World Journal of Gastroenterology

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DOI: 10.3748/wjg.v22.i44.9803

*World J Gastroenterol* 2016 November 28; 22(44): 9803-9812
ISSN 1007-9327 (print) ISSN 2219-2840 (online)


ORIGINAL ARTICLE

Retrospective Study

# Prognostic implications of *FGFR1* and *MYC* status in esophageal squamous cell carcinoma

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Author contributions: Kwon D and Yun JY contributed equally to this work; Kwon D and Yun JY collected and analyzed the data and wrote the manuscript; Keam B and Kim YT collected the clinical data; Jeon YK supervised and conducted the study; all authors have read and approved the final version of the manuscript.

Supported by the National Research Foundation for the Global Core Research Center, No. 2016005276; and the Korea Health Technology R&D Project through the Korea Health Industry Development Institute, No. HI14C0069.

Institutional review board statement: This study was reviewed and approved by Institutional Review Board of SNUH (H-1405-055-579).

Conflict-of-interest statement: The authors declare no conflict of interests.

Data sharing statement: No additional data are available.



Manuscript source: Unsolicited manuscript

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Received: August 21, 2016
Peer-review started: August 24, 2016
First decision: September 5, 2016
Revised: September 20, 2016
Accepted: September 28, 2016
Article in press: September 28, 2016
Published online: November 28, 2016

## Abstract

### *AIM*

To investigate the clinicopathological features and prognostic implications of combined MYC and fibroblast growth factor receptor 1 (*FGFR1*) status in esophageal squamous cell carcinomas (ESCCs).

### *METHODS*

All patients with ESCC ($n$ = 180) underwent surgical resection at Seoul National University Hospital sometime between 2000 and 2013. A tissue microarray was constructed using cores obtained from representative tumor areas of formalin-fixed, paraffin-embedded tissue blocks. *FGFR1* and *MYC* copy numbers were quantified using fluorescence *in situ* hybridization. The level of MYC expression was determined using immunohistochemistry. *FGFR1* and *MYC* amplification status was compared between primary and metastatic lymph nodes. Univariate and multivariate survival analyses were performed according to adjuvant therapy status.

***RESULTS***

*FGFR1* and *MYC* amplifications were observed in 21.4% (37/173) and 54.2% (91/168) of patients, respectively, while MYC expression was observed in 58.9% (106/180) of patients. There was a positive correlation between *MYC* amplification and overexpression ($P$ = 0.002). Although *FGFR1* amplification was not associated with *MYC* amplification or expression, 12.3% (20/163) of patients exhibited both *FGFR1* amplification and MYC expression. There was also a correlation in *FGFR1* amplification status between matched primary tumors and metastatic lymph nodes ($P$ < 0.001). MYC expression was higher in ESCCs with pT1 ($P$ < 0.001) and in those with no lymph node metastasis ($P$ = 0.023). MYC expression was associated with prolonged disease-free survival ($P$ = 0.036) and overall survival (OS) ($P$ = 0.017) but was not an independent prognostic factor. *FGFR1* amplification was an independent predictor for prolonged OS in all patients ($P$ = 0.029) and in those who did not receive adjuvant therapy ($P$ = 0.013). Combined *FGFR1* amplification and MYC expression predicted better OS in patients who did not receive adjuvant therapy ($P$ = 0.034) but not in those who did receive adjuvant therapy.

***CONCLUSION***

*FGFR1* amplification and MYC expression have prognostic implications in resected ESCCs with respect to adjuvant therapy. The role of FGFR1-targeted therapy in ESCC remains to be explored.

**Key words:** Receptor tyrosine kinase; Fibroblast growth factor receptor 1; MYC; Esophageal squamous cell carcinoma; Gene amplification; Prognosis; Fluorescent *in situ* hybridization



**Core tip:** MYC expression, together with fibroblast growth factor receptor 1 (*FGFR1)* amplification, was reported to modulate oncogenic transformation. We evaluated both *FGFR1* and MYC statuses in patients with resected esophageal squamous cell carcinoma (ESCC). *FGFR1* and *MYC* amplifications were observed in 21.4% and 54.2% of patients with ESCC, respectively, while 12.3% exhibited both *FGFR1* amplification and MYC expression. MYC expression and *FGFR1* amplification were significantly associated with prolonged survival. Combined *FGFR1* amplification and MYC expression was a predictor of better survival in patients who did not receive adjuvant therapy, but not in those who did. As such, *FGFR1* and MYC might have prognostic implications in resected ESCCs with respect to adjuvant therapy.

Kwon D, Yun JY, Keam B, Kim YT, Jeon YK. Prognostic implications of *FGFR1* and *MYC* status in esophageal squamous cell carcinoma. *World J Gastroenterol* 2016; 22(44): 9803-9812 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i44/9803.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i44.9803

## INTRODUCTION

Esophageal cancer is the eighth most common and the sixth leading cause of cancer-related mortality worldwide[1]. Esophageal squamous cell carcinoma (ESCC) accounts for the majority of esophageal cancers. Recently, genomic and molecular alterations have been discovered in ESCC, including activation of the receptor tyrosine kinase (RTK) pathway, cell cycle dysregulation, activation of Wnt and Notch signaling pathways and epigenetic modifications[2,3]. However, molecular targeted therapy for ESCC remains to be established[4].

Fibroblast growth factor receptors (FGFRs) are RTKs expressed in many different cell types and regulate cell proliferation, differentiation and survival. FGFRs also have oncogenic roles in many cancers[5,6]. In contrast, the FGFR signaling pathway can act as a tumor suppressor by promoting cell differentiation, regulating other oncogenic pathways, protecting cells from injury, or mediating immune surveillance[5,7]. *FGFR1* is one of the most frequently amplified genes in ESCC[2,8,9]. Additionally, new drugs targeting *FGFR* and its related pathways, including multi-kinase inhibitors and selected *FGFR* inhibitors, have been introduced for cancer treatment[5,10]. However, the prognostic significance of *FGFR1* amplification in patients with ESCC remains controversial[11,12].

Pulmonary squamous cell carcinoma (SCC) is another cancer frequently showing *FGFR1* amplification. Reportedly, *MYC* expression together with *FGFR1* amplification regulates oncogenic transformation of *FGFR1* and modulates responses to *FGFR* inhibitors in pulmonary SCC[13,14]. Those studies showed that *FGFR1*, located at 8p12, and *MYC*, located at 8q24, were frequently co-amplified in pulmonary SCC[13]. MYC plays an important role in cell proliferation and carcinogenesis in many types of cancer[15,16]. Additionally, a potential role of *MYC* as a predictor of the sensitivity to *FGFR* inhibitors in pulmonary SCC requires further investigation. However, the prevalence of *FGFR1* and *MYC* alterations and their relationship have not been addressed in patients with ESCC. Thus, we investigated *FGFR1* amplification and *MYC* amplification and expression in patients with resected ESCC and analyzed their clinicopathological features and prognostic significance.

## MATERIALS AND METHODS

### *Patients and samples*

Patients who underwent surgical resection for ESCC at Seoul National University Hospital (SNUH) from 2000 to 2013 were reviewed. Patients who received neoadjuvant chemo- and/or radiotherapy and those who

**Table 1 Clinicopathological characteristics of patients with esophageal squamous cell carcinoma**

| Variables | | *n* (%) |
|---|---|---|
| Age (yr) | ≤ 60 | 46 (25.6) |
| | > 60 | 134 (74.4) |
| Sex | Male | 169 (93.9) |
| | Female | 11 (6.1) |
| Smoking | No | 28 (15.6) |
| | Yes | 151 (84.4) |
| Histological grade | WD | 35 (19.4) |
| | MD | 119 (66.1) |
| | PD and basaloid | 26 (14.4) |
| Localization | Upper | 7 (3.9) |
| | Middle | 44 (24.9) |
| | Lower | 116 (65.5) |
| | EGJ | 10 (5.6) |
| T | 1a | 16 (8.9) |
| | 1b | 65 (36.1) |
| | 2 | 17 (9.4) |
| | 3 | 78 (43.3) |
| | 4 | 4 (2.2) |
| N | 0 | 92 (51.1) |
| | 1 | 52 (28.9) |
| | 2 | 29 (16.1) |
| | 3 | 7 (3.9) |
| Stage | Ⅰ A | 14 (7.8) |
| | Ⅰ B | 49 (27.2) |
| | Ⅱ A | 21 (11.7) |
| | Ⅱ B | 32 (17.8) |
| | Ⅲ A | 37 (20.6) |
| | Ⅲ B | 19 (10.6) |
| | Ⅲ C | 8 (4.4) |
| Adjuvant therapy | No | 112 (67.8) |
| | Yes | 58 (32.2) |
| *FGFR1* amplification | No amplification | 136 (78.6) |
| | Low amplification | 3 (1.7) |
| | High amplification | 34 (19.7) |
| *MYC* amplification | No amplification | 77 (45.9) |
| | Low amplification | 20 (11.9) |
| | High amplification | 71 (42.3) |
| MYC expression | 0 (none) | 74 (41.1) |
| | 1 (weak) | 54 (30.0) |
| | 2 (moderate) | 41 (22.8) |
| | 3 (strong) | 11 (6.1) |

*FGFR1*: Fibroblast growth factor receptor 1; WD: Well differentiated; MD: Moderately differentiated; PD: Poorly differentiated; EGJ: Esophagogastric junction.

had distant metastasis at the time of surgery were excluded. Finally, 180 total patients participated in this study. Clinical data including demographic features, treatment modalities and outcomes were obtained from medical records by an oncologist (B.K.). Overall survival (OS) was calculated from the date of diagnosis to the date of death from any cause or the last follow-up, and disease-free survival (DFS) was calculated from the date of diagnosis to the date of disease recurrence. Pathological tumor-node-metastasis (TNM) stage was based on the 7$^{th}$ American Joint Committee on Cancer. A tissue microarray was constructed from 2-mm diameter cores obtained from representative tumor areas of formalin-fixed paraffin-embedded tissue blocks and submitted for fluorescence *in situ* hybridization (FISH) and immunohistochemistry (IHC). This study followed the World Medical Association Declaration of Helsinki recommendations and was approved by the Institutional Review Board of SNUH (H-1405-055-579).

### *FGFR1* and *MYC* FISH

To evaluate *FGFR1* and *MYC* gene copies, FISH was performed using Vysis LSI *FGFR1* SpectrumRed probe, Vysis LSI c-MYC (8q24.12-q24.13) SpectrumOrange probe and Vysis CEP8 (D8Z2) SpectrumGreen probe as a chromosome enumeration probe (Abbott Molecular, Abbott Park, IL, United States), according to the manufacturer's protocol and as reported previously[17]. The entire tumor area was scanned for hot spots representing increased *FGFR1* copy numbers. Random areas were selected for evaluation if the signals were distributed homogeneously. A minimum of 60 non-overlapping tumor nuclei were counted for the number of *FGFR1* and CEP8 signals. *FGFR1* gene copy status was classified according to the criteria proposed by Schildhaus *et al*[18]. In brief, high-level *FGFR1* amplification was defined as follows: (1) an *FGFR1*/CEP8 ratio ≥ 2; (2) ≥ 15 *FGFR1* signals in ≥10% of tumor cells; or (3) average number of *FGFR1* signals/cell ≥ 6. Low-level *FGFR1* amplification was defined as ≥ 5 *FGFR1* signals/cell in ≥ 50% of tumor cells. The same methods and criteria were applied to evaluate *MYC* gene status.

### IHC

To evaluate MYC expression, IHC was performed using a rabbit monoclonal anti-*c-MYC* antibody (EP121, Cell Marque, Rocklin, CA, United States) and the Benchmark XT autostainer (Ventana Medical Systems, Tucson, AZ, United States). MYC expression was evaluated using a four-tier scoring system as follows: 0, none or any staining in < 10% of cells; 1, weak; 2, moderate; and 3, strong staining in ≥ 10% of tumor cells. Cases with a score of 1-3 were considered to express MYC.

### Statistical analysis

Statistical analysis was performed using SPSS (version 22.0; IBM Corp., Armonk, NY, United States). Differences between *FGFR1* or *MYC* status and clinicopathological variables were determined using Fisher's exact test or Student's *t*-test. The Kaplan-Meier method with the log-rank test and Cox proportional hazards regression analysis were used for survival analyses. Two-sided *P*-values < 0.05 were considered to indicate statistical significance.

## RESULTS

### Clinicopathological characteristics of patients with ESCC

The clinicopathological features of 180 patients with resected ESCC are summarized in Table 1. Briefly, the

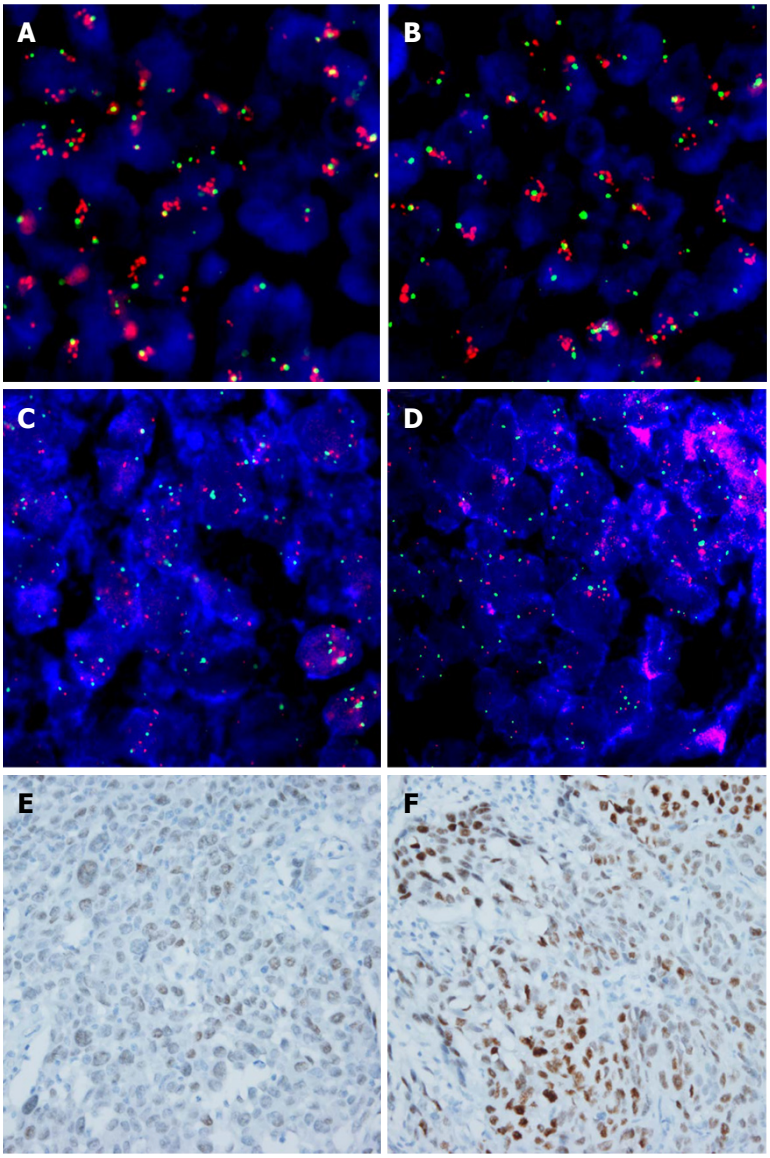



**Figure 1 Representative images of fluorescence *in situ* hybridization and immunohistochemistry for fibroblast growth factor receptor 1 and *MYC* in esophageal squamous cell carcinoma.** A and B: High amplification of *FGFR1* with increased gene copies of *FGFR1* (red signal), compared to chromosome 8 (CEP8, green signal), was observed; C and D: High amplification of *MYC* with increased gene copies of *MYC* (orange signal), compared to CEP8 was observed; E and F: MYC immunohistochemistry in the nuclei of tumor cells; E: Weak, F: Strong intensity (original magnification, × 400). *FGFR1*: Fibroblast growth factor receptor 1.

median age of the patients was 64.76 years (range, 41-83 years), and 74.4% were older than 60 years of age. Most patients were males (93.9%) and former or current smokers (84.4%). pT1b (36.1%) and pT3 (43.3%) diseases were common, and lymph node metastasis was observed in 48.9% of patients. Tumors were frequently localized in the lower esophagus (65.5%).

### *FGFR1 and MYC alterations in ESCC and the associated clinicopathological features*

In ESCCs, *FGFR1* amplification was detected in 21.4% (37/173) of patients (high amplification in 19.7%, $n$ = 34 and low amplification in 1.7%, $n$ = 3; Figure 1A and B). *MYC* amplification was found in 54.2% (91/168) of patients (high amplification in 42.3%, $n$ = 71 and low amplification in 11.9%, $n$ = 20; Figure 1C and D). MYC expression was observed in 58.9% (106/180) of patients (weak expression in 30%, moderate expression in 22.8% and strong expression in 6.1%; Figure 1E and F). *MYC* amplification was positively correlated with MYC expression ($P$ = 0.002; data not shown). *FGFR1* amplification status was not associated with *MYC* amplification or protein expression.

The relationships between *FGFR1* or MYC status and clinicopathological features are summarized in Table 2. ESCC patients with *FGFR1* amplification were younger than those without *FGFR1* amplification (mean ± SD, 62.3 ± 8.4 years versus 65.6 ± 7.4 years, $P$ = 0.022). Other clinicopathological parameters including sex, histological differentiation, smoking status and TNM stage were not significantly correlated with

**Table 2 Correlation among fibroblast growth factor receptor 1 amplification, MYC expression and clinicopathological features[1]**

| Variables | FGFR1, *n* (%) | | | MYC, *n* (%) | | |
|---|---|---|---|---|---|---|
| | No amplification | Amplification | *P* value | No expression | Expression | *P* value |
| Age (yr) | | | | | | |
| ≤ 60 | 30/44 (68.2) | 14/44 (31.8) | 0.058 | 18/46 (39.1) | 28/46 (60.9) | 0.862 |
| > 60 | 106/129 (82.2) | 23/129 (17.8) | | 56/134 (41.8) | 78/134 (58.2) | |
| Smoking | | | | | | |
| No | 21/28 (75) | 7/28 (25) | 0.619 | 12/28 (42.9) | 6/28 (57.1) | 0.836 |
| Yes | 115/145 (79.3) | 30/145 (20.7) | | 61/151 (40.4) | 90/151 (59.6) | |
| Histological grade | | | | | | |
| WD | 29/34 (85.3) | 5/34 (14.7) | 0.350 | 17/35 (48.6) | 18/35 (51.4) | 0.267 |
| MD | 90/117 (76.9) | 27/117 (23.1) | | 43/119 (36.1) | 76/119 (63.9) | |
| PD | 11/16 (68.8) | 5/16 (31.2) | | 10/18 (55.6) | 8/18 (44.4) | |
| Others | 6/6 (100) | 0/6 (0) | | 4/8 (50) | 4/8 (50) | |
| Localization | | | | | | |
| Upper | 6/7 (85.7) | 1/7 (14.3) | 0.981 | 4/7 (57.1) | 3/7 (42.9) | 0.688 |
| Middle | 34/42 (81) | 8/42 (19) | | 20/44 (45.5) | 24/44 (54.5) | |
| Lower | 86/111 (77.5) | 25/111 (22.5) | | 45/116 (38.8) | 71/116 (61.2) | |
| EGJ | 8/10 (80) | 2/10 (20) | | 4/10 (40) | 6/10 (60) | |
| T | | | | | | |
| 1 | 66/80 (82.5) | 14/80 (17.5) | 0.602 | 22/81 (27.2) | 59/81 (72.8) | < 0.001 |
| 2 | 12/16 (75) | 4/16 (25) | | 6/17 (35.3) | 11/17 (64.7) | |
| 3 | 55/73 (75.3) | 18/73 (24.7) | | 43/78 (55.1) | 35/78 (44.9) | |
| 4 | 3/4 (75) | 1/4 (25) | | 3/4 (75) | 1/4 (24) | |
| N | | | | | | |
| 0 | 73/90 (81.1) | 17/90 (18.9) | 0.460 | 30/92 (32.6) | 62/92 (67.4) | 0.023 |
| 1-3 | 63/83 (75.9) | 20/83 (24.1) | | 44/88 (50) | 44/88 (50) | |
| Stage | | | | | | |
| Ⅰ | 51/62 (82.3) | 11/62 (17.7) | 0.694 | 18/63 (28.6) | 45/64 (71.4) | < 0.001 |
| Ⅱ | 40/52 (76.9) | 12/52 (23.1) | | 17/53 (32.1) | 36/53 (67.9) | |
| Ⅲ | 45/59 (76.3) | 14/59 (23.7) | | 39/64 (60.9) | 25/64 (39.1) | |

[1]Differences in the proportions of fibroblast growth factor receptor 1 (*FGFR1*) amplification and MYC expression according to the clinicopathological variables were compared using Fisher's exact test. WD: Well differentiated; MD: Moderately differentiated; PD: Poorly differentiated; EGJ: Esophagogastric junction.

**Table 3 Correlation of fibroblast growth factor receptor 1 and MYC amplification status between the tumors of the primary and the metastatic lymph nodes, *n* (%)**

| | | Metastatic lymph nodes | | Total | *P* value |
|---|---|---|---|---|---|
| | | No amplification | Amplification | | |
| *FGFR1* Primary tumor | No amplification | 42 (91.3) | 3 (6.7) | 45 (100) | < 0.001 |
| | Amplification | 4 (36.4) | 7 (63.6) | 11 (100) | |
| | Total | 46 (82.1) | 10 (17.9) | 56 (100) | |
| *MYC* Primary tumor | No amplification | 17 (63.0) | 10 (37.0) | 27 (100) | 1.000 |
| | Amplification | 12 (60.0) | 8 (40.0) | 20 (100) | |
| | Total | 29 (61.7) | 18 (38.3) | 47 (100) | |

*FGFR1*: Fibroblast growth factor receptor 1.

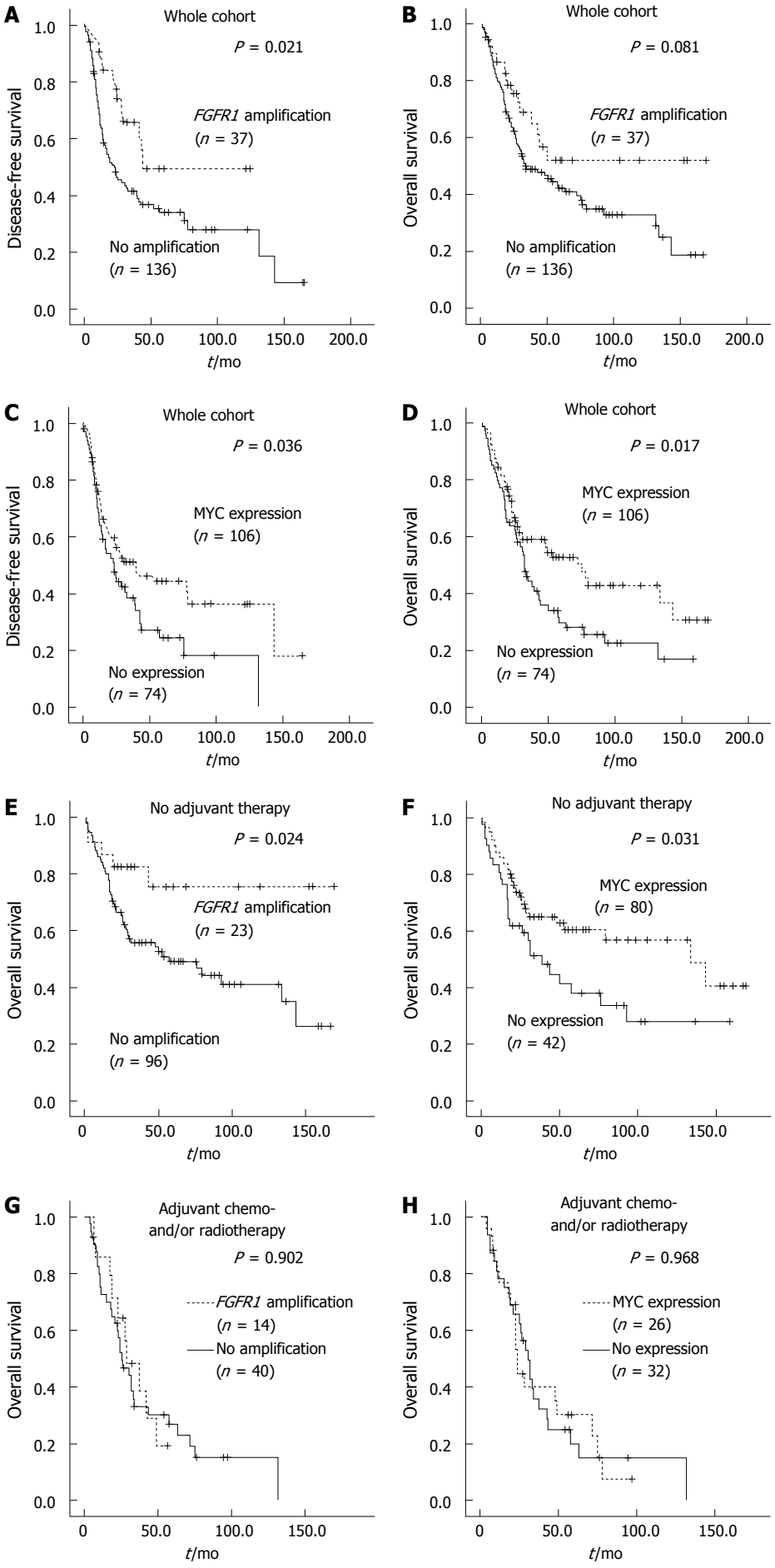
*FGFR1* amplification. In contrast, MYC expression was higher in patients with early pT stage disease ($P$ < 0.001), without lymph node metastasis ($P$ = 0.023) or with early TNM stage disease ($P$ < 0.001). In contrast, *MYC* amplification was not significantly correlated with clinicopathological features.

### Comparison of FGFR1 amplification status between primary and metastatic lesions in regional lymph nodes

*FGFR1* amplification was evaluated in matched primary tumors and metastatic lymph nodes of 56 patients, and a significantly positive correlation was found ($P$ < 0.001; Table 3). Briefly, *FGFR1* amplification in the primary tumor was observed in 11 of 56 cases, and 7 (63.6%) patients also showed *FGFR1* amplification in metastatic tumors of the regional lymph nodes. In contrast, only 3 (6.7%) of the 45 cases who did not have *FGFR1* amplification in the primary tumor exhibited *FGFR*1 amplification in metastatic tumors of the lymph nodes. In contrast, *MYC* gene copy status was not correlated with the primary tumors or metastatic tumors of the lymph nodes (Table 3).

### Prognostic significance of FGFR1 and MYC status in patients with ESCC

The mean and median follow-up times of 180 patients were 43.2 and 29.8 mo (range, 0.6-169.4 mo). The 5-year DFS and OS rates for all patients were 24% and 26%, respectively, depending on the stage as follows: 40.6% and 44.7% in stage Ⅰ, 22.9% and 27.6% in stage Ⅱ and 5.0% and 7.7% in stage Ⅲ, respectively. Kaplan-Meier analysis revealed that DFS of ESCC patients with *FGFR1* amplification was significantly prolonged compared with those without *FGFR1* amplification ($P$ = 0.021; Figure 2A). OS also tended to be longer in patients with *FGFR1* amplification compared with those without *FGFR1* amplification ($P$ = 0.081; Figure 2B). ESCC patients with *MYC*


A
Whole cohort
P = 0.021
FGFR1 amplification
(n = 37)
No amplification
(n = 136)
Disease-free survival
t/mo
B
Whole cohort
P = 0.081
FGFR1 amplification
(n = 37)
No amplification
(n = 136)
Overall survival
t/mo
C
Whole cohort
P = 0.036
MYC expression
(n = 106)
No expression
(n = 74)
Disease-free survival
t/mo
D
Whole cohort
P = 0.017
MYC expression
(n = 106)
No expression
(n = 74)
Overall survival
t/mo
E
No adjuvant therapy
P = 0.024
FGFR1 amplification
(n = 23)
No amplification
(n = 96)
Overall survival
t/mo
F
No adjuvant therapy
P = 0.031
MYC expression
(n = 80)
No expression
(n = 42)
Overall survival
t/mo
G
Adjuvant chemo-
and/or radiotherapy
P = 0.902
FGFR1 amplification
(n = 14)
No amplification
(n = 40)
Overall survival
t/mo
H
Adjuvant chemo-
and/or radiotherapy
P = 0.968
MYC expression
(n = 26)
No expression
(n = 32)
Overall survival
t/mo

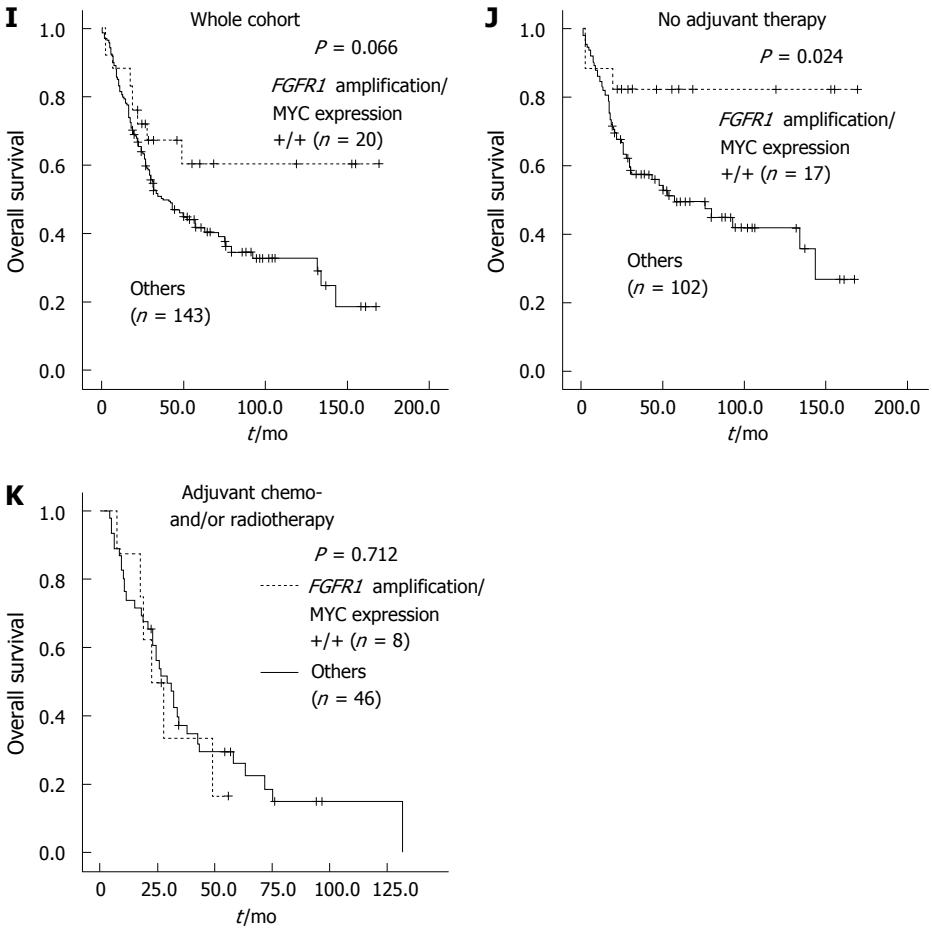



**Figure 2 Kaplan-Meier plots and log rank test results.** Disease-free survival (DFS) and overall survival (OS) in patients with resected esophageal squamous cell carcinoma (ESCC); A and B: According to *FGFR1* amplification; C and D: According to MYC expression status; OS was also plotted according to *FGFR1* amplification and MYC expression statuses; E and F: In patients with ESCC who did not receive adjuvant therapy; G and H: in those with ESCC who received adjuvant therapy; I-K: OS of patients with ESCC according to combined *FGFR1* amplification and MYC expression status was plotted and analyzed in all patients with resected ESCC, as well as in those with and without adjuvant therapy, respectively. *FGFR1*: Fibroblast growth factor receptor 1.

amplification tended to have a longer DFS and OS than did those without *MYC* amplification, but statistically insignificant ($P$ = 0.064 and 0.423, respectively; data not shown). However, patients with MYC expression had a significantly longer DFS ($P$ = 0.036) and OS ($P$ = 0.017) compared with those without MYC expression (Figure 2C and D).

### *Prognostic significance of FGFR1 amplification and MYC expression according to adjuvant chemo- and/or radiotherapy status in patients with ESCC*

Adjuvant chemo- and/or radiotherapy after surgical tumor resection was performed in 58 (32.2%) patients with ESCC. The mean OS of 112 patients who did not receive adjuvant chemo- and/or radiotherapy was 89.5 mo, which was significantly better than the OS of patients who received adjuvant therapy (42.5 mo). The patients who received adjuvant treatment showed a higher pT, nodal metastasis and a higher stage (all $P$ < 0.001). These data suggest that patients given adjuvant therapy have unfavorable clinical features and aggressive biological behavior, leading to adjuvant therapy. Thus, we performed survival analysis in patients with and without adjuvant therapy separately. In patients without adjuvant therapy, *FGFR1* amplification and MYC expression were significantly associated with prolonged OS ($P$ = 0.024 and 0.031, respectively; Figure 2E and F), but not in patients who received adjuvant chemo- and/or radiotherapy (Figure 2G and H).

Multivariate Cox analysis for OS incorporating age, T stage, lymph node metastasis, *FGFR1* amplification and MYC expression revealed that age, T and N stage were independent poor prognostic factors in all patients as well as in both groups of patients with and without adjuvant therapy (Table 4). In contrast, *FGFR1* amplification was found to be an independent favorable prognostic factor in all patients (HR = 0.532 with 95%CI: 0.302-0.937, $P$ = 0.029) and in patients without adjuvant therapy (HR = 0.301 with 95%CI: 0.117-0.774, $P$ = 0.013), but not in patients with adjuvant therapy (Table 4). MYC expression lost its prognostic significance in multivariate Cox analysis (Table 4).

**Table 4 Multivariate analysis for overall survival in patients with esophageal squamous cell carcinoma**

| Variables | Category | Whole cohort | | No adjuvant chemo- and/or radiotherapy | | Adjuvant chemo- and/or radiotherapy | |
|---|---|---|---|---|---|---|---|
| | | HR (95%CI) | *P* value | HR (95%CI) | *P* value | HR (95%CI) | *P* value |
| Age (yr) | ≤ 60 *vs* > 60 | 1.805 (1.102-2.955) | 0.019 | 2.371 (1.088- 5.167) | 0.030 | 1.81 (0.896-3.656) | 0.098 |
| T | 1, 2, 3, 4 | - | 0.005 | - | 0.091 | - | 0.009 |
| | 1 *vs* 2 | 1.204 (0.523-2.773) | 0.663 | 1.589 (0.591-4.269) | 0.358 | 1.260 (0.251-6.324) | 0.779 |
| | 1 *vs* 3 | 2.373 (1.454-3.872) | 0.001 | 2.115 (1.126-3.973) | 0.020 | 4.136 (1.691-10.119) | 0.002 |
| | 1 *vs* 4 | 1.902 (0.550-6.575) | 0.310 | 0.632 (0.078-5.130) | 0.667 | 4.256 (0.820-22.092) | 0.085 |
| N | 0 *vs* 1-3 | 1.981 (1.275-3.077) | 0.002 | 2.351 (1.338-4.133) | 0.003 | 0.319 (0.125-0.814) | 0.017 |
| *FGFR1* amplification | None *vs* Amplification | 0.532 (0.302-0.937) | 0.029 | 0.301 (0.117-0.774) | 0.013 | 0.830 (0.386-1.783) | 0.633 |
| MYC expression | None *vs* Expression | 0.993 (0.636-1.550) | 0.975 | 0.873 (0.478-1.595) | 0.659 | 1.566 (0.811-3.024) | 0.181 |

### *Prognostic significance of combined FGFR1 amplification and MYC expression status in patients with ESCC*

ESCC patients (25/173) with both *FGFR1* amplification and MYC expression (hereafter referred to as combined positivity) exhibited prolonged DFS ($P$ = 0.023; data not shown) and OS ($P$ = 0.066) in Kaplan-Meier analysis (Figure 2I). Combined positivity was significantly associated with longer OS ($P$ = 0.024) in patients who did not receive adjuvant therapy, but not in patients who received adjuvant therapy ($P$ = 0.712; Figure 2J and K). Combined positivity was also shown to be an independent favorable prognostic factor among patients who did not receive adjuvant therapy; this was determined when multivariate Cox analysis for OS was performed and incorporated age, T stage, lymph node metastasis, and combined positivity (HR = 0.275 with 95%CI: 0.083-0.97, $P$ = 0.034; data not shown).

## DISCUSSION

In this study, we comprehensively investigated *FGFR1* amplification and *MYC* amplification and expression in ESCC to elucidate the associated clinicopathological characteristics and explore the potential of FGFR1 and MYC as targets for cancer therapy.

Several previous studies reported the prognostic implication of *FGFR1* amplification in ESCC, but the results were controversial[11,12]. *FGFR1* amplification was associated with poor prognosis or had no prognostic significance in ESCC; however, the FISH criteria for *FGFR1* amplification were not identical[11,12]. In the present study, *FGFR1* amplification was a favorable prognostic indicator in patients with resected ESCC, which was in conflict with a previous report using the same FISH criteria[11]. In a study using FISH and different criteria, *FGFR1* amplification was not associated with clinical outcomes in patients with ESCC[12]. Similarly, in the case of pulmonary SCC, the prognostic implication of *FGFR1* amplification was controversial[19-21]. One study demonstrated that *FGFR1* amplification was an independent favorable prognostic factor in pulmonary SCC and large cell carcinoma[19], which contrasted with another study showing that *FGFR1* amplification was an independent negative prognostic factor in resected pulmonary SCC[20]. Consequently, a recent meta-analysis concluded that *FGFR1* amplification had no influence on the survival of patients with pulmonary SCC[22]. Notably, in this study, the association of *FGFR1* amplification with clinical outcome of resected ESCC patients was dependent on the status of adjuvant therapy; *i.e.*, *FGFR1* amplification was a favorable prognostic factor in patients with ESCC who did not receive adjuvant therapy. Adjuvant therapy after surgery for patients with stage Ⅲ-Ⅳ or lymph node metastasis prolonged survival compared with surgery alone in ESCC[23]. Therefore, adjuvant chemotherapy with or without radiotherapy is increasingly used for the treatment of advanced ESCC, although no definite criteria or regimen for adjuvant therapy has been established in ESCC. In this study, patients with adjuvant chemo- and/or radiotherapy tended to be in the advanced stage compared with those with no adjuvant therapy. Thus, FGFR1 might play variable biological roles during the progression of cancer and thereby have different prognostic significance depending on the stage and subsequent adjuvant therapy status of patients. Otherwise, it could be possible that ESCC with *FGFR1* amplification represents a biologically less aggressive group among ESCCs having variable genetic alterations. This could result in the prolonged survival of patients receiving no adjuvant therapy. FGFR1 could affect the efficacy of chemo- or radiotherapy in patients with ESCC, and thus be differently associated with the prognosis in those receiving adjuvant therapy.

To the best of our knowledge, this is the largest study to evaluate *MYC* status using IHC and FISH in ESCC. Kaplan-Meier analysis demonstrated that MYC expression, but not amplification, was associated with prolonged survival. This result might be contradictory to the role of MYC as an oncogene. In this study, MYC expression was more common in ESCC patients of younger age and in the early TNM stage, and it was not an independent prognostic factor. Thus, the favorable prognosis of patients with ESCC who showed combined *FGFR1* amplification and MYC expression in the group without adjuvant therapy might be due to the association of *FGFR1* amplification with prognosis. Based on this study, MYC status might have little, if any, prognostic implication in patients with ESCC.

However, more studies using a large cohort of patients are needed to validate the prognostic significance of MYC in ESCC.

However, this study had some limitations. First, it was a retrospective study and, as such, the specific regimen of adjuvant therapy may not have been well-controlled. Second, we used a TMA of 2 mm diameter, which may not reflect the intratumoral heterogeneity of FGFR1 and MYC status. However, comparative analysis showed that FGFR1 amplification status was not significantly different between primary and nodal metastatic tumors. In contrast, MYC amplification status was significantly different between these two groups. Third, small groups were compared as a result of subgroup analysis according to the different treatment modalities. Thus, another study using large prospective cohorts is required to validate the prognostic role of FGFR1 amplification in ESCC according to adjuvant therapy status.

Although ESCC is an aggressive cancer with poor clinical outcomes, treatment approaches remain limited, requiring the development of novel strategies including targeted molecular therapy. This study demonstrated that *FGFR1* was amplified in approximately 20% of ESCCs, and moreover, *FGFR1* amplification status was maintained during lymph node metastasis; hence, this group may benefit from therapeutic inhibition of *FGFR1*. *FGFR1* amplification is considered an adequate factor to predict sensitivity to FGFR inhibitors[24]. However, FGFR inhibitors resulted in insufficient clinical responses in patients with *FGFR1*-amplified lung cancer[25,26]. A recent study showed that MYC expression might modulate the sensitivity of *FGFR1*-amplified pulmonary SCC to FGFR1 inhibitors[13]. In that study, 40% of *FGFR1*-amplified pulmonary SCCs expressed high levels of MYC[13], which was similar to our results in that 54.1% (20/37) of *FGFR1*-amplified ESCCs expressed MYC. Among the all patients with resected ESCC, 12.3% (20/163) exhibited both *FGFR1* amplification and MYC expression. Based on the pulmonary SCC study, this population could be a potential candidate for FGFR inhibitor therapy in ESCC patients. The role of therapy targeting *FGFR1* or *MYC* in ESCC remains to be explored by further *in vitro* and clinical studies.

In conclusion, *FGFR1* amplifications were observed in 21.4% of patients and combined *FGFR1* amplification and MYC expression was observed in 12.3% of patients with resected ESCC. *FGFR1* amplification had prognostic implications in patients with resected ESCC with respect to adjuvant therapy. The role of targeted therapy against FGFR1 or MYC in ESCC remains to be explored.

## COMMENTS

### Background

It has been demonstrated that fibroblast growth factor receptor 1 (*FGFR1*) and *MYC* are frequently co-amplified and play a role in neoplastic transformation in pulmonary squamous cell carcinoma (SCC). Moreover, a potential role of MYC as a predictor of the sensitivity to FGFR inhibitors in pulmonary SCC has been reported. Although *FGFR1* and *MYC* alterations have been reported by genomic studies for esophageal squamous cell carcinoma (ESCC), the prevalence of *FGFR1* and *MYC* alterations and their relationship remains to be clarified in patients with ESCC. Thus, we investigated *FGFR1* amplification and *MYC* amplification and expression in patients with ESCC and analyzed their clinicopathological features and prognostic significance.

### Research frontiers

ESCC is one of the leading causes of cancer-related mortality worldwide and novel treatment strategies other than surgery and conventional chemo- and radio-therapy are required to improve clinical outcome. However, molecular targeted therapy for ESCC remains to be established. The results of this study contribute to clarifying the biological role of FGFR1 and MYC, and therapeutic potential of FGFR targeted therapy in patients with ESCC.

### Innovations and breakthroughs

In this study, *FGFR1* amplifications were observed in 21.4% of patients and combined *FGFR1* amplification and MYC expression was observed in 12.3% of patients with resected ESCC. *FGFR1* amplification had prognostic implications in patients with resected ESCC with respect to adjuvant therapy. The role of FGFR1-targeted therapy in ESCC remains to be explored.

### Applications

This study suggests that patients with ESCC harboring combined *FGFR1* amplification and MYC expression might benefit from therapies targeting FGFR1 and/or MYC, especially those with advanced disease requiring adjuvant therapies.

### Terminology

FGFR1 is a receptor tyrosine kinase playing an oncogenic role in many cancers and can be targeted for molecular therapy. MYC is an oncogene and contributes to sensitivity to FGFR inhibitor in pulmonary squamous cell carcinoma (SCC). Fluorescence *in situ* hybridization (FISH) is a tool useful to evaluate the gene amplification using tumor tissues from patients with solid tumor.

### Peer-review

It is a very interesting article presenting novel data on role of FGFR1 and MYC status in ESCC. All parts of the manuscript were composed correctly and they contain suitable information. Tables and figures were constructed appropriately. Statistical analysis of data was performed correctly with using the appropriate tests. All references are actual and relevant to the text of article.

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**P- Reviewer**: Alshehabi Z, Chen XL, Diakowska D, Ishiguro H
**S- Editor**: Gong ZM **L- Editor**: A **E- Editor**: Liu WX

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ISSN 1007-9327