Functional Role of S100A14 Genetic Variants and Their Association with Esophageal Squamous Cell Carcinoma

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Abstract

S100 proteins have been implicated in various human diseases, including certain types of cancer. Among them, S100A14 is down-regulated in esophageal squamous cell carcinoma (ESCC). In this study, we sought to identify functional genetic variants in the S100A14 locus and assessed their associations with susceptibility to ESCC. Thirty individual DNA samples were sequenced to search for genetic variations in S100A14, and the function of the variants was investigated by a set of biochemical assays. A case-control analysis was performed in 1,021 patients with ESCC and 1,253 control subjects. Odds ratios and 95% confidence intervals (95% CI) were computed by logistic regression model. Four single nucleotide polymorphisms, -43A>G, 461G>A, 1493A>G, and 1545A>T, were identified in the S100A14 locus and they are in absolute linkage disequilibrium. Among them, the 461G>A change was shown to diminish a P53-binding site and is therefore associated with decreased expression of S100A14 in vitro and in vivo in the target tissues. Case-control analysis showed that the 461A allele was associated with susceptibility to ESCC among smokers, with the ORs being 2.01 (95% CI, 1.50-2.69) or 2.10 (95% CI, 1.37-3.22) for the 461GA or 461AA genotype, respectively, compared with the 461GG genotype. These data constitute strong evidence in support of the notion that S100A14 might function as a cancer suppressor working in the P53 pathway and play a role in esophageal carcinogenesis. $4 \circ \circ 002$ 1

Introduction

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Materials and Methods

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Cancer Research



Figure 1. Reporter gene assays with constructs containing the *S100A14* promoter with different deletions or mutations. *A*, schematic representation of the *S100A14* 5'-flanking region and reporter gene constructs used in this study. *B*, luciferase expression of different constructs in HeLa or KYSE450 cells. *C*, luciferase expression in HCT116/P53^{-/-} or HCT116/P53^{-/-} cells of different constructs with mutations at the -43 and 461 positions. All constructs were cotransfected with pRL-SV40 to standardize transfection efficiency. Luciferase levels of pGL3-Basic and pRL-SV40 were determined in triplicate. Fold increase was measured by defining the activity of the empty pGL3-Basic vector as 1. *Columns*, mean of three independent transfection experiments, each performed in triplicate; *bars*, SE. *, *P* < 0.001, compared with P-AG; **, *P* < 0.001, compared with P-AG;

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Figure 3. The correlation between S100A14 expression and P53 expression. *A*, the S100A14 levels were significantly increased in HCT116/P53^{+/+} but not in HCT116/P53^{-/-} cells when P53 expression was stimulated by hydroxycamptothecin. *B*, the S100A14 levels were significantly elevated in EC9706 and HaCaT cells carrying the 461GG genotype but not in KYSE150 cells carrying the 461AA genotype when P53 was exogenously introduced to the cells by adenovirus hP53.

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Figure 4. Levels of *S100A14* RNA expression in human esophageal tissues as a function of *S100A14* 461G>A genotype. *Columns*, mean normalized to β -actin; *bars*, SE. Expression levels among the GA (*n* = 13) or AA (*n* = 2) genotypes were significantly lower than that among the GG genotype (*n* = 17). *, *P* = 0.026.

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