Biomarkers, 2009; 14(7): 480–485

informa
healthcare

ORIGINAL ARTICLE

CYFRA21-1 and CEA are useful markers for predicting the sensitivity to chemoradiotherapy of esophageal squamous cell carcinoma

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Abstract

Background: Chemoradiotherapy (CRT) is currently performed for patients with advanced esophageal carcinoma. Sensitivity of tumours to CRT differs from one case to another and may be influenced by the expression of biological molecules. The aim of this study was to identify biological markers which could predict sensitivities of esophageal squamous cell carcinoma (ESCC) to CRT.

Methods: A total of 84 patients with stage I–IV ESCC were evaluated. The cytokeratin 19 fragment antigen 21-1 (CYFRA21-1) and carcinoembryonic antigen (CEA) levels were measured before CRT by enzyme-linked immunosorbent assays in patients with primary ESCCs using 3.4 ng ml^{-1} and 3.3 ng ml^{-1}, respectively, as cut-off values. The relationships between pretreatment expression of CYFRA 21-1 and CEA and the effectiveness of CRT were analysed.

Results: The complete response (CR) rates of the primary tumours estimated by computed tomography in patients with high levels of CYFRA21-1 and CEA were 10% (3/30) and 4.2% (1/24), while in cases with low CYFRA21-1 and CEA the CR rates were 50% (27/54) and 48.3% (29/60), respectively ($p = 0.002$ and 0.003). The effective rates (CR+PR) in CYFRA21-1 high and low groups were 60% (18/30) and 96.3% (52/54), while in CEA high and low groups they were 58.3% (14/24) and 93.3% (56/60), respectively ($p = 0.013$ and 0.013).

Conclusion: CYFRA21-1 and CEA may be helpful in predicting the responsiveness in ESCC of primary lesions to CRT, although the results should be confirmed in larger, more homogeneous studies.

Keywords: *Esophageal squamous cell carcinoma; chemoradiotherapy; cytokeratin 19 fragment antigen 21-1; carcinoembryonic antigen*

Introduction

Esophageal carcinoma is a typical refractory cancer with a poor prognosis among malignant tumours of the gastrointestinal tract and esophageal squamous cell carcinoma (ESCC) is one of the most common malignant tumours in China (Su et al. 2001). For the early stage of esophageal cancer, a surgical approach is initially recommended. Unfortunately, the outcomes of surgery have been unsatisfactory in patients with advanced esophageal carcinoma (Ohtsu et al. 1999). Hironaka et al. (2003) reported that the 5-year survival rates were 46% and 51% in the chemoradiotherapy (CRT) and surgery groups, respectively ($p = 0.47$, log-rank test), which showed a trend for CRT in the treatment of esophageal carcinoma. Uncontrolled studies suggest that CRT has similar efficacy to surgery for esophageal cancer. Bedenne et al. (2007) carried out a randomised trial to compare CRT with CRT followed by surgery in patients with locally advanced tumours. They concluded that in patients with locally advanced thoracic esophageal cancers, especially epidermoid, who responded to CRT, there was no benefit for the addition of surgery after CRT compared with the continuation of additional CRT (Bedenne et al. 2007). Therefore, if factors that allow prediction of the effects of CRT are found, a more effective therapeutic strategy can be designed. However, whether or not primary complete response (CR) can be achieved

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(Received 28 April 2009; revised 08 June 2009; accepted 13 July 2009)

ISSN 1354-750X print/ISSN 1366-5804 online © 2009 Informa UK Ltd
DOI: 10.3109/13547500903180265

by CRT is an important point if CRT is intended to be a radical treatment. Due to the recent developments in molecular biology, various target molecules have been identified and their relations with chemo- or radiosensitivity and the prognosis have been evaluated (Wakatsuki et al. 2007, Kunisaki et al. 2006). Some reports revealed that patients who responded well to CRT had favourable outcomes, while poor responders conversely showed a worse prognosis. Therefore, molecular markers indicating responsiveness to CRT would be extremely helpful in selecting optimal treatment protocols for patients.

In this study, patients with detected serum levels of cytokeratin 19 fragment antigen 21-1 (CYFRA21-1) and carcinoembryonic antigen (CEA) before CRT were enrolled into our list. We investigated the relationships between the clinical effects of CRT on the primary lesions of ESCC and the serum levels of molecular markers through a review of previous cases diagnosed as ESCC, and evaluated whether these molecular markers could be used as predictors of sensitivity of esophageal carcinoma to CRT.

Patients and methods

Subjects

The source of the study data was a database of ESCC patients who received CRT between July 2002 and July 2008 at Shandong Tumor Hospital. The 84 patients fulfilled the following criteria: (1) histologically proven ESCC; (2) no previous treatment; (3) Karnofsky Performance Status (KPS) scale 70–100; (4) adequate organ, bone marrow, liver and renal functions; (5) no severe complications; (6) those with a computed tomography (CT) scan- and barium meal gastrointestinal tract-evaluable primary lesions pretreatment and posttreatment; (7) clinically diagnosed T1-4, N any, and M any on the International Union Against Cancer tumour-node-metastasis (TNM) classification; and (8) informed consents were obtained before treatment. All patients were given the same regimen of CRT.

Serum sampling, enzyme immunoassay for CYFRA21-1 and CEA assay

Blood samples were obtained by venipuncture before CRT. Each sample was centrifuged at 3000*g* for 5 min and then frozen at -80°C until use. Repeated thawing and freezing of samples was avoided. CYFRA21-1 and CEA levels were measured by CYFRA 21-1 enzyme immunoassay kit and CEA enzyme immunoassay kit (Boehringer-Mannheim GmbH, Mannheim, Germany). The cut-off values of CYFRA21-1 and CEA were defined as 3.4 ng ml^{-1} and 3.3 ng ml^{-1}, respectively, according to the 95% confidence intervals of non-cancer Chinese patients. Levels above the cut-off values were defined as high, while those below the value as low.

Treatment schedule

The treatment comprised two sources of protracted 5-fluorouracil (5-FU) infusion (400 mg m^{-2} daily on days 1–5 and 8–12), and a 2-h infusion of cisplatin (40 mg m^{-2} on days 1 and 8) combined with radiotherapy. Radiotherapy was administered using conformal radiotherapy or intensity modulated radiotherapy with 15-MV X-rays in 34 fractions with a total dose of 59.6 Gy (first phase: 40 Gy/20 f/2 Gy; second phase: 19.6 Gy/14 f/1.4 Gy twice a day with an interval of at least 6 h).

Evaluation of response concerning the primary site

Tumour responses were assessed as the following: CR for the primary tumour was defined as the complete disappearance of all measurable and assessable disease for more than 4 weeks, and partial response (PR) was defined as a subjective decrease with >50% tumour regression for more than 1 month; no change (NC) was defined as <50% reduction of the tumour, and progression of disease (PD) as a ≥25% enlargement of the tumour or the appearance of a new tumour. The evaluation of the response to treatment consisted of thoracic CT and barium esophagogram. Two kinds of categorised methods were employed in our evaluation: (1) patients who were evaluated as PR, NC and PD were considered to be non-CR, while patients with CR were classified into the CR group; (2) treatment of patients who were evaluated as CR and PR was regarded as effective while treatment of patients who were evaluated as NC and PD was defined as ineffective. The evaluation was performed 2 months after the treatment.

Statistical analysis

The χ^2 squared test and logistic regression analysis were used to evaluate the association between the responsiveness of primary lesions and clinical variables. Significance was defined as $p < 0.05$. Statistical analyses were conducted with SPSS 13.0.

Results

Patient characteristics and response

From July 2002 to July 2008, 84 patients fulfilled the inclusion criteria of our study. Table 1 shows characteristics of the patients. There were 69 men and 15 women with a median age of 64 years (range 40–88 years). Seventeen patients had tumours in the lower third of the esophagus,

38 in the middle third and 29 in the upper third. All had histologically proven ESCC. In terms of the T stage, two patients had T1, nine T2, 45 T3 and 28 T4. In terms of the N stage, 31 patients had N0 disease and 53 N1. In terms of the M stage 61 patients had M0 disease and 23 M1. Table 2 shows the correlations of serum CYFRA 21-1 and CEA levels before treatment according to T, N and M stages in all 84 patients. The levels of CYFRA 21-1 had a positive correlation with M stage ($p = 0.011$). Table 3 shows that the effectiveness of CRT was significantly associated with the serum levels of CYFRA 21-1 and CEA before treatment; the differences of the CR rates between CYFRA21-1 and CEA high and low groups were significant ($p = 0.000$ and 0.000, respectively). The differences of the CR+PR rates between CYFRA21-1 and CEA high and low groups were also significant ($p = 0.000$ and 0.000, respectively). By logistic regression analysis, the CR rates of CRT were significantly associated with the levels of CYFRA21-1 and CEA before treatment ($p = 0.002$ and 0.003, respectively) as shown in Table 4. From Table 5 we could detect that the CR+PR rates were significantly different between CYFRA21-1 and CEA high and low groups ($p = 0.013$ and 0.013, respectively). That is to say patients with low CYFRA21-1 and CEA levels were much more sensitive to CRT.

Table 1. Characteristics of 84 patients with esophageal squamous cell carcinoma (ESCC).

	Patients	
Characteristic	*n*	%
Sex		
Male	69	82
Female	15	18
Age (years)		
≤65	47	56
>65	37	44
KPS		
≤80	42	50
>80	42	50
Location		
Upper	29	35
Middle	38	45
Lower	17	20
Length (cm)		
≤5	63	75
>5	21	25
T stage		
T 1+2	11	13
T 3	45	54
T 4	28	33
N stage		
N 0	31	37
N 1	53	63
M stage		
M 0	61	73
M 1	23	27
CYFRA21-1 (ng ml^{-1})		
≤3.4	54	64
>3.4	30	36
CEA (ng ml^{-1})		
≤3.3	60	71
>3.3	24	29

KPS, Karnofsky Performance Status; CYFRA21-1, cytokeratin 19 fragment antigen 21-1; CEA, carcinoembryonic antigen.

Discussion

Tumour markers are expected to have five potential useful applications: screening, diagnosis, establishing prognosis, monitoring treatment and detecting relapse (Yamamoto et al. 1997, Ohno et al. 2003). With the development of molecular biology, tumour markers are becoming more and more widely used. In this study, we evaluated the usefulness of molecular biological markers for the prediction of the sensitivity to CRT in patients with ESCC.

Previous studies showed a positive correlation between the serum CYFRA 21-1 levels before treatment and TNM stage (Kawaguchi et al. 2000), and a significant relationship was found between elevated serum CEA levels and distant metastases in esophageal cancer (Kosugi et al. 2004). In this study, although a slight tendency was observed, a statistically significant difference was not found between CYFRA 21-1 or CEA high and low groups, only serum levels of CYFRA 21-1 showed a correlation with M stage ($p = 0.011$). This was partly because most of the patients enrolled were at an advanced stage and the sample was relatively small. However, further large-scale studies will be required for a final conclusion.

CYFRA21-1 was developed as a tumour marker for lung carcinoma (Stieber et al. 1993). It is expressed in the unstratified or pseudo-unstratified epithelium of the bronchial tree and is used as the most sensitive tumour

Table 2. Relationships between serum levels of CYFRA21-1 and CEA and TNM stage in 84 patients with esophageal squamous cell carcinoma.

		CEA (ng ml^{-1})		CYFRA21-1 (ng ml^{-1})	
Variables	*n*	Mean ± SD	*p*-Value	Mean ± SD	*p*-Value
T			0.522		0.538
T1	2	1.79 ± 0.86		1.82 ± 0.78	
T2	9	2.88 ± 1.33		3.16 ± 2.08	
T3	45	2.79 ± 1.35		2.68 ± 1.06	
T4	28	3.25 ± 2.22		2.91 ± 1.57	
N			0.229		0.172
N0	31	2.64 ± 1.28		2.52 ± 1.45	
N1	53	3.10 ± 1.87		2.94 ± 1.30	
M			0.622		0.011
M0	61	2.87 ± 1.75		2.56 ± 1.26	
M1	23	3.08 ± 1.52		3.39 ± 1.48	

CYFRA21-1, cytokeratin 19 fragment antigen 21-1; CEA, carcinoembryonic antigen.

Table 3. Relationships between effectiveness of chemoradiotherapy (CRT) and clinicopathological factors as well as serum levels of tumour markers.

Characteristics/ markers	Effectiveness		χ^2	*p*-Value	Effectiveness		χ^2	*p*-Value
	CR	Non-CR			CR+PR	NC+PD		
Sex			0.954	0.329			0.584	0.445
Male	23	46			59	10		
Female	7	8			11	4		
Age (years)			3.015	0.082			1.633	0.201
≤65	13	34			37	10		
>65	17	20			33	4		
KPS			0.830	0.362			0.000	1.000
≤80	17	25			35	7		
>80	13	29			35	7		
Location			1.626	0.443			0.040	0.980
Upper	13	16			24	5		
Middle	12	26			32	6		
Lower	5	12			14	3		
Length (cm)			1.728	0.189			0.000	1.000
≤5	25	38			53	10		
>5	5	16			17	4		
T stage			2.260	0.323			4.316	0.116
T 1 + 2	6	5			8	3		
T 3	16	29			41	4		
T 4	8	20			21	7		
N stage			1.910	0.167			0.501	0.479
N 0	14	17			27	4		
N 1	16	37			43	10		
M stage			7.090	0.008			9.388	0.002
M 0	27	34			56	5		
M 1	3	20			14	9		
CYFRA21-1 (ng ml^{-1})			13.440	0.000			18.293	0.000
≤3.4	27	27			52	2		
>3.4	3	27			18	12		
CEA (ng ml^{-1})			14.565	0.000			12.705	0.000
≤3.3	29	31			56	4		
>3.3	1	23			14	10		

CR, complete response; PR, partial response; NC, no change; PD, progression of disease; KPS, Karnofsky Performance Status; CYFRA21-1, cytokeratin 19 fragment antigen 21-1; CEA, carcinoembryonic antigen.

Table 4. Logistic regression analysis of the relationships between effectiveness of chemoradiotherapy (CRT) and clinicopathological factors as well as serum tumour markers.

Variables	*p*-Value	Hazard ratio	95% Confidence interval	
			Lower	Upper
Sex	0.274	0.384	0.069	2.132
Age	0.719	1.013	0.945	1.086
KPS	0.276	1.055	0.958	1.163
Location	0.313	1.579	0.650	3.834
Length (cm)	0.243	1.291	0.841	1.982
T	0.589	1.376	0.432	4.377
N	0.994	0.994	0.260	3.807
M	0.166	3.123	0.623	15.654
CYFRA21-1 (ng ml^{-1})	0.002	2.651	1.434	4.903
CEA (ng ml^{-1})	0.003	2.930	1.456	5.894
Constant	0.049	0.000		

KPS, Karnofsky Performance Status; CYFRA21-1, cytokeratin 19 fragment antigen 21-1; CEA, carcinoembryonic antigen.

Table 5. Logistic regression analysis of the relationships between effectiveness of chemoradiotherapy (CRT) and serum levels of CYFRA21-1 and CEA.

Variables	*p*-Value	Hazard ratio	95% Confidence interval	
			Lower	Upper
Sex	0.154	7.290	0.476	111.629
Age (years)	0.678	1.023	0.918	1.140
KPS	0.354	1.127	0.875	1.452
Location	0.237	0.353	0.063	1.981
Length (cm)	0.247	0.537	0.187	1.540
T	0.394	2.270	0.345	14.914
N	0.289	5.864	0.224	153.845
M	0.038	58.665	1.255	2741.272
CYFRA21-1 (ng ml^{-1})	0.013	5.579	1.446	21.529
CEA (ng ml^{-1})	0.013	5.683	1.453	22.231
Constant	0.113	0.000		

KPS, Karnofsky Performance Status; CYFRA21-1, cytokeratin 19 fragment antigen 21-1; CEA, carcinoembryonic antigen.

marker for lung carcinomas, except for small-cell carcinomas of the lung (Pujol et al. 1993). Van der Gaast et al. (1994) reported the usefulness of CYFRA 21-1 for disease monitoring in patients with small-cell lung cancer during and after chemotherapy. As for esophageal carcinoma, a previous study has shown that there is a correlation between CYFRA21-1 levels and clinical responses in patients who received chemotherapy or CRT (Nakamura et al. 1998). Yamamoto et al. (1997) reported that the levels of CYFRA21-1 were correlated with disease progression (including tumour size, tumour depth and pTNM stage), resectability and curability. The specificity, sensitivity and accuracy of CYFRA21-1 were 100%, 47.9% and 66.7%, respectively (Yamamoto et al. 1997). In this study, there was a statistically significant correlation between serum levels of CYFRA21-1 before CRT and the effectiveness of the treatment, the CR rates in CYFRA21-1 high and low groups were significantly different ($p = 0.002$), and the effective rates (CR+PR rate) were also significantly different ($p = 0.013$). Thus we showed that ESCC with a high level of CYFRA21-1 is less sensitive to CRT. However, this conclusion should be confirmed by study of larger and more homogeneous samples.

CEA was first isolated from human fetal intestine and adult colon cancer tissue by Gold and Freedman in 1965 (Gold & Freedman 1965a, b). Although the efficacy of CEA as a diagnostic and prognostic factor in patients with esophageal cancers has been demonstrated (Mroczko et al. 2008, Mao et al. 2003), there was no report to support its clinical significance as a predictor of sensitivity to CRT or effectiveness of the treatment until now. To our knowledge, this is the first study to detect the relationship between CEA levels before CRT and the effectiveness of the treatment; in other words, our study is the first to demonstrate that CEA is a biological marker of CRT. In our study, the effectiveness in patients with high CEA levels was markedly worse than that in patients with low CEA levels; the CR rates in CEA high and low groups were significantly different ($p = 0.013$), and the effective rates (CR+PR) were also significantly different ($p = 0.013$). Based on the study, we consider that patients with low serum levels of CEA may be more sensitive to CRT. However, the results should be confirmed by further studies.

In the present study, CYFRA21-1 and CEA levels were high in 35.7% (30/84) and 28.6% (24/84) of the patients before treatment, respectively. This is similar to the 20–48% that has previously been reported (Nakamura et al. 2004, Brockmann et al. 2000). Previous studies showed a positive correlation between the serum CYFRA21-1 levels before treatment and the effectiveness of CRT (Wakatsuki et al. 2007). Kunisaki also reported that CYFRA 21-1 is an independent predictor of radiosensitivity. Our results are similar to previous studies (Kunisaki et al. 2006).

In conclusion, CYFRA21-1 and CEA are useful predictors of sensitivity of ESCC to CRT. It is important to analyse the levels of CYFRA21-1 and CEA before CRT for predicting the response to the treatment, and effective therapeutic schedule can be made. However, larger and more homogeneous studies of these biomarkers for ESCC treated with CRT should be conducted.

Acknowledgments

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Bedenne L, Michel P, Bouché O, Milan C, Mariette C, Conroy T, Pezet D, Roullet B, Seitz JF, Herr JP, Paillot B, Arveux P, Bonnetain F, Binquet C. (2007). Chemoradiation followed by surgery compared with chemoradiation alone in squamous cancer of the esophagus: FFCD 9102. J Clin Oncol 25:1160–8.

Brockmann JG, St Nottberg H, Glodny B, Heinecke A, Senninger NJ. (2000). CYFRA 21-1 serum analysis in patients with esophageal cancer. Clin Cancer Res 6:4249–52.

Gold P, Freedman SO. (1965a). Demonstration of tumor-specific antigens in human colonic carcinoma by immunological tolerance and absorption techniques. J Exp Med 121:439–62.

Gold P, Freedman SO. (1965b). Specific carcinoembryonic antigen of the human digestive system. J Exp Med 122:467–81.

Hironaka S, Ohtsu A, Boku N, Muto M, Nagashima F, Saito H, Yoshida S, Nishimura M, Haruno M, Ishikura S, Ogino T, Yamamoto S, Ochiai A. (2003). Nonrandomized comparison between definitive chemoradiotherapy and radical surgery in patients with T(2-3)N(any) M(0) squamous cell carcinoma of the esophagus. Int J Radiat Oncol Biol Phys 57:425–33.

Kawaguchi H, Ohno S, Miyazaki M, Hashimoto K, Egashira A, Saeki H, Watanabe M, Sugimachi K. (2000). CYFRA 21-1 determination in patients with esophageal squamous cell carcinoma: clinical utility for detection of recurrences. Cancer 89:1413–17.

Kosugi S, Nishimaki T, Kanda T, Nakagawa S, Ohashi M, Hatakeyama K. (2004). Clinical significance of serum carcinoembryonic antigen, carbohydrate antigen 19-9, and squamous cell carcinoma antigen levels in esophageal cancer patients. World J Surg 28:680–5.

Kunisaki C, Imada T, Yamada R, Hatori S, Kinbara K, Watai K, Akiyama H, Nomura M, Matsuda G, Otsuka Y, Ono H, Shimada H. (2006). Prognostic factors after chemoradiotherapy for patients with inoperable esophageal squamous cell carcinoma. Hepatogastroenterology 53:366–71.

Mao YS, Zhang DC, Zhao XH, Wang LJ, Qi J, Li XX. (2003). Significance of CEA, SCC and Cyfra21-1 serum test in esophageal cancer. Zhonghua Zhong Liu Za Zhi 25:457–60.

Mroczko B, Kozłowski M, Groblewska M, Łukaszewicz M, Nikliński J, Jelski W, Laudański J, Chyczewski L, Szmitkowski M. (2008). The diagnostic value of the measurement of matrix metalloproteinase 9 (MMP-9), squamous cell cancer antigen (SCC) and carcinoembryonic antigen (CEA) in the sera of esophageal cancer patients. Clin Chim Acta 389:61–6.

Nakamura T, Ide H, Eguchi R, Hayashi K, Takasaki K, Watanabe S. (1998). CYFRA21-1 as a tumor marker for squamous cell carcinoma of the esophagus. Dis Esophagus 11:35–9.

Nakamura T, Matsunami K, Hayashi K, Ota M, Ide H, Takasaki K. (2004). Detection of bone marrow micrometastasis in esophageal cancer patients by immunomagnetic separation. Oncol Rep 11:999–1003.

Ohno T, Nakayama Y, Nakamoto S, Kato S, Imai R, Nonaka T, Ishikawa H, Harashima K, Suzuki Y. (2003). Measurement of serum squamous cell carcinoma antigen levels as a predictor of radiation response in patients with carcinoma of the uterine cervix. Cancer 97:3114–20.

Ohtsu A, Boku N, Muro K, Chin K, Muto M, Yoshida S, Satake M, Ishikura S, Ogino T, Miyata Y, Seki S, Kaneko K, Nakamura A. (1999). Definitive chemoradiotherapy for T4 and/or M1 lymph node squamous cell carcinoma of the esophagus. J Clin Oncol 17:2915–21.

Pujol JL, Grenier J, Daurès JP, Daver A, Pujol H, Michel FB. (1993). Serum fragment of cytokeratin subunit 19 measured by CYFRA 21-1 immunoradiometric assay as a marker of lung cancer. Cancer Res 53:61–6.

Stieber P, Hasholzner U, Bodenmüller H, Nagel D, Sunder-Plassmann L, Dienemann H, Meier W, Fateh-Moghadam A. (1993). CYFRA 21-1. A new marker in lung cancer. Cancer 72:707–13.

Su M, Lu SM, Tian DP, Zhao H, Li XY, Li DR, Zheng ZC. (2001). Relationship between ABO blood groups and carcinoma of esophagus and cardia in Chaoshan inhabitants of China. World J Gastroenterol 7:657–61.

van der Gaast A, Schoenmakers CH, Kok TC, Blijenberg BG, Cornillie F, Splinter TA. (1994). Evaluation of a new tumour marker in patients with non-small-cell lung cancer: Cyfra21-1. Br J Cancer 69:525–8.

Wakatsuki M, Suzuki Y, Nakamoto S, Ohno T, Ishikawa H, Kiyohara H, Kiyozuka M, Shirai K, Nakayama Y, Nakano T. (2007). Clinical usefulness of CYFRA 21-1 for esophageal squamous cell carcinoma in radiation therapy. J Gastroenterol Hepatol 22:715–19.

Yamamoto K, Oka M, Hayashi H, Tangoku A, Gondo T, Suzuki T. (1997). CYFRA 21-1 is a useful marker for esophageal squamous cell carcinoma. Cancer 79:1647–55.