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Single nucleotide polymorphism at codon 133 of the *RASSF1* gene is preferentially associated with human lung adenocarcinoma risk

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Abstract

The *RASSF1* gene, a putative tumor suppressor gene located on human chromosome 3p21, garners much attention for the frequent allelic loss and gene silencing via promoter hypermethylation in a variety of human malignancies. An association between a single nucleotide polymorphism (SNP) at codon 133 of the *RASSF1* gene, encoding either alanine (GCT) or serine (TCT), and human cancer risk remains undefined. We therefore, investigated the distribution of the Ala133Ser SNP in 101 patients with lung cancer, 63 with head and neck cancer, 72 with colorectal cancer, 56 with esophageal cancer and 110 healthy controls by polymerase chain reaction and restriction enzyme-digestion assay. The heterozygous *Ala/Ser* genotype was significantly more frequent in lung cancer patients than in healthy controls ($P=0.028$). The adjusted odds ratio (OR) for the patients with heterozygous *Ala/Ser* genotype as compared with the controls with the *Ala/Ala* genotype was 2.59 (95% confidence interval (CI); 1.11–6.04). The increased risk of the *Ala/Ser* genotype was found in lung cancer patients but not in other cancer patients we examined. The association was particularly strong in those lung cancer patients of male (adjusted OR; 3.33, 95% CI; 1.37–8.12), with adenocarinoma (adjusted OR; 3.33, 95% CI; 1.36–8.15), early stages (adjusted OR; 3.42, 95% CI; 1.33–8.75) and with smoking habit (adjusted OR; 2.70, 95% CI; 1.06–6.83). These results suggest that the RASSF1 *Ala133Ser* SNP is associated with development of lung cancer, especially of lung adenocarcinoma. The increased risk of the heterozygous genotype is intriguing, implying a close relation with the dimerization feature of RASSF1 proteins.

Keywords: SNP; RASSF1; Tumor suppressor gene (TSG); Non-small cell lung cancer (NSCLC); Cancer-predisposition

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1. Introduction

The short arm of human chromosome 3 is known to show a high prevalence of loss of heterozygosity (LOH) in a wide variety of human malignancies [1–8]. A particular region, 3p21.3, is the most prominent loss region and has been thought that this region may contain universal tumor suppressor genes (TSG). Eight putative TSGs have so far been identified in the narrow region of 3p21.3 [2,6,8,9]. The Ras association domain family protein 1-gene (*RASSF1*) is one of them, locating at 3p21.3 within a homozygous deletion region of 120 kb that frequently demonstrates LOH in both lung and breast cancers, and was found to be inactivated via promoter-hypermethylation in lung cancer [9]. Since then, many reports have described that the *RASSF1* gene is silenced by DNA methylation in over 50% of all solid tumors of different histological types ([10–17]; for review; [10,11]) as well as two subsets of hematological tumors [18,19]. Thus, RASSF1 has been established as a novel Ras-binding protein with tumor suppressor properties [9,13,14]. However, the function and its molecular mechanism have not been fully clarified. Just only a few facts have already been elucidated (for review; Ref. [20]). An early report described that RASSF1 is implicated in the RAS-induced apoptosis [21]. More recently, it was found that RASSF1 contributes to the cell cycle regulation [22] and its major isoform, RASSF1A protein, functions through forming homodimer and heterodimer with RASSF5 known as NORE1 [23]. The activity of RASSF1A is phosphorylation-dependent [22]. As for the transcripts of the *RASSF1* gene, at least three isoforms are generated due to the alternative splicing and differential promoter usage (Fig. 1A) [9,12,17,18]. RASSF1A and RASSF1C are two major protein products. RASSF1A, the longest isoform, is predicted as a 39-kd polypeptide that contains two putative functional domains including a diacylglycerol (DAG)-binding domain in the NH_2 terminus (Fig. 1B) and a Ras-association domain in the COOH terminus [9,12]. The amino-terminal domain (amino acid residues 1-119) is present exclusively in the RASSF1A isoform, and is not in the RASSF1C splicing variant. Ortiz-Vega et al. [23] reported that RASSF1A is able to dimerize, whereas RASSF1C has very much weaker or no ability to dimerize. Hence, the DAG-binding domain at the NH_2 terminus has crucial roles for dimerization.

A single nucleotide polymorphism (SNP) within the *RASSF1* gene at codon 133, encoding either alanine (Ala: GCT) or serine (Ser: TCT), has been already identified [12,13,15,16,22]. However, there has been no study done on the correlation between the *RASSF1* SNP, Ala133Ser, and predisposition to

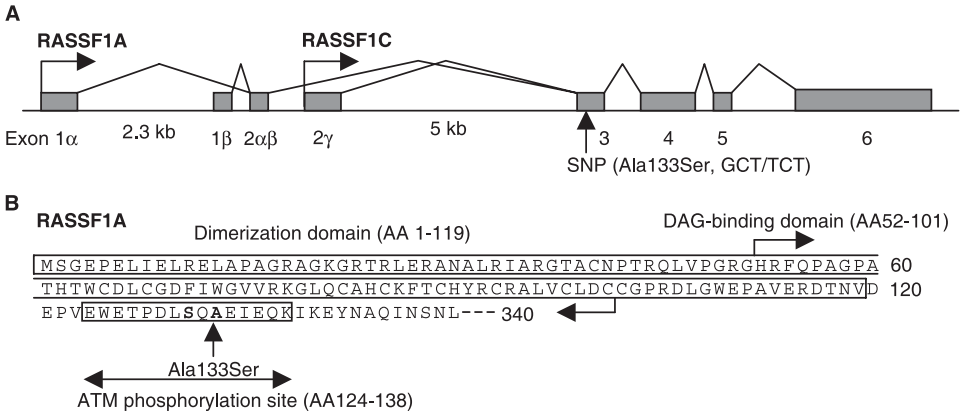

Fig. 1. Structure of the RASSF1 gene and proteins. (A)The *RASSF1* gene structure. The *RASSF1* gene has 6 exons and was identified to have three major transcripts (A, B and C). Two promoters (A and C) are indicated here. The numbering of exons is according to Spugnardi et al. [17]. (B) Peptide sequence of the N-terminal half of RASSF1A, showing some of the predicted domains, the dimerization domain, the DAG-binding domain and the ATM phosphorylation site (131Ser), and position of the SNP (133Ala) described in this study.