DJ-1 Could Predict Worse Prognosis in Esophageal Squamous Cell Carcinoma

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Abstract

Recent studies have revealed an oncogenic role of DJ-1 through its ability to transform normal cells, prevent oxidative damage, and inhibit apoptosis. However, its role in esophageal squamous cell carcinoma (ESCC) is unknown. In this study, by immunohistochemistry, we analyzed the expression of DJ-1 in 81 ESCC tumors, 31 paired nonneoplastic esophageal epithelia, and 19 paired ESCC lymph node metastases. We found that cytoplasmic DJ-1 expression was significantly higher in ESCC and ESCC lymph node metastases than in nonneoplastic esophageal epithelium. ESCC specimens with high distant metastatic potential also had a significantly higher level of nuclear DJ-1 expression (P = 0.018). By Kaplan-Meier analysis, we found that a high level of nuclear DJ-1 was significantly associated with poorer patient survival in our cohort (P = 0.028). To investigate whether DJ-1 promotes ESCC progression through phosphatidylinositol 3-kinase pathway and modulation of apoptosis, we performed immunohistochemistry of pAkt and Daxx. We found that DJ-1 expression was significantly associated with pAkt, whereas nuclear DJ-1 expression was significantly correlated with nuclear expression of Daxx. These results suggest that phosphatidylinositol 3-kinase pathway and Daxx-regulated apoptosis might be important in DJ-1-mediated ESCC progression. By using multivariate Cox regression, we further showed that T_4 stage (P = 0.003) and DJ-1 (P = 0.034) are independent predictors of patient survival. In conclusion, our results suggest that DJ-1 plays a very important role in transformation and progression of ESCC and may be used as a prognostic marker in ESCC. (Cancer Epidemiol Biomarkers Prev 2008;17(12):3593–602)

Introduction

Esophageal squamous cell carcinoma (ESCC) is common among Asian populations. Despite recent advances in the detection of premalignant lesions and the development of combination therapies, its incidence is increasing and its outcome remains poor (1). Proper clinical management should be given to patients with aggressive ESCC, which might improve survival. Identification of prognostic markers for ESCC might help to make the decision on which treatment regimen is the most suitable for a patient in terms of quality of living.

DJ-1 has been identified as an oncogene that can transform mouse NIH3T3 cells together with *ras* (2). Its oncogenic effect is partly attributed to its antiapoptotic ability. DJ-1 is an important factor governing apoptotic response in neuronal cells. It has been shown to protect neuronal apoptosis, whereas decreased expression of DJ-1 increases apoptosis of neuronal cells to various stresses (3, 4). Recent functional studies on DJ-1 revealed several important mechanisms on how it inhibits apoptosis. Sumoylation of the K130 residue of DJ-1 is essential for its antiapoptotic and transformation ability (5), whereas DJ-1 also interacts with and inhibits the function of Daxx, which binds to apoptosis signal-regulating kinase 1 to promote apoptosis induced by oxidative stress (6). The antioxidative activity of DJ-1 also links to the ability of DJ-1 to induce Nrf2 stability, which in turn activates the transcription of antioxidant and detoxification enzymes (7). In another study, DJ-1 has been shown to up-regulate glutathione synthesis, which is a downstream target of Nrf2, to protect cells from oxidative stress-induced apoptosis (8). More recently, DJ-1 has been shown to decrease the expression of Bax and inhibit caspase activation, suggesting a direct role of DJ-1 in inhibiting the apoptotic pathway (9). In cancer cells, decreased expression of DJ-1 enhances apoptosis in prostate (10) and lung cancer cells (9, 11). These lines of evidence suggest that DJ-1 is important in regulating apoptosis and hence plays an important role in carcinogenesis.

The roles and prognostic significance of DJ-1 have been studied in certain types of cancer. In breast cancer patients, serum level of DJ-1 is increased when compared with healthy subjects (12). DJ-1 negatively correlates with the expression of a potent tumor suppressor, PTEN, in human breast cancer specimens (13). DJ-1 is increased in primary lung cancer (11) and a high level of DJ-1 is correlated with poor prognosis of lung cancer patients (13). Reduced DJ-1 sensitizes prostate cancer cells toward apoptotic-inducing agents (10). DJ-1 has been shown to

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be an androgen receptor coactivator (14), which interacts with androgen receptor, and is up-regulated in human prostate cancer on androgen deprivation therapy (15). These results strongly suggest that DJ-1 promotes prostate cancer progression. Recently, DJ-1 has also been suggested to confer prognostic value to ovarian carcinoma with effusions (16).

There has not been any report concerning the significance of DJ-1 in ESCC. In this study, we analyzed, by immunohistochemistry, the expression of DJ-1 in 81 ESCC specimens with 31 paired nonneoplastic esophageal epithelium and 19 paired ESCC lymph node metastasis. The expression of DJ-1 was then correlated with the clinicopathologic data of the patients. In addition, as DJ-1 has been shown to promote cell survival through the phosphatidylinositol 3-kinase (PI3K) pathway by modulating PTEN and Akt activity (13, 17) and apoptotic response through Daxx (6), we further investigated the association between DJ-1 and pAkt or Daxx in the ESCC tumor specimens.

Materials and Methods

Patients and Specimens. Our cohort consists of 81 ESCC Chinese patients who underwent total esophagectomy for ESCC in Queen Mary Hospital from 1998 to 2005. The esophagectomy specimens were formalin-fixed and paraffin-embedded for routine histopathologic diagnosis. The specimens were collected consecutively. Patients who had received prior treatment directed against ESCC or specimens with insufficient tumor tissue for microarray incorporation or subsequent immunohistochemical study were excluded from this study. The extent of tumor at the time of operation was classified according to the tumor-node-metastasis (TNM) staging system, whereas tumor grades were classified based on the WHO classification (18). The clinical data were retrieved and are summarized in Table 1. Adjuvant therapies were given to the patient in a ''curative intent'' manner despite the heterogeneity of the treatment. Paired nonneoplastic esophageal epithelium was selected from the upper resection margin of esophagectomy for comparison with tumor. The ESCC tumors were regarded as having a high distant metastatic potential if distant metastasis could be detected within 1 year of esophagectomy, else they were considered to have a low distant metastatic potential. The mean follow-up time of our patients is 14.5 months (range, 0.7-65.2 months).

Tissue Microarray Construction. Tissue microarray (TMA) was constructed with a Beecher Instruments tissue microarrayer as described previously (19). Briefly, three representative areas of each specimen were selected by a pathologist (K.W.C.) and transferred to a TMA block using a 0.6 mm core diameter needle. H&E-stained sections from each TMA block were checked by K.W.C. to ensure that adequate targeted areas had been included. The sample numbers varied slightly among results of different markers because of variability in the number of interpretable tissue cores in TMA sections.

Immunohistochemical Staining. Immunohistochemical staining was done as described previously using DAKO EnVision$^+$ System HRP (19). DJ-1 polyclonal antibody (13) was applied at 1:5,000 dilution for overnight at 4°C. pAkt (Ser^{473}) antibody from Cell Signaling and Daxx antibody from Upstate were applied at a dilution of 1:50 and 1:100, respectively, for overnight at 4°C. The primary antibody was omitted in the negative control.

Table 1. Patient clinical and pathologic features

	No. (%) cases	Median (range)
Age (y)	81	67 (41-87)
Sex		
Male	62 (77)	
Female	19 (24)	
T stage		
T_1	2 (3)	
T_2	13 (16)	
T_3	49 (61)	
T_4	17 (21)	
N stage		
N_0	30 (37)	
N_1	51 (63)	
M stage		
M_0	68 (84)	
M_1	13 (16)	
pTNM staging		
Stage II	32 (40)	
Stage III	36 (44)	
Stage IV	13 (16)	
Histologic grade		
Well-differentiated	13 (16)	
Moderately differentiated	48 (59)	
Poorly differentiated	20 (25)	
Metastatic status		
Nonmetastatic	55 (68)	
Metastatic (within 1 y)	26 (32)	

Evaluation of Immunohistochemical Staining Results. The stained sections were reviewed by two independent observers (K.W.C. and Y.P.C.) who had no prior knowledge of the clinicopathologic data of the patients. All three cores of each specimen were scored and the ''hot spot'' core was selected for later statistical analysis. Cytoplasmic staining was graded by an arbitrary scale based on the extent and the intensity of the whole tissue core. A cytoplasmic staining score was given, ranging from 0 to 3, which represent negative, weak, moderate, and strong staining, respectively. For nuclear staining, nuclei from the whole tissue core were counted and only those cores with >100 nuclei of target cells were submitted for statistical analysis. The DJ-1 nuclear score was then divided into four groups according to the percentage of cells positively stained as negative (≤5% nuclear stained positive), weak (>5% and ≤20%), moderate (20% and ≤50%), and strong (≥50%). For pAkt and Daxx, their nuclear score was respectively graded into four groups as negative (≤5% nuclear stained positive), weak (>5% and ≤20%), moderate (20% and ≤30%), and strong (≥30%). In Kaplan-Meier and Cox regression analyses, the expression level was divided into two groups, with negative and weak expressions regarded as low-level expression and with moderate and strong expressions regarded as high-level expression. A combined score of cytoplasmic and nuclear staining was then given for pAkt and DJ-1 to investigate the overall expression of these two markers on ESCC progression. High level of both cytoplasmic and nuclear staining was graded as having a high combined score,

high level of cytoplasmic and low level of nuclear staining or vice versa was graded as having an intermediate combined score, and low level of both cytoplasmic and nuclear staining was graded as having a low combined score. As cytoplasmic expression of Daxx was similar across the entire cohort, statistical analysis of cytoplasmic Daxx expression was not carried out and a combined score was not assigned.

Statistical Analysis. Statistical analysis was done using SPSS 15.0. Differences in expression level among different clinicopathologic stages were analyzed by χ^2, Mann-Whitney U, or Fisher's exact tests where applicable. The association between the expression level of DJ-1 and the risk to develop distant metastasis within 1 year of esophagectomy was estimated by odds ratio and 95% confidence interval (95% CI) by using binary logistic regression analysis. Associations of the expression between different markers were analyzed by Spearman's rank test. Kaplan-Meier analysis was done to correlate between the expression of different markers and patient survival and the log-rank test was applied to test whether the correlation is statistically significant. Cox regression analysis was done to identify independent factors that could predict patient survival. Univariate Cox regression analysis was done for all the clinicopathologic variables including age, sex, TNM stage, tumor differentiation, cytoplasmic and nuclear expressions, and combined score of DJ-1, nuclear expression of Daxx, and combined score of pAkt. In multivariate Cox regression analysis, backward stepwise selection was used with an entry limit of $P < 0.1$ and a removal limit of P value of 0.05. $P < 0.05$ was considered significant in all statistical analyses.

Results

DJ-1 Expression in Different Esophageal Specimens. As shown in Figs. 1 and 2A, the cytoplasmic expressions of DJ-1 in both ESCC primary tumors and lymph node metastases were significantly increased when compared with nonneoplastic esophageal epithelium specimens ($P < 0.001$ and $P = 0.001$, respectively). There was no significant difference in cytoplasmic expressions of DJ-1 between ESCC primary tumors and lymph node metastases. Nuclear expression of DJ-1 was observed in all three types of esophageal specimens. We observed an increase of nuclear DJ-1 expression in ESCC primary tumors compared with nonneoplastic esophageal epithelium specimens, but the difference did not reach statistical significant level ($P = 0.059$). There were no differences in nuclear DJ-1 expression between nonneoplastic esophageal epithelium specimens and ESCC lymph node metastases and between ESCC primary tumor and lymph node metastases (Fig. 2B).

Correlation of DJ-1 Expression and Clinicopathologic Variables of ESCC Patients. We observed that more M_1-stage ESCC tumor stained with a high level of nuclear DJ-1 compared with M_0-stage ESCC tumor. However, the result was not statistically significant ($P = 0.064$). On the other hand, significantly more ESCC primary tumors of high distant metastatic potential had a high level of nuclear DJ-1 expression (46%, 11 of 24) compared with ESCC primary tumors of low distant metastatic potential (20%, 10 of 51; $P = 0.022$; Fig. 3A). By binary logistic regression, we found that patients with primary ESCC expressing high level of nuclear DJ-1 were more likely to develop distant metastasis within 1 year of esophagectomy (odds ratio, 3.469; 95% CI, 1.203-10.009; $P = 0.021$). These results suggest that a high level of nuclear DJ-1 expression might promote distant metastasis in ESCC. However, either the cytoplasmic DJ-1 expression or the combined DJ-1 score did not significantly correlate with metastatic status in our patient cohort ($P = 0.424$ and 0.396, respectively).

In addition, we observed significantly higher cytoplasmic expression of DJ-1 in T_4-stage ESCC tumors compared with tumors of other T stages ($P = 0.029$; Fig. 3B). There were 82% (14 of 17) T_4-stage tumors that stained

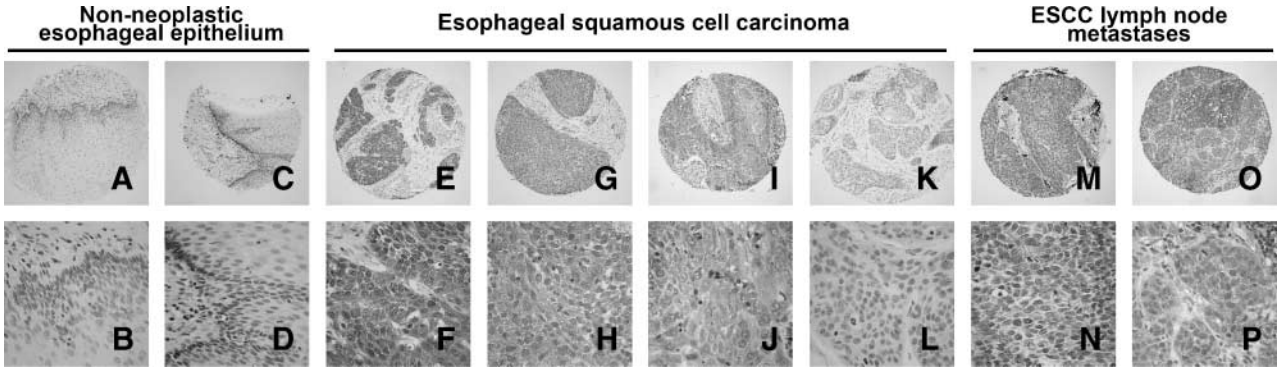


Figure 1. Representative results of immunohistochemical staining of DJ-1. *Top,* tissue cores at ×100 magnification; *bottom,* tissue cores at ×400 magnification. **A** and **B.** A nonneoplastic esophageal epithelium specimen shows negative staining of DJ-1 in both cytoplasmic and nuclear regions. **C** and **D.** A nonneoplastic esophageal epithelium specimen shows negative cytoplasmic and a high level of nuclear staining of DJ-1. **E** and **F.** An ESCC specimen shows high-level staining of DJ-1 in both cytoplasmic and nuclear regions. **G** and **H.** An ESCC specimen shows a moderate cytoplasmic and a strong nuclear staining of DJ-1. **I** and **J.** An ESCC specimen shows a high level of cytoplasmic and a low level of nuclear staining of DJ-1. **K** and **L.** An ESCC specimen shows a low level of DJ-1 staining in both cytoplasmic and nuclear regions. **M** and **N.** An ESCC lymph node metastasis specimen shows a high-level staining in both cytoplasmic and nuclear regions. **O** and **P.** An ESCC lymph node metastasis specimen shows a high level of cytoplasmic staining and a low level of nuclear staining of DJ-1.

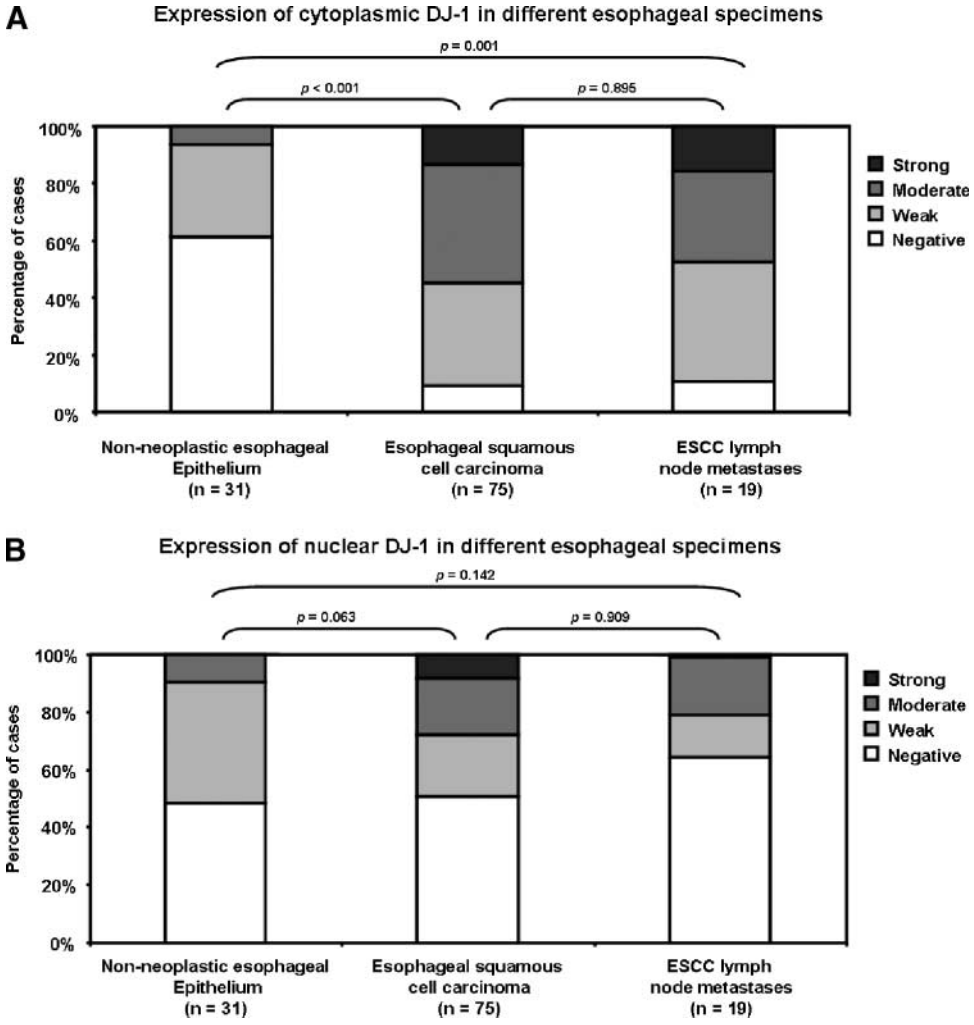


Figure 2. Summary of cytoplasmic and nuclear expression of DJ-1 in different esophageal specimens. **A.** Cytoplasmic staining of DJ-1 was significantly higher in both ESCC and lymph node metastases compared with nonneoplastic esophageal epithelium specimens. **B.** DJ-1 nuclear staining was not significantly different among the three types of esophageal specimens.

a high level of cytoplasmic DJ-1 compared with merely 47% of ESCC tumors of other T stages (27 of 58). We also found that tumors of T_4 stage had a significantly higher level of combined score of DJ-1 compared with those of other tumor stages ($P = 0.031$; Fig. 3C). Nuclear expression of DJ-1 was not different among specimens of different T stages ($P = 0.467$). Taken together, these results suggest that DJ-1 plays an important role in ESCC progression.

Our results showed that DJ-1 expression did not correlate with other clinicopathologic variables such as patient age, sex, N stage, pTNM pathologic staging, and degree of tumor differentiation. (Detailed results are shown in Supplementary Tables S1 to S3).

High Level of Nuclear DJ-1 Could Predict Patient Survival. High-level expression of nuclear DJ-1 was associated with a shorter mean patient survival (12.2 $\pm$ 1.6 months), whereas low level of nuclear DJ-1 was associated with a longer mean survival (23.9 $\pm$ 3.5 months). Figure 4A shows the Kaplan-Meier analysis of survival according to the level of expression of nuclear DJ-1 in the tumor cells ($P = 0.028$, log-rank test). When DJ-1 was analyzed as a possible differential prognostic factor in subgroups of our ESCC patient, we found that high level of nuclear DJ-1 also predicted a poorer survival in patients with N_1-stage ESCC ($P = 0.007$; mean survival: high level = 9.7 $\pm$ 1.6 versus low level = 26.9 $\pm$ 4.6; Fig. 4B) with pathologic stage III/IV ESCC ($P = 0.024$; mean survival: high level = 9.6 $\pm$ 1.6 versus low level = 22.7 $\pm$ 4.3; Fig. 4C) and with moderate/poor differentiated ESCC ($P = 0.003$; mean survival: high level = 11.6 $\pm$ 1.7 versus low level = 26.6 $\pm$ 4.1; Fig. 4D). These results suggest that DJ-1 might be a prognostic factor independent of these clinicopathologic variables. Considering the whole patient cohort, combined DJ-1 score was not significantly associated with patient survival ($P = 0.177$). However, significant associations were observed when the combined DJ-1 score was used in Kaplan-Meier analysis in groups of patients stratified by their clinicopathologic characteristics. In N_1-stage tumors, higher levels of DJ-1 were significantly correlated with shorter survival of patients ($P = 0.035$; mean survival: low level = 30.8 $\pm$ 7.1, intermediate level = 17.8 $\pm$ 3.3, and high level = 9.387 $\pm$ 1.9). Combined DJ-1 score was also a negative predictor in patients with a moderately/poorly differentiated tumor ($P = 0.028$; mean survival:

low level = 26.7 ± 5.4, intermediate level = 19.8 ± 2.7, and high level = 10.7 ± 2.2).

DJ-1 Expression Is Associated with Activated PI3K/Akt Pathway. To elucidate how DJ-1 might be involved in ESCC progression, we investigated the correlation between the expression of DJ-1 and its possible downstream effectors, including pAkt and Daxx. Previously, Kim et al. (13) showed that DJ-1 activates phosphorylation of Akt and thereby activating the PI3K pathway. In this study, we performed the immunohistochemistry on pAkt in our ESCC patient cohort. As shown in Fig. 5A, although combined score of pAkt was not significantly associated with any of the clinicopathologic data (Supplementary Table S4) and survival ($P = 0.727$), it was significantly associated with nuclear expression of DJ-1 ($r = 0.308$; $P = 0.007$) or combined score of DJ-1 ($r = 0.301$; $P = 0.009$). The activation of PI3K pathway by DJ-1 has also been described in other cellular context (17, 20, 21). These results suggest that DJ-1 might promote ESCC progression through activating the PI3K pathway.

Nuclear Expression of DJ-1 Was Associated with Nuclear Expression of Daxx. Recently, DJ-1-mediated apoptosis has been shown to be attributed by its ability to interact with Daxx, the death-associated protein, and retain Daxx in nuclear localization (6, 22). In this

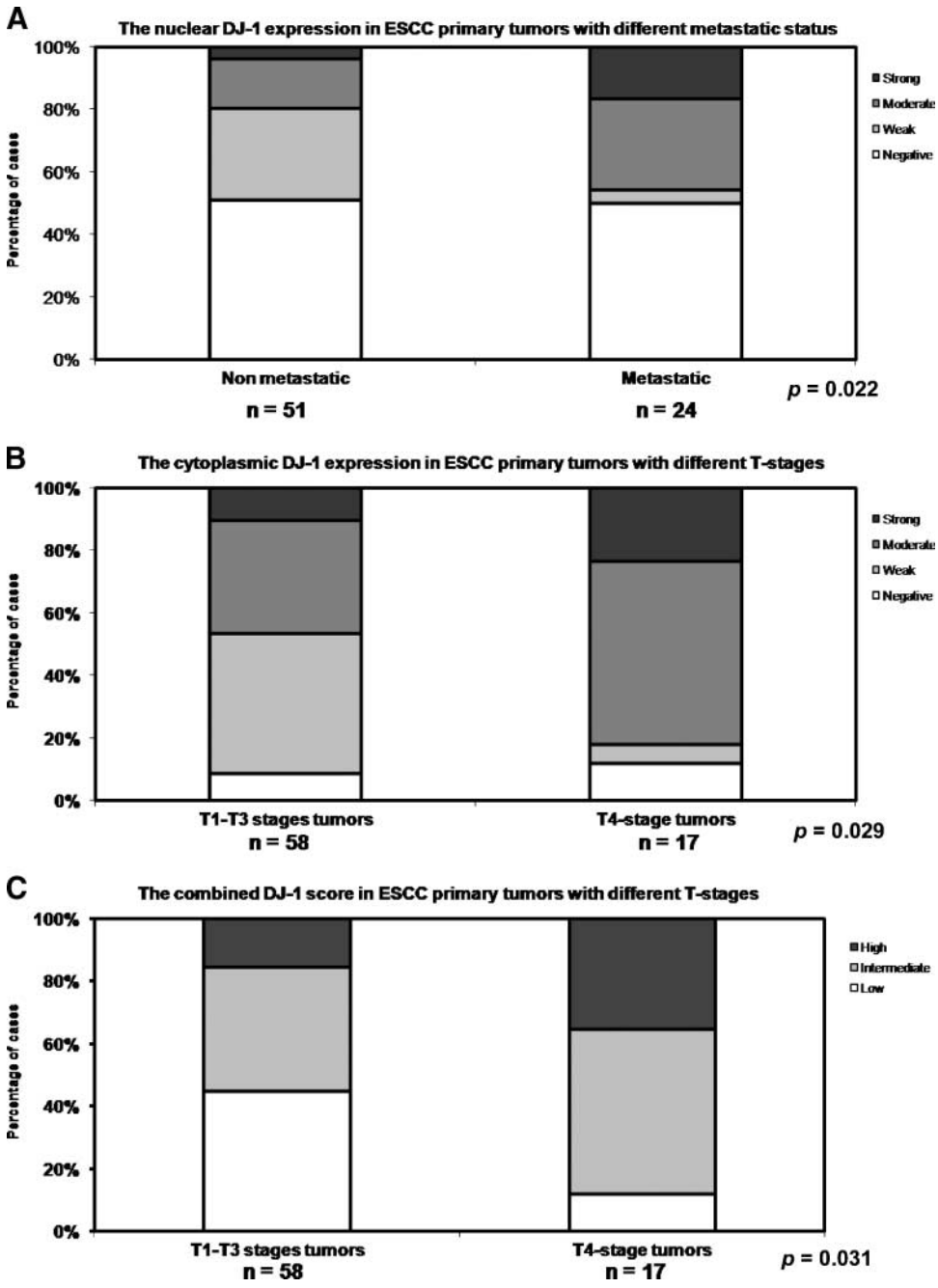


Figure 3. Summary of correlation between DJ-1 expressions and clinicopathologic variables of ESCC patients. **A.** Significantly more ESCC with high distant metastatic potential stained with a high level of nuclear DJ-1 compared with nonmetastatic ESCC. **B.** Significantly more T_4-stage ESCC stained with a high level of cytoplasmic DJ-1 compared with tumors of other T stages. **C.** Significantly more T_4-stage ESCC stained with high level of combined score of DJ-1 compared with tumors of other T stages.

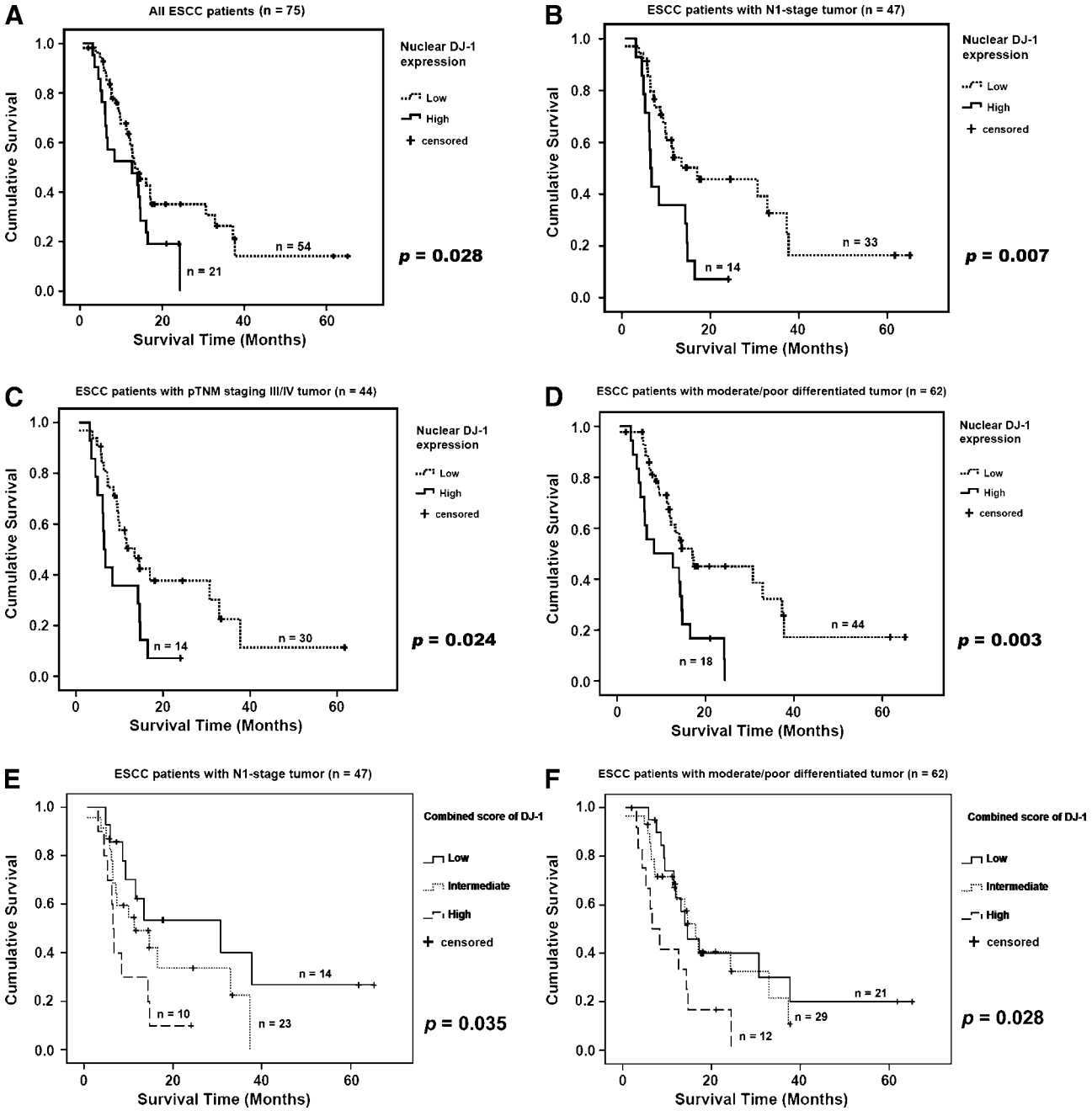


Figure 4. Kaplan-Meier analysis of the significance of DJ-1 in predicting patient survival. **A.** Patients with their primary ESCC stained a high level of nuclear DJ-1 had a significantly shorter survival. **B.** A high level of nuclear expression of DJ-1 in N1 primary tumor specimens is significantly correlated with a poor patient survival. **C.** A high level of nuclear expression of DJ-1 in pTNM stage III/IV primary tumors is significantly correlated with a poor patient survival. **D.** A high level of nuclear expression of DJ-1 in moderately or poorly differentiated ESCC is significantly correlated with a poor patient survival. **E.** Higher combined DJ-1 score significantly correlated with poorer patient survival in patients with N_1-stage tumor. **F.** High combined DJ-1 score significantly correlated with poor patient survival in patients with moderately or poorly differentiated tumor.

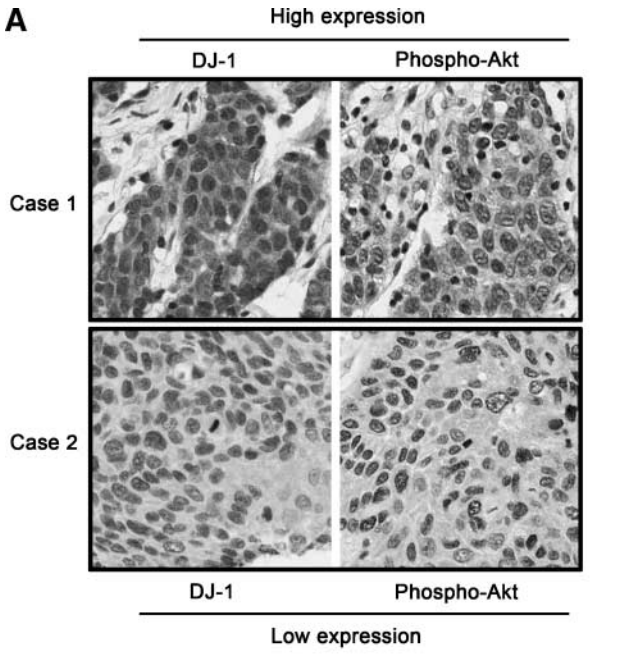
study, we found that nuclear DJ-1 (r = 0.296; P = 0.01) but not cytoplasmic DJ-1 (r = 0.079; P = 0.501) or combined DJ-1 score (r = 0.244; P = 0.057) was significantly associated with nuclear expression of Daxx (Fig. 5B). These results suggest that, in ESCC, nuclear DJ-1 might function to sequester Daxx through interacting with and retaining Daxx in a nuclear localization, thereby inhibiting its function to activate apoptosis through mediating Fas-mediated apoptosis. Nuclear expression of Daxx was neither significantly

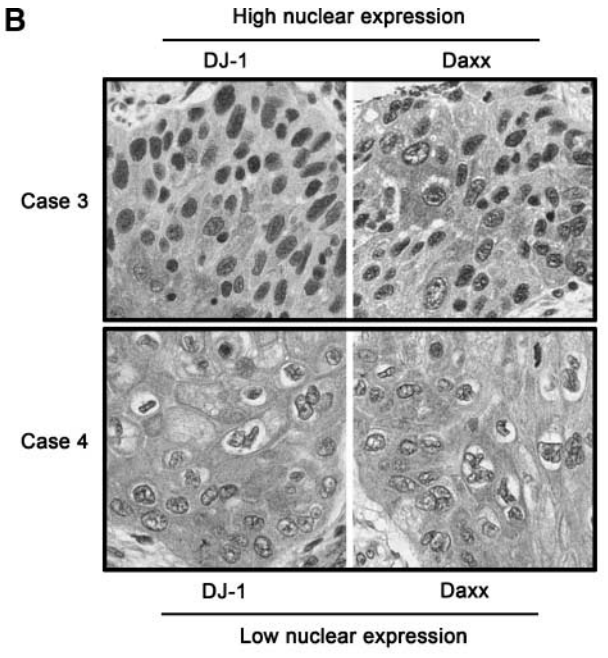
associated with any clinicopathologic variables (Supplementary Table S5) nor survival of our patients ($P = 0.388$).

DJ-1 Is an Independent Predictor for ESCC Patient Survival. By Cox regression univariate analysis (Table 2), we found that patients with a high level of nuclear DJ-1 expression had shorter survival (hazard ratio, 1.900; 95% CI, 1.062-3.399; $P = 0.031$). Apart from nuclear DJ-1 expression, only T_4 stage and sex, but not other clinicopathologic variables, could significantly predict poorer survival in our ESCC patients cohort (T_4 stage: hazard ratio, 2.529; 95% CI, 1.373-4.658; $P = 0.003$; sex: hazard ratio, 2.076; 95% CI, 1.039-4.149). By multivariate analysis (Table 2), we found that only T_4 stage ($P = 0.003$) and high nuclear DJ-1 expression ($P = 0.034$) are independent predictors for poorer survival in our ESCC patient cohort. Taken together, these results suggest that DJ-1 might be a useful prognostic marker for ESCC.

Discussions

DJ-1 has been shown previously to be expressed in both nuclear and cytoplasmic region (2, 23). DJ-1 might have different functions in different cellular compartments. For example, the localization of DJ-1 in mitochondria is important for its antioxidative function (24-26). Nuclear localization of DJ-1 is also important for it to carry out certain functions, such that it sequesters Daxx in the nucleus to prevent Daxx-mediated apoptosis (6). Nuclear localized DJ-1 might interact with other transcription cofactors to modify their transcription activity (4). In prostate cancer, the subcellular localization of DJ-1 is





Combined score of DJ-1 versus combined score of pAkt of the ESCC specimens

pAkt combined score	DJ-1 combined score: Low	Intermediate	High	Total
	n (%)	n (%)	n (%)	n (%)
Low	13 (65)	6 (30)	1 (5)	20 (100)
Intermediate	11 (28)	18 (45)	11 (28)	40 (100)
High	3 (21)	8 (57)	3 (21)	14 (100)

Nuclear expression of DJ-1 versus nuclear expression of Daxx of the ESCC specimens

Nuclear Daxx	Nuclear DJ-1: Negative	Weak	Moderate	Strong	Total
	n (%)	n (%)	n (%)	n (%)	n (%)
Negative	7 (88)	0 (0)	1 (13)	0 (0)	8 (100)
Low	6 (75)	0 (0)	1 (13)	1 (13)	8 (100)
Moderate	13 (65)	4 (20)	2 (10)	1 (5)	20 (100)
High	14 (36)	10 (26)	11 (28)	4 (10)	39 (100)

Figure 5. DJ-1 expression is positively correlated with pAkt and Daxx expression. **A.** Combined score of DJ-1 was positively correlated with combined score of pAkt ($r = 0.301$; $P = 0.009$). Two representative cases are presented with the uppercase showing high combined score of both DJ-1 and pAkt, whereas the lowercase showing low combined score of both DJ-1 and pAkt. A cross-tabulation of combined DJ-1 and pAkt score in our ESCC patient cohort is presented underneath the two representative cases. These results suggest that DJ-1 might promote ESCC progression through activation of PI3K pathway. **B.** Nuclear DJ-1 expression, but not cytoplasmic or combined score of DJ-1, was significantly correlated with nuclear Daxx expression ($r = 0.296$; $P = 0.01$). Two representative cases are presented with the uppercase showing high level of nuclear expression of DJ-1 and Daxx, whereas the lowercase showing low level of nuclear expression of DJ-1 and Daxx. A cross-tabulation of nuclear expression of DJ-1 and Daxx is presented underneath the two representative cases. These results suggest that DJ-1 might sequester Daxx in the nuclear region and inhibiting its function on apoptosis.

Table 2. Cox regression analyses of overall survival

Clinicopathologic variables	Univariate analysis		Multivariate analysis	
	Risk ratio (95% CI)	*P*	Risk ratio (95% CI)	*P*
Age (n = 81)	1.020 (0.996-1.045)	0.103		
Sex				
Female (n = 19)	1	Reference		
Male (n = 62)	2.076 (1.039-4.149)	0.039		
T stage				
$T_1/T_2/T_3$ (n = 64)	1	Reference	1	Reference
T_4 (n = 17)	2.503 (1.372-4.565)	0.003	2.503 (1.362-4.601)	0.003
M stage				
M_0 (n = 68)	1	Reference		
M_1 (n = 13)	1.848 (0.948-3.602)	0.071		
N stage				
N_0 (n = 30)	1	Reference		
N_1 (n = 51)	1.091 (0.629-1.892)	0.757		
pTNM staging				
Stage 0/I/II (n = 32)	1	Reference		
Stage III/IV (n = 49)	1.547 (0.893-2.678)	0.120		
Differentiation				
Well (n = 13)	1	Reference		
Moderate/poor (n = 68)	0.661 (0.340-1.285)	0.222		
Cytoplasmic expression of DJ-1				
Low level (n = 34)	1	Reference		
High level (n = 42)	0.967 (0.558-1.677)	0.906		
Nuclear expression of DJ-1				
Low level (n = 56)	1	Reference	1	Reference
High level (n = 21)	1.864 (1.050-3.310)	0.034	1.864 (1.048-3.314)	0.034
Combine expression of DJ-1				
Low (n = 28)	1	Reference		
Intermediate (n = 32)	0.929 (0.491-1.757)	0.821		
High (n = 15)	1.742 (0.853-3.557)	0.128		
Cytoplasmic expression of pAkt				
Low (n = 32)	1	Reference		
High (n = 48)	1.593 (0.909-2.790)	0.104		
Nuclear expression of pAkt				
Low (n = 56)	1	Reference		
High (n = 24)	0.745 (0.414-1.314)	0.326		
Combine expression of pAkt				
Low (n = 23)	1	Reference		
Intermediate (n = 42)	1.294 (0.686-2.441)	0.426		
High (n = 15)	1.178 (0.521-2.663)	0.694		
Nuclear expression of Daxx				
Low (n = 18)	1	Reference		
High (n = 61)	0.760 (0.406-1.422)	0.390		

regulated by androgens and antiandrogens, which in turn leads to differential binding to androgen receptor (15). These suggest that the subcellular localization of DJ-1 is tightly controlled in order for the protein to carry out its function. Based on these lines of evidence, expression levels of cytoplasmic and nuclear DJ-1 were separately correlated with clinicopathologic variables in the present study.

In this study, we found that cytoplasmic DJ-1 expression was relatively weak in nonneoplastic esophageal epithelium specimens compared with their ESCC counterparts, suggesting that an increase in cytoplasmic DJ-1 might be important in the neoplastic transformation of esophagus. On the other hand, nuclear expression of DJ-1 was significantly higher in specimens with higher metastatic potential, whereas we also observed that nuclear expression of DJ-1, but not its cytoplasmic expression, was a useful prognostic marker for ESCC. These results suggest that nuclear DJ-1 might play a more important role in ESCC progression compared with cytoplasmic DJ-1. These results also imply that DJ-1 in different cellular compartments might play different roles in ESCC tumorigenesis and progression. Further investigations are required to reveal how subcellular localization of DJ-1 is controlled and how this affects its activity in promoting ESCC tumorigenesis and progression.

DJ-1 functions to promote carcinogenesis through inhibition of PTEN, a potent tumor suppressor, which inhibits the survival pathway, PI3K pathway (13). Expression of DJ-1 rescues PTEN mutation while also inversely correlates with PTEN expression (13). These results suggest that DJ-1 might promote carcinogenesis through activation of PI3K pathway. Activation of Akt kinases, which are inhibited by PTEN, through PI3K on overexpression of epidermal growth factor receptor has been described in esophageal cancer cells (27). The activation of Akt by epidermal growth factor results in modulation of the proliferation and differentiation of esophageal epithelial cells (28). Activation mutation of PI3K p110α catalytic subunit has also been reported in ESCC (29). Previously, we have shown that Id-1 is correlated with development of distant metastasis in human ESCC specimens (19), whereas

Id-1 expression in ESCC cells protects them from tumor necrosis factor-α-induced apoptosis through PI3K pathway (30). These lines of evidence suggest that PI3K pathway plays a very important role in ESCC. Further investigations on how DJ-1 modulates the activity of PI3K pathway in ESCC might shed light on the functional role of DJ-1 in ESCC initiation and progression.

PTEN mutation is one of the mechanisms that result in activation of PI3K pathway, which is involved in the carcinogenesis of certain types of tumor (31). However, the frequency of PTEN mutation in many types of cancer is not as high as thought previously. In ESCC, PTEN mutation was only detected in 1 of 33 human ESCC specimens, whereas the mutation was located in intron 7 rather than the mutation hot spot region, exon 5 (32). However, another study have shown that negative or decreased nuclear expression of PTEN is a predictor for poor survival of ESCC patients, suggesting that decreased expression of PTEN might be an important event in ESCC progression (33). This result was further confirmed by another group studying a Chinese ESCC patient cohort (34). As PTEN mutation is not a common event in ESCC, whereas decreased expression of PTEN is correlated with poor survival of ESCC patients, it is possible that PTEN is suppressed in ESCC by mechanisms other than PTEN mutation, such as DJ-1 up-regulation. In this study, we found that DJ-1 expression in our ESCC tumor specimens was positively correlated with expression of pAkt, suggesting that an increased DJ-1 expression in ESCC might result in activation of PI3K pathway, thereby bypassing PTEN inhibition. This scenario has been described previously, such that DJ-1 plays its oncogenic role in a PTEN-dependent context (13).

Previously, DJ-1 has been shown to protect cells from apoptosis through sequestering Daxx in the nucleus, thereby inhibiting Daxx-induced apoptosis (6, 22). In the present study, we found that nuclear, but not cytoplasmic, expression of DJ-1 was positively associated with nuclear expression of Daxx, suggesting that DJ-1 might promote ESCC progression through the same mechanism. Interestingly, we have shown previously the importance of Fas-mediated apoptosis in ESCC progression (35). Taken together, our result suggests that DJ-1 might protect ESCC cells from apoptosis through sequestering Daxx in the nucleus region and thereby inhibiting Fas-mediated apoptosis.

The secretion of DJ-1 into serum in neoplastic and nonneoplastic diseases has been reported (12, 36). A potential use of serum DJ-1 as a tumor marker has been suggested in some cancers (12, 37). Together with the results in the present study, serum DJ-1 is an attractive target to be studied for its potential use as a noninvasive biomarker for ESCC progression and prognosis.

Taken together, our findings show that a high level of nuclear expression of DJ-1 in primary ESCC tumor predicts poor prognosis of ESCC patients and further studies are required to delineate the functional roles of DJ-1 in ESCC initiation and progression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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