [55].

DNMT1 is directly targeted by miR-342 [56]. miR-342 overexpression led to reduction in DNA methylation in promoter regions of RASSF1A, RECK and ADAM23 in SW480 cells (shown in Fig. 4). Injections of miR-342-overexpressing-SW480 cancer cells did not produce pulmonary metastatic nodules in any of the mice [56].

MBD2 (Methyl-CpG-binding domain protein-2) is also directly targeted by miR-373. miR-373-mediated targeting of MBD2 induced upregulation of tumor suppressive RASSF1A (shown in Fig. 4) [57].

Enforced expression of miR-152 and miR-148a in KMCH and Mz-ChA-1 cells caused significant increment in the levels of RASSF1A and p16INK4a concomitantly with suppression in DNMT1 levels [58]. Mz-ChA-1 cholangiocarcinoma cells were stably transfected with full-length IL-6 and inoculated in experimental mice. There was an evident increase in basal expression of DNMT1 and simultaneous reduction in the levels of p16INK4a and RASSF1A in Mz-IL-6 xenografts [58].

Regulation by LncRNAs: MiR-214-3p promoted invasion and migration of cancer cells. miR-214-3p directly targeted TET2 [59]. FENDRR blocked miR-214-3p mediated targeting of TET2. Importantly, TET2 stimulated the expression of RASSF1A (Fig. 3). MGC803 cells treated with the miR-214-3p mimics increased the methylation status of RASSF1A but MGC803 cells treated with miR-214-3p inhibitors caused significant reduction in the methylation levels of RASSF1A [59].

Intronic lncRNA (ANRASSF1) recruited PRC2 to the promoter region of RASSF1A, reducing the expression of RASSF1A (Fig. 3) [60]. Ectopically overexpressed ANRASSF1 reduced RASSF1A levels and increased the proliferative ability of HeLa cells, whereas silencing of ANRASSF1 led to a notable increase in the levels of RASSF1A. ANRASSF1 overexpression caused a robust increase in both histone H3K27me3 repressive marks and PRC2 occupancy, specifically at the promoter regions of RASSF1A [60].

Role of circular RNAs in the regulation of RASSF1A: circ_0078767 antagonized miR-330-3p-mediated targeting of RASSF1A [61]. circ_0078767 suppressed proliferation and invasion of cancer cells by blockade of miR-330-3p-mediated inhibition of RASSF1A pathway. Importantly, tumors overexpressing circ_0078767-A549 grew at a rapid rate and had larger volumes. circ_0078767 silencing mediated tumorigenesis was reversed by co-transfections with miR-330-3p inhibitors or RASSF1A. RASSF1A levels were lower in tumor tissues of experimental mice as compared to adjacently located tissues. miR-330-3p level in the tumors was considerably upregulated as compared to adjacently located tissues, but circ_0078767 expression was lower in the tumor tissues [61].

13. Regulation of RASSF2

hsa_circ_0059354 is derived from RASSF2 and located on chromosome 20 and thus termed as circRASSF2 [62]. miR-302b-3p targets IGF-1R to inhibit carcinogenesis. circRASSF2 potentiates the expression of IGF-1R by inhibition of miR-302b-3p-mediated targeting of IGF-1R. Depletion of circRASSF2 caused significant regression of growth rates and weight of xenografted tumors [62].

miR-200 family has been shown to demonstrate oncogenic effects in colorectal cancer by direct targeting of the tumor suppressor RASSF2 [63].

PAR4 is a direct binding partner of RASSF2 and any decline in the levels of RASSF2 severely impairs PAR4-driven apoptotic death in tumor cells [64]. PAR4 protein was enriched in the conditioned media of normal fibroblasts, but not in the CM of CAFs. Levels of RASSF2 and PAR4 were found to be reduced in miR-7 mimics-treated normal fibroblasts whereas notable increase in the levels of RASSF2 and PAR4 was noticed in miR-7-silenced-CAFs. HN13 cancer cells cultured in CAF-derived conditioned media demonstrated an increase in the proliferative activity as compared to conditioned-media derived from normal fibroblasts (NFs) [64]. Collectively, these findings indicated that cancer-associated fibroblasts promoted growth of cancer cells through a miR-7-RASSF2-PAR-4 axis.

14. Regulation of RASSF4

RASSF4 overexpression inhibited the proliferative potential and promoted the apoptotic death in HSC2 and Ca9-22 cancer cells, which was reversed by miR-626 mimics. RASSF4 overexpression blocked Wnt/β-catenin signaling cascade, accompanied by significant reduction in the levels of FZD1 and β-catenin [65].

M1 TAMs have tumor-suppressive functions but M2 TAMs can effectively promote metastasis [66]. M2 macrophages promoted the migratory and invasive capacities of A549 cells. Following treatment with GW4869 (inhibitors of exosome release), M2 macrophages failed to potentiate the migratory as well as invasive properties of A549 cancer cells. Overall, these findings indicated that exosomes derived from M2 macrophages played critical roles in augmenting the migratory and invasive features of A549 cancer cells. M2 macrophages-derived exosomes prompted invasion and epithelial-to-mesenchymal transition of A549 cancer cells through transportation of miRNA-155 and miRNA-196a-5p. Importantly, miR-155 and miR-196a-5p targeted RASSF4 in cancer cells. M2-derived exosomes markedly enhanced the lung metastasis. However, miR-155 and miR-196a-5p-knockdown exosomes did not promote pulmonary metastasis. miR-196a-5p and miR-155-knockdown M2-derived exosomes considerably reduced the metastatic spread of A549 cancer cells. Furthermore, M2-derived exosomes treated with inhibitors of miR-196a-5p and miR-155 caused reduction in vimentin and promoted E-cadherin for the inhibition of metastasis [66].

15. Regulation of RASSF5

Overexpression of MAPK8IP1P2 inhibited, while MAPK8IP1P2 silencing caused an increase in anchorage-independent growth rate of the thyroid cancer cells [67]. MAPK8IP1P2 overexpression enhanced, whereas MAPK8IP1P2 inhibition reduced the apoptotic death rate of thyroid cancer cells. MAPK8IP1P2 overexpression efficiently triggered the activities of caspase-9/caspase-3 and levels of pro-apoptotic BAD and BAX. Furthermore, levels of anti-apoptotic BCL2L1 and BCL2 were found to be notably reduced. MAPK8IP1P2 overexpression led to an increase in the levels of p-MST1/2, p-LATS1 as well as phosphorylated-YAP1 along with a reduction in nuclear accumulation of YAP1 and TAZ. LATS1/2-dependent phosphorylation of YAP resulted in YAP sequestration in the cytoplasm, consequent ubiquitination and degradation. MAPK8IP1P2 triggers the activation of Hippo pathway in thyroid cancer cells by sequestering away miR-146b-3p to relieve repressive effects of miRNA-146b-3p on RASSF1/RASSF5. miR-146b-3p mimics blocked MAPK8IP1P2 overexpression-mediated increase in the levels of RASSF1, RASSF5 and NF2. miR-146b-3p mimics also caused inactivation of Hippo pathway in MAPK8IP1P2-overexpressing K1

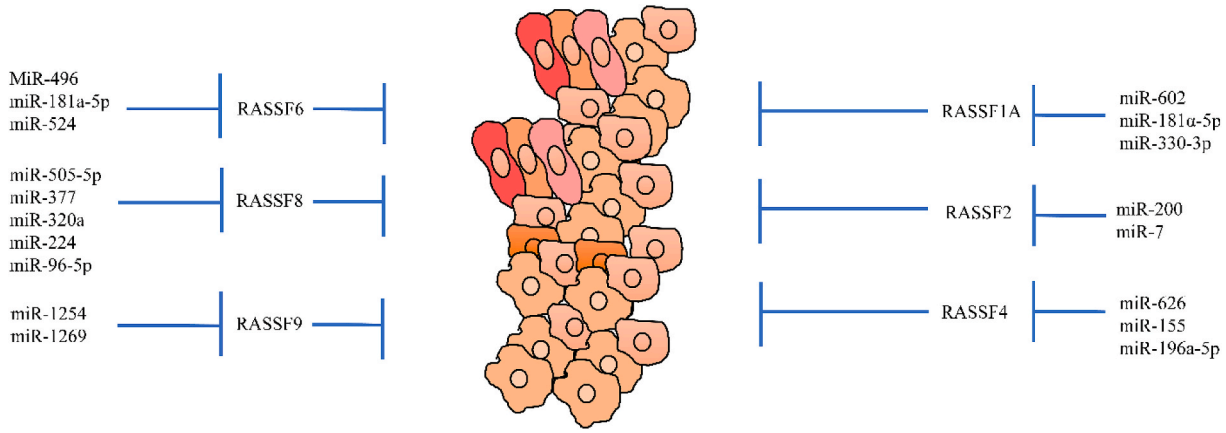

Fig. 5. Shows miRNA mediated inhibition of RASSF proteins.

cancer cells. Importantly, tumors in lymph nodes derived from MAPK8IP1P2-overexpressing cancer cells demonstrated evident reduction in the number of tumor cells and shrinkage of the tumor burden. Contrarily, the tumors in lymph nodes derived from MAPK8IP1P2-silenced cancer cells demonstrated enlarged tumor mass and increased number of tumor cells [67].

OIN1 knockdown caused a significant suppression of the proliferation of SKOV3 and A2780 cancer cells [68]. OIN1 overexpression considerably downregulated RASSF5 and ADORA1 levels in SKOV3 and A2780 cancer cells. Intra-tumoral injections of OIN1-specific siRNAs led to regression of the tumors in mice inoculated with A2780 cancer cells. Importantly, levels of ADORA1 and RASSF5 were found to be enhanced in dissected xenograft tumors injected with OIN1-specific siRNAs [68].

16. Regulation of RASSF6

RASSF6 has been shown to play a central role in tumor suppression. Different lncRNAs and circular RNAs have been reported to potentiate the expression of RASSF6.

Tumors derived from miR-181a-5p-overexpressing-gastric cancer cells were notably bigger in size [69]. Furthermore, there was an evident increase in the number of metastatic nodules within peritoneal cavities of tumor-bearing mice. miR-181a-5p directly targeted RASSF6 and activated MAPK signaling cascade. More importantly, knockdown or overexpression of RASSF6 partially counteracted the effects exerted by downregulation or upregulation of miR-181a-5p on the peritoneal metastatic capability of gastric cancer cells [69].

RASSF6 knockdown significantly enhanced the migration capacities of SW480 and LoVo cells [70]. RASSF6 downregulation caused activation of LEF1, SNAIL1, and TWIST1, while simultaneous repression of E-cadherin. MiR-496 targeted RASSF6 and triggered the activation of WNT pathway in colorectal cancer cells [70].

LncRNA TUSC7 effectively inhibited progression of osteosarcoma through the miR-181a/RASSF6 axis [71]. TUSC7 blocked miR-181a-induced targeting of RASSF6. Tumor volumes of TUSC7-overexpressing mice were found to be significantly reduced. RASSF6 expression was considerably enhanced in response to TUSC7 overexpression [71].

CASC2 acted as a sponge for miR-181a and enhanced the levels of RASSF6, ATM and PTEN in osteosarcoma cells [72]. Ectopic expression of CASC2 suppressed the colony formation and invasion of osteosarcoma cells [72].

circITCH not only reduced migration and invasion but also efficiently promoted apoptotic death of osteosarcoma cells. circITCH antagonized miR-524-mediated targeting of RASSF6 and induced apoptotic death. Tumor growth was found to be notably reduced in mice inoculated with circITCH-overexpressing MG-63 cells [73].

17. RASSF8

RASSF8 is another tumor suppressor reportedly involved in the inhibition of cancer progression. Different miRNAs have been shown to promote cancer via inhibition of RASSF8.

miR-505-5p directly targeted RASSF8 and promoted proliferation and colony formation of osteosarcoma cells. Tumor growth rates were found to be notably reduced in mice inoculated with miR-505-5p-silenced osteosarcoma cells [74].

In another study it was shown that miR-505 contributed to methotrexate resistance in colorectal cancer cells. RASSF8 overexpression blocked cell cycle of colorectal cancer cells, accelerated apoptotic death. Furthermore, miR-505 not only enhanced methotrexate-driven migration and invasiveness of LS174T cells but also reduced methotrexate-mediated cell apoptosis through RASSF8 downregulation [75].

RASSF8 overexpression significantly inhibited the proliferation and invasive abilities of BGC-823 gastric cancer cells [76]. miR-377-mediated targeting of RASSF8 in gastric cancer cells. RASSF8 overexpression caused significant suppression of the proliferation of BGC-823 cancer cells, whereas miR-377 abolished RASSF8-induced reduction in the proliferation properties of gastric cancer cells [76].

Likewise, miR-320a also directly targeted RASSF8 in ovarian cancer cells [77]. E-cadherin was considerably downregulated, while vimentin expression was increased in miR-320a-overexpressing-SKOV3 cancer cells. Growth of the tumors was noted to be remarkably enhanced in mice inoculated with miR-320a-overexpressing SKOV3 cancer cells [77].

HIF-1α transcriptionally upregulated miR-224 in gastric cancer cells. miR-224 acted as an oncogenic miRNA and directly inhibited RASSF8 [78]. NFκB-induced transcriptional activities were also found to be enhanced in RASSF8 knockdown-MGC-803 and SGC-7901 cancer cells. Tumor xenografts derived from miR-224 antagomir-treated cancer cells were smaller in size [78].

circPTPRA efficiently interfered with miR-96-5p-mediated targeting of RASSF8 in NSCLC cells [79]. Mice injected with circPTPRA-knockdown H23 cells demonstrated significant increase in lung metastatic lesions. Contrarily, mice injected with circPTPRA-overexpressing-H23 cells demonstrated fewer lung lesions [79].

18. RASSF9

RASSF9 overexpression caused reduction in the levels of p-AKT and concomitantly enhanced p53 levels in breast cancer cells. However, miR-1254 targeted RASSF9 and promoted cancer [80].

Likewise, miR-1269 also inhibited RASSF9 in gastric cancer cells and abolished apoptotic death. miR-1269 promoted pro-survival signaling

by activation of AKT in gastric cancer cells [81].

19. Concluding remarks

RASSF members have gained attention because of their characteristically unique ability to inhibit proliferation and invasion of cancer cells. More excitingly, regulation of RASSF members by non-coding RNAs is another are of intense and cutting-edge research (Fig. 5). However, this is the beginning of a new era in the field of RASSF-mediated pleiotropic roles in carcinogenesis. We still have to analyze how different non-coding RNAs regulate RASSF for cancer prevention and inhibition.

Declaration of competing interest

The authors declare that they do not have any conflict of interest.

References

[1] H. Donninger, M.L. Schmidt, J. Mezzanotte, T. Barnoud, G.J. Clark, Ras signaling through RASSF proteins, Semin. Cell Dev. Biol. 58 (2016 Oct) 86–95, https://doi.org/10.1016/j.semcdb.2016.06.007.

[2] A.M. Richter, G.P. Pfeifer, R.H. Dammann, The RASSF proteins in cancer; from epigenetic silencing to functional characterization, Biochim. Biophys. Acta 1796 (2) (2009 Dec) 114–128, https://doi.org/10.1016/j.bbcan.2009.03.004.

[3] S. Volinia, G.A. Calin, C.G. Liu, S. Ambs, A. Cimmino, F. Petrocca, R. Visone, M. Iorio, C. Roldo, M. Ferracin, R.L. Prueitt, N. Yanaihara, G. Lanza, A. Scarpa, A. Vecchione, M. Negrini, C.C. Harris, C.M. Croce, A microRNA expression signature of human solid tumors defines cancer gene targets, Proc. Natl. Acad. Sci. U. S. A. 103 (7) (2006 Feb 14) 2257–2261.

[4] J.R. Lytle, T.A. Yario, J.A. Steitz, Target mRNAs are repressed as efficiently by microRNA-binding sites in the 5' UTR as in the 3' UTR, Proc. Natl. Acad. Sci. U. S. A. 104 (23) (2007 Jun 5) 9667–9672.

[5] B. Khraiwesh, M.A. Arif, G.I. Seumel, S. Ossowski, D. Weigel, R. Reski, W. Frank, Transcriptional control of gene expression by microRNAs, Cell 140 (1) (2010 Jan 8) 111–122.

[6] A. Ahmad, Epigenetic regulation of immunosuppressive tumor-associated macrophages through dysregulated microRNAs, Semin. Cell Dev. Biol. 124 (2022 Apr) 26–33, https://doi.org/10.1016/j.semcdb.2021.09.001.

[7] M.N. Cabili, M.C. Dunagin, P.D. McClanahan, A. Biaesch, O. Padovan-Merhar, A. Regev, J.L. Rinn, A. Raj, Localization and abundance analysis of human lncRNAs at single-cell and single-molecule resolution, Genome Biol. 16 (1) (2015 Jan 29) 20, https://doi.org/10.1186/s13059-015-0586-4.

[8] M.K. Iyer, Y.S. Niknafs, R. Malik, U. Singhal, A. Sahu, Y. Hosono, T.R. Barrette, J. R. Prensner, J.R. Evans, S. Zhao, A. Poliakov, X. Cao, S.M. Dhanasekaran, Y.M. Wu, D.R. Robinson, D.G. Beer, F.Y. Feng, H.K. Iyer, A.M. Chinnaiyan, The landscape of long noncoding RNAs in the human transcriptome, Nat. Genet. 47 (3) (2015) 199–208.

[9] M. Guttman, I. Amit, M. Garber, C. French, M.F. Lin, D. Feldser, M. Huarte, O. Zuk, B.W. Carey, J.P. Cassady, M.N. Cabili, R. Jaenisch, T.S. Mikkelsen, T. Jacks, N. Hacohen, B.E. Bernstein, M. Kellis, A. Regev, J.L. Rinn, E.S. Lander, Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals, Nature 458 (7235) (2009) 223–227.

[10] H.S. Chiu, S. Somvanshi, E. Patel, T.W. Chen, V.P. Singh, B. Zorman, S.L. Patil, Y. Pan, S.S. Chatterjee, , Cancer Genome Atlas Research Network, A.K. Sood, P. H. Gunaratne, P. Sumazin, Pan-cancer analysis of lncRNA regulation supports their targeting of cancer genes in each tumor context, Cell Rep. 23 (1) (2018 Apr 3) 297–312, e12.

[11] I. Gareev, Y. Gileva, A. Dzidzaria, O. Beylerli, V. Pavlov, M. Agaverdiev, B. Mazorov, I. Biganyakov, A. Vardikyan, M. Jin, A. Ahmad, Long non-coding RNAs in oncourology, Noncoding RNA Res. 6 (3) (2021 Aug 26) 139–145, https://doi.org/10.1016/j.ncrna.2021.08.001.

[12] S. Ahmad, M. Abbas, M.F. Ullah, M.H. Aziz, O. Beylerli, M.A. Alam, M.A. Syed, S. Uddin, A. Ahmad, Long non-coding RNAs regulated NF-κB signaling in cancer metastasis: micromanaging by not so small non-coding RNAs, Semin. Cancer Biol. (2021 Jul 24), https://doi.org/10.1016/j.semcancer.2021.07.015. S1044-579X(21)00210-8.

[13] S. Memczak, M. Jens, A. Elefsinioti, F. Torti, J. Krueger, A. Rybak, L. Maier, S. D. Mackowiak, L.H. Gregersen, M. Munschauer, A. Loewer, U. Ziebold, M. Landthaler, C. Kocks, F. le Noble, N. Rajewsky, Circular RNAs are a large class of animal RNAs with regulatory potency, Nature 495 (7441) (2013 Mar 21) 333–338, https://doi.org/10.1038/nature11928.

[14] T.B. Hansen, T.I. Jensen, B.H. Clausen, J.B. Bramsen, B. Finsen, C.K. Damgaard, J. Kjems, Natural RNA circles function as efficient microRNA sponges, Nature 495 (7441) (2013 Mar 21) 384–388, https://doi.org/10.1038/nature11993.

[15] J. Salzman, C. Gawad, P.L. Wang, N. Lacayo, P.O. Brown, Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types, PLoS One 7 (2) (2012), e30733, https://doi.org/10.1371/journal.pone.0030733.

[16] C. Zhang, Y. Kang, F. Kong, Q. Yang, D. Chang, Hotspots and development frontiers of circRNA based on bibliometric analysis, Noncoding RNA Res. 7 (2) (2022 Mar 21) 77–88, https://doi.org/10.1016/j.ncrna.2022.03.001.

[17] M.L. Schmidt, K.R. Hobbing, H. Donninger, G.J. Clark, RASSF1A deficiency enhances RAS-driven lung tumorigenesis, Cancer Res. 78 (10) (2018 May 15) 2614–2623, https://doi.org/10.1158/0008-5472.CAN-17-2466.

[18] Y.Y. Liang, X.B. Deng, X.T. Lin, L.L. Jiang, X.T. Huang, Z.W. Mo, Y.W. Yuan, M. T. Teh, RASSF1A inhibits PDGFB-driven malignant phenotypes of nasopharyngeal carcinoma cells in a YAP1-dependent manner, Cell Death Dis. 11 (10) (2020 Oct 14) 855, https://doi.org/10.1038/s41419-020-03054-z.

[19] S.H. Kim, H. Jin, R.Y. Meng, D.Y. Kim, Y.C. Liu, O.H. Chai, B.H. Park, S.M. Kim, Activating hippo pathway via Rassf1 by ursolic acid suppresses the tumorigenesis of gastric cancer, Int. J. Mol. Sci. 20 (19) (2019 Sep 23) 4709, https://doi.org/10.3390/ijms20194709.

[20] S.A. Stoner, K.T.H. Liu, E.T. Andrews, M. Liu, K.I. Arimoto, M. Yan, A.G. Davis, S. Weng, M. Dow, S. Xian, R.C. DeKelver, H. Carter, D.E. Zhang, The RUNX1-ETO target gene RASSF2 suppresses t(8;21) AML development and regulates Rac GTPase signaling, Blood Cancer J. 10 (2) (2020 Feb 6) 16, https://doi.org/10.1038/s41408-020-0282-9.

[21] H. Song, S. Oh, H.J. Oh, D.S. Lim, Role of the tumor suppressor RASSF2 in regulation of MST1 kinase activity, Biochem. Biophys. Res. Commun. 391 (1) (2010 Jan 1) 969–973, https://doi.org/10.1016/j.bbrc.2009.11.175.

[22] W.N. Cooper, R.E. Dickinson, A. Dallol, E.V. Grigorieva, T.V. Pavlova, L.B. Hesson, I. Bieche, M. Broggini, E.R. Maher, E.R. Zabarovsky, G.J. Clark, F. Latif, Epigenetic regulation of the ras effector/tumour suppressor RASSF2 in breast and lung cancer, Oncogene 27 (12) (2008 Mar 13) 1805–1811, https://doi.org/10.1038/sj.onc.1210805.

[23] I.C. Jacquemart, A.E. Springs, W.Y. Chen, Rassf3 is responsible in part for resistance to mammary tumor development in neu transgenic mice, Int. J. Oncol. 34 (2) (2009 Feb) 517–528.

[24] H. Peng, H. Liu, S. Zhao, J. Wu, J. Fan, J. Liao, Silencing of RASSF3 by DNA hypermethylation is associated with tumorigenesis in somatotroph adenomas, PLoS One 8 (3) (2013), e59024, https://doi.org/10.1371/journal.pone.0059024.

[25] E. De Smedt, K. Maes, S. Verhulst, H. Lui, A. Kassambara, A. Maes, N. Robert, C. Heirman, A. Cakana, D. Hose, K. Breckpot, L.A. van Grunsven, K. De Veirman, E. Menu, K. Vanderkerken, J. Moreaux, E. De Bruyne, Loss of RASSF4 expression in multiple myeloma promotes RAS-driven malignant progression, Cancer Res. 78 (5) (2018 Mar 1) 1155–1168, https://doi.org/10.1158/0008-5472.CAN-17-1544.

[26] L.E. Crose, K.A. Galindo, J.G. Kephart, C. Chen, J. Fitamant, N. Bardeesy, R. C. Bentley, R.L. Galindo, J.T. Chi, C.M. Linardic, Alveolar rhabdomyosarcoma-associated PAX3-FOXO1 promotes tumorigenesis via Hippo pathway suppression, J. Clin. Invest. 124 (1) (2014 Jan) 285–296, https://doi.org/10.1172/JCI67087.

[27] D. Lee, S.J. Park, K.S. Sung, J. Park, S.B. Lee, S.Y. Park, H.J. Lee, J.W. Ahn, S. J. Choi, S.G. Lee, S.H. Kim, D.H. Kim, J. Kim, Y. Kim, C.Y. Choi, Mdm2 associates with Ras effector NORE1 to induce the degradation of oncoprotein HIPK1, EMBO Rep. 13 (2) (2012 Feb 1) 163–169, https://doi.org/10.1038/embor.2011.235.

[28] J. Park, S.I. Kang, S.Y. Lee, X.F. Zhang, M.S. Kim, L.F. Beers, D.S. Lim, J. Avruch, H. S. Kim, S.B. Lee, Tumor suppressor ras association domain family 5 (RASSF5/NORE1) mediates death receptor ligand-induced apoptosis, J. Biol. Chem. 285 (45) (2010 Nov 5) 35029–35038, https://doi.org/10.1074/jbc.M110.165506.

[29] K.P. Ko, S.I. Jeong, J.S. Lim, K.W. Lee, M.G. Lee, S.G. Chi, NORE1A directs apoptotic switch of TNF signaling through reciprocal modulation of ITCH-mediated destruction of TNFRI and BAX, Oncogene 39 (34) (2020 Aug) 5675–5689, https://doi.org/10.1038/s41388-020-01392-y.

[30] K.K. Slemmons, C. Yeung, J.T. Baumgart, J.O.M. Juarez, A. McCalla, L.J. Helman, Targeting hippo-dependent and hippo-independent YAP1 signaling for the treatment of childhood rhabdomyosarcoma, Cancer Res. 80 (14) (2020 Jul 15) 3046–3056, https://doi.org/10.1158/0008-5472.CAN-19-3853.

[31] N. Zhu, M. Si, N. Yang, Y. Jing, Y. Fu, X. Zhao, Z. Lin, G. Yang, Overexpression of RAS-association domain family 6 (RASSF6) inhibits proliferation and tumorigenesis in hepatocellular carcinoma cells, Oncol. Res. 25 (6) (2017 Jul 5) 1001–1008, https://doi.org/10.3727/096504016X14796039599926.

[32] E. Chen, F. Yang, H. He, L. Lei, R. Liu, L. Du, J. Dong, M. Wang, J. Yang, Decreased level of RASSF6 in sporadic colorectal cancer and its anti-tumor effects both in vitro and in vivo, Oncotarget 7 (15) (2016 Apr 12) 19813–19823, https://doi.org/10.18632/oncotarget.7852.

[33] Y.Y. Liang, X.B. Deng, L.S. Zeng, X.T. Lin, X.F. Shao, B. Wang, Z.W. Mo, Y.W. Yuan, RASSF6-mediated inhibition of Mcl-1 through JNK activation improves the anti-tumor effects of sorafenib in renal cell carcinoma, Cancer Lett. 432 (2018 Sep 28) 75–83, https://doi.org/10.1016/j.canlet.2018.05.048.

[34] L. Zheng, Z. Zhao, L. Rong, L. Xue, Y. Song, RASSF6-TRIM16 axis promotes cell proliferation, migration and invasion in esophageal squamous cell carcinoma, J. Genet. Genom. 46 (10) (2019 Oct 20) 477–488, https://doi.org/10.1016/j.jgg.2019.10.004.

[35] A. Kumaraswamy, A. Mamidi, P. Desai, A. Sivagnanam, L.R. Perumalsamy, C. Ramakrishnan, M. Gromiha, K. Rajalingam, S. Mahalingam, The non-enzymatic RAS effector RASSF7 inhibits oncogenic c-Myc function, J. Biol. Chem. 293 (40) (2018 Oct 5) 15691–15705, https://doi.org/10.1074/jbc.RA118.004452.

[36] X. Zheng, Q. Dong, X. Zhang, Q. Han, X. Han, Y. Han, J. Wu, X. Rong, E. Wang, The coiled-coil domain of oncogene RASSF 7 inhibits hippo signaling and promotes non-small cell lung cancer, Oncotarget 8 (45) (2017 Aug 12) 78734–78748, https://doi.org/10.18632/oncotarget.20223.

[37] L. Zhang, J.H. Wang, R.X. Liang, S.T. Huang, J. Xu, L.J. Yuan, L. Huang, Y. Zhou, X. J. Yu, S.Y. Wu, R.Z. Luo, J.P. Yun, W.H. Jia, M. Zheng, RASSF8 downregulation promotes lymphangiogenesis and metastasis in esophageal squamous cell carcinoma, Oncotarget 6 (33) (2015 Oct 27) 34510–34524, https://doi.org/10.18632/oncotarget.5923.

[38] L. Shi, J. Middleton, Y.J. Jeon, P. Magee, D. Veneziano, A. Laganà, H.S. Leong, S. Sahoo, M. Fassan, R. Booton, R. Shah, P.A.J. Crosbie, M. Garofalo, KRAS induces

lung tumorigenesis through microRNAs modulation, Cell Death Dis. 9 (2) (2018 Feb 13) 219, https://doi.org/10.1038/s41419-017-0243-9.
[39] F.E. Lock, N. Underhill-Day, T. Dunwell, D. Matallanas, W. Cooper, L. Hesson, A. Recino, A. Ward, T. Pavlova, E. Zabarovsky, M.M. Grant, E.R. Maher, A. D. Chalmers, W. Kolch, F. Latif, The RASSF8 candidate tumor suppressor inhibits cell growth and regulates the Wnt and NF-kappaB signaling pathways, Oncogene 29 (30) (2010 Jul 29) 4307–4316, https://doi.org/10.1038/onc.2010.192.
[40] H. Shi, Q. Ju, Y. Mao, Y. Wang, J. Ding, X. Liu, X. Tang, C. Sun, TAK1 phosphorylates RASSF9 and inhibits esophageal squamous tumor cell proliferation by targeting the RAS/MEK/ERK Axis, Adv. Sci. 8 (5) (2021 Jan 6) 2001575, https://doi.org/10.1002/advs.202001575.
[41] J. Yuan, Q. Ju, J. Zhu, Y. Jiang, X. Yang, X. Liu, J. Ma, C. Sun, J. Shi, RASSF9 promotes NSCLC cell proliferation by activating the MEK/ERK axis, Cell Death Dis. 7 (1) (2021 Jul 31) 199, https://doi.org/10.1038/s41420-021-00583-0.
[42] A.M. Richter, M.L. Woods, M.M. Küster, S.K. Walesch, T. Braun, T. Boettger, R. H. Dammann, RASSF10 is frequently epigenetically inactivated in kidney cancer and its knockout promotes neoplasia in cancer prone mice, Oncogene 39 (15) (2020 Apr) 3114–3127, https://doi.org/10.1038/s41388-020-1195-6.
[43] A.M. Richter, M.M. Küster, M.L. Woods, S.K. Walesch, M.Y. Gökyildirim, M. Krueger, R.H. Dammann, RASSF10 is a TGFβ-target that regulates ASPP2 and E-cadherin expression and acts as tumor suppressor that is epigenetically downregulated in advanced cancer, Cancers 11 (12) (2019 Dec 8) 1976, https://doi.org/10.3390/cancers11121976.
[44] X. Han, Q. Dong, J. Wu, Y. Luo, X. Rong, Q. Han, X. Zheng, E. Wang, RASSF10 suppresses lung cancer proliferation and invasion by decreasing the level of phosphorylated LRP6, Mol. Carcinog. 58 (7) (2019 Jul) 1168–1180, https://doi.org/10.1002/mc.23000.
[45] Ch NP. Lakshmi, A. Sivagnanam, S. Raja, S. Mahalingam, Molecular basis for RASSF10/NPM/RNF2 feedback cascade-mediated regulation of gastric cancer cell proliferation, J. Biol. Chem. 297 (2) (2021 Aug) 100935, https://doi.org/10.1016/j.jbc.2021.100935.
[46] F. Wang, Y. Feng, P. Li, K. Wang, L. Feng, Y.F. Liu, H. Huang, Y.B. Guo, Q.S. Mao, W.J. Xue, RASSF10 is an epigenetically inactivated tumor suppressor and independent prognostic factor in hepatocellular carcinoma, Oncotarget 7 (4) (2016 Jan 26) 4279–4297, https://doi.org/10.18632/oncotarget.6654.
[47] C. Zhou, Y. Huang, Y. Chen, Y. Xie, H. Wen, W. Tan, C. Wang, miR-602 mediates the RASSF1A/JNK pathway, thereby promoting postoperative recurrence in nude mice with liver cancer, OncoTargets Ther. 13 (2020 Jul 10) 6767–6776, https://doi.org/10.2147/OTT.S243651.
[48] Z.P. Han, D.B. Liu, L.Q. Wu, Q. Li, Z.G. Wang, X.F. Zang, IL-1β secreted by macrophage M2 promotes metastasis of osteosarcoma via NF-κB/miR-181α-5p/RASSF1A/Wnt pathway, Transl. Cancer Res. 9 (4) (2020 Apr) 2721–2733, https://doi.org/10.21037/tcr.2020.02.52.
[49] J. Yu, J. Qi, X. Sun, W. Wang, G. Wei, Y. Wu, Q. Gao, J. Zheng, MicroRNA-181a promotes cell proliferation and inhibits apoptosis in gastric cancer by targeting RASSF1A, Oncol. Rep. 40 (4) (2018 Oct) 1959–1970, https://doi.org/10.3892/or.2018.6632.
[50] D. Bräuer-Hartmann, J.U. Hartmann, A.A. Wurm, D. Gerloff, C. Katzerke, M. V. Verga Falzacappa, P.G. Pelicci, C. Müller-Tidow, D.G. Tenen, D. Niederwieser, G. Behre, PML/RARα-Regulated miR-181a/b cluster targets the tumor suppressor RASSF1A in acute promyelocytic leukemia, Cancer Res. 75 (16) (2015 Aug 15) 3411–3424, https://doi.org/10.1158/0008-5472.CAN-14-3521.
[51] Y. Huang, C. Zhou, H. Wen, Y. Chen, Y. Xie, X. Lan, J. Lin, X. Huang, Y. Mo, C. Yang, Q. Wang, C. Wang, Jianpi-huayu formula inhibits development of hepatocellular carcinoma by regulating expression of miR-602, which targets the RASSF1A gene, Integr. Cancer Ther. 19 (2020 Jan-Dec), https://doi.org/10.1177/1534735419900804, 1534735419900804.
[52] A. Liao, G. Tan, L. Chen, W. Zhou, H. Hu, RASSF1A inhibits gastric cancer cell proliferation by miR-711- mediated downregulation of CDK4 expression, Oncotarget 7 (5) (2016 Feb 2) 5842–5851, https://doi.org/10.18632/oncotarget.6813.
[53] L. Yang, Z. Ma, D. Wang, W. Zhao, L. Chen, G. Wang, MicroRNA-602 regulating tumor suppressive gene RASSF1A is overexpressed in hepatitis B virus-infected liver and hepatocellular carcinoma, Cancer Biol. Ther. 9 (10) (2010 May 15) 803–808, https://doi.org/10.4161/cbt.9.10.11440.
[54] Y.G. Amaar, M.E. Reeves, RASSF1C regulates miR-33a and EMT marker gene expression in lung cancer cells, Oncotarget 10 (2) (2019 Jan 4) 123–132, https://doi.org/10.18632/oncotarget.26498.
[55] Q. Li, F. Zhu, P. Chen, 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 (1) (2012 Jul 20) 28–33, https://doi.org/10.1016/j.bbrc.2012.06.028.
[56] H. Wang, J. Wu, X. Meng, X. Ying, Y. Zuo, R. Liu, Z. Pan, T. Kang, W. Huang, MicroRNA-342 inhibits colorectal cancer cell proliferation and invasion by directly targeting DNA methyltransferase 1, Carcinogenesis 32 (7) (2011 Jul) 1033–1042, https://doi.org/10.1093/carcin/bgr081.
[57] Y. Chen, J. Luo, R. Tian, H. Sun, S. Zou, miR-373 negatively regulates methyl-CpG-binding domain protein 2 (MBD2) in hilar cholangiocarcinoma, Dig. Dis. Sci. 56 (6) (2011 Jun) 1693–1701, https://doi.org/10.1007/s10620-010-1481-1.
[58] C. Braconi, N. Huang, T. Patel, MicroRNA-dependent regulation of DNA methyltransferase-1 and tumor suppressor gene expression by interleukin-6 in human malignant cholangiocytes, Hepatology 51 (3) (2010 Mar) 881–890, https://doi.org/10.1002/hep.23381.
[59] Z. He, X. Wang, C. Huang, Y. Gao, C. Yang, P. Zeng, Z. Chen, The FENDRR/miR-214-3P/TET2 axis affects cell malignant activity via RASSF1A methylation in gastric cancer, Am. J. Transl. Res. 10 (10) (2018 Oct 15) 3211–3223.
[60] F.C. Beckedorff, A.C. Ayupe, R. Crocci-Souza, M.S. Amaral, H.I. Nakaya, D. T. Soltys, C.F. Menck, E.M. Reis, S. Verjovski-Almeida, The intronic long noncoding RNA ANRASSF1 recruits PRC2 to the RASSF1A promoter, reducing the expression of RASSF1A and increasing cell proliferation, PLoS Genet. 9 (8) (2013), e1003705, https://doi.org/10.1371/journal.pgen.1003705.
[61] T. Chen, Z. Yang, C. Liu, L. Wang, J. Yang, L. Chen, W. Li, Circ_0078767 suppresses non-small-cell lung cancer by protecting RASSF1A expression via sponging miR-330-3p, Cell Prolif 52 (2) (2019 Mar), e12548, https://doi.org/10.1111/cpr.12548.
[62] L. Tian, J. Cao, H. Jiao, J. Zhang, X. Ren, X. Liu, M. Liu, Y. Sun, CircRASSF2 promotes laryngeal squamous cell carcinoma progression by regulating the miR-302b-3p/IGF-1R axis, Clin. Sci. (Lond.) 133 (9) (2019 May 9) 1053–1066, https://doi.org/10.1042/CS20190110.
[63] J.V. Carter, S.J. O'Brien, J.F. Burton, B.G. Oxford, V. Stephen, J. Hallion, C. Bishop, N.J. Galbraith, M.R. Eichenberger, H. Sarojini, E. Hattab, S. Galandiuk, The microRNA-200 family acts as an oncogene in colorectal cancer by inhibiting the tumor suppressor RASSF2, Oncol. Lett. 18 (4) (2019 Oct) 3994–4007, https://doi.org/10.3892/ol.2019.10753.
[64] Z. Shen, X. Qin, M. Yan, R. Li, G. Chen, J. Zhang, W. Chen, Cancer-associated fibroblasts promote cancer cell growth through a miR-7-RASSF2-PAR-4 axis in the tumor microenvironment, Oncotarget 8 (1) (2017 Jan 3) 1290–1303, https://doi.org/10.18632/oncotarget.13609.
[65] S.H. Cui, X.D. Hu, Y. Yan, Wnt/β-catenin signaling pathway participates in the effect of miR-626 on oral squamous cell carcinoma by targeting RASSF4, J. Oral Pathol. Med. 50 (10) (2021 Nov) 1005–1017, https://doi.org/10.1111/jop.13216.
[66] X. Li, Z. Chen, Y. Ni, C. Bian, J. Huang, L. Chen, X. Xie, J. Wang, Tumor-associated macrophages secret exosomal miR-155 and miR-196a-5p to promote metastasis of non-small-cell lung cancer, Transl. Lung Cancer Res. 10 (3) (2021 Mar) 1338–1354, https://doi.org/10.21037/tlcr-20-1255.
[67] X. Liu, Q. Fu, X. Bian, Y. Fu, J. Xin, N. Liang, S. Li, Y. Zhao, L. Fang, C. Li, J. Zhang, G. Dionigi, H. Sun, Long non-coding RNA MAPK8IP1P2 inhibits lymphatic metastasis of thyroid cancer by activating hippo signaling via sponging miR-146b-3p, Front. Oncol. 10 (2021 Jan 7) 600927, https://doi.org/10.3389/fonc.2020.600927.
[68] T. Takeiwa, Y. Mitobe, K. Ikeda, K. Hasegawa, K. Horie, S. Inoue, Long intergenic noncoding RNA OIN1 promotes ovarian cancer growth by modulating apoptosis-related gene expression, Int. J. Mol. Sci. 22 (20) (2021 Oct 18) 11242, https://doi.org/10.3390/ijms222011242.
[69] Y. Mi, D. Zhang, W. Jiang, J. Weng, C. Zhou, K. Huang, H. Tang, Y. Yu, X. Liu, W. Cui, M. Zhang, X. Sun, Z. Zhou, Z. Peng, S. Zhao, Y. Wen, miR-181a-5p promotes the progression of gastric cancer via RASSF6-mediated MAPK signalling activation, Cancer Lett. 389 (2017 Mar 28) 11–22, https://doi.org/10.1016/j.canlet.2016.12.033.
[70] H. Wang, B. Yan, P. Zhang, S. Liu, Q. Li, J. Yang, F. Yang, E. Chen, MiR-496 promotes migration and epithelial-mesenchymal transition by targeting RASSF6 in colorectal cancer, J. Cell. Physiol. 235 (2) (2020 Feb) 1469–1479, https://doi.org/10.1002/jcp.29066.
[71] A. Zhao, W. Liu, X. Cui, N. Wang, Y. Wang, L. Sun, H. Xue, L. Wu, S. Cui, Y. Yang, R. Bai, lncRNA TUSC7 inhibits osteosarcoma progression through the miR 181a/RASSF6 axis, Int. J. Mol. Med. 47 (2) (2021 Feb) 583–594, https://doi.org/10.3892/ijmm.2020.4825.
[72] Z. Ba, L. Gu, S. Hao, X. Wang, Z. Cheng, G. Nie, Downregulation of lncRNA CASC2 facilitates osteosarcoma growth and invasion through miR-181a, Cell Prolif 51 (1) (2018 Feb), e12409, https://doi.org/10.1111/cpr.12409.
[73] W. Zhou, Y. Liu, X. Wu, Down-regulation of circITCH promotes osteosarcoma development and resistance to doxorubicin via the miR-524/RASSF6 axis, J. Gene Med. 23 (10) (2021 Oct), e3373, https://doi.org/10.1002/jgm.3373.
[74] T. Wang, H. Zhang, H. Wang, C. Chang, F. Huang, L. Zhang, MiR-505-5p inhibits proliferation and promotes apoptosis of osteosarcoma cells via regulating RASSF8 expression, J. BUON 26 (2) (2021 Mar-Apr) 599–605.
[75] Y. Chen, L. Bian, Y. Zhang, MiR-505 mediates methotrexate resistance in colorectal cancer by targeting RASSF8, J. Pharm. Pharmacol. 70 (7) (2018 Jul) 937–951, https://doi.org/10.1111/jphp.12913.
[76] X. Bo, Y. Chen, W. Sheng, Y. Gong, H. Wang, W. Gao, B. Zhang, The regulation and function of microRNA-377/RASSF8 signaling axis in gastric cancer, Oncol. Lett. 15 (3) (2018 Mar) 3630–3638, https://doi.org/10.3892/ol.2018.7740.
[77] L. Zhang, H. Chen, F. He, S. Zhang, A. Li, A. Zhang, A. Zhang, MicroRNA-320a promotes epithelial ovarian cancer cell proliferation and invasion by targeting RASSF8, Front. Oncol. 11 (2021 Feb 25) 581932, https://doi.org/10.3389/fonc.2021.581932.
[78] C. He, L. Wang, J. Zhang, H. Xu, Hypoxia-inducible microRNA-224 promotes the cell growth, migration and invasion by directly targeting RASSF8 in gastric cancer, Mol. Cancer 16 (1) (2017 Feb 7) 35, https://doi.org/10.1186/s12943-017-0603-1.
[79] S. Wei, Y. Zheng, Y. Jiang, X. Li, J. Geng, Y. Shen, Q. Li, X. Wang, C. Zhao, Y. Chen, Z. Qian, J. Zhou, W. Li, The circRNA circPTPRA suppresses epithelial-mesenchymal transitioning and metastasis of NSCLC cells by sponging miR-96-5p, EBioMedicine 44 (2019 Jun) 182–193, https://doi.org/10.1016/j.ebiom.2019.05.032.
[80] B. Li, P. Chen, J. Wang, L. Wang, M. Ren, R. Zhang, J. He, MicroRNA-1254 exerts oncogenic effects by directly targeting RASSF9 in human breast cancer, Int. J. Oncol. 53 (5) (2018 Nov) 2145–2156, https://doi.org/10.3892/ijo.2018.4530.
[81] W.L. Liu, H.X. Wang, C.X. Shi, F.Y. Shi, L.Y. Zhao, W. Zhao, G.H. Wang, MicroRNA-1269 promotes cell proliferation via the AKT signaling pathway by targeting RASSF9 in human gastric cancer, Cancer Cell Int. 19 (2019 Nov 21) 308, https://doi.org/10.1186/s12935-019-1026-4.