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Regular Article

Association of Single Nucleotide Polymorphisms in *STAT3*, *ABCB1*, and *ABCG2* with Stomatitis in Patients with Metastatic Renal Cell Carcinoma Treated with Sunitinib: A Retrospective Analysis in Japanese Patients

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Signal transducer and activator of transcription (STAT) 3 is a key factor in homeostasis of the oral mucosa by regulating the production of inflammatory cytokines. Sunitinib is a substrate of P-glycoprotein (multidrug resistance (MDR)-1/*ABCB1*) and breast-cancer resistance protein (BCRP/*ABCG2*). In this retrospective study, we evaluated the association between sunitinib-induced stomatitis and *STAT3*, *ABCB1*, and *ABCG2* polymorphisms in patients with metastatic renal cell carcinoma (mRCC). Fifty-two Japanese patients with RCC treated with sunitinib were retrospectively genotyped to elucidate a potential association between *STAT3*, *ABCB1*, and *ABCG2* polymorphisms and stomatitis development. Stomatitis occurred in 22 out of 52 patients. The TT+TC genotypes at *STAT3* rs744166 had an odds ratio of 5.00 against CC genotype for the stomatitis development (95% confident interval, 0.97–25.8). In the Kaplan–Meier method for the cumulative incidence of stomatitis, a statistically significant difference was observed between the TT+TC and CC genotypes in *STAT3* rs744166 ($p=0.037$). Both multiple logistic regression analysis and Cox proportional-hazards regression analysis show *STAT3* rs744166 TT+TC genotypes and serum creatinine in each patient were significant independent factors for stomatitis development. In conclusion, *STAT3* polymorphism may be a novel risk factor for sunitinib-induced stomatitis in patients with mRCC.

Key words sunitinib; polymorphism; stomatitis; adverse effect; renal cell carcinoma

Molecular-targeted drugs represent a standard first-line therapeutic option for patients with metastatic renal cell carcinoma (mRCC).[1)] However, stomatitis is a very frequent complication associated with molecular-targeted drugs. Sunitinib, one of multiple tyrosine-kinase inhibitors currently used as first-line treatment for mRCC, is associated with stomatitis and has a reported incidence of 52% at any grades.[2)] It was reported that substantial differences in the incidence of any-grade adverse events (AEs) between all Asian and non-Asian patients were observed for stomatitis (39 *vs.* 26%, respectively).[3)] Asian patients treated with sunitinib developed stomatitis more frequently than did non-Asian patients. There were major differences in the incidence of AEs depending on where the Asian patients were treated. For example, Asian-A patients (treated at sites in Asia) and Asian-O patients (treated at sites outside Asia) showed substantial differences in the incidences of stomatitis (47 *vs.* 23%, respectively).[3)] Stomatitis is the cause of significantly decreased QOL. Furthermore, 10% of the reported stomatitis cases were associated with dose reduction, drug interruption, or cessation.[4)] Because stomatitis is a clinically important symptom, elucidation of its pathogenic mechanism is an important challenge.

It is well known that stomatitis induced by chemotherapy and radiotherapy is generated by reactive oxygen species (ROS). ROS activate nuclear factor-kappaB (NF-κB) and release pro-inflammatory cytokines, such as tumor necrosis factor (TNF), interleukin (IL)-6, and IL-1β. These pro-inflammatory cytokines trigger stomatitis *via* apoptosis and tissue damage.[5,6)] However, molecular-targeted drug-induced stomatitis may not be mediated by these mechanisms.[7)] Chemotherapy-induced stomatitis is characterized by local tissue damage and an inflammatory reaction; however, sunitinib-induced stomatitis, in contrast, appears to be primarily a "functional" mucosal irritation.[8)]

Signal transducer and activator of transcription (STAT) 3 is a point of convergence for the signaling pathways downstream of numerous cytokine receptors or tyrosine kinases, including vascular endothelial growth factor (VEGF) receptor, platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor, and Src.[1,9,10)] STAT3 regulates the expression of several genes involved in a variety of cellular responses including proliferation, differentiation, apoptosis, and wound healing.[11,12)] Furthermore, it was reported that activating STAT3 can promote oral mucosal wound healing,[13)] STAT3 may play a key role for regulating homeostasis of oral mucosa.

Sunitinib is a substrate of P-glycoprotein (Pgp/multidrug resistance (MDR)-1/*ABCB1*) and breast-cancer resistance protein (BCRP/*ABCG2*).[14–16)] Interestingly, *ABCB1* polymorphism influences drug concentrations in saliva.[17)] Pgp and BCRP are expressed in various sites, including salivary glands.[17–19)] However, the association between sunitinib-induced stomatitis and genomic functional changes of *ABCB1* or *ABCG2* has not yet been studied.

There are ethnic differences in *STAT3*, *ABCB1*, and *ABCG2* polymorphisms. In addition, their polymorphisms affect the function or the expression of each protein. Differences in

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polymorphism may be one of the risk factors for sunitinib-induced stomatitis. In this study, we selected *STAT3*, *ABCB1*, and *ABCG2* polymorphisms, which are known to occur with greater frequency in the Japanese population. The frequency of stomatitis in the present study was similar to that in patients treated in Asia. In this retrospective study, we evaluated the relationship between *STAT3*, *ABCB1*, and *ABCG2* polymorphisms and the development of sunitinib-induced stomatitis in patients with mRCC. Our findings suggest that the *STAT3* polymorphism contribute to a risk factor for stomatitis.

PATIENTS AND METHODS

Patients The present series consisted of RCC patients who had been diagnosed with metastatic disease, and were subsequently treated with sunitinib at the Department of Urology, Kobe University Hospital from April 2008 to March 2016. In case of AEs, dose reduction, interruption, or cessation of sunitinib therapy was implemented. Eligible patients were those whose blood sample was available for DNA extraction and those who were available for follow-up. Incidence of stomatitis was followed up until 1 year since the start of therapy. Patients were excluded if sunitinib treatment failure were occurred within 28 d for any reasons other than stomatitis. Patients who developed stomatitis before the sunitinib therapy were excluded. Laboratory data were collected at the start of sunitinib therapy. All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was approved by the hospital's Institutional Ethical Committee. This study was a retrospective analysis, and written informed consent for the use of blood samples for research purposes was obtained from all patients; under approval, records were reviewed, and follow-up data were collected in cooperation with the assigned urologist and/or the clinical pharmacist.

Evaluation of Stomatitis Stomatitis was graded according to different levels of severity and was determined retrospectively using the Common Terminology Criteria for Adverse Events version 4.0 of the National Cancer Institute according to the patients' medical records. Stomatitis developer was defined as a patient who developed stomatitis by sunitinib of grade $\geq$1 in the period from initiation of sunitinib therapy to termination of follow-up. Toxicity was assessed on the day when the patient initially developed stomatitis after starting sunitinib. If patients developed stomatitis at ambulatory practice, the day of stomatitis occurrence was defined as the date of ambulatory visit.

Blood Sampling and Genotyping Blood samples were the residue of blood collected for the diagnostic or clinical laboratory tests. DNA was isolated from peripheral blood mononuclear cells using the NucleoSpin® Blood kit (MACHEREY-NAGEL, GmbH, Düren, Germany), according to the manufacturer's protocol. Samples were subjected to genotyping to detect *STAT3* polymorphisms (rs744166 and rs4796793) using TaqMan SNP Genotyping Assay purchased from Applied Biosystems (ABI; Carlsbad, CA, U.S.A.). Reactions were conducted on an ABI PRISM 7900HT Sequence Detection System, according to the following protocol: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The post-PCR plates were read on the 7900HT and used to determine the genotype. Samples were subjected to genotyping to detect *ABCB1* polymorphisms (rs2032582 G2677T/A, rs1045642 C3435T, and rs1128503 C1236T) and *ABCG2* polymorphism (rs2231142 C421A) using the PCR-restriction fragment length polymorphism (RFLP) technique or TaqMan SNP Genotyping Assay.[20–22] In brief, PCR was performed using TaKaRa Ex Taq® (TaKaRa, Shiga, Japan) according to the manufacturer's instructions. The rs1045642, rs1128503, and rs2231142 polymorphic sites were amplified by PCR using the following pair of primers: forward (5′-TTG ATG GCA AAG AAA TAA AGC-3′) and reverse (5′-CTT ACA TTA GGC AGT GAC TCG-3′), forward (5′-ATC CTG TGT CTG TGA ATT GC-3′) and reverse (5′-TCA GAA AGA TGT GCA ATG TG-3′), and forward (5′-GTT GTG ATG GGC ACT CTG ATG GT-3′) and reverse (5′-CAA GCC ACT TTT CTC ATT GTT-3′), respectively. After amplification, PCR products underwent digestion with the indicated allele-specific restriction enzyme. RFLP for rs1046542 SNP was performed using Mbo I restriction enzyme, rs1128503 SNP was performed using EcoO109 I restriction enzyme, and rs2231142 SNP was performed using Taa I restriction enzyme. Cleaved DNA fragments were then subjected to electrophoresis in either 3 or 7.5% agarose gel.

Statistical Analysis All genotype frequencies were compared with the reported Japanese frequencies using the chi-square test. Comparisons of patient characteristics between the non-stomatitis and stomatitis groups were made using the Fisher's exact test and the Mann–Whitney *U* test. Associations between genotypes and stomatitis development were evaluated using the Fisher's exact test and estimated using odds ratio (ORs) and 95% confident intervals (CIs). The Kaplan–Meier method and log-rank test were used to estimate and compare the cumulative incidence of stomatitis between two groups.

In the multivariate analysis, the stepwise logistic regression analysis was performed to evaluate the predictive significance of SNPs for stomatitis development using variables of patient's characteristics with a *p*-value less than 0.1 in the univariate analysis. Cox proportional-hazards regression analysis was performed to evaluate the significance of cumulative incidence of stomatitis in SNPs using variables of patient's characteristics with a *p*-value less than 0.1 in the univariate analysis by the step-down procedure. $p<0.05$ was considered statistically significant. All statistical analyses were performed using EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan),[23] which is a graphical user interface for R version 3.2.2 or more precisely, a modified version of R Commander designed to aid statistical functions frequently used in biostatistics (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Distribution of *STAT3*, *ABCB1*, and *ABCG2* Polymorphisms and Subject Characteristics Four patients experienced treatment failure within 28 d of starting sunitinib. Six patients were lost to follow-up. One patient was unable to collect blood sampling. Thus, the final analysis included 52 patients (Fig. 1). Patient characteristics at the start of sunitinib therapy are shown in Table 1. Sunitinib therapy was initiated at a dose of 50, 37.5, or 25 mg/d as 4 weeks on and 2 weeks off or 2 weeks on and 1 week off. Serum creatinine in sto-

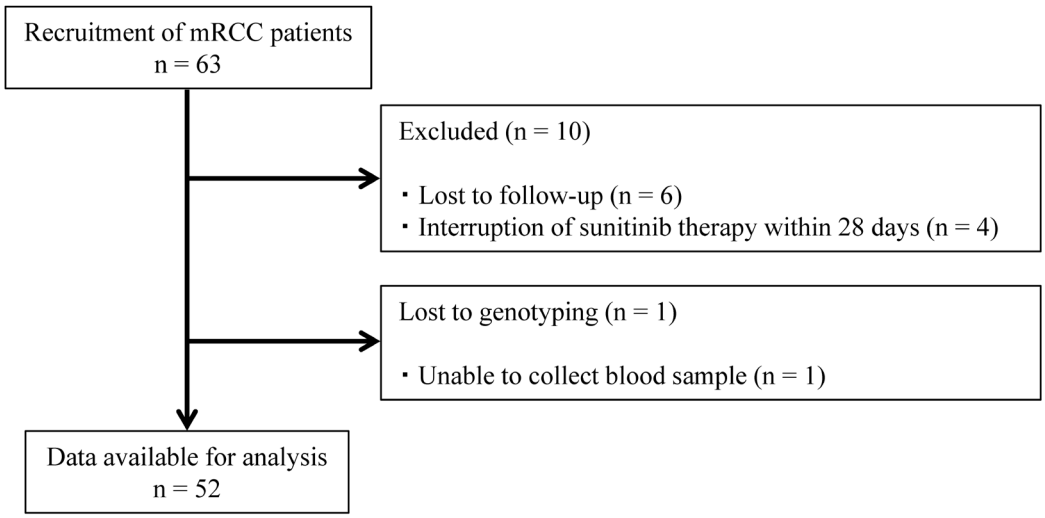

Fig. 1. Flowchart of Study Selection Process

Table 1. Association between Stomatitis Development and Patient Characteristics

	Total patients (n=52)	Non-stomatitis (n=30)	Stomatitis (n=22)	p Value
Gender: Male[a)]	36 (69.2%)	23 (76.7%)	13 (59.1%)	0.23[c)]
Body weight (kg)[b)]	58.7 (36.0–87.1)	59.8 (36.0–78.2)	56.5 (40.9–87.1)	0.89[d)]
Dose density (mg/d)[b)]	28.1 (10.7–50.0)	29.0 (10.7–50.0)	27.5 (18.8–50.0)	0.80[d)]
Dose density (mg/d/kg)[b)]	0.49 (0.19–0.90)	0.51 (0.19–0.88)	0.49 (0.31–0.90)	0.61[d)]
POD (d)[b)]	1179.5 (0–8087)	979 (0–7467)	1519.2 (10–8087)	0.60[d)]
Therapy line: 1st line[a)]	40 (76.9%)	21 (70.0%)	19 (86.4%)	0.20[c)]
Smoking[a)]	23 (44.2%)	13 (43.3%)	10 (45.5%)	1.00[c)]
Diabetes[a)]	13 (25.0%)	7 (23.3%)	6 (27.3%)	0.76[c)]
Radiotherapy[a)]	15 (28.8%)	9 (30.0%)	6 (27.3%)	1.00[c)]
Age (years)[b)]	67.5 (41–89)	68 (41–89)	66.5 (52–79)	0.50[d)]
AST (IU/L)[b)]	19 (10–92)	20 (10–40)	19 (14–92)	0.97[d)]
ALT (IU/L)[b)]	18.5 (7–99)	18 (7–52)	19 (7–99)	0.88[d)]
γ-GTP (IU/L)[b)]	30.5 (10–288)	31 (12–146)	24 (10–288)	0.61[d)]
Serum creatinine (mg/dL)[b)]	1.1 (0.6–1.6)	1.18 (0.68–1.61)	1.03 (0.64–1.55)	0.034*[,d)]
eGFR (mL/min/1.73 m^2)[b)]	50.3 (26.8–83.6)	45.2 (28.7–83.6)	53.9 (26.8–69.9)	0.21[d)]
Albumin (g/dL)[b)]	4.1 (2.0–5.0)	4.0 (2.8–5.0)	4.2 (2.0–4.8)	0.21[d)]
BMI (kg/m^2)[b)]	22.2 (14.5–30.4)	21.9 (16.0–28.7)	22.2 (14.5–30.4)	0.35[d)]

Dose density: cumulative dose for 28 d after the start of sunitinib therapy divided by 28 d. POD: Postoperative day for the initiation of sunitinib therapy. *a*) Number (%). *b*) Median (range). *c*) p Value by Fisher's exact test. *d*) p Value by Mann–Whitney U test. *$p<0.05$.

matitis patients was significantly lower compared with that in non-stomatitis patients (p=0.034; Table 1).

Samples from all 52 patients were successfully subjected to *STAT3* (rs744166 and rs4796793), *ABCB1* (rs2032582 G2677T/A, rs1045642 C3435T, and rs1128503 C1236T), and *ABCG2* (rs2231142 C421A) polymorphism analysis. All analyzed genotype frequencies did not show significant deviation compared with those in the Japanese population according to the NCBI database (http://www.ncbi.nlm.nih.gov/snp/).

Association of *STAT3*, *ABCB1*, and *ABCG2* Polymorphisms with Stomatitis Development Stomatitis was observed in 22 patients. Among the six candidate polymorphisms, a significant association with stomatitis development was observed with the *STAT3* rs744166 genotype. The *STAT3* rs744166 T allele frequencies were 50.0 and 70.5% in the non-stomatitis and stomatitis groups, respectively (OR, 2.39; 95% CI, 1.05–5.43; p=0.045; Table 2). The *STAT3* rs744166 CT+TT had a tendency to participate in sunitinib-induced stomatitis (OR, 5.00; 95% CI, 0.97–25.8; p=0.051). Furthermore, the *ABCB1* rs1128503 CT+TT genotypes exhibited a significant association with stomatitis development (OR and 95% CI, not applicable (NA); p=0.033). Meanwhile, the *STAT3* rs4796793 C allele frequencies had a tendency to participate in sunitinib-induced stomatitis (OR, 2.23; 95% CI, 0.98–5.08; p=0.069).

Furthermore, multiple logistic regressions considering patient characteristics in addition to the *STAT3* and *ABCB1* genotypes ($p<0.1$ in the univariate analysis) as independent variables were examined. After the stepwise deletion of an insignificant factor one by one, rs744166 TT+TC genotypes at *STAT3* and serum creatinine were proved to be significant associated factors with stomatitis development (OR for *STAT3*, 6.91, 95% CI, 1.20–39.7, p=0.030; OR for serum creatinine, 0.059, 95% CI, 0.0051-0.69, p=0.024; Table 3).

Association of *STAT3*, *ABCB1*, and *ABCG2* Polymorphisms with Cumulative Incidence of Stomatitis Development Kaplan–Meier analysis demonstrated a statistically significant difference in the median time to stomatitis development between patients with the CC genotype and those with

Table 2. Association between Stomatitis Development and *STAT3*, *ABCB1* and *ABCG2* Polymorphisms

		Number (%)		Allele or genotype comparison			
		Non-stomatitis (n=30)	Stomatitis (n=22)		Odds ratio	95% CI	p Value
STAT3 rs744166							
Allele	T	30 (50.0%)	31 (70.5%)	T *vs.* C	2.39	1.05–5.43	0.045*
	C	30 (50.0%)	13 (29.5%)				
Genotype	TT	10 (33.3%)	11 (50.0%)	TT *vs.* TC *vs.* CC	—	—	0.12
	TC	10 (33.3%)	9 (40.9%)	TT *vs.* TC+CC	2.00	0.65–6.19	0.26
	CC	10 (33.3%)	2 (9.1%)	TT+TC *vs.* CC	5.00	0.97–25.8	0.051
STAT3 rs4796793							
Allele	C	31 (51.7%)	31 (70.5%)	C *vs.* G	2.23	0.98–5.08	0.069
	G	29 (48.3%)	13 (29.5%)				
Genotype	CC	10 (33.3%)	11 (50.0%)	CC *vs.* CG *vs.* GG	—	—	0.17
	CG	11 (36.7%)	9 (40.9%)	CC *vs.* CG+GG	2.00	0.65–6.19	0.26
	GG	9 (30.0%)	2 (9.1%)	CC+CG *vs.* GG	4.29	0.82–22.3	0.092
ABCB1 rs2032582: G2677T/A							
Allele	G	26 (43.3%)	18 (40.9%)	G *vs.* T *vs.* A	—	—	0.058
	T	13 (21.7%)	10 (22.7%)				
	A	21 (35.0%)	16 (36.4%)				
Genotype	GG	3 (10.0%)	4 (18.2%)	GG *vs.* GT *vs.* GA *vs.* TT *vs.* AA *vs.* TA	—	—	0.062
	GT	12 (40.0%)	4 (18.2%)	GG *vs.* GT+GA *vs.* TT+AA+TA	—	—	0.31
	GA	8 (26.7%)	6 (27.3%)	GG *vs.* GT+GA+TT+AA+TA	2.00	0.40–10.0	0.44
	AA	6 (20.0%)	2 (9.1%)	GG+GT+GA *vs.* TT+AA+TA	0.53	0.16–1.79	0.53
	TA	1 (3.3%)	6 (27.3%)				
	TT	0 (0.0%)	0 (0.0%)				
ABCB1 rs1045642: C3435T							
Allele	C	34 (56.7%)	26 (59.1%)	C *vs.* T	1.11	0.50–2.43	0.84
	T	26 (43.3%)	18 (40.9%)				
Genotype	CC	11 (36.7%)	7 (31.8%)	CC *vs.* CT *vs.* TT	—	—	0.53
	CT	12 (40.0%)	12 (54.5%)	CC *vs.* CT+TT	0.81	0.25–2.58	0.77
	TT	7 (31.8%)	3 (13.6%)	CC+CT *vs.* TT	1.93	0.44–8.49	0.49
ABCB1 rs1128503: C1236T							
Allele	C	25 (41.7%)	14 (31.8%)	C *vs.* T	0.65	0.29–1.48	0.41
	T	35 (58.3%)	30 (68.2%)				
Genotype	CC	6 (20.0%)	0 (0.0%)	CC *vs.* CT *vs.* TT	—	—	0.067
	CT	13 (43.3%)	14 (63.6%)	CC *vs.* CT+TT	—	—	0.033*
	TT	11 (36.7%)	8 (36.4%)	CC+CT *vs.* TT	1.01	0.32–3.18	1.00
ABCG2 rs2231142: C421A							
Allele	C	43 (71.7%)	36 (81.8%)	C *vs.* A	1.78	0.69–4.60	0.26
	A	17 (28.3%)	8 (18.2%)				
Genotype	CC	16 (53.3%)	14 (63.6%)	CC *vs.* CA *vs.* AA	—	—	0.30
	CA	11 (36.7%)	8 (36.4%)	CC *vs.* CA+AA	1.53	0.50–4.73	0.57
	AA	3 (10.0%)	0 (0.0%)	CC+CA *vs.* AA	—	—	0.25

*$p<0.05$.

the TT+TC genotypes at *STAT3* rs744166 (CC, NA; TT+TC, 315 d; p=0.037, log-rank test; Fig. 2A) and between patients with the CC genotype and the CT+TT genotypes at *ABCB1* rs1128503 (NA; $p<0.050$, log-rank test; Fig. 2B). Seven patients developed stomatitis after changing from hospitalization therapy to the ambulatory therapy. The intervals of hospital visit were 1 week (1 patient), 2 weeks (2 patients), 3 weeks (2 patients), and 6 weeks (2 patients). Patients with hospital visit interval of 6 weeks developed stomatitis in 306 and 315 d after start of sunitinib therapy.

The Cox proportional-hazards regression analyses were performed considering patient characteristics in addition to the *STAT3* and *ABCB1* genotypes as independent variables ($p<0.1$ in the univariate analysis). As a result of the stepwise deletion, rs744166 TT+TC genotypes at *STAT3* and serum creatinine were significantly associated with the cumulative incidence of stomatitis development (Hazard ratio for *STAT3*, 5.21, 95% CI, 1.20–22.6, p=0.028; Hazard ratio for serum creatinine, 0.11, 95% CI, 0.020-0.66, p=0.015; Table 3).

DISCUSSION

Oral mucositis develops almost exclusively on the buccal and labial mucosa, lateral aspects of the tongue, floor of mouth, and soft palate.[7] As STAT3 regulates oral mucosal wound healing,[13] activation of STAT3 might protect oral mucosa. It is known that the C allele of rs744166 leads to a higher basal expression level of STAT3.[24] In this study, we clarified that patients with CC genotypes at *STAT3* rs744166 had a significantly lower incidence of stomatitis compared

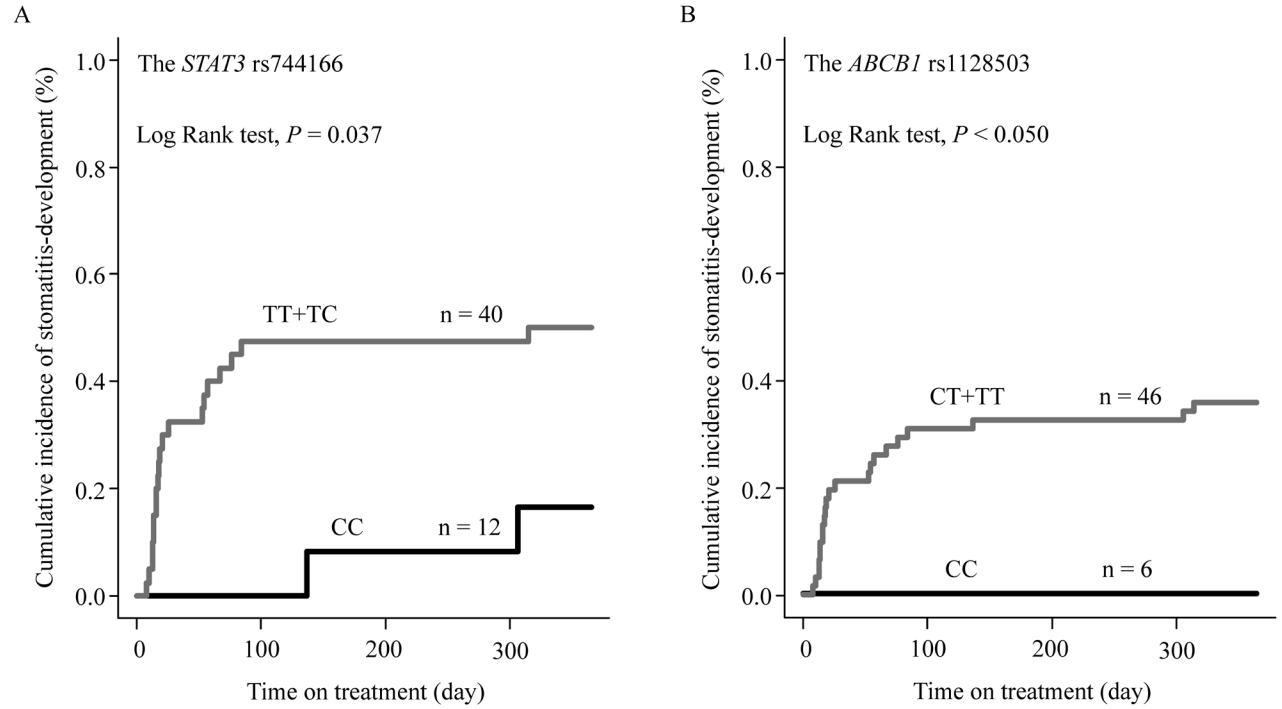

Fig. 2. Cumulative Incidence of Stomatitis for All 52 Patients with mRCC on rs744166 and rs1128503 Genotypes
A) The *STAT3* rs744166 and B) the *ABCB1* rs1128503.

Table 3. Multiple Analyses for Stomatitis Development

Logistic regression analysis	Odds ratio	95% CI	*p* Value
STAT3 rs744166 (TT+TC *vs.* CC)	6.91	1.20–39.7	0.030*
Serum creatinine (mg/dL)	0.059	0.0051–0.69	0.024*
Cox proportional-hazards regression analysis	Hazard ratio	95% CI	*p* Value
STAT3 rs744166 (TT+TC *vs.* CC)	5.21	1.20–22.6	0.028*
Serum creatinine (mg/dL)	0.11	0.020–0.66	0.015*

*$p<0.05$.

with those with TT+TC.

In recent works, sunitinib was reported to induce RCC cell apoptosis and to decrease immunosuppressive cell numbers as a result of STAT3 inhibition.[1,24–27] STAT3 is activated by the stimulation of IL-6 and growth factors, and is then involved in the regulation of cellular differentiation, survival, and proliferation.[28,29] Immune systems such as Toll-like receptor signaling and B-cell receptor signaling and inflammatory cytokines relate to stomatitis development.[5,6] The following underlying mechanisms for sunitinib-induced stomatitis have been suggested: dendritic-cell proliferation by sunitinib and cytokine production by increased Toll-like receptor are caused by the immunodeficiency and increased inflammatory cytokine production. It is necessary to elucidate the relationship between the rs744166 and inflammatory cytokines in the future.

The rs1128503 (C1236T) TT-genotype at the encoding site of *ABCB1* could lead to increased activity of the efflux pump or increased affinity of the pump for sunitinib.[30] Moreover, in mRCC patients treated with sunitinib, the TT-variant of *ABCB1* rs1128503 was associated with a higher plasma clearance of sunitinib.[30] In the present study, although there were no patients with stomatitis who had the *ABCB1* rs1128503 CC genotype, the *ABCB1* rs1128503 CT+TT genotypes exhibited a significant association with stomatitis development. Pgp and BCRP are expressed in various sites across the body, and they are also expressed in the salivary glands.[17–19,31] In addition, *ABCB1* polymorphism influences drug concentrations in the saliva.[17] Our results suggested the relationship between the *ABCB1* rs1128503 polymorphism and sunitinib-induced stomatitis. However, our results did not find the link between *ABCB1* rs2032582, *ABCB1* rs1045642, or *ABCG2* rs2231142 polymorphisms and sunitinib-induced stomatitis. The *ABCB1* rs1128503 CT+TT genotypes might excrete a drug to the saliva more extensively compared with CC genotype. Therefore, there is a possibility that the difference in saliva secretion amount depending on the *ABCB1* rs1128503 polymorphism affects the occurrence of stomatitis. If it is possible to evaluate the salivary concentration of sunitinib relative to *ABCB1* polymorphism, it might be possible to examine *ABCB1*'s effect on salivary secretion of sunitinib within the oral cavity.

The *ABCB1* 3435TT (rs1045642) polymorphism affects the activation or affinity of the efflux pump for sunitinib, leading to increased plasma concentration of sunitinib in mRCC patients.[32] In addition, the *ABCG2* 421C>A (rs2231142) polymorphisms significantly affect the pharmacokinetics of sunitinib in Japanese RCC patients.[33] However, in the pres-

ent patient group, there was no relationship between these polymorphisms or dose density in 28 d per body weight and sunitinib-induced stomatitis. Although the effects of *ABCB1* TTT haplotype on pharmacokinetics and pharmacodynamics were often examined, our study cannot consider these effects because there were no patients with the TT-variant of the rs2032582.

The positive predictive value of genetic polymorphisms in determining stomatitis was 50.0% in the *STAT3* rs744166; thus, there is a probability that approximately half positive results could be false positive. We believe that the development of stomatitis depends on a variety of factors, such as infections in immunocompromised patients with low white blood cell and neutrophil counts, poor oral hygiene, nutritional deficiency, or steroid administration. Furthermore, the VEGF and the MAP-kinase pathway may be involved in mucosal integrity/defense and repair.[8] Data from the present study indicate a high probability that these factors affect sunitinib-induced stomatitis.

There are no significant differences in patient characteristics except for serum creatinine concentration between sunitinib-induced stomatitis patients and non-stomatitis patients. The exact reasons why serum creatinine concentrations in stomatitis patients were significantly lower compared with those in non-stomatitis patients were unknown. However, the estimated glomerular filtration rate (eGFR)-values were not significantly different between in sunitinib-induced stomatitis and non-stomatitis patients, and it was reported that pharmacokinetics and sunitinib safety was not affected by impaired renal function.[34] Creatinine production depends on muscle mass.[35] In the recent studies, low serum creatinine, a surrogate of muscle mass, has prognostic importance for patient mortality in the peritoneal dialysis patients or general hospital population.[36,37] One possible explanation was that sunitinib-induced stomatitis might be associated with the muscle mass reflected by serum creatinine. In the present study, the stomatitis group included more female patients compared with the non-stomatitis group did (40.9 *vs.* 23.3%), although there was no statistical significance. Furthermore, Karnofsky performance status (KPS) was often used as an index of patient functional impairment in cancer patients, where 100% is perfect health and 0% is death.[38] The number of patients who has 80% and over KPS was lower in the stomatitis group than in the non-stomatitis group (50 *vs.* 69% excluding the three patients whose KPS were unknown). Since female patients and/or patients with lower KPS who may have less muscle mass were considered to be easy to develop sunitinib-induced stomatitis, the serum creatinine, but not eGFR, might be a good predictor for the development of stomatitis. A more precise study about this phenomenon is needed in the future.

We should emphasize that a limited number of patients were retrospectively investigated in the present study. Sample size was preferentially considered with feasibility according to the number of ambulatory patients; therefore, it would appear that the statistical power of the hypotheses in this study was inadequate. Large exploratory clinical trials are required to confirm our hypothesis. Furthermore, outpatients could not be specified the exact day of the onset of stomatitis. However, in this study, patients with hospital visit of 6-week intervals developed stomatitis after 300 d since the initiation of sunitinib. Therefore, we believe that this concern did not affect the conclusion of this study.

In conclusion, our retrospective analysis indicates that *STAT3* polymorphism may be a significant risk factor for sunitinib-induced stomatitis in Japanese patients with mRCC. In the future, we believe that *STAT3* polymorphism can be used to establish dedicated measures against drug side effects and mechanism-based prophylaxis in stomatitis.

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REFERENCES

1) Yamamoto K, Shinomiya K, Ioroi T, Hirata S, Harada K, Suno M, Nishioka T, Kume M, Makimoto H, Nakagawa T, Hirano T, Bito T, Nishigori C, Miyake H, Fujisawa M, Hirai M. Association of single nucleotide polymorphisms in STAT3 with hand-foot skin reactions in patients with metastatic renal cell carcinoma treated with multiple tyrosine kinase inhibitors: A retrospective analysis in Japanese patients. *Target. Oncol.*, **11**, 93–99 (2016).
2) Uemura H, Shinohara N, Yuasa T, Tomita Y, Fujimoto H, Niwakawa M, Mugiya S, Miki T, Nonomura N, Takahashi M, Hasegawa Y, Agata N, Houk B, Naito S, Akaza H. A phase II study of sunitinib in Japanese patients with metastatic renal cell carcinoma: insights into the treatment, efficacy and safety. *Jpn. J. Clin. Oncol.*, **40**, 194–202 (2010).
3) Lee SH, Bang YJ, Mainwaring P, Ng C, Chang JW, Kwong P, Li RK, Sriuranpong V, Toh CK, Yuan J, Pitman Lowenthal S, Chung HC. Sunitinib in metastatic renal cell carcinoma: an ethnic Asian subpopulation analysis for safety and efficacy. *Asia Pac. J. Clin. Oncol.*, **10**, 237–245 (2014).
4) Yoo C, Kim JE, Lee JL, Ahn JH, Lee DH, Lee JS, Na S, Kim CS, Hong JH, Hong B, Song C, Ahn H. The efficacy and safety of sunitinib in korean patients with advanced renal cell carcinoma: high incidence of toxicity leads to frequent dose reduction. *Jpn. J. Clin. Oncol.*, **40**, 980–985 (2010).
5) Sonis ST. Pathobiology of oral mucositis: novel insights and opportunities. *J. Support. Oncol.*, **5** (Suppl. 4), 3–11 (2007).
6) Blijlevens N, Sonis S. Palifermin (recombinant keratinocyte growth factor-1): a pleiotropic growth factor with multiple biological activities in preventing chemotherapy- and radiotherapy-induced mucositis. *Ann. Oncol.*, **18**, 817–826 (2007).
7) Boers-Doets CB, Epstein JB, Raber-Durlacher JE, Ouwerkerk J, Logan RM, Brakenhoff JA, Lacouture ME, Gelderblom H. Oral adverse events associated with tyrosine kinase and mammalian target of rapamycin inhibitors in renal cell carcinoma: a structured literature review. *Oncologist*, **17**, 135–144 (2012).
8) Kollmannsberger C, Bjarnason G, Burnett P, Creel P, Davis M, Dawson N, Feldman D, George S, Hershman J, Lechner T, Potter A, Raymond E, Treister N, Wood L, Wu S, Bukowski R. Sunitinib in metastatic renal cell carcinoma: recommendations for management

of noncardiovascular toxicities. *Oncologist*, **16**, 543–553 (2011).
9) Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao Y, Pestell RG, Albanese C, Darnell JE Jr. Stat3 as an oncogene. *Cell*, **98**, 295–303 (1999).
10) Kujawski M, Zhang C, Herrmann A, Reckamp K, Scuto A, Jensen M, Deng J, Forman S, Figlin R, Yu H. Targeting STAT3 in adoptively transferred T cells promotes their *in vivo* expansion and antitumor effects. *Cancer Res.*, **70**, 9599–9610 (2010).
11) Rocha GA, Rocha AM, Gomes AD, Faria CL Jr, Melo FF, Batista SA, Fernandes VC, Almeida NB, Teixeira KN, Brito KS, Queiroz DM. STAT3 polymorphism and *Helicobacter pylori* CagA strains with higher number of EPIYA-C segments independently increase the risk of gastric cancer. *BMC Cancer*, **15**, 528 (2015).
12) Cury PR, Furuse C, de Araujo NS, de Araujo VC. Signal transducer and activator of transcription-3 expression and activation is dysregulated in actinic cheilitis. *J. Cutan. Pathol.*, **34**, 606–611 (2007).
13) Yu R, Ding Y, Zhu L, Qu Y, Zhang C, Liu L, Chen L. IL-22 mediates the oral mucosal wound healing *via* STAT3 in keratinocytes. *Arch. Oral Biol.*, **72**, 14–20 (2016).
14) Thomas-Schoemann A, Blanchet B, Bardin C, Noe G, Boudou-Rouquette P, Vidal M, Goldwasser F. Drug interactions with solid tumour-targeted therapies. *Crit. Rev. Oncol. Hematol.*, **89**, 179–196 (2014).
15) Low SK, Fukunaga K, Takahashi A, Matsuda K, Hongo F, Nakanishi H, Kitamura H, Inoue T, Kato Y, Tomita Y, Fukasawa S, Tanaka T, Nishimura K, Uemura H, Hara I, Fujisawa M, Matsuyama H, Hashine K, Tatsugami K, Enokida H, Kubo M, Miki T, Mushiroda T. Association study of a functional variant on ABCG2 gene with sunitinib-induced severe adverse drug reaction. *PLoS ONE*, **11**, e0148177 (2016).
16) Oberoi RK, Mittapalli RK, Elmquist WF. Pharmacokinetic assessment of efflux transport in sunitinib distribution to the brain. *J. Pharmacol. Exp. Ther.*, **347**, 755–764 (2013).
17) Drozdzik M, Mysliwiec K, Lewinska-Chelstowska M, Banach J, Drozdzik A, Grabarek J. P-glycoprotein drug transporter MDR1 gene polymorphism in renal transplant patients with and without gingival overgrowth. *J. Clin. Periodontol.*, **31**, 758–763 (2004).
18) Uematsu T, Yamaoka M, Doto R, Tanaka H, Matsuura T, Furusawa K. Expression of ATP-binding cassette transporter in human salivary ducts. *Arch. Oral Biol.*, **48**, 87–90 (2003).
19) Li W, Tamamura R, Wang B, Liu Q, Liu H, Liu T, Katase N, Xiao J, Nagatsuka H. Expressions of ABCG2, CD133, and podoplanin in salivary adenoid cystic carcinoma. *Biomed. Res. Int.*, **2014**, 132349 (2014).
20) Dong L, Luo R, Tong Y, Cai X, Mao M, Yu D. Lack of association between ABCB1 gene polymorphisms and pharmacoresistant epilepsy: an analysis in a western Chinese pediatric population. *Brain Res.*, **1391**, 114–124 (2011).
21) Tanabe M, Ieiri I, Nagata N, Inoue K, Ito S, Kanamori Y, Takahashi M, Kurata Y, Kigawa J, Higuchi S, Terakawa N, Otsubo K. Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. *J. Pharmacol. Exp. Ther.*, **297**, 1137–1143 (2001).
22) Kobayashi D, Ieiri I, Hirota T, Takane H, Maegawa S, Kigawa J, Suzuki H, Nanba E, Oshimura M, Terakawa N, Otsubo K, Mine K, Sugiyama Y. Functional assessment of ABCG2 (BCRP) gene polymorphisms to protein expression in human placenta. *Drug Metab. Dispos.*, **33**, 94–101 (2005).
23) Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant.*, **48**, 452–458 (2013).
24) Yuan K, Liu H, Huang L, Ren X, Liu J, Dong X, Tian W, Jia Y. rs744166 polymorphism of the STAT3 gene is associated with risk of gastric cancer in a Chinese population. *Biomed. Res. Int.*, **2014**, 527918 (2014).
25) Tai WT, Cheng AL, Shiau CW, Huang HP, Huang JW, Chen PJ, Chen KF. Signal transducer and activator of transcription 3 is a major kinase-independent target of sorafenib in hepatocellular carcinoma. *J. Hepatol.*, **55**, 1041–1048 (2011).
26) Xin H, Zhang C, Herrmann A, Du Y, Figlin R, Yu H. Sunitinib inhibition of Stat3 induces renal cell carcinoma tumor cell apoptosis and reduces immunosuppressive cells. *Cancer Res.*, **69**, 2506–2513 (2009).
27) Wongkajornsilp A, Wamanuttajinda V, Kasetsinsombat K, Duangsaard S, Sa-ngiamsuntorn K, Hongeng S, Maneechotesuwan K. Sunitinib indirectly enhanced anti-tumor cytotoxicity of cytokine-induced killer cells and CD3(+)CD56(+) subset through the co-culturing dendritic cells. *PLoS ONE*, **8**, e78980 (2013).
28) Takeda K, Kaisho T, Yoshida N, Takeda J, Kishimoto T, Akira S. Stat3 activation is responsible for IL-6-dependent T cell proliferation through preventing apoptosis: generation and characterization of T cell-specific Stat3-deficient mice. *J. Immunol.*, **161**, 4652–4660 (1998).
29) Willson TA, Jurickova I, Collins M, Denson LA. Deletion of intestinal epithelial cell STAT3 promotes T-lymphocyte STAT3 activation and chronic colitis following acute dextran sodium sulfate injury in mice. *Inflamm. Bowel Dis.*, **19**, 512–525 (2013).
30) Diekstra MH, Klumpen HJ, Lolkema MP, Yu H, Kloth JS, Gelderblom H, van Schaik RH, Gurney H, Swen JJ, Huitema AD, Steeghs N, Mathijssen RH. Association analysis of genetic polymorphisms in genes related to sunitinib pharmacokinetics, specifically clearance of sunitinib and SU12662. *Clin. Pharmacol. Ther.*, **96**, 81–89 (2014).
31) Uematsu T, Yamaoka M, Matsuura T, Doto R, Hotomi H, Yamada A, Hasumi-Nakayama Y, Kayamoto D. P-glycoprotein expression in human major and minor salivary glands. *Arch. Oral Biol.*, **46**, 521–527 (2001).
32) Teo YL, Wee HL, Chue XP, Chau NM, Tan MH, Kanesvaran R, Wee HL, Ho HK, Chan A. Effect of the CYP3A5 and ABCB1 genotype on exposure, clinical response and manifestation of toxicities from sunitinib in Asian patients. *Pharmacogenomics J.*, **16**, 47–53 (2016).
33) Mizuno T, Fukudo M, Terada T, Kamba T, Nakamura E, Ogawa O, Inui K-i, Katsura T. Impact of genetic variation in breast cancer resistance protein (BCRP/ABCG2) on sunitinib pharmacokinetics. *Drug Metab. Pharmacokinet.*, **27**, 631–639 (2012).
34) Khosravan R, Toh M, Garrett M, La Fargue J, Ni G, Marbury TC, Swan SK, Lunde NM, Bello CL. Pharmacokinetics and safety of sunitinib malate in subjects with impaired renal function. *J. Clin. Pharmacol.*, **50**, 472–481 (2010).
35) Hoek FJ, Kemperman FA, Krediet RT. A comparison between cystatin C, plasma creatinine and the Cockcroft and Gault formula for the estimation of glomerular filtration rate. *Nephrol. Dial. Transplant.*, **18**, 2024–2031 (2003).
36) Park J, Mehrotra R, Rhee CM, Molnar MZ, Lukowsky LR, Patel SS, Nissenson AR, Kopple JD, Kovesdy CP, Kalantar-Zadeh K. Serum creatinine level, a surrogate of muscle mass, predicts mortality in peritoncal dialysis patients. *Nephrol. Dial. Transplant.*, **28**, 2146–2155 (2013).
37) Thongprayoon C, Cheungpasitporn W, Kittanamongkolchai W, Harrison AM, Kashani K. Prognostic importance of low admission serum creatinine concentration for mortality in hospitalized patients. *Am. J. Med.* (2016), in press.
38) Yates JW, Chalmer B, McKegney FP. Evaluation of patients with advanced cancer using the Karnofsky performance status. *Cancer*, **45**, 2220–2224 (1980).