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Original Article

SDF-1α predicts poor prognosis in patients with locally advanced esophageal squamous cell carcinoma receiving definitive concurrent chemoradiotherapy

Yen-Hao Chen [a,b,c,f], Hung-I Lu [d,f], Yu-Ming Wang [e,f], Chien-Ming Lo [d,f], Shang-Yu Chou [e,f], Shau-Hsuan Li [a,f,*]

[a] Department of Hematology-Oncology, Chang Gung Memorial Hospital at Kaohsiung, Kaohsiung, Taiwan
[b] School of Medicine, Chung Shan Medical University, Taichung, Taiwan
[c] Department of Nursing, Meiho University, Pingtung, Taiwan
[d] Department of Thoracic & Cardiovascular Surgery, Chang Gung Memorial Hospital at Kaohsiung, Kaohsiung, Taiwan
[e] Department of Radiation Oncology, Chang Gung Memorial Hospital at Kaohsiung, Kaohsiung, Taiwan
[f] College of Medicine, Chang Gung University, Taoyuan, Taiwan

ARTICLE INFO

Article history:
Received 27 November 2020
Accepted 13 May 2021
Available online 19 May 2021

Keywords:
SDF-1α
Esophageal cancer
Squamous cell carcinoma
Concurrent chemoradiotherapy

ABSTRACT

Background: Stromal cell-derived factor-1α (SDF-1α) is a chemokine associated with tumor progression in various types of cancers. The current study aimed to evaluate whether pre-treatment or kinetics of SDF-1α can predict the prognosis in patients with esophageal squamous cell carcinoma (ESCC) receiving definitive concurrent chemoradiotherapy (CCRT).

Methods: A total of 97 patients with ESCC were identified at Kaohsiung Chang Gung Memorial Hospital between January 2010 and December 2015. Serum concentration of SDF-1α was measured at day 0 (pre-treatment) and chemotherapy day 28 to determine its kinetics and the cut-off level of pre-chemotherapy SDF-1α was 1.5 ng/mL. Two ESCC cell lines, TE1 and KYSE30, were selected to evaluate the function of SDF-1α.

Results: Univariate and multivariate analyses showed that pre-treatment SDF-1α ≥ 1.5 ng/mL and an increased SDF-1α level after treatment were significantly associated with worse progression-free survival ($p = 0.021$ and $p = 0.008$, respectively) and overall survival ($p = 0.005$ and $p < 0.001$, respectively). In addition, patients with pre-treatment SDF-1α ≥ 1.5 ng/mL and increased SDF-1α levels after treatment were found to have poor response to CCRT. Moreover, these cell lines were treated with chemotherapeutic agents (cisplatin or 5-FU) and SDF-1α, alone or in combination. Our in vitro study results showed SDF-1α promoted the proliferation of tumor cells and overcame the cytotoxic effect of chemotherapy ($p < 0.001$).

* *Corresponding author*. Department of Hematology-Oncology, Chang Gung Memorial Hospital at Kaohsiung, 123, Dapi Rd., Niaosong Dist., Kaohsiung 833, Taiwan.
E-mail address: lee.a0928@msa.hinet.net (S.-H. Li).
Peer review under responsibility of Chang Gung University.
https://doi.org/10.1016/j.bj.2021.05.004

Conclusion: Our study suggested that SDF-1α plays an important role in ESCC disease progression and that pre-treatment SDF-1α level and kinetics of SDF-1α are the independent prognostic factors for ESCC patients receiving definitive CCRT. Periodic determinations of serum SDF-1α level may be valuable to predict prognosis of ESCC in clinical practice.

At a glance commentary

Scientific background on the subject

Esophageal squamous cell carcinoma (ESCC) is an aggressive human malignancy and the outcome is very poor. Therefore, identifying signal pathways involving in the tumor progression is an important research priority. Chemokines are small, secreted proteins and mediate the host-response to cancer cells. Stromal cell-derived factor-1α (SDF-1α) is a homeostatic chemokine and have prominent roles in common malignancies. However, the role of SDF-1α in ESCC remains unclear. The present study was to investigate the role of SDF-1α in the clinical outcome of ESCC patients receiving definitive concurrent chemoradiotherapy (CCRT).

What this study adds to the field

Our study showed pre-treatment SDF-1α $\geq$ 1.5 ng/mL and an increased SDF-1α level after treatment were significantly associated with worse progression-free survival and overall survival in the univariate and multivariate analyses. Our in vitro study results also demonstrated that SDF-1α promoted the proliferation of tumor cells and overcame the cytotoxic effect of chemotherapy.

Esophageal squamous cell carcinoma (ESCC) is one of the most aggressive malignancies and ranks the ninth leading cause of cancer-related deaths in Taiwan [1]. The major risk factors for ESCC include alcohol, tobacco, betel quid chewing, chronic mechanical irritation of the mucosa, hot tea drinking and achalasia [2–4]. In addition, the long-term use of smoking and alcohol abuse frequently results in secondary primary head and neck cancer [5–7]. Because it is usually asymptomatic in early stage, the majority of ESCC patients have locally advanced disease when they are diagnosed. Despite there have been noticeable improvements in surgical and medical treatments, the outcomes for patients with ESCC are still dissatisfactory [8–12]. Therefore, identifying a novel mechanism involved in disease progression of ESCC is an important issue.

Chemotherapy is one of the standard therapies for patients with clinically unresectable ESCC and platinum-based treatment is a frequent first-line chemotherapy in clinical practice. However, acquired chemotherapy resistance commonly results in treatment failure and high incidences of tumor relapse and mortality. Cellular resistance to chemotherapy can arise through multiple mechanisms, but the specific molecular mechanism of chemotherapy resistance in ESCC remain largely unknown. Therefore, a better understanding of these mechanisms is crucial for improving esophageal cancer treatment.

Chemokines are a family of small, secreted proteins that direct tumor cell proliferation, apoptosis, and epithelial–mesenchymal transition, resulting in tumor invasion, migration and distant metastasis. Stromal cell-derived factor-1α (SDF-1α) is a kind of homeostatic chemokines and found to be expressed in multiple organs, including brain, liver, lung, heart, kidney, skeletal muscle and bone marrow. The secretion of SDF-1α is related to tissue damage such as myocardial infarction, excessive blood loss, ischemic change of organs, toxic liver damage, irradiation and chemotherapy related tissue damage. C-X-C chemokine receptor type 4 (CXCR4), the receptor of SDF-1α, and the SDF-1α/CXCR4 activates the downstream signaling pathway, leading to retention and homing of hematopoietic stem cells, lymphocyte trafficking, actin polymerization, cell skeleton rearrangement, and cell migration [13,14]. SDF-1α has a prominent role in common malignancies, including breast, ovarian, prostate, kidney, brain, and lung cancers [15–17]. The binding of SDF-1α to CXCR4, the receptor of SDF-1α, induces several downstream signaling pathways, resulting in anti-apoptosis, tumor cell growth, chemotaxis, and gene transcription [18,19]. Our previous study has shown that higher post-treatment vascular endothelial growth factor (VEGF) level and kinetic change of serum VEGF are prognostic factors for ESCC patients who received chemotherapy [20]. We found lower post-treatment VEGF levels and decreasing levels of VEGF during concurrent chemoradiotherapy (CCRT) are significantly associated with better clinical outcomes. However, to the best of our knowledge, the role of serum SDF-1α in ESCC remains unclear. The aim of the current study was to investigate whether pre-treatment or kinetics of SDF-1α can predict the prognosis in ESCC patients receiving definitive CCRT.

Materials and methods

Patient selection

Records of patients with ESCC who received definitive CCRT at Kaohsiung Chang Gung Memorial Hospital were retrospectively reviewed between January 2010 and December 2015. Exclusion criteria included a history of a second primary malignancy, chemotherapy or radiotherapy alone, and distant metastasis. Finally, a total of 97 patients were finally

identified. Clinical tumor stage was determined by chest computed tomography, endoscopic ultrasonography, and positron emission tomography scans for each ESCC patient, according to the system outlined in the 8th edition of the American Joint Committee on Cancer (AJCC) staging system [21].

Chemotherapy consisted of cisplatin (75 mg/m^2; 4-h infusion) on day 1 and 5-fluorouracil (1000 mg/m^2; continuous infusion) on days 1–4, every 4 weeks. Chemotherapy was arranged concurrently with radiotherapy. For patients with creatinine clearance <60 mL/min, carboplatin was used instead of cisplatin. Chemotherapy and radiotherapy were administered according to the protocol described in previously published studies [20,22–24].

The treatment response to CCRT was assessed in accordance with the guidelines of the modified Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [25].

Serum chemokine measurement

In our hospital, we collected a series of blood samples from some ESCC patients who received CCRT or chemotherapy alone in accordance with the approval by the Chang Gung Medical Foundation Institutional Review Board. Among the ESCC database in our hospital, we identified 30 patients who had sufficient blood sample volume, including 15 patients with a good response to chemotherapy (tumor shrinkage more than 50% or complete remission) and 15 patients with a poor response to chemotherapy (tumor progression more than 20%). Blood samples were collected from each patient at three time points, including day 0 (pre-treatment), day 5 (post chemotherapy cycle 1), and day 28 (between chemotherapy cycles 1 and 2). We hypothesized that the response to chemotherapy is associated with changes in chemokines over time. We used the ratio of pre-chemotherapy and post-chemotherapy chemokine levels to compare the two study groups. For example, a good response to chemotherapy was indicated by a decrease in chemokine levels, resulting in day 5/day 0 and day 28/day 5 ratios being less than 1. However, for patients with a poor response to chemotherapy was indicated by an increase in chemokine levels, resulting in day 5/day 0 and day 28/day 5 ratios being greater than 1. Subsequently, the 45-Plex Cytokine/Chemokine/Growth Factor Panel 1 (EPX450-12171-901; Affymetrix, Santa Clara, CA, USA), consisting of 45 biomarkers, was used to identify differences in chemokine levels between these two groups.

In addition, Zajac et al. reported that the median concentration of serum SDF-1α in ESCC was 1.501 ng/mL, so the 97 ESCC patients were divided into two groups, pre-treatment SDF-1α < 1.5 ng/mL group and pre-treatment SDF-1α ≥ 1.5 ng/mL group [26].

Table 1 Characteristics of 97 patients with locally advanced esophageal squamous cell carcinoma patients underwent curative concurrent chemoradiotherapy.

Characteristics	
Age	56 years old (32–77)
Sex	
Male	96 (99%)
Female	1 (1%)
T status	
1	4 (4%)
2	11 (11%)
3	29 (30%)
4	53 (55%)
N status	
0	4 (4%)
1	27 (28%)
2	39 (40%)
3	27 (28%)
Stage	
II	4 (4%)
III	26 (27%)
IVA (locally advanced)	67 (69%)
Grade	
1	23 (24%)
2	48 (49%)
3	26 (27%)
Location	
Upper	40 (41%)
Middle	34 (35%)
Lower	23 (24%)
Salvage esophagectomy	
Yes	31 (32%)
No	66 (68%)

Cell culture and viability assay

ESCC cell lines, TE1 and KYSE-30, have previously been established. KYSE-30 cells were purchased from Public Health England (Porton Down, Salisbury, United Kingdom) and TE1 cells were obtained from the Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer (Tohoku University, Sendai, Japan). Cells were cultured in RPMI 1640 or PRMI1640/F12 (1:1) media with 5% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL Amphotericin B and 2 mmol/L L-glutamine.

To examine the role of SDF-1α in determining the malignant properties of ESCC cells, we treated these ESCC cell lines with chemotherapeutic agents (cisplatin or 5-FU), SDF-1α, or in combination. Each cell line (2500 cells) was incubated in a 200 μL solution containing SDF-1α or chemotherapeutic agent (cisplatin or 5-FU) in triplicate in a 96-well, flat-bottomed plate. To investigate the cell proliferative activity of SDF-1α in ESCC cells, an MTT (3-[4,5-dimenthylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; 0.5 mg/mL; Sigma–Aldrich, St. Louis, MO, USA) assay was performed using these cell lines. Each cell line (7000 cells) was incubated, along with control cells, in triplicate in a 96-well flat-bottomed plate. After incubation for 96 h at 37 °C, 100 μL of MTT was added to each well and cells were incubated for 4 h. The supernatant was then discarded and the crystalline products were eluted with DMSO (100 μL/well, Sigma). Colorimetric evaluation was performed at 570 nm using a spectrophotometer.

Statistical analysis

Statistical analyses were performed using the SPSS 19 software package (IBM, Armonk, NY, USA). Differences between groups were performed using a chi-square test was used to

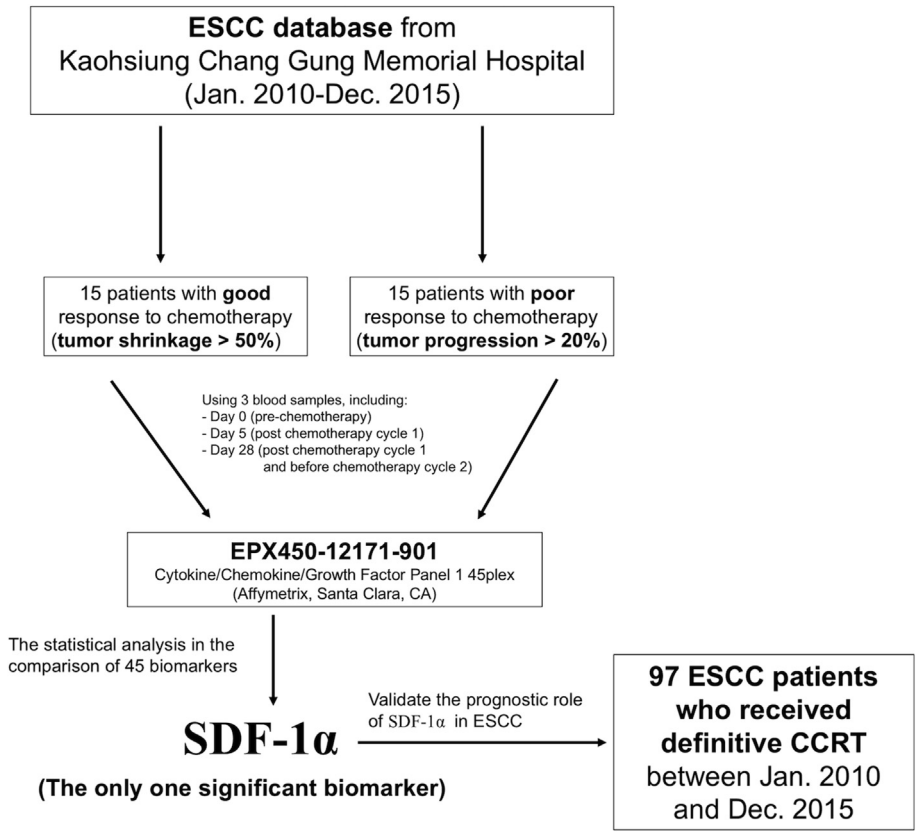

Fig. 1 The protocol of identification of potential chemokines in the mechanism of chemotherapy resistance in esophageal squamous cell carcinoma patients.

analyze categorical variables. For baseline SDF-1α level in ESCC patients with different treatment response, a one-way ANOVA was used for the statistical analysis. A Student's t-test was used for the statistical analysis in the cell line experiments. All experiments were performed in triplicate wells for each condition and repeated at least twice. Progression-free survival (PFS) was defined as the time from starting treatment to disease progression or death from any cause and overall survival (OS) was calculated from the date of CCRT initiation to the date of death from any cause or to the date of the last follow-up.

PFS and OS were analyzed by the Kaplan–Meier method and a log-rank test was performed to evaluate the differences between groups for univariate analysis. All variables were entered into Cox regression model to analyze their relative prognostic importance. Hazard ratios (HRs) with 95% confidence intervals (CIs) and P values were calculated to quantify the strength of the associations between the prognostic parameters and survival. All tests were two-sided and $p < 0.05$ was considered statistically significant.

Ethics statement

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and was approved by the Chang Gung Medical Foundation Institutional Review Board (201701862B0 and 201801383B0).

Results

Patient characteristics

We retrospectively reviewed our ESCC database and a total of 97 ESCC patients who underwent definitive CCRT were identified finally. The patients included 96 males and 1 female, with a mean age of 56 years (range: 32–77 years). More than 80% of ESCC patients had T3-4 status and most patients had positive lymph node metastasis. Tumor stage was determined by the 8th edition of the AJCC staging system, and near 70% of ESCC patients were diagnosed as stage IVA (locally advanced). There were 31 patients (32%) who received salvage esophagectomy after definitive CCRT. The clinicopathological characteristics of these patients are shown in Table 1.

Analysis of SDF-1α kinetics and clinical outcomes

The 45-Plex Cytokine/Chemokine/Growth Factor Panel 1 (EPX450-12171-901; Affymetrix, Santa Clara, CA, USA), consisting of 45 biomarkers, was used to identify differences in chemokine levels between these two groups [Fig. 1]. Finally,

Table 2 The statistical analysis in the comparison of 45 biomarkers between esophageal squamous cell carcinoma patients with good and poor response to chemotherapy.

Biomarkers	Day 5/Day 0 ratio			Day 28/Day 5 ratio		
	Good response to chemotherapy group	Poor response to chemotherapy group	*p* value	Good response to chemotherapy group	Poor response to chemotherapy group	*p* value
IFN-γ	0.54–1.53	0.02–2.43	0.3369	0.44–2.33	0.67–1.40	0.4720
IL-12p70	0.95–1.66	0.76–1.87	0.3963	0.70–1.32	0.39–1.08	0.1358
IL-13	0.56–8.43	0.33–1.71	0.1990	0.31–2.08	0.49–1.52	0.0559
IL-1beta	0.71–5.38	0.06–2.51	0.0613	0.37–1.89	0.42–1.11	0.2475
IL-2	0.71–5.63	0.12–2.01	0.1034	0.57–1.35	0.68–1.21	0.2720
IL-4	2.23–2.70	0.28–4.31	0.8445	0.69–1.86	0.36–1.54	0.7543
IL-5	0.90–1.84	0.72–6.58	0.2471	0.69–1.35	0.45–0.80	0.0214
IL-6	0.68–4.25	0.21–3.60	0.1292	0.25–1.68	1.07–5.06	0.5259
TNF-α	0.62–9.59	0.06–2.49	0.1257	0.33–1.95	0.43–1.23	0.6069
GM-CSF	0.45–4.14	0.18–2.24	0.5428	0.04–5.35	0.46–1.27	0.2216
IL-18	0.51–1.82	0.05–2.59	0.3247	0.53–3.12	0.37–1.51	0.1667
IL-10	0.79–2.33	0.39–2.30	0.3736	0.45–2.70	0.69–2.02	0.1113
IL-17A	0.35–3.61	0.16–6.15	0.6225	0.04–1.32	0.29–2.30	0.5719
IL-21	0.87–1.20	1.03–9.00	0.7532	0.67–1.77	0.78–1.75	0.5761
IL-22	0.68–2.47	0.44–2.43	0.9947	0.56–2.11	0.62–1.45	0.1009
IL-23	0.35–3.95	0.29–7.32	0.5119	0.20–2.40	0.57–0.98	0.0033
IL-27	0.52–4.06	0.19–6.76	0.3727	0.15–2.18	0.96–2.00	0.5908
IL-9	0.43–3.17	0.34–2.57	0.4766	0.73–1.45	0.72–1.27	0.5821
IFN-α	0.59–3.21	0.65–4.67	0.2436	0.54–1.98	0.32–1.27	0.3219
IL-31	0.52–3.19	0.83–3.90	0.4838	0.08–2.34	0.66–1.93	0.5817
IL-15	0.57–4.93	0.80–1.93	0.4045	0.18–1.69	0.25–4.05	0.6095
IL-1α	0.58–1.13	1.94–2.08	0.0105	1.25–4.80	0.47–1.94	0.3609
IL-1RA	0.07–2.08	0.04–2.16	0.3113	0.35–7.87	0.79–2.63	0.1688
IL-7	0.52–3.92	0.40–1.72	0.0595	0.27–1.63	0.63–1.58	0.9503
TNF-β	0.67–2.84	0.59–2.59	0.2451	0.97–2.47	0.86–2.83	0.3162
Eotaxin	0.36–1.86	0.36–1.48	0.8014	0.48–2.14	0.46–1.91	0.2965
GRO-α	0.62–3.76	0.65–2.25	0.5104	0.41–1.81	0.83–1.79	0.1810
IL-8	0.40–5.17	0.04–2.90	0.2027	0.13–6.80	0.24–9.21	0.8732
IP-10	0.44–1.00	0.21–1.69	0.5980	0.77–1.75	1.16–2.39	0.0377
MCP-1	0.29–5.05	0.22–2.42	0.4115	0.28–2.95	0.33–2.33	0.6786
MIP-1α	0.63–1.84	0.17–2.77	0.7828	0.73–1.91	0.84–2.69	0.2181
MIP-1β	0.41–2.35	0.39–2.68	0.4584	0.65–2.22	0.49–2.41	0.2386
SDF-1α	**0.79–0.95**	**0.96–1.28**	**<0.0001**	**1.03–1.31**	**1.12–1.43**	**0.0135**
RANTES	0.38–1.24	0.71–4.45	0.0985	0.53–2.03	0.25–1.74	0.0299
NGF-β	0.72–6.27	0.09–1.35	0.1607	0.29–1.32	0.36–1.54	0.4407
BDNF	0.33–4.89	0.12–2.02	0.1421	0.21–1.42	0.46–2.44	0.7342
EGF	0.52–5.81	0.08–4.27	0.5019	0.16–2.90	0.68–1.85	0.3686
FGF-2	0.09–8.61	0.27–4.25	0.5952	0.46–1.80	0.35–3.20	0.5342
HGF	0.47–1.75	0.39–2.39	0.6148	0.70–2.01	0.36–1.99	0.2405
LIF	0.71–5.38	0.04–2.16	0.0446	0.27–1.84	0.48–1.73	0.5214
PDGF-BB	0.58–2.05	0.43–1.39	0.0594	0.59–1.44	0.79–2.62	0.8059
PlGF-1	0.39–3.27	0.38–2.07	0.1549	0.25–1.55	0.59–2.06	0.6798
SCF	0.36–1.43	0.43–2.35	0.4142	0.86–2.54	1.23–2.45	0.0761
VEGF-A	0.12–4.85	0.10–2.80	0.4562	0.19–2.03	0.75–1.60	0.1731
VEGF-D	1.66–3.55	0.36–1.19	0.3030	1.05–4.56	0.41–0.83	0.9648

Bold means that SDF-1α is the only one biomarker with statistically significant for both day 5/day 0 and day 28/day 5 ratios.

only one chemokine, SDF-1α, showed significant differences in the day 5/day 0 and day 28/day 5 ratios between the two groups [Table 2]. According to the kinetic change of serum SDF-1α between chemotherapy day 0 and day 28, these patients were divided into two groups: 68 patients with decreased SDF-1α levels after treatment and the other 29 patients with increased SDF-1α levels after treatment.

In our study, the response to CCRT showed partial response (PR) in 63 patients (65%), stable disease (SD) in 24 patients (25%) and progressive disease (PD) in the rest 10 patients (10%). There was no significant difference of pre-treatment SDF-1α level among these three groups; however, there were higher percentage of PR in the patients with pre-treatment SDF-1α level <1.5 ng/mL than those with pre-treatment SDF-1α level ≥1.5 ng/mL ($p = 0.020$); in addition, higher PR was found in the decreased SDF-1α level after treatment group ($p = 0.014$). The correlation between response rate and SDF-1α is shown in Table 3.

Table 3 Correlation between response rate and SDF-1α in 97 patients with locally advanced esophageal squamous cell carcinoma patients underwent curative concurrent chemoradiotherapy.

Treatment response	Pre-treatment SDF-1α level (ng/mL), mean ± SD	Pre-treatment SDF-1α level (ng/mL), median	*p* value	Pre-treatment SDF-1α level < 1.5 ng/mL (n = 17)	Pre-treatment SDF-1α level ≥ 1.5 ng/mL (n = 80)	*p* value	Decreased SDF-1α level after treatment (n = 68)	Increased SDF-1α level after treatment (n = 29)	*p* value
Partial response (n = 63)	2.02 ± 0.57	1.98	0.38	16 (94%)	47 (59%)	0.020*	50 (73%)	13 (45%)	0.014*
Stable disease (n = 24)	2.18 ± 0.49	2.16		1 (6%)	23 (29%)		14 (21%)	10 (34%)	
Progressive disease (n = 10)	2.18 ± 0.44	2.10		0 (0%)	10 (12%)		4 (6%)	6 (21%)	

Abbreviations: SD: standard deviation. *Statistically significant.

A univariate analysis of PFS showed that neither T status, N status, stage, tumor grade, nor tumor location were statistically significant predictors of PFS. Meanwhile, the 64 patients aged <60 years had longer PFS times than the 33 patients aged ≥60 years (15.6 months versus 11.5 months, $p = 0.044$). Better PFS was mentioned in the 31 patients who received salvage esophagectomy than the others who not (18.9 months versus 10.1 months, $p = 0.005$). The 17 patients with pre-treatment SDF-1α level <1.5 ng/mL had superior PFS (24.7 months versus 12.3 months, $p = 0.034$) compared to the other 80 patients with pre-treatment SDF-1α level ≥ 1.5 ng/mL [Fig. 2A]. Moreover, significantly improved PFS (15.6 months versus 6.8 months, $p = 0.007$) was found in the 68 patients who had decreased SDF-1α levels after treatment than in the rest 29 patients who had increased SDF-1α levels after treatment [Fig. 3A]. Multivariate analysis showed that salvage esophagectomy ($p = 0.005$, HR: 0.52, 95% CI: 0.33–0.82), pre-treatment SDF-1α level < 1.5 ng/mL ($p = 0.021$, HR: 0.51, 95% CI: 0.28–0.90) and an increased SDF-1α level after treatment ($p = 0.008$, HR: 0.54, 95% CI: 0.34–0.85) represented an independent predictive factor of superior PFS.

With respect to OS, univariate analysis showed that there were no significant effects of age, T status, stage, tumor location, and tumor grade. The 32 patients with N0-1 status were found to have superior OS (25.5 months versus 12.9 months, $p = 0.021$) compared with the 65 patients with N2-3 status. Better OS was mentioned in the 31 patients who received salvage esophagectomy than the others who not (22.2 months versus 11.1 months, $p = 0.009$). The 17 patients with pre-treatment SDF-1α level <1.5 ng/mL had superior OS (31.8 months versus 13.2 months, $p = 0.010$) compared to the other 80 patients with pre-treatment SDF-

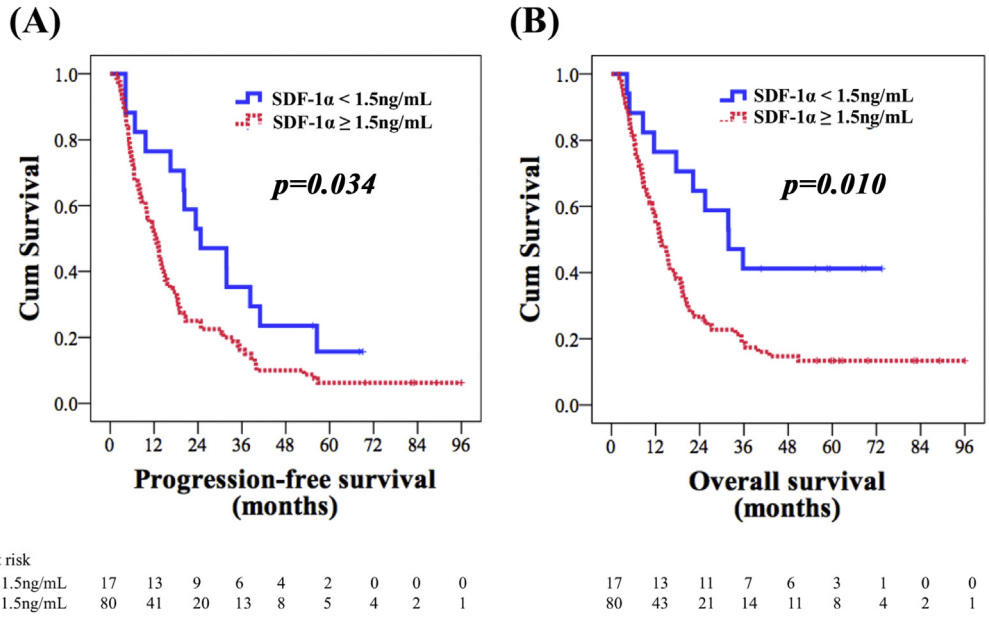

Fig. 2 Comparison of survival curves in esophageal squamous cell carcinoma patients receiving definitive concurrent chemoradiotherapy according to pre-treatment SDF-1α level. (A) Progression-free survival and (B) overall survival.

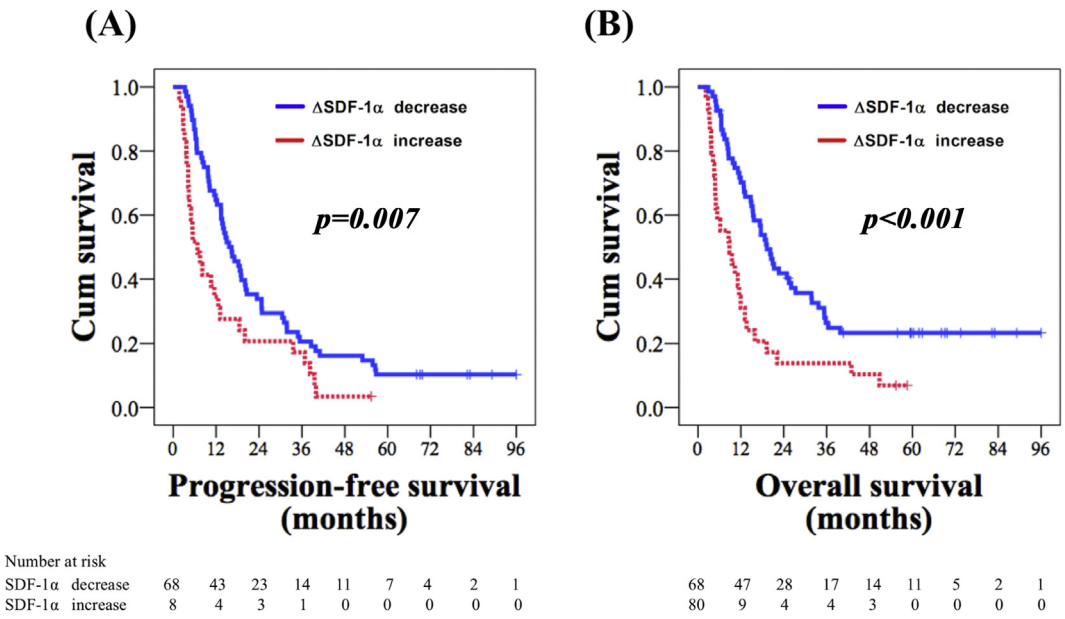

Fig. 3 Kaplan–Meier curves of progression-free survival (PFS) and overall survival (OS) of esophageal squamous cell carcinoma patients receiving definitive concurrent chemoradiotherapy. (A) PFS and (B) OS. ΔSDF-1α decrease: the level of SDF-1α after chemotherapy (day 28) is lower than that before chemotherapy (day 0). ΔSDF-1α increase: the level of SDF-1α after chemotherapy (day 28) is higher than that before chemotherapy (day 0).

Table 4 Univariate and multivariate analysis of progression-free survival (PFS) in 97 patients with locally advanced esophageal squamous cell carcinoma patients underwent curative concurrent chemoradiotherapy.

Characteristics	No. of patients	No. of events	Univariate analysis			Multivariate analysis	
			Median PFS (months)	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Age							
< 60 years	64 (66%)	57	15.6	0.64 (0.41–0.99)	0.044*		
≥ 60 years	33 (34%)	32	11.5				
T status							
1 + 2	15 (16%)	14	19.1	0.76 (0.43–1.35)	0.35		
3 + 4	82 (84%)	75	11.9				
N status							
0 + 1	32 (33%)	29	20.3	0.82 (0.52–1.28)	0.38		
2 + 3	65 (67%)	60	11.9				
Stage							
II + III	32 (33%)	29	16.5	0.98 (0.63–1.53)	0.98		
IVA (locally advanced)	65 (67%)	60	11.5				
Grade							
1 + 2	71 (73%)	65	13.3		0.49		
3	26 (27%)	24	14.0	0.85 (0.53–1.36)			
Location							
Upper	40 (41%)	35	14.6	0.81 (0.53–1.23)	0.76		
Middle + Lower	57 (59%)	54	12.3				
Salvage esophagectomy							
Yes	31 (32%)	26	18.9	0.52 (0.33–0.83)	0.005*	0.52 (0.33–0.82)	0.005*
No	66 (68%)	63	10.1				
Pre-treatment SDF-1α level							
<1.5 ng/mL	17 (18%)	14	24.7	0.54 (0.31–0.96)	0.034*	0.51 (0.28–0.90)	0.021*
≥1.5 ng/mL	80 (82%)	75	12.3				
Kinetic change of SDF-1α#							
Increase	29 (30%)	28	6.8		0.007*		
Decrease	68 (70%)	61	15.6	0.54 (0.34–0.85)		0.52 (0.33–0.83)	0.005*

Abbreviations: SDF-1α: stromal cell-derived factor-1α; HR: hazard ratio; CI: confidence interval; #change of SDF-1α level from chemotherapy day 0 to day 28; *Statistically significant.

Table 5 Univariate and multivariate analysis of overall survival (OS) in 97 patients with locally advanced esophageal squamous cell carcinoma patients underwent curative concurrent chemoradiotherapy.

Characteristics	No. of patients	No. of events	Univariate analysis			Multivariate analysis	
			Median OS (months)	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Age							
< 60 years	64 (66%)	48	17.3	0.67 (0.42–1.06)	0.09		
≥ 60 years	33 (34%)	30	13.1				
T status							
1 + 2	15 (16%)	11	19.3	0.71 (0.38–1.35)	0.30		
3 + 4	82 (84%)	67	13.6				
N status							
0 + 1	32 (33%)	22	25.5	0.56 (0.34–0.92)	0.021*	0.51 (0.31–0.85)	0.010*
2 + 3	65 (67%)	56	12.9				
Stage							
II + III	30 (31%)	25	19.3	0.84 (0.52–1.35)	0.60		
IVA (locally advanced)	67 (69%)	53	13.1				
Grade							
1 + 2	71 (73%)	57	15.6		0.76		
3	26 (27%)	21	13.6	0.93 (0.56–1.53)			
Location							
Upper	40 (41%)	33	19.3	0.83 (0.53–1.30)	0.42		
Middle + Lower	57 (59%)	45	13.2				
Salvage esophagectomy							
Yes	31 (32%)	22	22.2	0.52 (0.31–0.85)	0.009*	0.45 (0.27–0.74)	0.002*
No	66 (68%)	56	11.1				
Pre-treatment SDF-1α level							
<1.5 ng/mL	17 (18%)	10	31.8	0.43 (0.22–0.83)	0.010*	0.40 (0.20–0.79)	0.008*
≥1.5 ng/mL	80 (82%)	68	13.2				
Kinetic change of SDF-1α#							
Increase	29 (30%)	27	8.8		<0.001*		
Decrease	68 (70%)	51	19.3	0.44 (0.27–0.70)		0.33 (0.20–0.55)	<0.001*

Abbreviations: SDF-1α: stromal cell-derived factor-1α; HR: hazard ratio; CI: confidence interval; #change of SDF-1α level from chemotherapy day 0 to day 28; *Statistically significant.

1α level ≥ 1.5 ng/mL [Fig. 2B]. Moreover, longer OS time (19.3 months versus 8.8 months, $p < 0.001$) was found in the 70 patients with a decreased SDF-1α level after treatment, compared to the remaining 27 patients with increased SDF-1α levels after treatment [Fig. 3B]. According to a multivariate comparison, N0-1 status ($p = 0.018$, HR: 0.55, 95% CI: 0.33–0.90), salvage esophagectomy ($p = 0.002$, HR: 0.45, 95% CI: 0.27–0.74), pre-treatment SDF-1α level < 1.5 ng/mL ($p = 0.008$, HR: 0.40, 95% CI: 0.20–0.79) and a decreased SDF-1α level after treatment ($p < 0.001$, HR: 0.43, 95% CI: 0.27–0.69) represented independent predictive factors of superior OS. Results of the univariate and multivariate analyses of PFS and OS in 97 ESCC patients who underwent definitive CCRT are shown in Tables 4 and 5.

SDF-1α and tumor cell proliferation in vitro

In the current study, the two ESCC cell lines, TE1 and KYSE-30, were test the proliferation of tumor cells in vitro. In order to investigate the significance of SDF-1α in vitro, these two ESCC cell lines were treated with chemotherapeutic agents (cisplatin or 5-FU), SDF-1α, or in combination, to determine the dependence of tumor proliferation on SDF-1α. The results demonstreated that SDF-1α could promote tumor cell growth and overcome the cytotoxic effect of chemotherapy (cisplatin or 5-FU) at 48 h after SDF-1α treatment [Fig. 4].

Discussion

The current study showed pre-treatment SDF-1α level and kinetic change of SDF-1α before and after treatment are independent prognostic factors of ESCC. Patients with lower pre-treatment SDF-1α level or decreased SDF-1α levels after treatment are found to have better PFS and OS than others. In addition, higher percentage of response rate to CCRT were also mentioned in the same patient group. As stated above, these results of the present study demonstrated that SDF-1α may play an important role in the disease progression of ESCC.

Our previous study focused on the kinetic change of serum VEGF in ESCC patients and there were two important findings: first, a decreased VEGF level after treatment may predict better outcome; and second, VEGF level more than 80 pg/mL after treatment is a poor prognostic factor regardless of the pre-treatment VEGF level [20]. A decreased VEGF level after treatment means tumor responds to treatment, tumor shrinkage and angiogenesis decreases, contributing to superior clinical outcome. Therefore, the significant finding of serum VEGF kinetics in ESCC may support the rationale of SDF-1α in our study. However, different from VEGF, the pre-treatment SDF-1α is valuable in ESCC. The classic tumor markers for esophageal cancer,

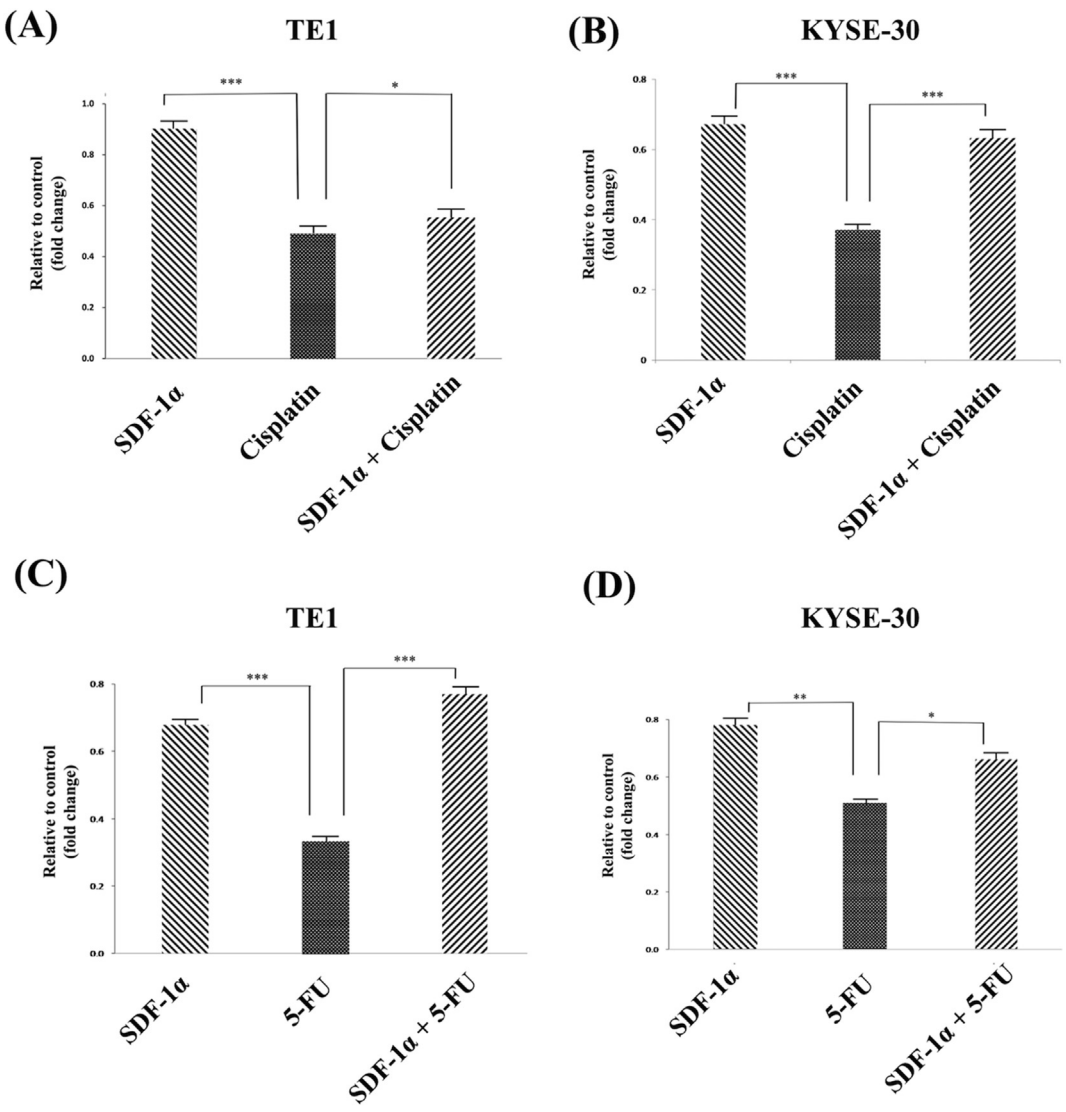

Fig. 4 Effects of SDF-1α and chemotherapy in esophageal squamous cell carcinoma cell lines using MTT assay. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

such as carcinoembryonal antigen (CEA) and squamous cell carcinoma antigen, are generally higher than healthy humans and frequently used in clinical practice. Zajac et al. reported that the serum concentrations of SDF-1α in ESCC patients were significantly higher than healthy controls, and the median of serum SDF-1α in ESCC is 1.501 ng/mL [26]. As we know, a decreased SDF-1α after treatment may indicate regression of tumor size, reduced tumor burden and inhibition of tumor cell growth. In our study, patients with decreased SDF-1α levels after treatment had higher response rate to CCRT and better PFS/OS than others who not; however, patients with pre-treatment SDF-1α $\geq$ 1.5 ng/mL had lower response rate to CCRT and worse PFS/OS, suggesting SDF-1α may be defined as not only a diagnostic marker but also a prognostic factor.

Our in vitro study showed the proliferation of ESCC cells were inhibited by chemotherapy, whether cisplatin or 5-FU; however, addition of SDF-1α could significantly enhance tumor cell growth and overcome the cytotoxic effect of chemotherapy. Moreover, patients with higher pre-treatment SDF-1α level or increased SDF-1α levels after treatment were found to have poor response to CCRT, suggesting SDF-1α may exist the potential function of chemoresistance.

The expression of SDF-1α, as detected by immunohistochemical staining, has been reported to be associated with ESCC disease severity, including lymph node metastasis, tumor stage, and clinical outcome [27,28]. A Japanese study showed that ESCC patients with high levels of SDF-1α expression had a higher percentage of lymph node metastasis and more advanced tumor stage compared to those with low levels of SDF-1α expression. Moreover, shorter disease-free survival and OS times were found in the SDF-1α-positive group than in the SDF-1α-negative group. Uchi et al. demonstrated that positive SDF-1α expression is associated with lower recurrence-free survival rates and is an independent prognostic factor for recurrence in ESCC patients [28]. In our

study, patients with pre-treatment SDF-1α ≥ 1.5 ng/mL were mentioned to have poor response to CCRT and worse survival outcome. As mentioned above, whether high expression of SDF-1α by immunohistochemical staining or high serum concentration of pre-treatment SDF-1α, both predict poor prognosis of ESCC, indicating the significance of SDF-1α in the disease progression of ESCC.

Salvage esophagectomy has been regarded as a viable option for ESCC patients who got recurrent or persistent tumor after CCRT; however, this procedure usually contributes to excessive mortality and morbidity, resulting in decreased willingness of operation by surgeons. Kumagai et al. reported a meta-analysis which included four studies, and enrolled 219 ESCC patients who had recurrent or persistent tumor. The results of this analysis showed there were an OS benefit following salvage esophagectomy in comparison with second-line chemoradiotherapy [29]. A total of 36 patients died from salvage esophagectomy and treatment-related mortality was 10.3%. In our previous published study, synchronous ESCC/head and neck squamous cell carcinoma patients (HNSCC) had worse survival outcome than isolated ESCC patients; even for synchronous ESCC/HNSCC patients with a serious medical condition, patients who underwent salvage esophagectomy had superior OS compared to those who not [30]. In our study, salvage esophagectomy was an independent prognostic factor of better PFS and OS, indicating the importance of salvage operation for ESCC patients with recurrent or persistent tumor after CCRT.

Our study had several limitations. Firstly, the study was a retrospectively analysis at a single institution and therefore, the sample size was relatively small. Secondly, since there was only one female patient in our study, the significance of sex was not able to be fully evaluated. Third, the effect of radiotherapy was not evaluated in our study. Nevertheless, sex is not commonly regarded as a prognostic factor for survival in the literature. However, to the best of our knowledge, this study is the first to investigate the role of SDF-1α kinetics in ESCC patients who underwent definitive CCRT. Further studies in a larger population and animal studies are warranted to validate the findings of our study.

Conclusions

The results of our study suggested that SDF-1α plays an important role in ESCC disease progression through chemoresistance and the pre-treatment SDF-1α level and kinetics of SDF-1α are independent prognostic factors for ESCC patients receiving definitive CCRT. Periodic determinations of serum SDF-1α level may be valuable to predict prognosis of ESCC in clinical practice. Further studies in a larger population or a prospective study are warranted to validate these findings.

Funding

This research was funded by the Ministry of Science and Technology, grant numbers MOST 107-2314-B-182A-156-MY3 and the Chang Gung Memorial Hospital, grant numbers CMRPG8I0201, CMRPG8J1061, CMRPG8J0403, CMRPG8K0492 and CMRPG8K1291.

Conflicts of interest

The authors have declared that no competing interests exist.

Acknowledgements

We appreciated the Biostatistics Center, Kaohsiung Chang Gung Memorial Hospital for statistics work.

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