

Identifying a Novel Pathogenic Mutation in Exon 3 of ARID1A Gene in Colon Cancer

Mehran Erfani*, Pooneh Mokarram**†, Maral Mokhtari**, Jafar Fallahi***

*Department of Biochemistry, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

**Autophagy Research Center and Department of Pathology, Shiraz University of Medical Sciences, Shiraz, Iran

***Molecular Medicine Department, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract

ARID1A, a bona fide tumor suppressor, is the third most mutated gene in human colorectal cancer. In this paper, we reported the identification of a novel mutation in one of 20 primary human colorectal cancer tumors. Sanger sequencing of the exon 3 of the ARID1A gene revealed a novel heterozygous non-sense mutation of the c.1653C>G. This resulted in the premature termination of the 2285 amino acid protein at the 551st codon. Mutation Taster predicted this novel mutation to be pathogenic. Immunohistochemical analysis revealed the loss of ARID1A protein expression in cancerous tissues harboring ARID1A mutation. As far as we know, this is the first report on the mutation of ARID1A gene in an Iranian patient. This mutation can expand the spectrum of ARID1A gene pathogenic mutations among colorectal cancer patients.

Keywords: ARID1A, Colon cancer, Novel mutation

Introduction

The AT-rich interactive domain 1A (ARID1A) (also known as BAF250) is located on chromosome 1p. It is widely expressed and is present in the nucleus.¹ The protein encoded by ARID1A is a key subunit of the chromatin remodeling complex called SWItch/Sucrose Non-Fermentable (SWI/SNF). This complex employs integral helicase activities to regulate certain gene transcriptions through changing the

structure of chromatin around these genes.² ARID1A is deemed to give specificity to the SWI/SNF complex and might recruit the complex to its target genes through either protein-protein or protein-DNA interactions. Therefore, ARID1A seems to be involved in regulating a variety of cellular processes, including development, differentiation, and DNA repair.³ ARID1A has recently been identified as a bona fide tumor suppressor in colorectal cancer and

***Corresponding Author:**

Pooneh Mokarram, PhD
Department of Biochemistry,
School of Medicine, Shiraz
University of Medical Sciences,
Shiraz, Iran
Tel/Fax: +98 7112303029
Email: mokaram2@gmail.com;
mokaramp@sums.ac.ir

various cancer types. ARID1A has one of the highest mutation rates across multiple cancer types with the most frequency reported in ovarian clear-cell carcinomas (46–57%).⁴ A relatively high ARID1A mutation rate was reported in the colorectal cancer (10–40%).^{1,3,5,6} ARID1A gene is the third most significantly mutated gene in colorectal cancer in humans with the most prevalence observed in MSI type cancers (~39%).^{7,8} Low ARID1A expression is associated with more aggressive colorectal cancer phenotypes.⁹ These findings underscore the significance of ARID1A expression in colorectal carcinoma, suggesting that ARID1A expression loss is a significant driver event in an important proportion of colorectal carcinoma. Accordingly, as a potential therapeutic and diagnostic marker in colorectal carcinoma, it warrants further investigation.

Multiple genes and diverse molecular mechanisms are involved in the changes in the function of genes regarding colorectal cancer transformation over different populations.^{10–13} Recently, mutation of multiple genes in the

colorectal cancer has been reported amongst African-Iranian patients, showing that MSH3, MSH6, APC, BRAF, and PIK3CA mutations are superior in Iranian patients.¹⁴ However, despite the importance of ARID1A expression in colorectal cancer and other cancer types, there is no research on the prevalence of ARID1A mutation in this population. Therefore, the current study, aimed to identify the possible mutations of ARID1A gene amongst Iranian patients with colorectal cancer for the first time. Previous studies showed the high frequency of mutation in exon 3 of ARID1A gene in several tumor types.³ Therefore, we analyzed exon 3 of ARID1A gene for the presence of possible mutations in colorectal cancer patients. In this paper, we reported a novel mutation of ARID1A gene in an Iranian patient with colon carcinoma associated with definite cancer risk. In addition, immunohistochemistry examined the impact of this mutation on ARID1A expression changes.

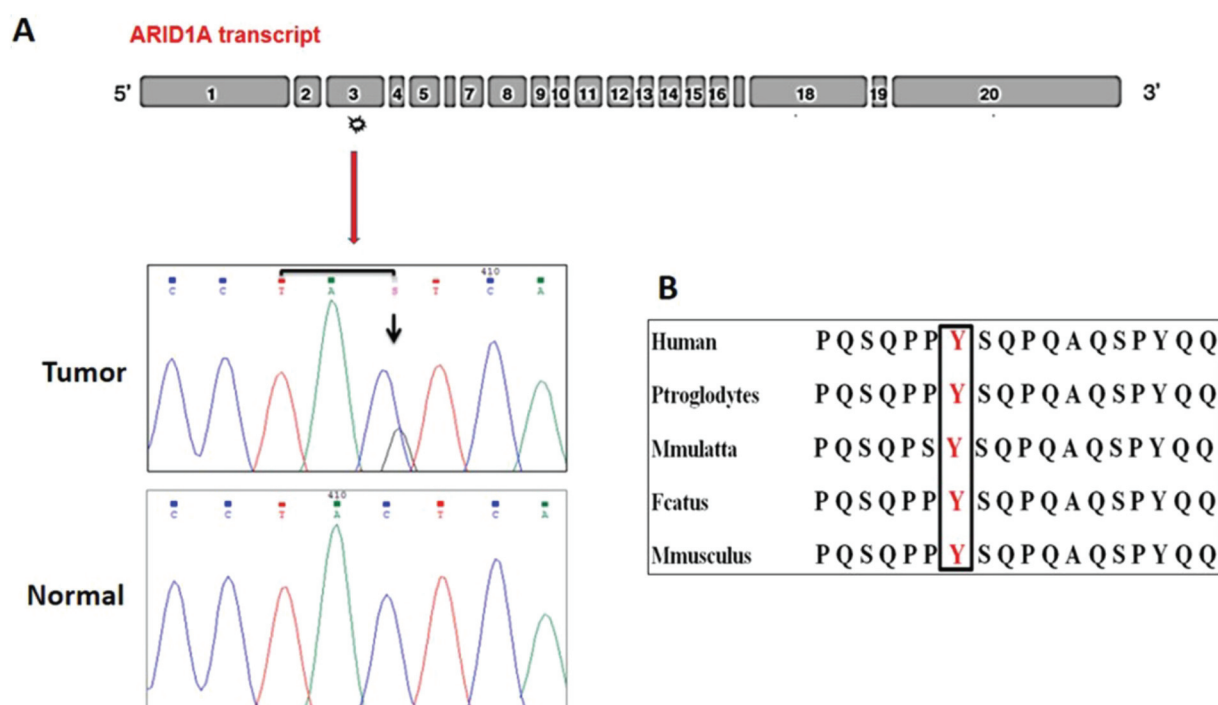


Figure 1. Direct DNA sequence analysis of exon 3 of ARID1A gene and conservation analysis of ARID1A. (A) Sequence chromatogram shows the identified mutation and its wild type. Direct DNA sequencing revealed a novel heterozygous nonsense mutation (c.1653C>G) in a patient with colon cancer. (B) The amino acid mutated in the affected individual are conserved in different species.

Materials and Methods

Faghihi Hospital (Affiliated to Shiraz University of Medical Sciences) provided 20 colorectal cancer tumor tissue samples with their matched non-tumor adjacent tissues. This was done after the patients provided a written informed consent in accordance with the declaration of Helsinki. Afterwards, we extracted genomic DNA specimens from the tissues according to the standard phenol/chloroform method. By sequencing the exon 3 of the ARID1A gene, we further evaluated the tissues to identify possible mutations. PCR amplified the exon 3 of the ARID1A gene with the forward primer (5'- ACC-CTGGGCCTCCTAAGTATG -3'), reverse primer (5'-TGCACGTTAGAGAACCACTCTG -3'), and PCR cycling conditions previously described in detail.¹⁵ Sanger sequencing of PCR products was performed using the forward primer of PCR reaction. To ensure the accuracy of sequence results, the Sanger sequencing and all the molecular tests were repeated twice for samples.

Results

We evaluated the results of Sanger sequencing using the chromas software (Technelysium Pty Ltd) and compared them with the reference

sequence in NCBI (NG_029965.1). A novel mutation occurred in colon cancer tissue from a patient. As illustrated in figure 1A, Sanger sequencing revealed that a transversion mutation in ARID1A gene caused the formation of a stop codon. This mutation substituted cytosine 1653 in guanine (c.1653C>G) and converted TAC to TAG (TAC>TAG). Mutation Taster predicted the c.1653C>G mutation to be pathogenic and disease-causing (<http://www.mutationtaster.org>). This mutation was in heterozygous state (Figure. 1A) and the affected allele had an early stop codon at the site 551 (p.Tyr551X). Multiple sequence alignment showed that tyrosine in codon 551 was conserved in various species by Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) (Figure 1B). With this stop codon, a truncated protein with disrupted function was generated. We observed that this novel mutation was somatic because the sequencing result pertaining to the extracted DNA of non-tumorous tissue, as the control sample, showed the nucleotide sequence of the wild type of ARID1A gene.

Discussion

The type and status of our novel mutation is

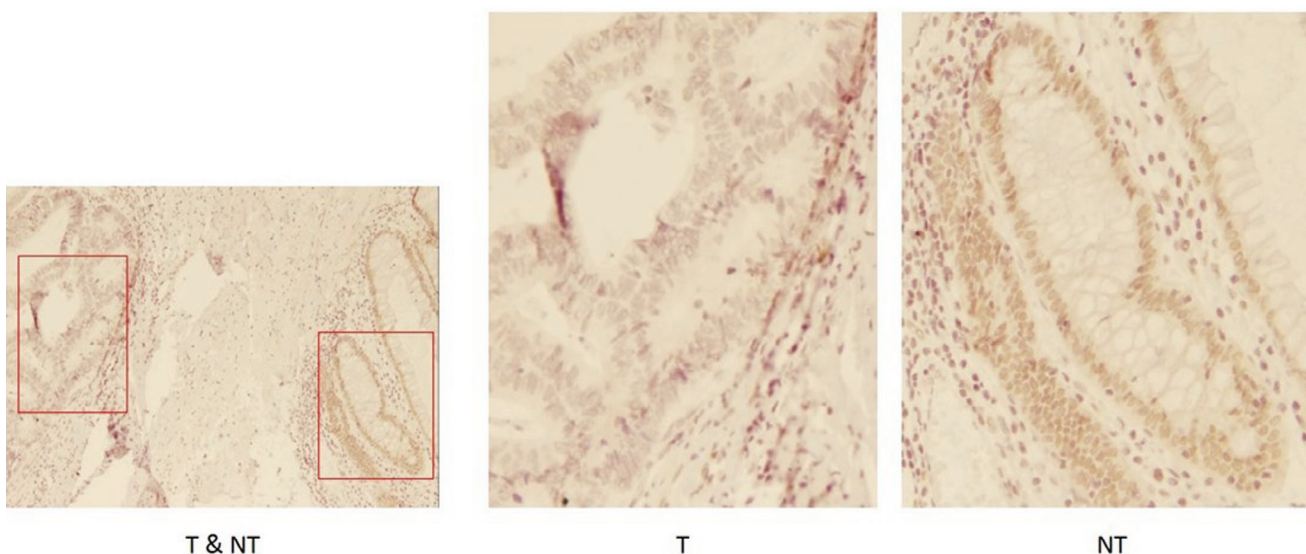


Figure 2. Immunohistochemistry of ARID1A protein expression in colon cancer surgical specimen. Positive ARID1A nuclear staining in non-tumorous tissue (NT) is presented in the right panel while negative staining of ARID1A in colon cancer (T) is shown in the middle panel. Red boxes in the left panel indicate the area enlarged in the middle and right panels.

in line with previous studies where the preponderance of ARID1A mutations in cancer (>97%) were frame-shift or non-sense (rather than missense or silent) mutations resulting in mRNA decay, hence protein loss.^{1,3} In addition, the previous studies reported that the majority of ARID1A mutations were heterozygous.¹ It was previously noted that our novel mutation was in heterozygous state. We found this mutation in a patient with stage I colon cancer. This finding showed that this mutation could occur in the early stages of tumor development. Table 1 shows the patients' sex, age at diagnosis, tumor size, location, and histology and stage of colon cancer with c.1653C>G mutation of ARID1A. Comparison with other clinical specimens revealed no clinical diagnostic features or stages unique to this mutation.

To investigate the impact of this novel mutation on ARID1A expression changes, we performed immunohistochemical analysis on ARID1A protein expression on 4 µm thick sections of paraffin-embedded cancerous tissue samples; these samples harbored ARID1A mutation and matched adjacent non-tumorous tissue samples from the patient as previously described.⁹ The immunohistochemical analysis of ARID1A revealed positive nuclear staining of ARID1A in adjacent non-tumorous tissues and loss of ARID1A protein expression in cancerous tissues (Figure 2). These observations showed that there had been an ARID1A expression loss during the development of colorectal cancer. Given the critical role of ARID1A expression loss in colorectal cancer tumorigenesis through numerous mechanisms such as loss of normal cell cycle arrest and apoptosis,⁸ our novel mutation is likely to possess high oncogenic activity. Also, it might potentially contribute to the formation and progression of colon cancer in this patient.

An interesting finding in the present research was the complete loss of ARID1A protein expression in cancerous tissues harboring ARID1A heterozygous mutation. This is not surprising because previous studies showed that in certain cancer types, 75% of tumors with heterozygous mutations of the ARID1A gene

Table 1. Clinicopathological features of colon cancer patient with c.1653C>G mutation of ARID1A

| Characteristics | |
|----------------------------|---------------------|
| Age | 57 |
| Sex | Male |
| Tumor location | Colon |
| Tumor size | 4×3.5 cm |
| Histological type | Adenocarcinoma |
| Pathologic differentiation | Well differentiated |
| TNM stage (AJCC) | I |
| Distant metastasis | Absent |

TNM: Tumor-node metastasis; AJCC: American Joint Committee on Cancer

lacked protein expression and no mutations occurred in ARID1A coding sequence in the wild-type allele.¹ Several possible mechanisms can be postulated to account for this interesting observation. One group stated that epigenetic silencing might be a contributing factor. Some studies have hypothesized that post-transcriptional and/or post-translational mechanisms account for ARID1A protein loss in cancerous tissues harboring heterozygous mutations.¹

As far as we know, this mutation has never been reported in the literature or recorded in mutation databases. Therefore, we submitted the c.1653C>G mutation in the NCBI database where it received the ClinVar accession no. SCV000743092 (<https://www.ncbi.nlm.nih.gov/clinvar/variation/523633/>). This novel mutation can broaden the pathological mutation spectrum of the ARID1A gene, form the foundation of genetic counseling, and augment the role of ARID1A as a tumor suppressor. Further studies are required to delineate the exact effect of this novel mutation on downstream signals and, ultimately, colon tumorigenesis.

As far as limitations are concerned, DNA sequencing was not carried out on the entire length of the gene. Moreover, the mutation frequency of ARID1A gene in colorectal cancer patients needs to be further investigated over a larger population as well as on the entire length of the gene.

Acknowledgment

This article was extracted from a part of the PhD dissertation written by Mehran Erfani (grants

No, 94-9136) and was funded by Shiraz University of Medical Sciences. The authors wish to thank Mr. H. Argasi at the Research Consultation Center (RCC) of Shiraz University of Medical Sciences for his invaluable assistance in editing this manuscript.

Conflict of Interest

None declared.

References

1. Wu JN, Roberts CW. ARID1A mutations in cancer: another epigenetic tumor suppressor? *Cancer Discov.* 2013;3(1):35-43. doi: 10.1158/2159-8290.CD-12-0361.
2. Masliah-Planchon J, Bièche I, Guinebretière JM, Bourdeaut F, Delattre O. SWI/SNF chromatin remodeling and human malignancies. *Annu Rev Pathol.* 2015;10:145-71. doi: 10.1146/annurev-pathol-012414-040445.
3. Jones S, Li M, Parsons DW, Zhang X, Wesseling J, Kristel P, et al. Somatic mutations in the chromatin remodeling gene ARID1A occur in several tumor types. *Hum Mutat.* 2012;33(1):100-3. doi: 10.1002/humu.21633.
4. Guan B, Mao TL, Panuganti PK, Kuhn E, Kurman RJ, Maeda D, et al. Mutation and loss of expression of ARID1A in uterine low-grade endometrioid carcinoma. *Am J Surg Pathol.* 2011;35(5):625-32. doi: 10.1097/PAS.0b013e318212782a.
5. Lee SY, Kim DW, Lee HS, Ihn MH, Oh HK, Park DJ, et al. Loss of AT-rich interactive domain 1A expression in gastrointestinal malignancies. *Oncology.* 2015;88(4):234-40. doi: 10.1159/000369140.
6. Yan HB, Wang XF, Zhang Q, Tang ZQ, Jiang YH, Fan HZ, et al. Reduced expression of the chromatin remodeling gene ARID1A enhances gastric cancer cell migration and invasion via downregulation of E-cadherin transcription. *Carcinogenesis.* 2014;35(4):867-76. doi: 10.1093/carcin/bgt398.
7. Testa U, Pelosi E, Castelli G. Colorectal cancer: genetic abnormalities, tumor progression, tumor heterogeneity, clonal evolution and tumor-initiating cells. *Med Sci (Basel).* 2018;6(2). pii: E31. doi: 10.3390/medsci6020031.
8. Mathur R, Alver BH, San Roman AK, Wilson BG, Wang X, Agoston AT, et al. ARID1A loss impairs enhancer-mediated gene regulation and drives colon cancer in mice. *Nat Genet.* 2017;49(2):296-302. doi: 10.1038/ng.3744..
9. Wei XL, Wang DS, Xi SY, Wu WJ, Chen DL, Zeng ZL, et al. Clinicopathologic and prognostic relevance of ARID1A protein loss in colorectal cancer. *World J Gastroenterol.* 2014;20(48):18404-12. doi: 10.3748/wjg.v20.i48.18404.
10. Sjöblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, et al. The consensus coding sequences of human breast and colorectal cancers. *Science.* 2006;314(5797):268-74.
12. Brim H, Mokarram P, Naghibalhossaini F, Saberi-Firoozi M, Al-Mandhari M, Al-Mawaly K, et al. Impact of BRAF, MLH1 on the incidence of microsatellite instability high colorectal cancer in populations based study. *Mol Cancer.* 2008 21;7:68. doi: 10.1186/1476-4598-7-68.
13. Naghibalhossaini F, Hosseini HM, Mokarram P, Zamani M. High frequency of genes' promoter methylation, but lack of BRAF V600E mutation among Iranian colorectal cancer patients. *Pathol Oncol Res.* 2011;17(4):819-25. doi: 10.1007/s12253-011-9388-5.
14. Ashktorab H, Mokarram P, Azimi H, Olumi H, Varma S, Nickerson ML, et al. Targeted exome sequencing reveals distinct pathogenic variants in Iranians with colorectal cancer. *Oncotarget.* 2017;8(5):7852-66. doi: 10.18632/oncotarget.13977.
15. Zhang X, Sun Q, Shan M, Niu M, Liu T, Xia B, et al. Promoter hypermethylation of ARID1A gene is responsible for its low mRNA expression in many invasive breast cancers. *PLoS One.* 2013;8(1):e53931. doi: 10.1371/journal.pone.0053931.