Evaluation of a novel clinicopathological marker *JK-1* for human oesophageal carcinoma

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KEY MESSAGE

The expression level of *JK-1* in oesophageal squamous cell carcinoma may serve as a clinicopathological marker for tumour differentiation, metastasis, and patient survival.

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Introduction

Oesophageal cancer ranks the ninth most common malignancy and the sixth most frequent cause of cancer death in the world, with geographic variations in incidence and histological subtypes. In China, oesophageal squamous cell carcinoma (ESCC) accounts for >90% of the total incidence of oesophageal cancer; approximately 300 000 new cases of oesophageal cancer are diagnosed every year in the world of which almost half originate in the high-incidence regions of China.[1] According to the Hong Kong Cancer Registry in 2012, ESCC ranked the tenth most frequent cancer death among all other cancers for both sexes. The current treatment modalities for ESCC achieve relatively suboptimal survival and cure rates.[2] To further improve the management of this disease, novel prognostic markers for ESCC need to be identified.

Gene amplification and overexpression have been suggested to be the major genomic aberrations involved in the pathogenesis of ESCC. Previously, our group reported a novel oncogene *JK-1* in ESCC located in the chromosomal region 5p15.1-2; it frequently shows amplification in ESCC and other solid tumours.[3] Our collaborators also reported the overexpression of *JK-1* mRNA in colorectal tumours, providing the first evidence of the significance of *JK-1* mRNA overexpression in gastrointestinal cancer.[4] Nonetheless, there are no studies of *JK-1* protein expression in ESCC or its correlation with clinicopathological features.

Identifying novel histopathologial tumour markers for ESCC is important. Correlating the levels of these protein signals in tissues with the respective clinicopathological features enables better management of the disease. Detection of oncoprotein *JK-1* level in ESCC may improve the current protocols for cancer detection, prognosis, and treatment in the long run. This study may provide the ground work for future application of *JK-1* level detection in other human cancers.

Methods

A total of 303 paraffin-embedded archival paired samples of tumour and non-tumour tissues of ESCC with clinicopathological data collected after oesophagectomy since June 1996 at Queen Mary Hospital, Hong Kong were included for construction of the tissue microarray. The selection of tumour areas was assisted by an experienced histopathologist. Clinicopathological data were available for correlation with the detection of *JK-1* protein level in tissues. Moreover, 16 biopsy samples including one normal control of oesophageal epithelia were collected to assess the feasibiltiy of detecting *JK-1* protein level with dysplastic lesions or tumours.

Dewaxed paraffin sections (8 μm) of the tissue microarray from the ESCC archival cases comprising non-tumours and tumours or oesophageal biopsies were immunostained using the streptavidin-biotin-peroxidase complex method with the use of polyclonal antibody against the *JK-1* protein (Santa Cruz Biotechnology, USA). As pre-treatment, microwave-based antigen retrieval was performed in 10 mM citrate buffer (pH 6.0). Immunoreactions were visualised with diaminobenzidine, and the sections counterstained with 3% methylgreen. The NE1 cell-line blocks prepared from the *JK-1* transfected cells with overexpression of *JK-1* transcripts served as positive controls, and those without *JK-1* transfection as negative controls. In each section, five high-

TABLE 1. The clinicopathological features of the surgical specimens of oesophageal squamous cell carcinoma

Characteristics	All specimens (n=303)*	*JK-1* low expression (n=194)*	*JK-1* high expression (n=109)*	P value
Mean±SD patient age, y	64.19±8.71	63.81±6.19	65.67±7.44	0.476
Sex				0.409
Male	248 (81.8)	160	88	
Female	55 (18.2)	34	21	
Tumour depth				0.595
T1-3	238 (78.5)	157	81	
T4	65 (21.5)	37	28	
Lymph node metastasis				0.261
N0	122 (40.3)	75	47	
N1	181 (59.7)	119	62	
Distant metastasis				0.036
M0	253 (83.5)	156	97	
M1	50 (16.5)	38	12	
TNM stage				0.364
0/I/II	117 (38.6)	73	44	
III/IV	186 (61.4)	121	65	
Differentiation				0.005
Well & moderate	220 (72.6)	151	69	
Poor	83 (27.4)	43	40	

* Data are presented as No. (%) or No., unless otherwise stated

power fields were selected and a total of at least 700 cells were evaluated. The results were expressed as the percentage of cells with positive staining. The intensity of staining was estimated on a scale from 0 to 3 (negative, weak, moderate, and strong). The low expression group had scores of either 0 or 1, and the high expression group had scores of either 2 or 3.

The correlation between the expression level of *JK-1* and the clinicopathological features was analysed. The quantitative differences in protein expression in the different ESCC pathologic loci among *JK-1* positive cases was evaluated using the Chi-square test or Fisher's exact test. The association between *JK-1* protein expression and the patient's clinicopathological features was assessed using the Chi-square test. A P value of <0.05 was considered statistically significant.

Results

The expression level of *JK-1* was associated with tumour features in which the low-expression group was associated with less aggressive tumours with well and moderate differentiation (P=0.005) and absence of distant metastasis (P=0.036) [Table 1]. Survival was shorter in the high-expression than low-expression group (36.09 months vs 61.02 months, P=0.022, Fig]. Moreover, 14 (87.5%) of 16 cases with premalignant epithelia showed high expression of *JK-1*, suggesting the role of *JK-1* in early transformation of oesophageal epithelia (Table 2).

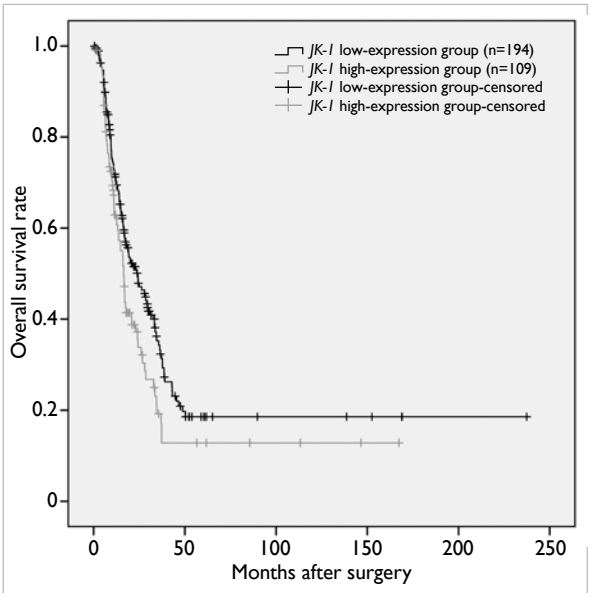

FIG. The overall 5-year survival of patients with oesophageal squamous cell carcinoma is higher in those with low expression than high expression of *JK-1* (P=0.022).

TABLE 2. Histopathological features of the 16 oesophageal biopsy samples

Patient sex/ age, y	Histological features	*JK-1* low expression	*JK-1* high expression
M/57	Squamous cell carcinoma		✓
M/75	Squamous cell carcinoma		✓
M/59	Squamous cell carcinoma		✓
M/59	Squamous cell carcinoma (in situ)		✓
M/72	Squamous cell carcinoma (in situ)		✓
M/76	Squamous cell carcinoma (poorly differentiated)		✓
F/62	Squamous cell carcinoma (moderately differentiated)		✓
M/74	Squamous cell carcinoma (moderately differentiated)		✓
M/57	Squamous cell carcinoma (moderately differentiated)	✓	
M/56	Squamous cell carcinoma (moderately differentiated)		✓
F/62	Minor dysplasia	✓	
M/57	Severe dysplasia		✓
M/76	Severe dysplasia		✓
M/57	Moderate dysplasia		✓
M/76	Moderate dysplasia	✓	
M/57	Normal epithelia	✓	

A similar pattern was observed in the oesophageal biopsy samples, although not statistically viable, in which a high expression of *JK-1* was detected in severe (2 out of 2) and moderate (1 out of 2) dysplastic lesions, whereas 9 out of 10 (90.0%) of the biopsy samples with tumours also showed high expression of *JK-1*.

Discussion

This is the first study to correlate the expression level of *JK-1* with the clinicopathological features of ESCC using the tissue microarray–immunohistochemistry method. Less-aggressive ESCC (well and moderately differentiated) and absence of distant metastasis were correlated with low-expression of *JK-1*, indicating the possibility of functional needs. This finding supports further investigation of the functional roles of *JK-1* in the process of molecular carcinogenesis in ESCC and other tumours, and development of treatments to target the *JK-1* protein using pharmacological or gene-therapy approaches. One example is the development of imatinib (Gilvec) that targets the tyrosine kinase functions of the *bcr-abl* fusion protein.[5] Moreover, the survival was shorter in samples with high-expression than low-expression of *JK-1* (36.09 months vs 61.02 months). High-expression of *JK-1* was also observed in 14 (87.5%) of 16 premalignant epithelia from surgical specimens, severe (2 out of 2) and moderate (1 out of 2) dysplastic lesions from biopsy samples, and 9 (90.0%) of 10 biopsy samples with early tumours. This suggests the possible involvement of *JK-1* in the early transformation of oesophageal epithelia. Similar observations were also reported for the role of DEC1 and connective tissue growth factors (CTGF and CCN2) in ESCC as prognostic markers for early detection.

Limitations of this study are the inaccessibility of a control population and lack of multivariate analysis that would involve observation and analysis of more than one variable at a time. Further studies should include analysis of other oncoproteins such as cyclooxygenase-2 that have been shown to have a close clinicopathological correlation with ESCC.

Conclusion

The findings of the present study provide the first evidence of the prognostic significance of *JK-1* expression in ESCC and may be beneficial to the management of ESCC. Extending the detection of *JK-1* expression in other cancers to study its prognostic significance is warranted.

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Declaration

The authors have no conflicts of interest to disclose.

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