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

Hongyan Chen,[1] Dianke Yu,[1,2] Aiping Luo,[1] Wen Tan,[1,2] Chunpeng Zhang,[1,2] Dan Zhao,[2] Ming Yang,[1,2] Junniao Liu,[1,2] Dongxin Lin,[1,2] and Zhihua Liu[1]

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

Note:

Requests for reprints:

Materials and Methods

SNP identification.

Study subjects.

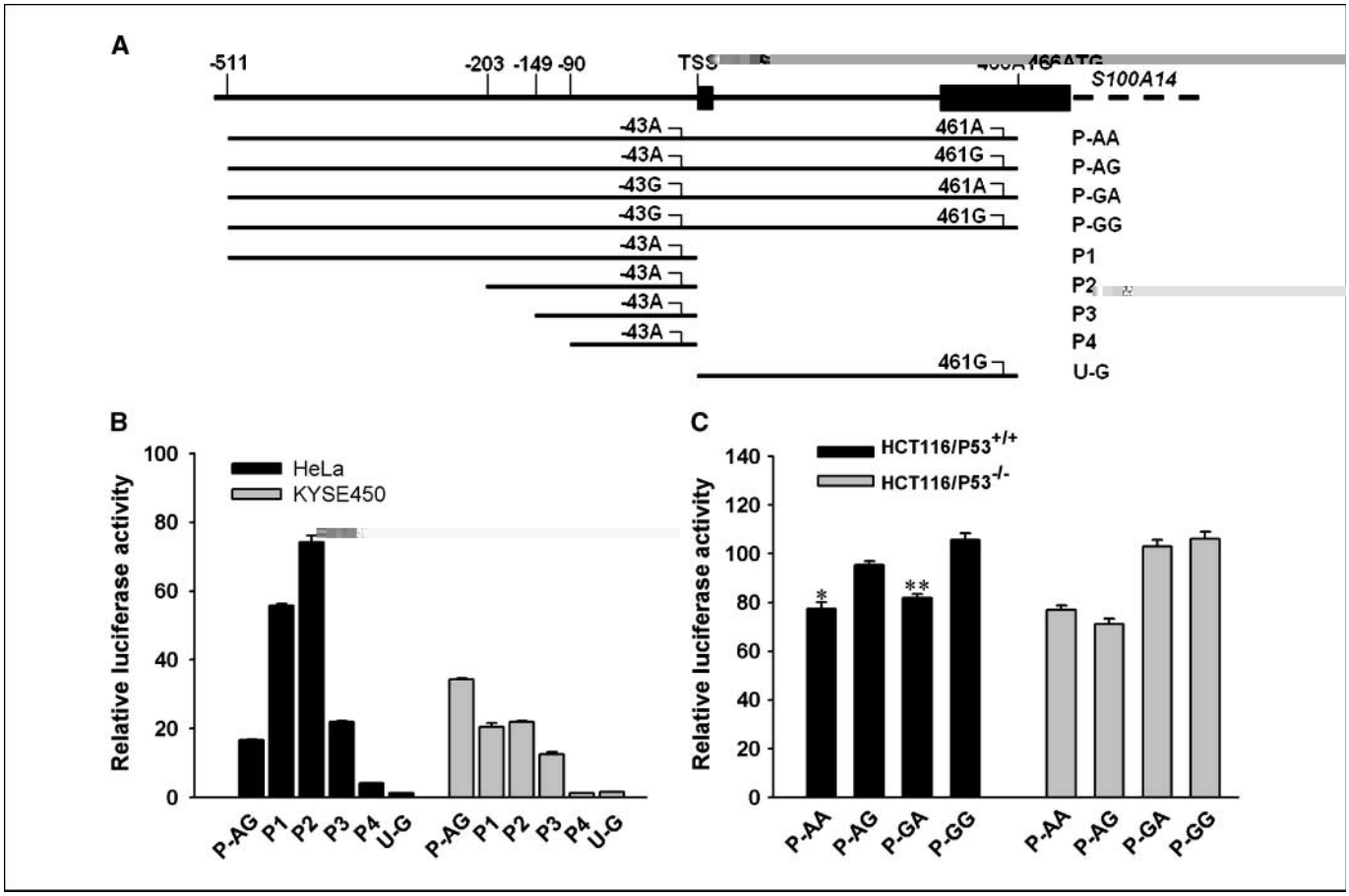

Figure 1. Reporter gene assays with constructs containing the *S100A14* promoter with different deletions or mutations. *A,* schematic representation of the *S100A14* 5′-flanking region and reporter gene constructs used in this study. *B,* luciferase expression of different constructs in HeLa or KYSE450 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.

( ) . - . .

Genotyping of *S100A14* polymorphisms. — > (0), 1 > , 1 , > , 1 > . - . 1. 10% , .

Construction of reporter gene plasmids. , — 11 + , — 0 + , —1 , + , —, 0 + , +1 , ( ). 1, , , , - , , - . — 1 . - / 1 , - , - , - . ( ) , - - , 1 , /1 1 , /1 *S100A14* ′- , . .

Cell culture. 11 / +/+ 11 / −/− . . (,). , 0 , 1 0 . . (,), , 0 . , 11 / +/+, 11 / −/− , , 0, , 1 0, , 0 1 0 10% , 100 / , 100 / .

Transient transfection and luciferase assay. , , 0, 11 / +/+, 11 / −/− . 0% 0% . (100) ( ). 1.0 : 0 ( ). ( ). , - 1.

Electrophoretic mobility shift assays. - *S100A14* — , — , 1 , 1 ,

- ( ) -
 .
11 / −/− 11 / +/+ ,
  (10 µ /) 1 ,
- ° 0
  ( ) ( ).
  %
 / ( ).
  , 00-
  ( )
  - .

Chromatin immunoprecipitation assays. 11 / +/+ ,
  (10 µ /) 1 ,
- 1% 10 . -
  ( ) ( )
 - .
 - ′ ′- -′, -
1 - *S100A14* ′- 0 1

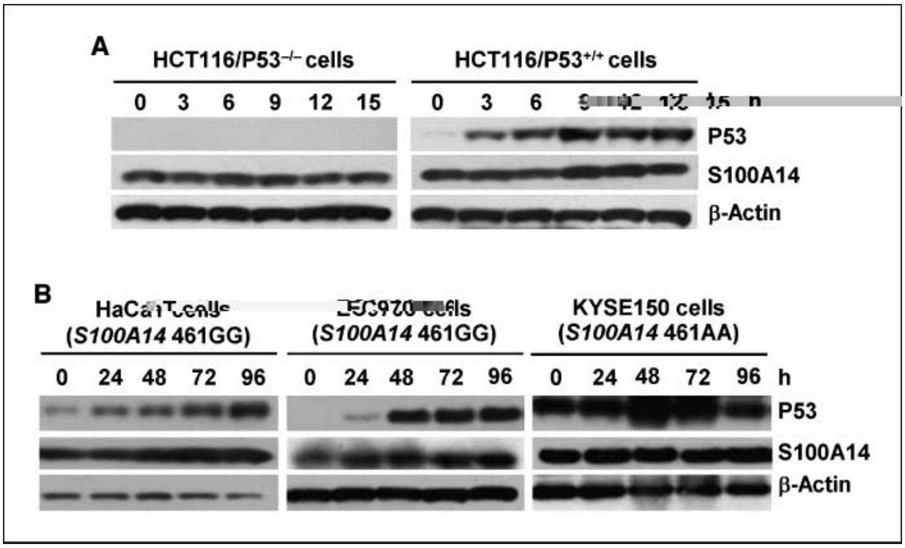

Figure 3. The correlation between S100A14 expression and P53 expression. *A,* the S100A14 levels were significantly increased in HCT116/P53$^{+/+}$ but not in HCT116/P53$^{-/-}$ cells when P53 expression was stimulated by hydroxycamptothecin. *B,* the S100A14 levels were significantly elevated in EC9706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

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

Effects of 461G>A change on *S100A14* expression.

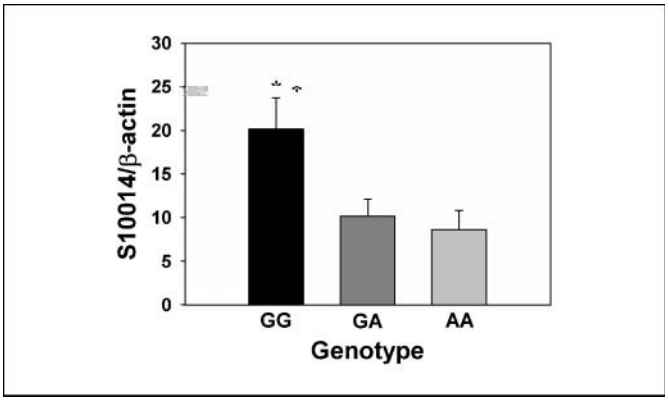

Figure 4. Levels of *S100A14* RNA expression in human esophageal tissues as a function of *S100A14* 461G>A genotype. *Columns,* mean normalized to β-actin; *bars,* SE. Expression levels among the GA (*n* = 13) or AA (*n* = 2) genotypes were significantly lower than that among the GG genotype (*n* = 17).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

.t .t .t .t .t
, .t .
.t *S100A14*
.t .t .t *S100A14*
1 .t (.), .t .t .t .t .t
.t 1 .t .t *S100A14*
(±,) .t .t .t .t .t 1 0.1 ± .
(*n* 1) ,, ± 1. (, *n* 1 , *n*) *P* 0.0 .

***S100A14* variants and the risk of developing ESCC.** -
.t 1,0 1 .t .t .t , 1, .t
.t .t .t .t .t .t *S100A14* ,
, . , .t
, , .t .t .t .t
. , .t .t .t .t
.t (1. % . % , 1. , % , 1.1 1. *P* 0.001).
.t .t 1. .t — > ,
1 > , 1 , > , 1 > .t
.t .t , .t 1 > .t .t
. .t 1 .t 0. ,
.t 0. 1 . .t 1 , ,
.t .t .t (. %, . %, 10. %, .t)
.t .t .t (0%, 0. %, , . %,

Table 1. Allele and genotype frequencies of *S100A14* 461G>A polymorphism among patients and controls and their associations with the risk of ESCC

Genotype	Controls (*n* = 1,253)	Patients (*n* = 1,021)	OR* (95% CI)	*P*
	No. (%)	No. (%)		
	1, , (0.)	1, (,)		0.01,
	(, .)	(.1)		
	(0.0)	(.)	1.00 ( )	
	0 (0.)	(.)	1. (1.0 1.)	0.01
	1 1 (, .)	10, (10.)	1. (0, 1.)	0.1
+	(0.0)	(.)	1. (1.0 1.)	0.00

* .t .t .t , .t , ,
.t .t .

.t χ . *P* 0.0 *df*). .t .t .t
.t .t .t .t .t 1
(.t) 1. - (, % , 1.0 1.)
, .t .t .t .t
1 .t (1). .t
.t .t , , .t , .t .t .t
1 > .t .t .t .t .t
.t .t ( ). 1 >
.t .t , .t .t . .t , .t
- .t .t .t 1 >
, .t .01 (, % , 1. 0 . ,) .10 (, % ,
1. .) .t .t 1 .t
1. (, % , 1.0 1. 0) .t .t 1 .t
1.1 (, % , 0. , 1.) 1.1 (, % , 0. 1.)
.t .t 1 .t , .t (.01 >
1. × 1.1 .10 > 1. × 1.1 *P* 0.0 , .t .t
.t). .t .t - ,
.t .t .t .t .t .t
.t 1 .t (>
-). .t .t .t
1 > .t , / .t .t (.t .t)
.t .t (.t) .t .t .t .
, .t .t .t .t (.t .t).

Discussion

.t .t .t , .t .t .t
.t *S100A14* .t .t .t .t .t , .
, .t , .t *S100A14* ,
.t . .t , .t
1 > , .t .t ′- .t .t .t
.t .t .t .t .t .t
S100A14. - .t .t *S100A14*
, (1), - .t .t .t
.t .t .t , .t .t .t -
.t .t .t . , .t .t %
, .t 1 .t .t .t
, .t .t .t 1, %
.t .t 1 .t . .t
.t .t .t .t .t .t .t .t .t
.t .t .t .t .t .t .
.t .t .t .t *S100A14* .t
.t .t .t .t , .t .t .t
.t .t ′- .t .t .t . .t
.t .t ′- .t .t .t .t .t
.t .t .t .t .t 1 > .t .t
.t .t *S100A14* . .t
.t .t .t 1 - .t .t
.t .t .t .t .t .t 1 - .t
.t 11 / +/+ .t .t 11 / −/− ,
.t .t .t .t .t .t .t .t .t .t
.t .t .t .t . .t .t .t
.t .t 1 > , - .t .t
S100A14 ′- .t .t .t .t .t
.t , , .t .t .t 1
.t , .t 1 .t
.t .t .t , .t
.t .t .t , 100 1
.t .t .t *S100A14* 1 .t .t .t
S100A14 1 .t . .t , .t .t .t .t .t

S100A14 ′-

( ).

11 / +/+

S100A14

(, ,)

(0).

S100A14

11 / −/−

11 / +/+

>

>

( ).

S100A14 —

Grant support: (00 0 7), (00 1 01 00 ,10 0), (0 11 0 0).

References

1. l,9 -l,,,. 00 10 1 .

2. 00 ,9 1 .

3. 7 00 ,9 1 ,9.

4. 00 11 ,9 .

5. , 7 00 .

6. , 7 .00 ,9 .

7. 7 00 1 ,9 .

8. 00 ,9 0 1 .

9. 001 .

10. 00 1111 .

11. 00 0 1 .

12. 00 .

13. 00 0 ,1 ,9 .

14. 001 ,9 0 .

15. 00 ,9 0 1 .

16. 001 1 1 0.

17. 00 ,9 1 .

18. , 7 (7 (00(0 0(, (0((00 +0((1 - .0(. ,0 **2614.**