The Role of RASSF1 Methylation in Lung Carcinoma

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Tingting Zhang, Yanjun Li, Hui Zhang, Xiangdong Wang, Xiaozhuan Liu, and Li Li

Abstract

Lung carcinoma is the most frequently diagnosed malignant neoplasms and mainly consists of small-cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC). Large number of lung carcinoma patients have poor outcomes due to the late diagnosis and the limited therapeutic options. Previous attempts have proved that the evolution of lung carcinoma is a multistep molecular aberration which various genetic or epigenetic alterations may be take part in. Among these molecular aberrations, the inactivation of tumor suppressor gene has been widely observed in all types of carcinoma including lung carcinoma. As a vital inactivated mechanism, DNA methylation of tumor suppressor gene is frequently found in lung cancer. To gain exhaustive comprehension of the carcinogenesis of lung carcinoma, we summarize our current knowledge on DNA methylation of RASSF1 (RAS-Association Domain Family 1) and its clinical significance in lung carcinoma.

Keywords

Lung carcinoma · RASSF1 · Tumor-suppressor gene · DNA methylation

Abbreviations

SCLC	Small-cell lung carcinoma
NSCLC	Non-small-cell lung carcinoma
RASSF1	RAS-Association Domain Family 1
TEADs	Transcriptional enhancer factors
TAZ	WW domain-containing transcriptional coactivators
YAP	TAZ paralog
hMOB1	MOB kinase activator 1B
hSAV1	The adaptor/scaffold proteins Salvador homolog 1

Tingting Zhang, Yanjun Li, and Hui Zhang contributed equally to this work.

T. Zhang · Y. Li · H. Zhang · X. Liu (✉)
Clinical Center for Single Cell Biomedicine, Henan Provincial People's Hospita, Zhengzhou, Henan, China

People's Hospital of Zhengzhou University, Zhengzhou, Henan, China

People's Hospital of Henan University, Zhengzhou, Henan, China

X. Wang
Zhongshan Hospital, Fudan University, Shanghai, Shanghai, China

L. Li
Department of Scientific Research and Discipline Construction, Henan Provincial People's Hospital, Zhengzhou, Henan, China

Zhengzhou University People's Hospital, Zhengzhou, Henan, China

Henan University People's Hospital, Zhengzhou, Henan, China

Clinical Center for Single Cell Biomedicine, Henan Provincial People's Hospita, Zhengzhou, Henan, China
e-mail: lili@henu.edu.cn

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LATS1 and LATS2	Large tumor suppressor 1 and 2
MST1 and MST2	Mammalian sterile 20-like kinase 1 and 2
LOH	Heterozygosity

8.1 Introduction

Lung carcinoma is the leading cause of cancer-related death, with an estimated 388,000 deaths in Europe in 2018 [1, 2]. Based upon the data of smoking prevalence from the population-based Adult Health Survey in 2003, the estimated lung cancer mortality was 15.0 and 7.1 per 100,000 among men and women in 2018 [1], respectively. In China, the incidence of lung carcinoma is also high, with the highest mortality rate as compared with other countries [2]. The uptake of tobacco among males and exposure to unventilated cooking fumes among females are the predominant non-genetic risk factors for lung carcinoma [3–7]. The 5-year survival rate of lung carcinoma is very low, especially in Eastern Asia, due to the large proportion of lung carcinoma patients present with advanced metastatic tumors when diagnosed [8, 9].

Lung carcinoma mainly consists of small-cell lung carcinoma (SCLC) as the most aggressive lung carcinoma accounted for about 25% of bronchogenic carcinomas and non-small-cell lung carcinoma (NSCLC) as the most common lung carcinoma subtype for approximately 85% of lung cancer cases [10, 11]. The major histological subtypes of NSCLC are represented by lung adenocarcinoma, squamous cell carcinoma, and large cell carcinoma [12], of which the resection is performed in the early stage and chemo-combination in the late stage, with the mean 5-year survival of 15%. The large number of lung cancer patients have poor outcomes due to the late diagnosis, acquired multidrug resistance, and complex mechanisms [13]. This chapter aims at exploring the comprehensive mechanisms on the carcinogenesis of lung cancer by furthermore understanding DNA methylation of RAS-Association Domain Family 1 (RASSF1) and its clinical significance in lung carcinoma. We pay more specific attention on the potential mechanisms and new specific molecular markers of lung cancer, especially DNA methylation of tumor suppressor genes and inactivated genes in the development of lung carcinoma.

8.2 The RASSF1 Gene

RASSF1 is one of the key tumor-suppressor genes allocated in chromosome 3p21.3 and spans about 11,151 bp [14, 10]. RASSF1 promotes apoptosis, microtubule stability and polymerization, and mitotic progression [15]. The protein encoded by RASSF1 can participate in RAS-related cellular signal pathways and regulate oncogenesis, cell proliferation, differentiation, and apoptosis in a wide variety of cancer types [16]. Eight transcripts, i.e., RASSF1A, B, C, D, E, F, G, and H, are generated by RASSF1 gene and contain a Ras-Association (RA) domain in the carboxyterminal segments, except for RASSF1F-H which is similar to the RAS effector proteins, Raf1. Raf1 is associated with Ras-GTP to activate Ras proteins, suppress cell growth, and promote proapoptotic effects.

RASSF1A and RASSF1C are two predominant common isoforms and encode an ATM-kinase phosphorylation site and a conserved carboxyterminal SARAH (Sav/RASSF/Hpo) domain as a key component of the Hippo signaling pathway, except for the RA domain. RASSF1A has a diacylglycerol/phorbol ester-binding (DAG) domain containing a central zinc finger which is also known as the protein kinase C conserved domain (C1 domain). RASSF1C variant is shorter than RASSF1A and lacks the amino terminal C1 domain. RASSF1D and E have the RA, SARAH, C1 domains and ATM-kinase phosphorylation site similar to RASSF1A in structure. RASSF1B contains one RA and SARAH domain, respectively. Isoforms

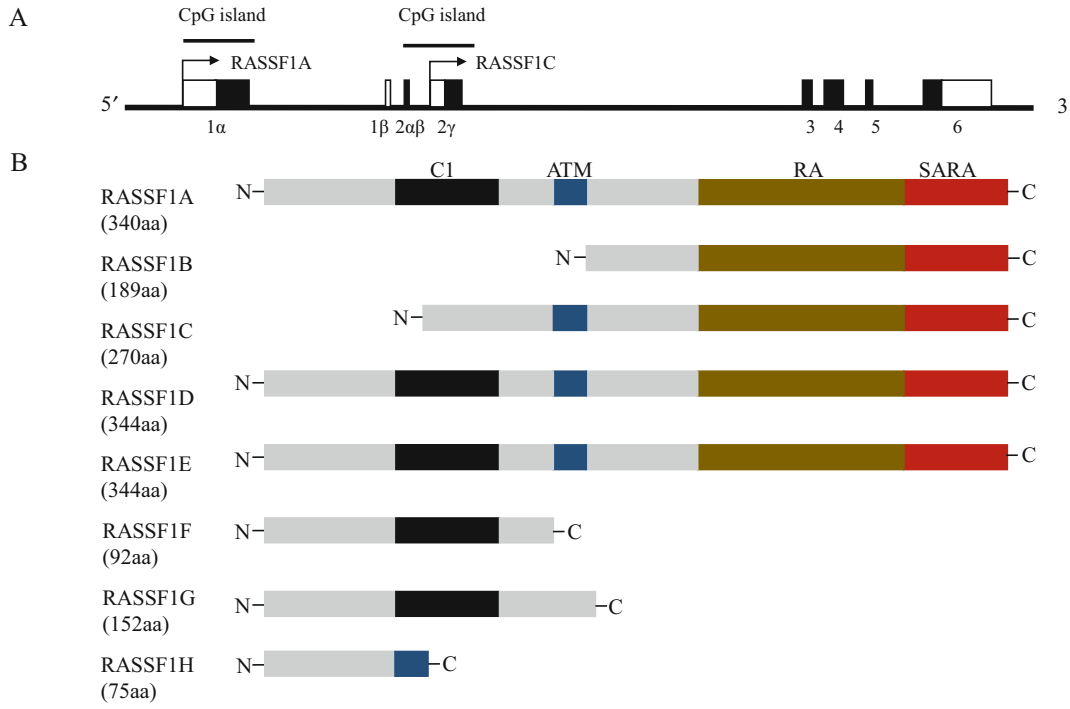

Fig. 8.1 (**a**) Schematic map of RASSF1 locus. Black boxes indicate exons and open boxes indicate untranslated regions, respectively. Two CpG islands are shown by black lines. The transcription start sites of isoform A and isoform C are indicated with black arrows. (**b**) The domain structures of polypeptides encoded by RASSF1. C1, DAG/diacylglycerol binding domain (black) putative ATM kinase phosphorylation consensus sequence motif (blue) RA, Ras-association domain (brown) and SARAH, Sav/RASSF/Hpo interaction domain (red)

F/G and H have a C1 domain and an ATM-kinase phosphorylation site, respectively (Fig. 8.1).

RASSF1C appears to share many of the biological characteristics of RASSF1A. On basis of the similar structure to Ras effector, RASSF1 gene regulates cell proliferation, differentiation, and apoptosis. RASSF1A functions as a negative regulator of cell proliferation by blocking the cell cycle progression at the level of G1/S-phase [17] and has the dual role in the coordination of p53 and p73 responses [18], while RASSF1C exhibits growth inhibitory potency [19], although there is little known on functions of other variants. RASSF1B, D, and E are found poorly expressed in hemopoietic, cardiac, and pancreatic cells, respectively. RASSF1F, E, D, and G share the same promoter region with RASSF1A, although the biological significance remains unclear.

RASSF2, RASSF3, NORE1, and RASSF6 were identified as the homolog of RASSF1, which share similar Ras-association domain with RASSF1. These genes have SARAH domain and code multiple transcripts. RASSF2 shares a lower homology (29% identity) with RASSF1, while acting as a tumor suppressor gene and undergoing promoter methylation at high frequency similar to RASSF1 [20]. The inactivation of RASSF2 may be associated with tumor progression [20], and RASSF3 suppresses tumor formation through interacting with MDM2 and inducing NSCLC cell apoptosis [21]. NORE1 shares about 50% sequence identities with RASSF1 and has similar pattern of mRNA transcript expression and function as a tumor-suppressor gene [22–24]. RASSF6 is found frequently suppressed in several human cancers [20, 25–27].

8.3 Inactivation of RASSF1A by DNA Methylation in Lung Carcinoma

The genomic mutational landscape provided evidences that genetic alterations taken part in the tumorigenesis of lung cancer. The epigenetic regulation provides a novel insight in the progression and evolution of lung cancer [28, 29]. Of the epigenetic modifications, DNA methylation mainly occurs in C-G dinucleotide-rich regions, also named CpG islands [30], where the methyl group is added by DNA methyltransferase at the 5-position cytosine and erased by demethylase. DNA methylation mainly occurs at the cytosine--phosphate-guanine (CpG) island which locates in promoter region of a gene and regulates the expression of gene, which plays a vital role in genomic imprint erasure, instability of chromatin structure, and X-chromosome inactivation. The aberrant DNA methylation interacts with gene expression in the early stage of human cancers and dynamically during lung carcinogenesis. A lot of methylated genes have been identified in lung carcinoma, including RASSF1, major tumor suppressor 1, fragile histidine triad, methylguanine-DNA methyltransferase, and adenomatosis polyposis coli tumor suppressor.

The loss of heterozygosity (LOH) is the most frequent event during lung tumorigenesis [31], while rarely attributed to somatic mutations, except for one frame-shift and missense mutation identified in nasopharyngeal carcinomas [32]. RASSF1 is inactivated frequently by the hypermethylation of the promoter CpG island in cancers [33–37]. RASSF1 methylation was originally reported in lung cancer and then shown as the common event in cancers [33, 38]. RASSF1A was methylation-inactivated in SCLC, while aberrant methylation of the RASSF1C CpG island promoter was not observed in lung cancer [33].

8.4 Signaling Pathway Involving RASSF1 in Lung Cancer

Among signaling pathways, RASSF1A contributes to the carcinogenesis of lung cancer mainly through Hippo signaling pathways. The Hippo pathway (i.e., Salvador-Warts-Hippo pathway) in a kinase cascade regulates the organ size through regulating cell proliferation, differentiation, and apoptosis [39–41]. The core components of the pathway encompass the mammalian sterile 20-like kinase 1 and 2 (MST1 and MST2) and the large tumor suppressor 1 and 2 (LATS1 and LATS2), and cooperate with the adaptor/scaffold proteins, Salvador homolog 1 (hSAV1), and MOB kinase activator 1A and 1B (hMOB1). The downstream effectors of Hippo pathway are two WW domain-containing transcriptional coactivators TAZ and its paralog YAP. Mst1/2 phosphorylates hSAV1 and forms the activated Mst1/2-hSAV1 complex which cooperates with hMOB1 and activates LATS1/2. After then LATS1/2 phosphorylates YAP/TAZ which is prevented from entering to the nucleus. Then the complex with transcriptional enhancer factors (TEADs) is formed and the expression of anti-apoptotic and pro-proliferative genes are activated [42–44].

During DNA damage, RASSF1A activated by ATM can induce apoptosis through the interaction of Hippo pathway with MST1/MST2 via the C-terminus to prevent the autophosphorylation of those protein kinases [18, 44, 45]. The components of the Hippo pathway are intimately involved in lung morphogenesis and tumorigenesis [46–48]. The abnormal expression of those components is associated with the clinical classification, poor differentiation, metastasis, and poor prognosis and survival in lung cancer [47, 49–52]. The DNA methylation of promoter results in the inactivation of RASSF1A, RASSF1A-MST1/MST2 complex, and dysfunction of the Hippo pathway. RASSF1A can enhance the transcription of proapoptotic genes

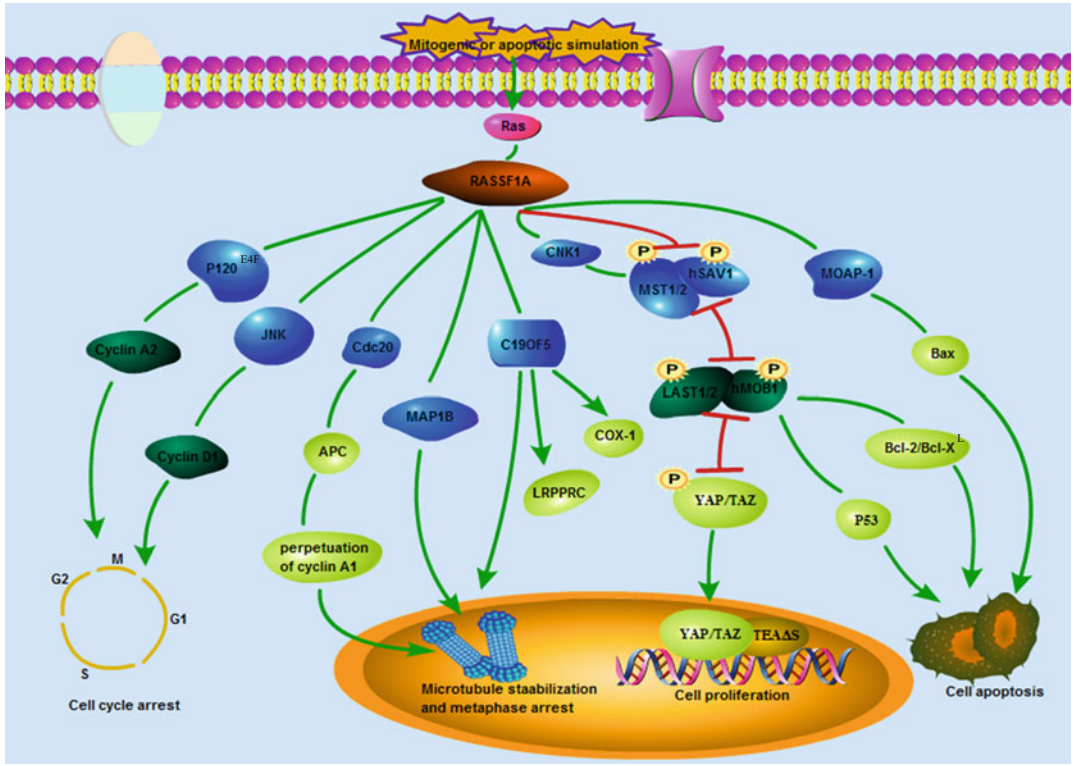

Fig. 8.2 A summary of RASSF1A pathways in cancergenesis of lung cancer. RASSF1A regulates cell apoptosis through its interactions with the connector enhancer of KSR (CNK1), the proapoptotic kinase MST1, and the modulator of apoptosis-1 (MAP-1). The CNK1–MST1 complex is also thought to play important role in cell proliferation. RASSF1A can regulate the microtubule network by recruiting effectors of the microtubule-associated protein 1B (MAP1B), C19ORF5, and the Cdc20. RASSF1A also induced G1 and S-phase cell cycle arrest through inhibiting the transcription factor $p120^{E4F}$ (RASSF1A was enhanced by $p120^{E4F}$) and JNK

through the formation of a complex with YAP and p73 in the nucleus (Fig. 8.2).

RASSF1A may contribute to the carcinogenesis of lung cancer through microtubules and binding of Cdc20 via an N-terminal region. Cdc20 cannot bind with APC and fail to form the complex Cdc20-APC for the spindle assembly checkpoint during mitosis [53]. RASSF1A is required for stabilizing the microtubule. RASSF1A controls the motility and invasion of lung cancer cells through the modulation of tubulin dynamics [54, 55]. The promoter hypermethylation of RASSF1A activates premature APC, following by accelerated cell division, mitotic spindle abnormalities, and chromosome misalignment [53] (Fig. 8.2). The exogenous expression of RASSF1A modulates levels of cyclin D1 and induces cell cycle arrest in lung carcinoma cells [17]. RASSF1A inhibits lung cancer cell growth through reducing the phosphorylation of JNK [56] (Fig. 8.2).

8.5 Clinical Significance of RASSF1 in Lung Carcinoma

RASSF1 methylation in cancer may serve an important role in clinical utilities, especially in lung cancer. For example, the aberrant RASSF1A methylation may be an ideal biomarker for early diagnostic and prognostic due to the non-invasive, high sensitivity, and high

specificity characteristics. It is questionable whether RASSF1A methylation can be a powerful marker for patient prognosis at early stage of lung cancer. RASSF1A exhibited lung cancer-specific methylation pattern, with the hypermethylation level up to 100% in SCLC and 63% in NSCLC [57, 58]. RASSF1A methylation can be detected in body fluids including blood, urine, sputum, and bronchial alveolar lavages [56–59]. For example, RASSF1A methylation is observed in the blood of patients with NSCLC [59]. The RASSF1A methylation of bronchial aspirates was 21% in patients with lung cancer and smoking and 1% in patients with lung cancer alone, respectively. The methylation level of RASSF1A was associated with the number of cigarette packs and smoking years during the lifetime of patients with lung cancer [60]. The RASSF1 methylation of bronchial washings was found to have diagnostic sensitivity [61], which has the great potential to screen risk populations of patients with lung cancer. DNA methylation of RASSF1A is correlated with poor clinicopathological characteristics in nearly all solid tumors [62], which also includes lung cancer. RASSF1 promoter methylation was found in poorly differentiated tumors [63–65], associated with tumor grades, stages, and survival. For example, RASSF1A methylation was associated with patient survival time in lung adenocarcinoma [66]. Decreased survival time was observed in NSCLC patients with RASSF1A methylation, irrespective of whether patients have received adjuvant radio therapy or surgical treatment [58, 64, 67, 68]. On basis of those evidence, RASSF1 and isoforms as disease biomarkers should be furthermore evaluated, since disease biomarkers are expected to have the clear specificity for disease per se, disease stage, phase, severity, duration, or response to therapy [69–76]. Several natural compounds can regulate DNMT activity or expression to re-activate RASSF1A [77]. Peperomin E, as a natural bioactive secolignan polyphenol extracted from the plant peperomia dindygulensis, could demethylate RASSF1A and upregulate the expression of RASSF1A by reducing the level of DNMT1 in lung cancer cells [78].

8.6 Conclusion

Epigenetics changes especially DNA methylation has been proved to take part in the carcinogenesis of cancers. The DNA methylation of the tumor suppressor genes may be exploitable for the biologic and clinical significance of cancers. Overall, as the common tumor suppressor gene of lung cancer, evidence have suggested the DNA methylation of RASSF1 can be an essential potential clinic diagnostic or prognostic marker and may provide new therapeutic strategies for future successful treatment of lung cancer. It will be very interesting to further explore how to develop non-invasive, rapid and less cost detection methods for DNA methylation and to confirm the reliability and sensitivity of DNA methylation.

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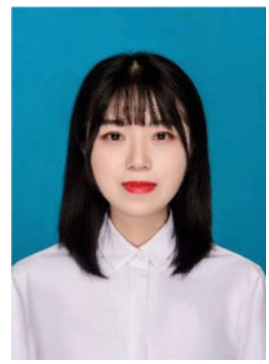
Tingting Zhang received her postgraduate degree in People's Hospital of Zhengzhou University. She has strong background of epigenetic modification of oocytes. Currently, Tingting Zhang is interested in next generation sequencing data analysis and interpretation, especially on single-cell analysis and cancer research. She has published three SCI papers in the past 3 years.



Yanjun Li did her Master's degree in Cell Biology at Zhengzhou University. Now, she works as a research assistant at Center for Clinical Single Cell Biomedicine in Henan Provincial People's Hospital. Her main research direction is focused on lung cancer. Currently, Yanjun Li is interested in the epigenetic alterations in lung cancer, especially in DNA methylation.

Hui Zhang is an editor-in-chief assistant at Henan Provincial People's Hospital and a postgraduate in Zhengzhou University. She has read a large number of scientific articles on topics such as single-cell sequencing, gene editing, molecular drug targeting, stem cells, immune therapy, and heterogeneity during her editing work. In addition, years of experience in clinical surgery and ethics committee gave her a deep understanding of medical practice ethics and scientific research ethics.

Xiangdong Wang is a Distinguished Professor of Medicine, Director of Shanghai Institute of Clinical Bioinformatics, Executive Director of Clinical Science Institute of Fudan University Zhongshan Hospital, Director of Fudan University Center of Clinical Bioinformatics, Deputy Director of Shanghai Respiratory Research Institute, and visiting professor of King's College of London. His main research is focused on clinical bioinformatics, disease-specific biomarkers, lung chronic diseases, cancer immunology, and molecular and cellular therapies. He is the author of more than 200 scientific publications with the impact factor about 900, citation number about 6918, h-index 46, i10-index 181, and cited journal impact factor about 7000.

Xiaozhuan Liu received her PhD on Epidemiology and Health Statistics at Zhengzhou University, and work as Researcher in the Center for Clinical Single Cell Biomedicine of Henan Provincial People's Hospital. She mainly engaged in the research of tumor pathogenesis. Currently, Xiaozhuan Liu is interested in the role of gene methylation in cancers.

Li Li is a Director of Department of Science Research and Discipline Construction, Henan Provincial people's Hospital. She is Member of clinical research group of Chinese Medical Association's Scientific Research Management Branch, Standing Committee member of Chinese Medical Association's Henan Research and Management Branch, and Vice-chairman of Henan Discipline Management Branch of Chinese Hospital Management Society. She has engaged in the management of medical scientific research for 30 years and her main research is focused on health management scientific research big data, laboratory biosafety, and medical ethics. She published more than 20 scientific papers.