

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.

Introduction

Esophageal squamous cell carcinoma (ESCC) is one of the most common and aggressive malignancies in the world. The etiology of ESCC is multifactorial, involving both environmental and genetic factors. Smoking and alcohol consumption are well-established risk factors for ESCC. In addition, several genetic variants have been associated with susceptibility to ESCC. S100 proteins, a family of calcium-binding proteins, have been implicated in various human diseases, including certain types of cancer. S100A14, a member of the S100 protein family, is down-regulated in ESCC. In this study, we sought to identify functional genetic variants in the *S100A14* locus and assess their associations with susceptibility to ESCC.

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Materials and Methods

SNP identification. Thirty individual DNA samples were sequenced to search for genetic variations in *S100A14*. 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.

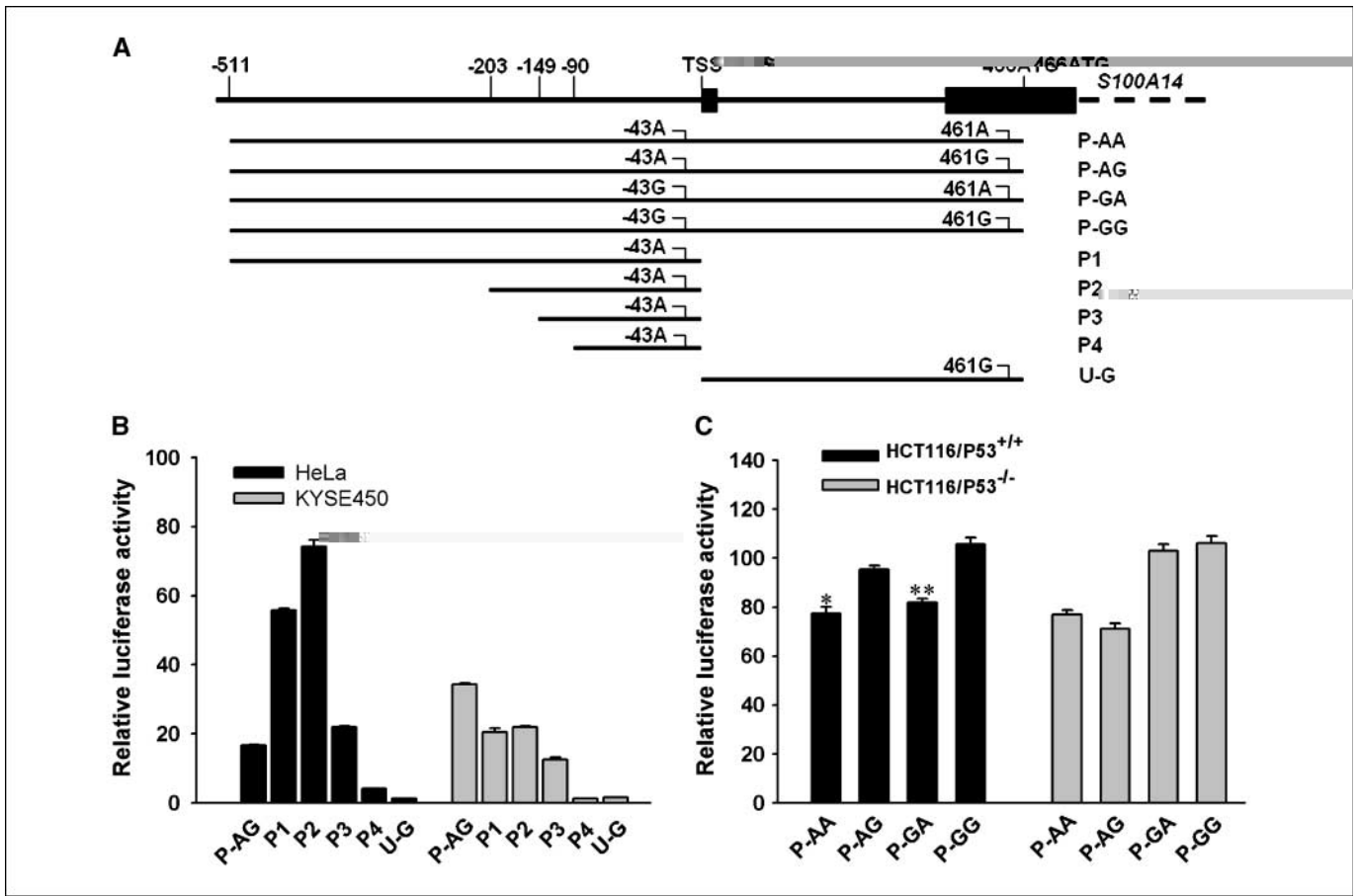


Figure 1. Reporter gene assays with constructs containing the *S100A14* promoter with different deletions or mutations. **A**, schematic representation of the *S100A14* 5'-flanking region and reporter gene constructs used in this study. **B**, luciferase expression of different constructs in HeLa or 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-GG.

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Genotyping of *S100A14* polymorphisms.

Construction of reporter gene plasmids.

Cell culture.

Transient transfection and luciferase assay.

Electrophoretic mobility shift assays.

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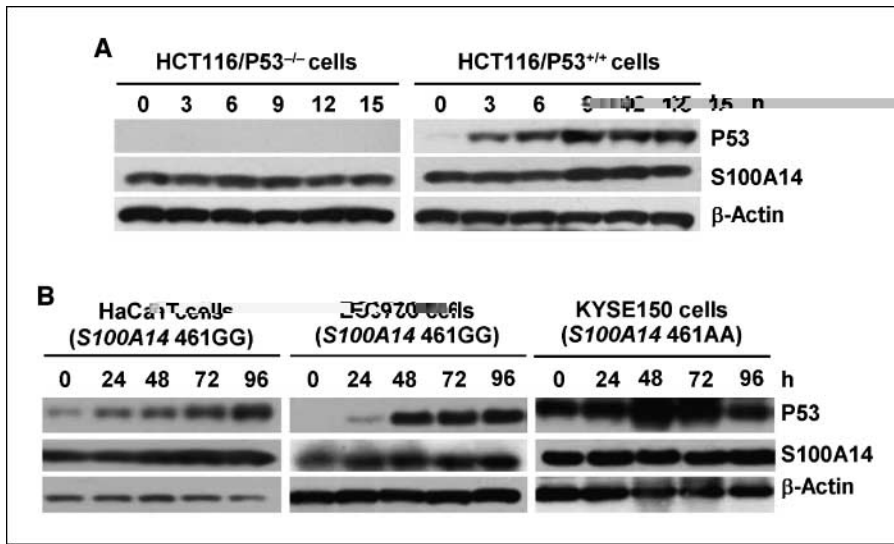


Figure 3. The correlation between S100A14 expression and P53 expression. **A**, the S100A14 levels were significantly increased in HCT116/P53^{+/+} but not in HCT116/P53^{-/-} cells when P53 expression was stimulated by hydroxycamptothecin. **B**, the S100A14 levels were significantly elevated in EC9706 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.

Western blot analysis of P53, S100A14, and β-actin in HCT116/P53^{-/-} and HCT116/P53^{+/+} cells treated with hydroxycamptothecin (0, 3, 6, 9, 12, 15 h). In HCT116/P53^{+/+} cells, P53 and S100A14 levels increased over time, while β-actin levels remained constant. In HCT116/P53^{-/-} cells, P53 levels increased but S100A14 levels did not.

Western blot analysis of P53, S100A14, and β-actin in HaCaT cells (S100A14 461GG), EC970 cells (S100A14 461GG), and KYSE150 cells (S100A14 461AA) treated with adenovirus hP53 (0, 24, 48, 72, 96 h). In HaCaT and EC970 cells, P53 and S100A14 levels increased over time, while β-actin levels remained constant. In KYSE150 cells, P53 levels increased but S100A14 levels did not.

Effects of genetic variants on S100A14 promoter activity and P53-binding ability.

Luciferase reporter assays were performed in HCT116 cells transfected with S100A14 promoter constructs (S100A14⁻⁹⁰ and S100A14⁻⁹⁰Δ461G>A) and P53 expression vector. The results are shown in Figure 4A and 4B. The S100A14 promoter activity was significantly increased in HCT116/P53^{+/+} cells transfected with S100A14⁻⁹⁰Δ461G>A construct compared with S100A14⁻⁹⁰ construct (P < 0.001). The P53-binding ability of S100A14 promoter was significantly increased in HCT116/P53^{+/+} cells transfected with S100A14⁻⁹⁰Δ461G>A construct compared with S100A14⁻⁹⁰ construct (P < 0.001).

Effects of 461G>A change on S100A14 expression.

Western blot analysis was performed in HCT116 cells transfected with S100A14⁻⁹⁰Δ461G>A and S100A14⁻⁹⁰ constructs. The results are shown in Figure 4C. The S100A14 protein levels were significantly increased in HCT116/P53^{+/+} cells transfected with S100A14⁻⁹⁰Δ461G>A construct compared with S100A14⁻⁹⁰ construct (P < 0.001).

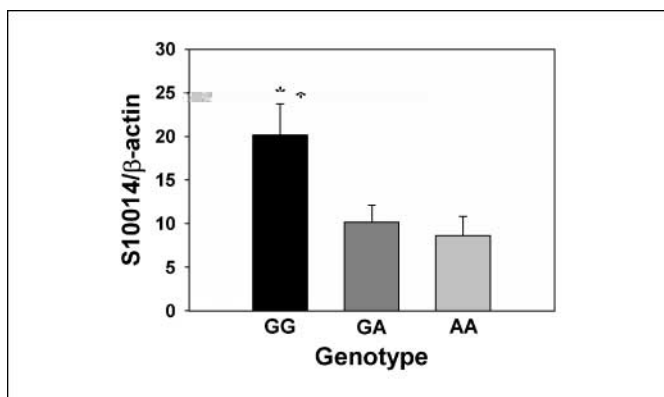


Figure 4. Levels of *S100A14* RNA expression in human esophageal tissues as a function of *S100A14* 461G>A genotype. Columns, mean normalized to β-actin; bars, SE. Expression levels among the GA (n = 13) or AA (n = 2) genotypes were significantly lower than that among the GG genotype (n = 17). *, P =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

S100A14 variants and the risk of developing ESCC.

The association between *S100A14* genotype and the risk of developing ESCC was analyzed. The results are summarized in Table 1. The GA genotype was significantly associated with an increased risk of ESCC (OR = 1.1, 95% CI = 1.0-1.2, P = 0.001). The AA genotype was also significantly associated with an increased risk of ESCC (OR = 1.1, 95% CI = 1.0-1.2, P = 0.001).

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Genotype	Controls	Patients	OR* (95% CI)	P
	(n = 1,253)	(n = 1,021)		
	No. (%)	No. (%)		
G	1,199 (95.7%)	1,019 (99.8%)	1.00 (0.99-1.01)	0.01
A	54 (4.3%)	2 (0.2%)	1.1 (1.0-1.2)	0.001
GG	1,111 (88.7%)	1,019 (100%)	1.0 (0.99-1.01)	0.1
GA	88 (7.0%)	10 (1.0%)	1.1 (1.0-1.2)	0.001
AA	54 (4.3%)	1 (0.1%)	1.1 (1.0-1.2)	0.001

* OR = Odds Ratio; CI = Confidence Interval.

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Discussion

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Grant support: (00 0 7), advertisement
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References

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