Brief Report

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Cytokine expression profiles in the sera of cutaneous squamous cell carcinoma patients

Saori Yamada, Masatoshi Jinnin*, Ikkou Kajihara, Taiji Nakashima, Jun Aoi, Miho Harada, Toshikatsu Igata, Shinichi Masuguchi, Satoshi Fukushima, Hironobu Ihn

Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan.

Summary **We focused on the interaction of cytokines in squamous cell carcinoma (SCC), and determined the expression profile of multiple cytokines in the serum of each patient with SCC in the present study. Serum samples were obtained from 12 SCC patients and 7 normal subjects. Four cytokines (IFN-γ, IL-6, GM-CSF, and TGF-β) were selected because they are reported to be involved in keratinocyte proliferation and SCC progression. Serum levels were measured using ELISA. We found a statistically significant increase of serum IFN-γ levels in SCC patients compared to those in normal subjects, and areas under the curve (AUC) of 0.82 for the serum levels of IFN-γ were higher than those for other cytokine levels according to ROC curve analysis. Patients with increased IFN-γ levels had a significantly more severe cancer stage. Furthermore, the combination of IFN-γ levels and TGF-β levels could improve the AUC to 0.84. We also found there was a significant correlation between IFN-γ levels and GM-CSF levels or between GM-CSF levels and TGF-β levels only in SCC patients. Our results suggest that the combination of IFN-γ levels and TGF-β levels is more effective to diagnose SCC, while serum levels of IFN-γ alone are useful to evaluate tumor progression. Furthermore, expression of these cytokines was not independent, but may be regulated by common upstream factors (*e.g.* cytokines or methylation) in SCC patients, and such factors may play some roles in the pathogenesis of SCC.**

Keywords: Squamous cell carcinoma, IFN-γ, IL-6, GM-CSF, TGF-β

1. Introduction

Squamous cell carcinoma (SCC) is one of the most frequent skin neoplasms. Compared to SCC seen in many other organs including esophagus, lungs and urinary bladder, cutaneous SCC is characterized by its strong correlation with cumulative ultraviolet exposure. Most cutaneous SCCs are usually low risk and treatable, but they have potential to recur and metastasize when they progress. To date, SCC antigen (SCC-Ag), tumor antigen that was originally purified from SCC, is the only reliable serum marker to diagnose SCC or to monitor the progress of the tumor. However, SCC-Ag has a drawback that the serum levels usually remain within normal limits at their early stage whereas they start to elevate only at the late stage (*1*). Therefore, it is mandatory to develop novel diagnostic methods for early detection and new therapeutic strategies. However, the pathogenesis of this malignant tumor is still to be clarified.

At present, several cytokines such as IFN-γ, IL-6, GM-CSF, and TGF-β have been reported to be involved in keratinocyte proliferation of the skin, and in the pathogenesis of SCC (*2-6*). In this study, we focused on the interaction of cytokines, and showed the expression profile of multiple cytokines in the serum of each patient with SCC.

2. Materials and Methods

2.1. *Clinical assessment and patient material*

Serum samples were obtained from 12 SCC patients (6

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**Address correspondence to:*
Dr. Masatoshi Jinnin, Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto, Japan.
E-mail: mjin@kumamoto-u.ac.jp

Table 1. Clinical and laboratory features of 12 patients with SCC

Age	Gender	Location	Diameter (cm)	Stage	SCC antigen (ng/mL)	IFN-γ (pg/mL)	IL-6 (pg/mL)	GM-CSF (pg/mL)	TGF-β (ng/mL)	IFN-γ + TGF-β
70	M	face	0.5	0	1.0	20.5	59.0	19.6	1.6	22.1
91	F	face	0.7	0	1.0	16.6	59.0	21.0	2.1	18.7
83	F	lip	1.5	I	1.1	21.0	73.7	25.3	2.4	23.4
85	F	nose	1.5	I	0.8	24.9	125.1	26.8	2.5	27.4
89	M	lip	1.5	I	2.2	25.8	110.5	14.3	2.0	27.8
73	F	jaw	2.9	II	1.9	45.0	176.4	24.4	1.9	46.9
71	M	lip	4.5	II	1.6	52.5	165.4	27.2	2.6	55.1
83	M	lip	2.0	II	1.0	125.9	172.7	84.1	2.8	128.7
77	M	face	2.5	II	1.4	35.9	70.1	26.8	2.3	38.2
75	M	face	5.0	III	2.0	48.5	70.1	32.5	2.1	50.6
79	F	lip	2.0	III	3.2	8.3	95.8	29.2	2.3	10.6
64	F	head	8.0	III	0.5	8.7	143.5	24.4	2.4	11.1

M, male; F, female; SCC, squamous cell carcinoma; IFN-γ, interferon-γ; IL-6, interleukin-6; GM-CSF, granulocyte macrophage colony-stimulating factor; TGF-β, transforming growth factor-β.

males and 6 females; age range, 64-91 years) (Table 1). Control serum samples were obtained from 7 normal subjects with seborrheic keratosis. Institutional review board approval and written informed consent were obtained according to the Declaration of Helsinki.

2.2. *Statistical analysis*

Statistical analysis was carried out with Mann-Whitney's *U* test for the comparison of medians, and Fisher's exact probability test for the analysis of frequency. Correlations were assessed using Pearson's correlation coefficient. *p* values less than 0.05 were considered significant.

3. Results and Discussion

First, we measured the serum concentrations of multiple cytokines by ELISA to determine a cytokine profile for SCC sera. Four cytokines were selected because they are reported to be involved in keratinocyte proliferation of the skin, and in the pathogenesis of SCC (2-6). As a result, we found a statistically significant increase of the serum IFN-γ levels in SCC patients compared to normal subjects (Figure 1a). On the other hand, there was no significant difference in levels of the other cytokines between SCC patients and normal subjects (Figures 1b-1d).

Next, we performed receiver operating characteristic (ROC) curve analysis to evaluate the usefulness of the concentration of each cytokine for diagnosis of SCC. The areas under the curve (AUC) of 0.82 (95% CI, 0.61 to 1.03) for serum levels of IFN-γ (Figure 2a) was higher than those for other cytokine levels (Figures 2b-2d), indicating that serum IFN-γ might serve as a more useful biomarker for differentiating SCC patients and normal subjects than other cytokines. Furthermore, the combination of IFN-γ levels and TGF-β levels could improve the AUC to 0.84, suggesting that the combination is more effective to diagnose SCC.

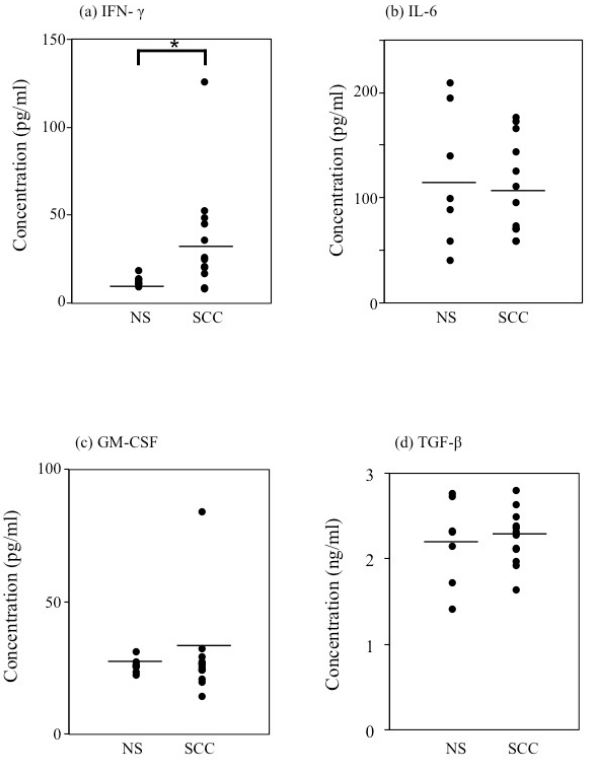

Figure 1. The concentrations of four cytokines (IFN-γ, IL-6, GM-CSF, and TGF-β) measured by ELISA using sera of normal subjects (NS) and SCC patients are shown on the ordinate. Bars show means. * $p < 0.05$.

Also, we examined the correlation among the levels of four cytokines. We could not find significant correlation among them in normal subjects, but there was mild and significant correlation between IFN-γ and GM-CSF ($r = 0.89$, $p < 0.01$) or between GM-CSF and TGF-β ($r = 0.64$, $p = 0.03$) in SCC patients (Figure 3). Therefore, expression of these cytokines was not independent in the sera of SCC patients.

When the cut-off value was set at mean + 6SD of normal subjects (= 30.4 pg/mL), serum IFN-γ levels were increased in 5 of 12 SCC patients. Patients with increased IFN-γ levels tended to have a significantly

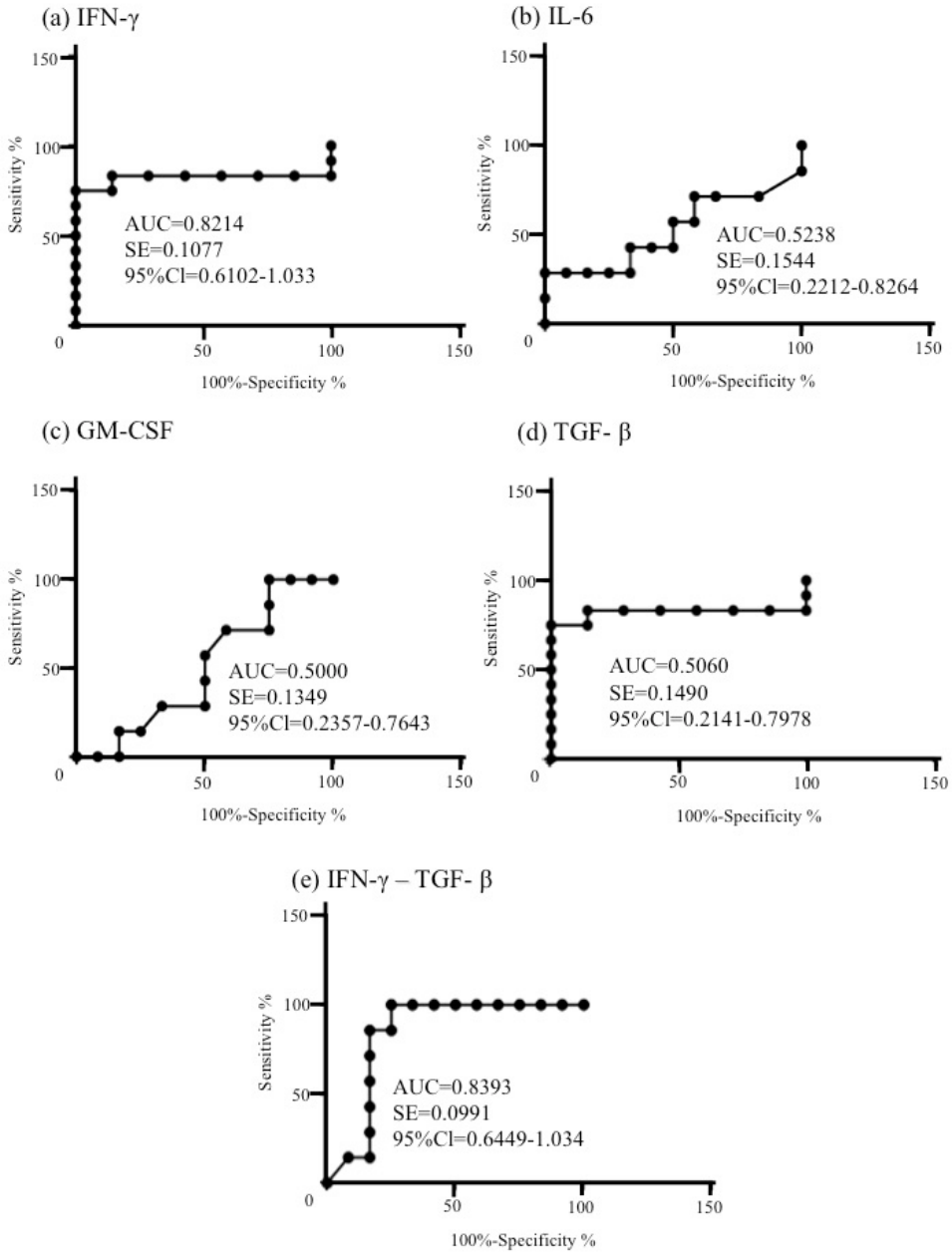

Figure 2. Receiver operating characteristic (ROC) curve for serum levels of indicated cytokines to distinguish SCC patients from normal subjects. AUC, areas under curves: SE, standard error: CI, confidence interval.

more severe cancer stage (II and III) than those with normal levels (100% vs. 28%, $p < 0.05$, Table 2).

Serum SCC-Ag was increased (> 1.5 ng/mL) in 1 of the 3 stage I patients, 2 of the 4 stage II patents, and 2 of the 3 stage IV patients (Table 1). Of note, increased serum IFN-γ levels were seen in all of the 4 stage II patients and 1 of the 3 stage IV patients. These results suggest that serum IFN-γ levels serve as a biomarker of tumor progression from early to middle stage, reflecting its contribution to tumor growth of SCC.

On the other hand, patients with increased IFN-γ levels also had increased levels of IFN-γ and TGF-β combination (> mean + 6SD, see Table 1), and analysis of correlation of the combination with clinical manifestations showed the same result as Table 2. Thus, the clinical significance of IFN-γ and TGF-β combination to evaluate tumor progression seems to be similar to that of IFN-γ alone in SCC patients.

In this study, we presented two novel findings: first, we found a statistically significant increase of serum IFN-γ levels in SCC patients compared to those in normal subjects. Patients with increased IFN-γ levels had a significantly more severe cancer stage. IFN-γ levels tended to be increased around stage II in SCC patients. Furthermore, the AUC of the combination of IFN-γ levels and TGF-β levels was higher than that of IFN-γ alone according to ROC curve analysis. Given that patients with increased levels of IFN-γ also showed increased levels of IFN-γ and TGF-β combination, our results suggest that the combination of IFN-γ levels and

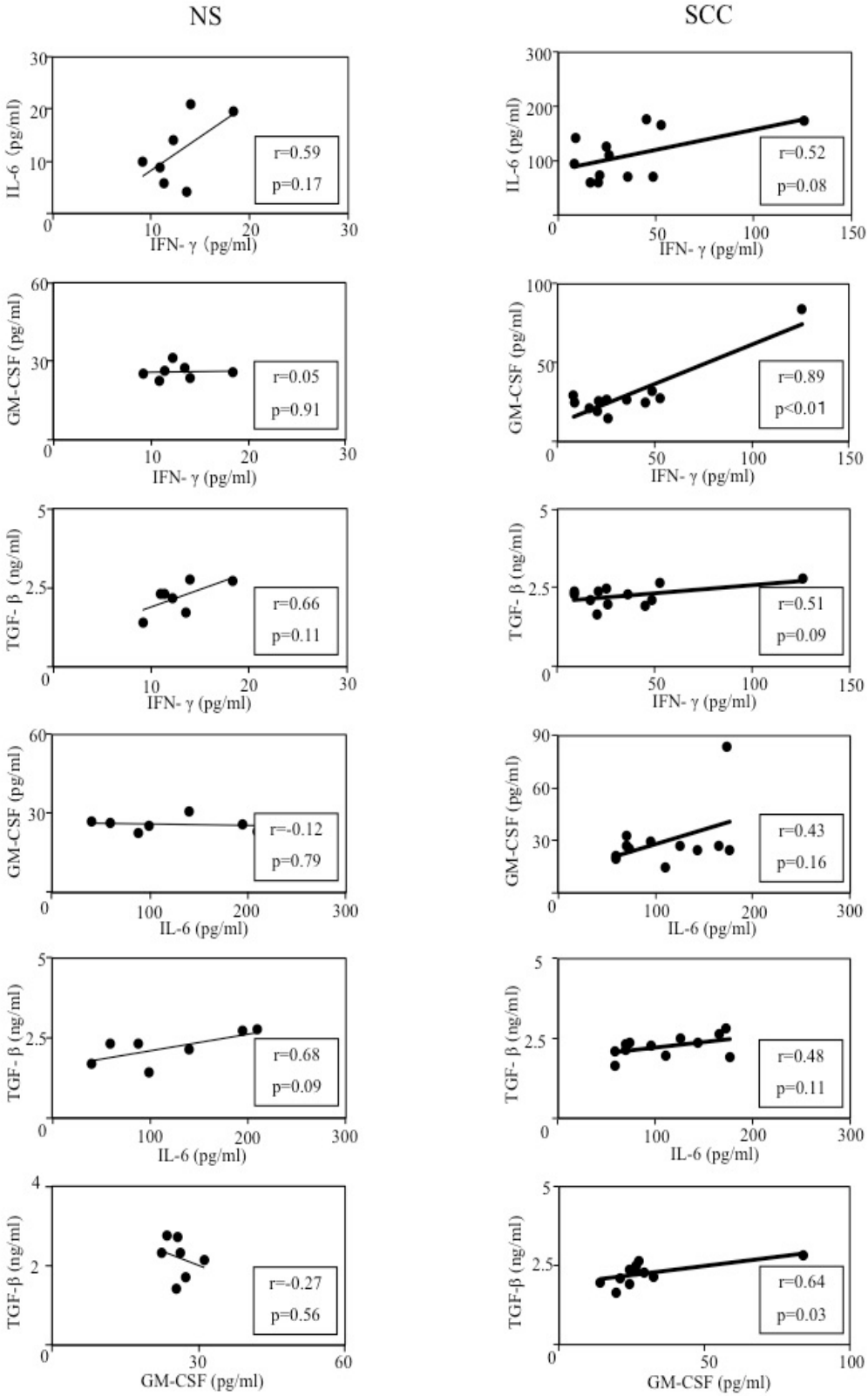

Figure 3. Correlation among the levels of four cytokines (IFN-γ, IL-6, GM-CSF and TGF-β) in each individual of normal subjects (NS) and SCC patients. Correlations were assessed by Pearson's correlation coefficient.

TGF-β levels is more effective to diagnose SCC, while serum levels of IFN-γ alone is sufficient to evaluate tumor progression from early to middle stage.

Second, we found there was significant correlation between IFN-γ levels and GM-CSF levels or between GM-CSF levels and TGF-β levels only in SCC patients. Therefore, expression of these cytokines was not independent, but regulated by common upstream factors (*e.g.* cytokines or methylation) in SCC patients, and such factors may play some roles in pathogenesis

Table 2. The association of serum IFN-γ levels with clinical and serological features of patients with SCC

Items	Patients with normal Interferon-γ levels (n = 7)	Patients with increased Interferon-γ levels (n = 5)
Age at the time of serum sampling (mean years)	80.14	75.80
Gender (M:F)	2:5	1:4
Clinical features		
Mean diameter (cm)	2.89	3.38
% of patients with stage II/III	28.57	100*
Laboratory features		
SCC antigen (ng/mL)	1.40	1.58

* $p < 0.05$ versus patients with normal IFN-γ levels using Fisher's exact probability test.

of SCC. Further studies are also needed to determine whether the upstream factors are the key molecules in SCC.

This is the first report focusing on the interaction of multiple cytokines, and demonstrating their expression profile in each patient with SCC. We suggest the possibility that the balance among multiple cytokines contribute to the pathogenesis of SCC, and indicate its clinical significance. This is a pilot study with a small number of patients. Although we could not find statistically significant correlation between cytokine levels and specific features of SCC (*e.g*. location or diameter), this may be because of the small patient number. Lager studies are needed in the future. Clarifying the involvement of the cytokine network in pathogenesis of SCC may lead to development of new diagnostic tools or new therapeutic strategies, and may contribute to the understanding of the mechanism of SCC.

References

1. Yagi H, Danno K, Maruguchi Y, Yamamoto M, Imamura S. Significance of squamous cell carcinoma (SCC)-related antigens in cutaneous SCC. A preliminary report. Arch Dermatol. 1987; 123:902-906.
2. Lathers DM, Young MR. Increased aberrance of cytokine expression in plasma of patients with more advanced squamous cell carcinoma of the head and neck. Cytokine. 2004; 25:220-228.
3. Skrinjar I, Brailo V, Vidovic-Juras D, Vucicevic-Boras V, Milenovic A. Evaluation of pretreatment serum interleukin-6 and tumour necrosis factor alpha as a potential biomarker for recurrence in patients with oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal. 2015; 20:e402-407.
4. Glick AB. The role of TGFβ signaling in squamous cell cancer: Lessons from mouse models. J Skin Cancer. 2012; 2012:249063.
5. Smith CW, Chen Z, Dong G, Loukinova E, Pegram MY, Nicholas-Figueroa L, Van Waes C. The host environment promotes the development of primary and metastatic squamous cell carcinomas that constitutively express proinflammatory cytokines IL-1alpha, IL-6, GM-CSF, and KC. Clin Exp Metastasis. 1998; 16:655-664.
6. Naganawa K, Takayama E, Adachi M, Mitsudo K, Iida M, Kamiya-Mizuno M, Kawaki H, Ichinose M, Motohashi M, Muramatsu Y, Tohnai I, Sumitomo S, Shikimori M, Kondoh N. Producing capabilities of interferon-γ and interleukin-10 in peripheral blood from oral squamous cell carcinoma patients. Open Dent J. 2015; 9:120-124.
7. Kudo H, Jinnin M, Yamane K, Makino T, Kajihara I, Makino K, Honda N, Nakayama W, Fukushima S, Ihn H. Intravenous immunoglobulin treatment recovers the down-regulated levels of Th1 cytokines in the sera and skin of scleroderma patients. J Dermatol Sci. 2013; 69:77-80.

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