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S100A2 Overexpression is Frequently Observed in Esophageal Squamous Cell Carcinoma

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Abstract. *Background: We previously detected that ΔNp63, a human p53 homologue, is an oncogene amplified in squamous cell carcinomas (SCC) including esophageal SCC. Subsequently, we examined global patterns of gene expression in cancer cells following ΔNp63 gene introduction using an oligonucleotide microarray approach. We identified S100A2, a Ca^{2+}-binding protein, as a novel downstream mediator of ΔNp63. Materials and Methods: In this study, we examined S100A2 expression in esophageal SCC cell lines and primary SCCs using Northern analysis. Results: We found that 2 out of 8 (25%) cell lines and 14 out of 30 primary esophageal cancers (47%) showed S100A2 gene overexpression compared to paired normal tissues. To examine a possible relationship between S100A2 overexpression and clinicopathological features, we proceeded with statistical analysis. S100A2 overexpression was significantly associated with higher age in esophageal SCC (p=0.0434). Interestingly, S100A2-overexpressing cancers showed a trend toward preferentially developing lymph node metastases and distant metastases (p=0.111 and 0.178, respectively). Conclusion: These results suggested that S100A2 might be related to the progression of esophageal SCC.*

Esophageal squamous cell carcinoma (SCC) is one of the most aggressive cancers occuring at a high incidence in certain countries (1). Treatment of this fatal cancer, involves surgery and subsequent chemotherapy and radiotherapy. For this purpose, it is important to search for novel genetic changes that might indicate the malignancy of esophageal SCC.

Accumulating evidence indicates that a series of genetic changes in dominant oncogenes such as *bcl-2, cyclin D1* and *c-myc*, together with the inactivation of tumor suppressor genes such as *p53*, are involved in the pathogenesis of human esophageal SCC (2-5). Several other candidate oncogenes have also been implicated in reports (6, 7). Recently, Hibi *et al.* proved that *ΔNp63*, a human p53 homologue, is an oncogene amplified in SCC (8). Subsequently, we examined *ΔNp63* status in 8 esophageal SCC cell lines and found that all (100%) showed *ΔNp63* gene overexpression, whereas most gastric and colorectal carcinoma cell lines did not (9). These results suggested that *ΔNp63* might be oncogenic in esophageal SCC, though its mode of action remains unknown. In an effort to gain further insight into the tumorigenic pathway, we examined global patterns of gene expression in cancer cells following *ΔNp63* gene introduction using an oligonucleotide microarray approach. We identified S100A2, a Ca^{2+}-binding protein, as a novel downstream mediator of *ΔNp63* (10).

These results prompted us to examine *S100A2* status in esophageal SCC, which is overexpressed in other SCCs such as skin and head and neck SCCs (11, 12). In this study, we examined *S100A2* expression in esophageal SCC cell lines and primary SCCs using Northern analysis.

Materials and Methods

Tissue specimens and RNA extraction. Five cell lines were established in our laboratory (NUEC1, 2, 3, 4 and TT). The other cell lines were purchased from the American Type Culture Collection. Cultured cell lines were lysed in guanidine buffer, and total RNA was isolated using the CsCl gradient method. For primary tissues, the collected samples were grossly dissected, quickly frozen or lysed immediately in the guanidine buffer, and the RNA was isolated as described (13).

Northern analysis. Northern blot hybridization using the cDNA probe was performed as described previously (14). cDNA included the 3‘ part of the *S100A2* gene. The human β-actin gene was used as an internal control to standardize the relative amount of RNA in each lane.

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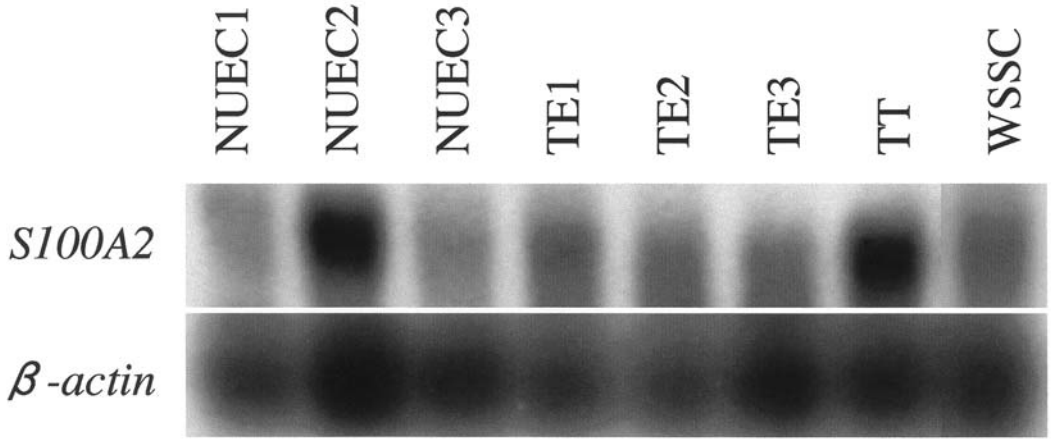

Figure 1. *Detection of S100A2 expression in esophageal SCC cell lines. Two out of 8 esophageal SCC cell lines (25%) (NUEC2 and TT) showed S100A2 gene overxpression. A human β-actin probe was used as an internal control.*

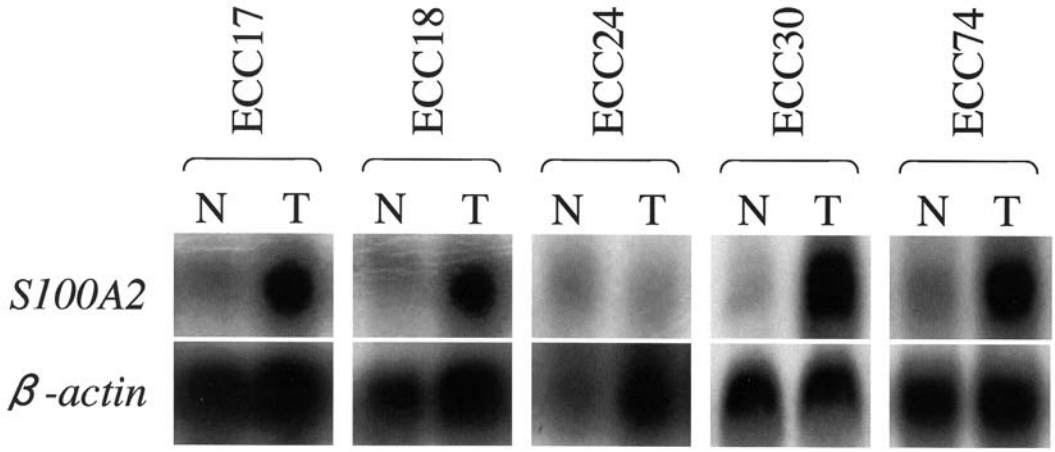

Figure 2. *Detection of S100A2 expression in primary esophageal SCC. Five μg of total RNA extracted from tumor (T) and normal (N) tissues of 30 different patients with primary esophageal cancers were hybridized with a ^{32}P-labelled probe for S100A2 and β-actin. Although normal esophageal tissues showed low-grade expression, higher expression of S100A2 was observed in cases ECC-17, 18, 30 and 74.*

Statistical analysis. Mann-Whitney's *U*-test was used to examine the association between the *S100A2* expression and clinicopathological features.

Results

We first examined *S100A2* expressions in esophageal SCC cell lines by Northern analysis. We found that 2 out of 8 (25%) cell lines showed *S100A2* gene overexpression (Figure 1). This result suggested that the *S100A2* gene was overexpressed in esophageal SCC, as well as other SCCs reported previously (11, 12).

We then tested for *S100A2* expressions in paired esophageal normal tissues and SCCs. Fourteen out of 30 primary esophageal SCCs (47%) showed overexpression of the *S100A2* gene while normal esophageal tissues only showed low-grade expression. A representative result is shown in Figure 2. Frequent overexpression of the *S100A2* gene suggested that this gene is related to the progression of esophageal SCC.

To examine a possible relationship between *S100A2* overexpression and clinicopathological features, we proceeded with statistical analysis. *S100A2* overexpression was significantly associated with higher age in esophageal

Table I. *Clinicopathological features and S100A2 overexpression in primary esophageal SCC.*

Clinicopathological feature	Variable	No. of cases	*S100A2* overexpression		*p* value[a]
			+	−	
Age	50 to 77	30	66.7±6.9	61.2±7.5	0.0434
Sex	Male	22	10	12	0.828
	Female	8	4	4	
Size	1.5 to 14	30	4.96±1.9	5.33±2.5	0.851
Histological	Well[b]	8	5	3	0.324
Type	Moderate[c]	14	6	8	
	Poor[d]	8	3	5	
Lymph node	-	11	3	8	0.111
Metastasis	+	19	11	8	
Distant	-	28	14	14	0.178
Metastasis	+	2	0	2	
TNM stage	1	2	1	1	0.333
	2	10	3	7	
	3	16	9	7	
	4	2	1	1	

[a]Mann-Whitney's *U*-test
[b]Well, well-differentiated
[c]Moderate, moderately-differentiated
[d]Poor, poorly-differentiated squamous cell carcinoma

SCC ($p=0.0434$, Table I. Interestingly, *S100A2*-overexpressing cancers showed a trend toward preferentially developing lymph node metastases and distant metastases ($p=0.111$ and 0.178, respectively). This result suggested that *S100A2* might be useful as a marker for advanced esophageal SCC.

Discussion

The expression of *ΔNp63* in SCC was first detected in the head and neck, lung and esophagus in previous studies (8, 9). Moreover, we found that increased expression of *ΔNp63* in mouse fibroblast cells led to a transformed phenotype. To gain additional insight into this pathway, we previously examined global patterns of gene expression in cancer cells after *ΔNp63* gene introduction using an oligonucleotide microarray approach, and identified *S100A2* as a novel downstream mediator of *ΔNp63* (10). The S100A2 protein is a member of the S100 family of Ca^{2+}-binding proteins, which are involved in signal transduction processes and consequently in the regulation of proliferation and differentiation (15). It has been reported that basal cell and SCCs showed strong S100A2 immunoreactivity in neoplastic cells corresponding to basal cells, but were non-reactive or faintly reactive for other S100 proteins (16). This indicated that S100A2 exhibited the same distribution as ΔNp63 in human tissues and suggested that S100A2 might be a target of the *ΔNp63* pathway. Xia *et al.* (11) reported that *S100A2* was strongly expressed in bulk specimens of basal and SCCs of the skin and oral cavity. Moreover, Villaret *et al.* (12) found, using subtractive and microarray technology, that the *S100A2* gene was significantly overexpressed in head and neck SCC compared with normal tissue. These results indicated that *S100A2* has an oncogenic function, especially in SCC.

In this study, we tested for the expression status of the *S100A2* gene in esophageal SCC. Fourteen out of 30 primary esophageal cancers (47%) showed obviously higher expressions of the *S100A2* gene compared to paired normal tissues. Subsequently, we found that *S100A2* gene expression showed a trend toward preferentially developing lymph node metastases and distant metastases. Although a larger study is needed to assess the precise relationship between *S100A2* expression and clinicopathological features, the present study showed the possibility that *S100A2* might be a marker for the estimation of malignancy in this cancer.

Although the mechanism of *S100A2* on oncogenicity remains to be proven, our results indicated that it might be related to the oncogenic pathway of esophageal SCC. The present emerging model supports the presence of transcriptional cross-talk among *p53*, *ΔNp63* and *S100A2.* This critical interaction may balance the oncogenic and growth-stimulating activity in tumorigenesis with their abilities to induce epithelial proliferation during development.

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