Predicting Skin Toxicity According to *EGFR* Polymorphisms in Patients with Colorectal Cancer Receiving Antibody Against EGFR

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Abstract. *Background/Aim: Monoclonal antibodies against epidermal growth factor receptor (EGFR) can extend progression-free survival (PFS) and overall survival (OS) in patients with unresectable colorectal cancer; however, skin toxicity often interferes with therapy continuation. Patients and Methods: We analyzed the polymorphisms in EGFR and IgG fragment C receptor (FCGR) genes and determined their associations with clinical outcomes including PFS, OS, and skin toxicity. Five polymorphisms in EGFR and FCGR genes in 32 patients with unresectable colorectal cancer who were treated with antibodies against EGFR were examined. Results: Patients carrying the C/C genotype of the EGFR D994D polymorphism displayed significantly less skin toxicity than those with other genotypes, although no significant differences in PFS and OS were noted and no significant interactions were detected for other gene polymorphisms. Conclusion: These results suggest that the EGFR D994D polymorphism is a useful biomarker for predicting the severity of skin toxicity in patients receiving antibody against EGFR.*

Cetuximab and panitumumab are immunoglobulin G (IgG) antibodies against epidermal growth factor receptor (EGFR), and they have exhibited clinical activity both as monotherapies and in combination with chemotherapeutics in metastatic colorectal cancer (mCRC). The principal mechanism of action of these antibodies is based on the inhibition of ligand-induced *EGFR* activation, resulting in reduced cell proliferation, cell survival, and angiogenesis. In addition, cetuximab, and possibly panitumumab, may induce antibody-dependent cell cytotoxicity (ADCC) *via* the recruitment of cytotoxic host effector cells such as

monocytes and natural killer cells (1, 2). The efficacy of ADCC may depend on the degree of activation of effector cells after IgG fragment C receptor (FCGR) IIa and IIIa engagement (3). The level of expression of *EGFR* in tumors has been considered a biomarker for the efficacy of therapy with antibody against EGFR; however, recent clinical studies revealed that the *v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog* (*KRAS*) status in tumors is the most useful biomarker to predict for efficacy of this therapy (4).

Although anti-EGFR therapy has greatly influenced the treatment of patients with mCRC, the therapy is associated with some side-effects that cannot be ignored. A major dose-limiting side-effect of anti-EGFR therapy is skin toxicity such as acne and paronychia, which often results in dose reduction or longer intervals between doses. The severity of acneiform skin rashes is associated with the efficacy of cetuximab (5), but as this adverse event occurs after therapy is initiated, it cannot be predicted before starting treatment. Several reports have shown an association of single nucleotide polymorphisms (SNPs) in *EGFR* and *FCGR* with clinical outcomes including therapeutic efficacy and side-effects (6, 7, 8), and some of these SNPs may be predictive biomarkers.

In this study, we investigated the influence of polymorphisms in *EGFR* and *FCGR* genes on the clinical response, skin toxicity, and survival of patients with mCRC who were treated with anti-EGFR, and identified genetic polymorphisms that may be useful biomarkers before treatment.

Patients and Methods

Patients and data collection. Thirty-two patients with unresectable recurrent CRC or mCRC who received chemotherapy including cetuximab (Bristol-Myers Squibb, NY, USA) or panitumumab (Takeda, Osaka, Japan) at the University of Tsukuba Hospital (Tsukuba, Ibaraki, Japan) were analyzed. The patients had histologically-confirmed CRC, and all patients had wild-type *KRAS*. Cetuximab or panitumumab was administered alone or in combination with chemotherapeutics such as 5-fluorouracil or irinotecan as a first-, second-, or third-line treatment between 2009 and 2012. Skin toxicity was evaluated by the National Cancer

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Institute Common Toxicity criteria (version 4.0)(http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5×7.pdf). The grade of skin toxicity at eight weeks after anti-EGFR administration was used for the evaluation. The tumor response to chemotherapy was evaluated by computed tomography every 2-3 months and defined according to the Response Evaluation Criteria in Solid Tumors (RECIST version 1.1) criteria (9) as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). Progression-free survival (PFS) and overall survival (OS) were calculated from the date of anti-EGFR administration to the date of progression and death, respectively. These data were retrospectively collected from the patients' medical records. This study was approved by the Institutional Review Board of the University of Tsukuba Hospital (#H21-483).

DNA extraction and genotyping. Genomic DNA was extracted from peripheral blood lymphocytes taken before anti-EGFR therapy using a Puregene Blood Core Kit (Qiagen, CA, USA). The *EGFR* polymorphisms analyzed in this study are shown in Table I. A Taqman 5' nuclease assay was performed using the ABI 7500 Sequence Detection System and SDS 2.3 software (Applied Biosystems, CA, USA) according to the manufacturer's protocol.

Statistical analysis. The interaction between polymorphisms in *EGFR* and *FCGR* genes and clinical outcomes was calculated by using Fisher's exact test. Comparisons of PFS according to genotype were performed using the Kaplan Meier method, and significance was determined using the log-rank test. Statistical analysis was conducted using SPSS 13.0 for Windows (IBM, NY, USA). Differences corresponding to $p<0.05$ were considered statistically significant.

Results

Patients' characteristics. The patients' demographics and genotype distribution are shown in Table II. All SNPs were amplified successfully in 91-100% of the samples. The genotypic frequency of each SNP was found to be in Hardy-Weinberg equilibrium (chi-square $p>0.05$). Patients were divided into two groups for each polymorphism of *EGFR* and *FCGR* genes such as C/C and C/T+T/T for the *EGFR D994D* SNP. There were no statistically significant differences in patients' characteristics between these groups.

Effects of genotype on clinical outcome and therapy-induced skin toxicity. As shown in Table III, there were no statistical differences in therapeutic response (CR+PR *vs*. SD+PD) according to polymorphisms in *EGFR* and *FCGR* genes. In addition, OS and PFS were not associated with any SNP in the examined genes (data not shown). The patients were divided into two groups according to the severity of skin rash as follows: grade 0-2 and grade 3. As shown in Table IV, the *EGFR D994D* C/C genotype was found to be significantly associated with less toxicity than the genotypes C/T and T/T ($p=0.038$). There were no significant differences in the response rate, PFS and OS between these genotypes (data not shown).

Table I. *SNPs evaluated*.

Gene	Location	Function	RS no.	Genotypic frequency	
				This study	Pacific Rim*
EGFR	5'-UTR		4444903	A/A=2 (6.3%)	16.7%
				A/G=13 (40.6%)	33.3%
				G/G=17 (53.1%)	50%
	Exon 13	R521K	2227983	A/A=12 (37.5%)	
				A/G=16 (50%)	
				G/G=4 (12.5%)	
	Exon 25	D994D	2293347	C/C=13 (40.6%)	45.8%
				C/T=16 (50%)	45.8%
				T/T=3 (9.4%)	8.3%
FCGRIIa	Exon 4	H166R	1801274	A/A=1 (3.4%)	50%
				A/G=17 (58.6%)	41.7%
				G/G=11 (38%)	8.3%
FCGRIIIa	Exon 5	F212V	396991	C/C=19 (61.3%)	8.3%
				C/A=11 (35.5%)	87.5%
				A/A=1 (3.2%)	4.2%

*The reported genotypic frequency (Pacific Rim) was obtained from the National Cancer Institute SNP500 cancer website (http://variantgps.nci.nih.gov/cgfseq/pages/snp500.do).

Discussion

Previous reports revealed a relationship between *EGFR* gene polymorphisms and clinical status. Graziano *et al*. reported that anti-EGFR-treated patients with fewer *EGFR* intron 1 and *EGF* 61 G/G genotypes experienced longer survival (10). Bibeau *et al*. reported that the *FCGR* IIIa polymorphism is associated with better PFS in patients with mCRC treated with cetuximab (7). In our study, although the number of patients may not be sufficient, polymorphisms in *EGFR* and *FCGR* genes did not display any significant associations with response to anti-EGFR therapy, and no significant effect of these polymorphisms on PFS and OS was detected. The relationship between polymorphisms in *EGFR* and *FCGR* genes and therapy-derived clinical outcome remains controversial. Concerning skin toxicity, Graziano *et al*. reported that EGFR intron-1 S/S carriers more frequently exhibited serious skin toxicity than L/L carriers (10). By contrast, Klinghammer *et al*. identified the *EGFR R521K* SNP but not the *EGFR* intron-1 CA repeats polymorphism as an attractive predictor of the occurrence of skin-related side-effects (8). Although only the *EGFR D994D* SNP was found to be related to skin toxicity in this study, the effect of this SNP on clinical outcome is controversial. Ma *et al*. reported that the *EGFR D994D* SNP is a predictive biomarker in patients with advanced non-small cell lung cancer treated with gefitinib (11). On the contrary, Shitara *et al*. reported that the EGFR 8227 G/A polymorphism, but not the *EGFR D994D* polymorphism, might be associated with clinical outcome in

Table II. *Patient characteristics and distribution by genotype (n=32).*

Factor	n	%	*EGFR* 5'-UTR 61A>G			*EGFR* R521K			*EGFR* D994D			*FCGRIIa* 131G>A			*FCGRIIIa* 158 T>G		
			A/A+A/G	G/G	*p*-Value	A/A	A/G+G/G	*p*-Value	C/C	C/T+T/T	*p*-Value	A/A+A/G	G/G	*p*-Value	C/C	C/A+A/A	*p*-Value
Gender					0.6			0.85			0.78			0.6			0.58
Male	21	65.6	10	11		7	14		8	13		11	7		12	8	
Female	11	34.4	5	6		5	6		5	6		7	4		7	4	
Age.years																	
Median (range)		61 (34-84)	60±10	58±13	0.61	64±13	56±10	0.09	60±8	58±12	0.76	57±10	63±14	0.24	61±13	55±8	0.18
ECOG PS					0.81			0.17			0.91			0.43			0.89
0	21	65.6	9	12		7	14		8	13		11	10		8	12	
1	9	28.1	5	4		3	6		4	5		5	4		3	6	
2	2	6.3	1	1		2	0		1	1		2	0		1	1	
Therapy					0.34			0.68			0.46			0.2			0.94
CPT11+cetuximab	8	25	6	2		2	6		4	4		3	5		4	4	
Cetuximab	3	9.4	1	2		2	1		2	1		1	2		1	2	
FOLFOX+panitumumab	3	9.4	1	2		2	1		0	3		3	0		1	2	
FOLFIRI+panitumumab	9	28.1	3	6		3	6		4	5		6	1		2	6	
CPT11+panitumumab	2	6.2	0	2		1	1		0	2		1	0		1	1	
Panitumumab	7	21.9	4	3		2	5		3	4		4	3		3	4	
Therapy line					0.57			0.71			0.51			0.52			0.88
First	3	9.4	1	2		2	1		0	3		3	0		1	2	
Second	11	34.4	4	7		4	7		5	6		5	4		4	7	
Third	14	43.7	7	7		5	9		6	8		8	5		6	7	
Fourth	4	12.5	3	1		1	3		2	2		2	2		1	3	

ECOG, Eastern Cooperative Oncology Group; FOLFOX, 5-fluorouracil, leucovorin, and oxaliplatin; FOLFIRI, 5-fluorouracil, leucovorin, and irinotecan; CPT-11, camptothecin 11.

Table III. *Response rate by genotype.*

	EGFR 5'-UTR 61A>G		*EGFR* R521K		*EGFR* D994D		*FCGRIIa* 131G>A		*FCGRIIIa* 158 T>G	
Response	A/A,A/G	G/G	A/A	A/G,G/G	C/C	C/T,T/T	A/A,A/G	G/G	C/C	C/A,A/A
CR,PR	3	6	3	6	5	4	6	3	6	2
SD,PD	12	11	9	14	8	15	12	8	13	10
p-Value*	0.287		0.546		0.248		0.534		0.313	

*Fisher's exact test. CR, Complete Response; PR, Partial Response; SD, Stable Disease; PD, Progressive Disease.

Table IV. *Skin toxicity by genotype.*

	EGFRV-VTR 61A>G		*EGFR* R521K		*EGFR* D994D		*FCGRIIa* 131G>A		*FCGRIIIa* 158 T>G	
Grade	A/A,A/G	G/G	A/A	A/G,G/G	C/C	C/T,T/T	A/A,A/G	G/G	C/C	C/A,A/A
0,1,2	11	12	9	14	12	11	12	10	14	8
3	4	5	3	6	1	8	6	1	5	4
p-Value*	0.589		0.546		0.038		0.151		0.489	

*Fisher's exact test. The skin toxicity grade was scored from 0 to 3 according to National Cancer Institute Common Toxicity Criteria (version 4.0) (httpV/evs.nci.nih.gov/ftp 1/CTCAE/CTCAE_4.03_2010-06- 14_QuickReference_5×7.pdf).

EGFR tyrosine kinase inhibitor-treated patients with non-small cell lung cancer (12). There is no report describing an association between the *EGFR D994D* polymorphism and skin toxicity in patients treated with anti-EGFR antibodies. Although the *EGFR D994D* SNP is synonymous and considered not to change the amino acid sequence of the protein nor affect the biological function of the protein itself, the SNP may have functional significance because it is located in the coding region in exon 25 of the *EGFR* gene (11). Indeed, recent studies revealed it affected the stability, splicing and the translational kinetics of the mRNA, resulting in changes in the amount, structure and function of proteins (13-15). Further research at the molecular level is expected to clarify the influence of this SNP on biological functions.

There are some limitations to this study. Firstly, our findings were obtained from a relatively small number of patients. Secondly, we examined only five polymorphisms of genes within the *EGFR* pathway. Thirdly, there is therapeutic bias for the clinical outcome because different chemotherapies were used and two antibodies against EGFR, cetuximab and panitumumab, were regarded as a single therapy.

In conclusion, the *EGFR D994D* polymorphism is a candidate biomarker to predict for severity of skin toxicity in patients receiving anti-EGFR therapy. As the detailed functions of this SNP are unknown, larger sample sizes and further investigations at the molecular level are required.

Conflicts of Interest

None.

Acknowledgements

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