The Role of Methylation in the CpG Island of the ARHI Promoter Region in Cancers

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Abstract

Hypermethylation can downregulate many tumor suppressor gene expressions. Aplasia Ras homologue member I (ARHI, DIRAS3) is one of the maternally imprinted tumor suppressors in the RAS superfamily. This chapter overviewed the importance of ARHI methylation and expression phenomes in various types of cancers, although the exact mechanisms remain unclear. As an imprinted gene, aberrant DNA methylation of the paternal allele of ARHI was identified as a primary inhibitor of ARHI expression. The role of methylation in the CpG islands of the ARHI promoter region vary among ovarian cancers, breast cancers, hepatocellular carcinoma, colon cancers, pancreatic cancer osteosarcoma, glial tumors, follicular thyroid carcinoma, or lung cancers. The methylation of ARHI provides a new insight to understand molecular mechanisms of tumorigenesis and progression of cancers.

Keywords

ARHI · Methylation · Cancer · Disease · Inhibitor

10.1 Introduction

Aplasia Ras homologue member I (ARHI, DIRAS3) is the first tumor suppressor gene identified in the Ras superfamily [1] and allocated in chromosome 1p31 where there is loss of heterozygosity. ARHI has a distinctive N terminal extension for the suppression of tumor growth and is one of 40 genes to be imprinted in the human genome. ARHI is expressed in cells from the paternal allele during the process of embryonic development [1]. The protein coding region is located within exon 2 and encodes a 229-residue small GTP binding protein belonging

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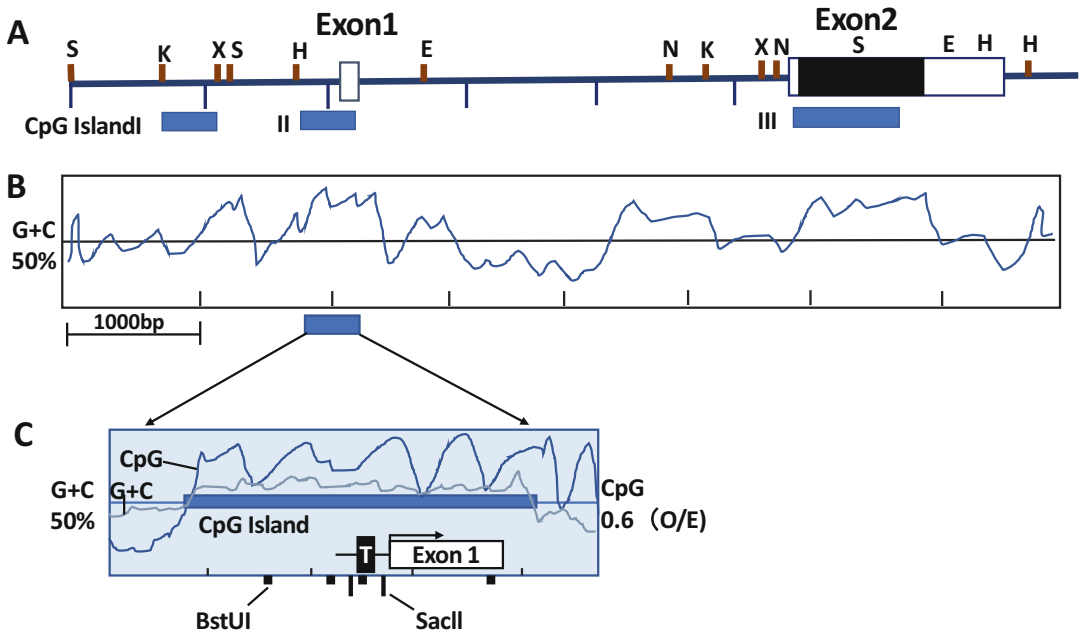

Fig. 10.1 Structure of the human ARHI gene. (**a**) Structural organization of the ARHI gene and schematic drawing of the ARHI cDNA. The ARHI gene contains two exons interrupted by large intron. The blocked and opened boxes represent the coding and noncoding regions. Shadowed boxes are CpG island regions. Restriction enzyme sites are designated as: *S* SmaI, *K* KpnI, *X* XbaI, *H* HindIII, *E* EcorI, *N* NcoI. (**b**) The GC content per 100 bp across the entire ARHI locus. (**c**) The G + C content per 100 bp and CpG density per 100 bp for the CpG island II spanning the region upstream of and encompassing the entire exon1

to the Ras superfamily [2]. Three potential CpG islands about 300 base pairs were found in the promoter and exons of the ARHI gene (Fig. 10.1). CpG island I, II, and III are located about 1 kb upstream of the transcription initiation site, and in the region of exon 2, respectively (Fig. 10.1b). Of those, CpG island II spans the 5′-up-stream region of ARHI, including the transcription initiation site and a portion of exon 1 (Fig. 10.1b) [3].

The imprinted gene ARHI undergoes the dysfunction with a "single hit" during carcinogenesis by inactivating single functional allele [4]. ARHI silencing in cancers can be caused by multiple mechanisms, including LOH, DNA methylated, histone deacetylation, histone methylation, and transcriptional regulation. The acetylation and methylation of chromatin lead to the downregulation of ARHI expression and ability to suppress tumor growth [4]. The histone deacetylation and H3 lysine 9 methylation contribute to the silence of ARHI by DNA methylation-dependent pathway (Fig. 10.2) and the binding of transcriptional repressors to recruit relevant enzymes onto chromatin (Fig. 10.3). Human oncogenesis may be due to the change of DNA methylation. About 50% of human genes have clusters of CpG islands in the 5′-regulatory sequences, of which the most are not methylated. In human cancers, the aberrant methylation includes hypomethylation, hypermethylation, and increased DNA methyltransferase activity [5, 6].

Aberrant methylation of CpG islands acts as a distinct molecular mechanism, leading to malignant transformation and providing the epigenetic equivalent of mutation/deletion during oncogenesis [7, 8]. Such DNA methylation is also recognized as potential driver of carcinogenesis [9]. CpG methylation lead to gene transcription declining in the promoter region in ARHI genes [10]. The downregulation of ARHI is found in many types of cancer, including ovarian cancer, hepatocellular carcinoma, and others [11]. This

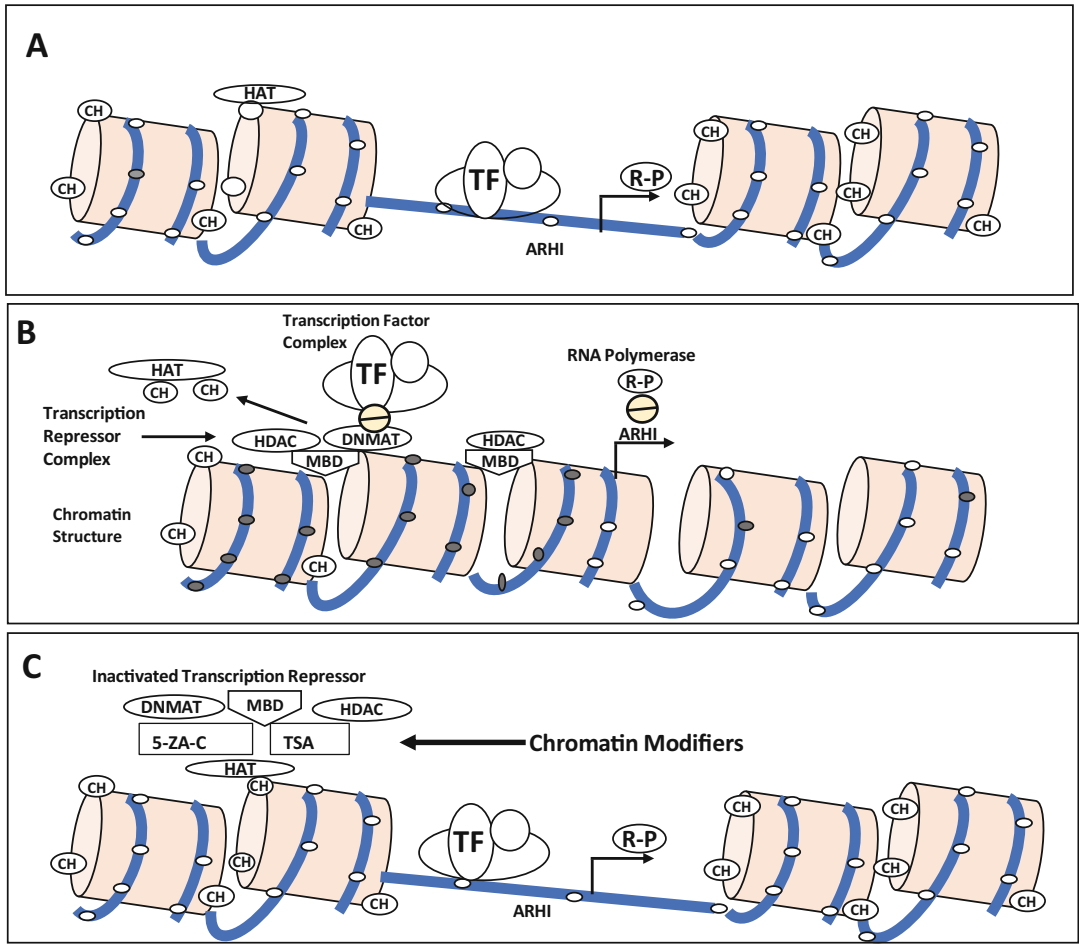

Fig. 10.2 Methylation-dependent model for the silencing of the ARHI gene in cancer cell. (**a**) A transcriptionally active CpG island promoter is depicted with positioned nucleosomes, consisting of acetylated (CH) histone and unmethylated CpG residue (white circle). Histone acetyltransferase (HAT) creates an accessible chromatin configuration that facilitates transcriptional activity. (**b**) Silenced ARHI gene. Transcriptional repressor complex including methyl-CpG binding domain (MBD) protein, DNA methyltransferase (DNMT), histone deacetylase (HDAC), and other repressors binds to methylated CpG (gray circles) and inactivates the ARHI gene. (**c**) Chemical inhibitors such as 5-AZA and TSA can inhibit DNMT and reactivate the ARHI gene

chapter aims at overviewing the correlation between ARHI CpG methylation and the tumor in the development of cancer.

10.2 ARHI and Ovarian Cancer

Of malignancies, the highest expression ARHI is expressed in ovarian tissues [1]. The ARHI expression was downregulated in ovarian tumor tissues, as compared with the normal ovarian tissues [12, 13]. The ARHI expression was reduced in ovarian serous papillary carcinomas [14] and ARHI protein consistently expressed in epithelial cells of ovarian surface [4]. The levels of ARHI expression were correlated with the malignancy of tumors [14], of which ARHI was reduced in 88% of ovarian cancer tissues. The overexpression of ARHI can inhibit the proliferation of ovarian tumor cells and induced autophagy and tumor dormancy and other phenomena [15].

The ARHI expression is regulated by CpG island methylation in the ARHI promoter region

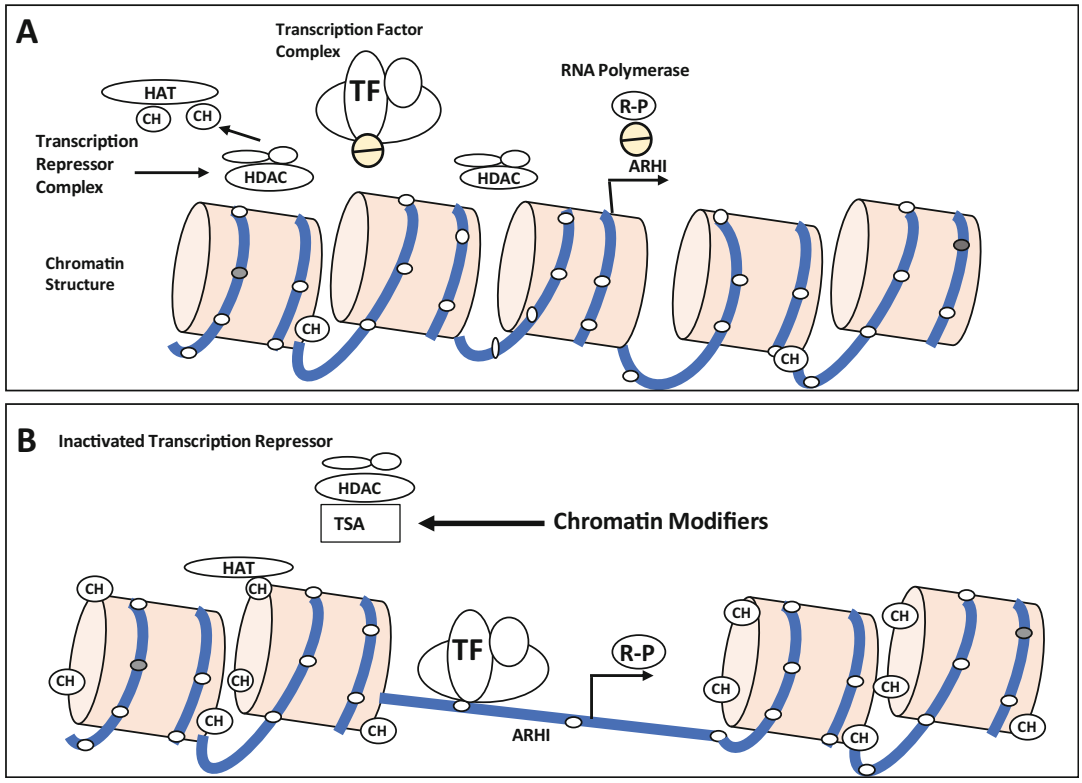

Fig. 10.3 Methylation-independ model for silencing the ARHI gene in cancer. (**a**) Silence ARHI gene. Transcriptional repressor complex, including histone deacetylase (HDAC) and other transcription repressors, inhibits HAT and inactivates the ARHI gene. (**b**) TSA can inhibit HDAC and reactive the ARHI gene

and other way. ARHI CpG islands I and II were hypermethylated in 31% and 12% of ovarian cancers, respectively, associated with reduced ARHI expression [16]. ARHI expression reduced in ovarian cancer epithelial and modified cancer cells (SKOV-3 and HO-8910), where CpG islands I and II were partially methylated or hypermethylated, enhancing the proliferation of tumor cells. Such proliferation was reversed by the administration of 5-aza-2′-deoxycytidine [17].

10.3 ARHI and Breast Cancer

ARHI expression is lost or downregulated in most breast cancers, while the ARHI overexpression inhibits the growth of tumor cells and induces the apoptosis of tumor cells [18]. Transcriptional repression of ARHI is closely related to breast cancer progression [19]. The expressions of ARHI were detected in normal breast epithelia, downregulated in 41% of ductal carcinoma in situ (DCIS) and 70% of invasive carcinomas [20]. Compared with DCIS in the same sample, ARHI was further downregulated in 26% of invasive carcinomas. About 17% of invasive carcinoma lost ARHI protein expression. Other investigators reported that ARHI mRNA expression decreased in 46–48% of human breast cancer specimens [20, 21], correlated with lymph node metastases [21] and involved with the progression of breast tumor.

ARHI expression can be downregulated by various mechanisms. For example, hypermethylation of both alleles in the CpG island II of the ARHI promoter region was closely correlated with silencing of ARHI expression in 10–15% of patients with breast cancers [4, 10]. Aberrant methylation was accompanied with decreased ARHI expression in breast cancer

cells. Hypermethylation was detected at CpG island I of 67% breast cancer cells, 33% at CpG island II, and 56% at CpG island III, while hypomethylation at CpG island II of 44% breast cancer cells. Treatment with 5-aza-2deoxycytidine, a methyltransferase inhibitor, can demethylate and partially restore ARHI expression with hypermethylation of CpG islands [10]. ARHI expression was partially upregulated in cells with hypermethylation of CpG islands. CpG islands methylation was studied in 20 human tissues. On the other hand, no hypermethylation was found in CpG island I of surgical specimen, 15% hypermethylation in CpG island II, and 20% in CpG island III [10]. During imprinting, CpG islands are consistently methylated and silenced in the maternal allele of normal cells, whereas not in paternal alleles. CpG island II hypermethylation of both alleles completely eliminated ARHI promoter activity. The degree of ARHI methylation is related to the survival of patients [22], which provides a new mechanism for the breast tumors [10].

10.4 ARHI and Colon Cancer

ARHI expression was also downregulated in colon cancer cells, while overexpression could reduce the number of invaded cells and the adhesive ability [23] and promote colon cancer cell apoptosis [24]. ARHI expression was downregulated in 62% of colon cancer specimens, associated with worse differentiation degree and Dukes' stage. Methylation-specific PCR assay revealed that the methylation rates of ARHI were 53% and 47% in CpG Island I and CpG Island II, respectively. The promoter methylation may downregulate ARHI expression in colon cancer, which can be a therapeutic potential for the disease [25].

10.5 ARHI and Hepatocellular Carcinoma (HCC)

ARHI gene expression was found to be related to hepatocellular carcinoma, evidenced by the fact that ARHI expression was downregulated in 78.6% HCC specimens, accompanied by reduced levels of ARHI protein [26]. The overexpression of ARHI inhibited HCC growth and colony formation, while the silencing of endogenous ARHI promoted cell growth [26]. Upregulated ARHI expression inhibited tumor growth and angiogenesis in hepatocellular carcinoma, which were prevented by 5-aza-20-deoxycytidine [27, 28]. ARHI hypermethylation occurred in 47% of patients with HCC without ARHI expression. The downregulated expression of ARHI in HCCs acts as a tumor suppressor role, which was mainly stimulated by the epigenetic modification in HCC [26].

10.6 ARHI and Pancreatic Cancer

Overexpression of ARHI can inhibit the cell cycle and apoptosis in pancreatic tumor cells [29]. Compared with normal pancreatic tissues, ARHI is downregulated in approximately 50% in pancreatic cancer tissues. The immediate reason for this downregulation or loss of ARHI expression in pancreatic cancer cells was due to the aberrant methylation of ARHI locus. Hypermethylation was detected at CpG island I of 100% pancreatic cancer cells, at CpG island II of 40%, and at CpG island III of 80%, respectively. The growth of pancreatic cancer cells can be suppressed by the overexpression of ARHI which is involved with the apoptosis of cancer cells. The upregulation of ARHI mRNA expression induced by the demethylation of ARHI can obviously inhibit cell growth and increase apoptosis in human pancreatic cancer cells. It was evidenced that ARHI serves as a gene that inhibits growth in pancreatic cancers [30].

10.7 ARHI and Osteosarcoma (OS)

ARHI protein and RNA levels were downregulated in OS cells [31]. The knockdown of ARHI could promote OS cell proliferation and attenuate apoptosis. Zebularine may upregulate the tumor suppressor genes through a

demethylation function, which inhibits the growth and promotes apoptosis in OS cells. The ARHI expression was upregulated by Zebularine due to the downregulation of ARHI methylation and the function of DNA methyltransferase 1 (DNMT1) and histone methyltransferase G9a. The distinct reduction of ARHI methylation can be induced by knockdown of DNMT1 or G9a. Zebularine may directly repress DNMT1 alone, while G9a through regulating DNMT1 function on ARHI methylation, which were restored by knockdown of ARHI [32].

10.8 ARHI and Glial Tumors

Experimental studies demonstrated that expression of ARHI was downregulated in human glioma tumors as compared with normal brain tissue as well as four different glioma cells [33]. The proliferation and invasion of glioma cell can be suppressed by up-expression of ARHI [33]. The expression and methylation status of ARHI were evaluated in tissue and peripheral blood [34]. The expression of ARHI RNA increased in 67% of patients with glial tumor and decreased in 33% [34]. Methylation of the CpG island at ARHI was detected using the combined bisulfite restriction analysis and the restriction fragment length polymorphism in glial tumors as compared with hypermethylated healthy volunteers. Hypermethylation was detected at CpG island I in two glial tumors, indicating that the progression of glial tumor may be due to the downregulation of ARHI [34].

ARHI can be influenced by a large number of genetic events and epigenetic mechanisms [3, 22, 35, 36], while ARHI expression may be firstly silenced by the aberrant DNA methylation of ARHI, varying among cell types [10].

10.9 ARHI and Follicular Thyroid Carcinoma (FTC)

The global gene expression analysis showed that ARHI expression was low in **FTC**. Studies revealed that a complete methylation pattern was exist in ARHI in **FTC** shows [37]. The silencing of ARHI, primarily by large genomic deletion is involved with hypermethylation of the genomically imprinted allele, which may be an important early event in FTC [37].

10.10 ARHI and Lung Cancer

Studies demonstrated that overexpression of ARHI gene can inhibit the growth, proliferation and invasion of lung cancer cells, and promote the apoptosis of lung cancer cells [38]. Aberrant DNA methylation was observed in non-small cell lung cancers. The methylation status of 245 CpG positions in 59 candidate genes was examined in different types of lung cancer and normal adjacent lung tissues from smokers, which found that the DNA-methylation levels were different among different histological types of tumor tissues and normal adjacent tissue [39]. The highest degree of DNA methylations in squamous cell carcinoma was observed in ARHI, GP1Bbeta, RAR beta genes, etc. It was proposed that methylation profiles of specific genes may be used to distinguish histological types of lung cancer [39].

10.11 Conclusion and Perspectives

This chapter overviewed the importance of ARHI methylation and expression phenomes in various types of cancers, although the exact mechanisms remain unclear. As an imprinted gene, aberrant DNA methylation of the paternal allele of ARHI was identified as a primary inhibitor of ARHI expression. The role of methylation in the CpG islands of the ARHI promoter region vary among ovarian cancers, breast cancers, hepatocellular carcinoma, colon cancers, pancreatic cancer osteosarcoma, glial tumors, follicular thyroid carcinoma, or lung cancers. The methylation of ARHI provides a new insight to understand molecular mechanisms of tumorigenesis and progression of cancers.

There are further needs to explore whether ARHI methylation and expression can be defined

as disease-specific biomarkers with the specificity of disease duration, severity, stage, phase, phenome, and response to therapy as requested [40–46]. It is questioned whether the heterogeneity of ARHI methylations exists among cells of the same cancer. The single-cell sequencing was widely applied for the identification of the intra- and inter-heterogeneity among cancer locations, types, and durations within the cancer [47, 48]. Dynamic three-dimensional chromatin conformation and the potential association between cell-type specific chromatin conformation and differential DNA methylations should be considered in the understanding of ARHI methylation, since altered 3D genome controls gene regulation during development and disease [49–51]. Roles of ARHI methylation and expression in the development and diseases are furthermore specifically clarified by gene editing technologies, e.g., CRISPR [52–55]. Thus, we believe that the deep understanding of ARHI methylation and expression will provide new opportunities for future diagnosis and therapy.

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