Epidermal Growth Factor Receptor R497K Polymorphism Is a Favorable Prognostic Factor for Patients with Colorectal Carcinoma

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Abstract

Purpose: It has been shown that the R497K polymorphism of the epidermal growth factor receptor (EGFR) has attenuated functions in ligand binding, tyrosine kinase activation, and growth stimulation. Because the activation of EGFR results in an unfavorable prognosis of patients with colorectal carcinoma, a pilot study was conducted to assess the influence of this polymorphism on colorectal carcinoma patients.

Experimental Design: We retrospectively analyzed the effect of the R497K polymorphism of EGFR on clinicopathologic features in 209 colorectal carcinoma patients, including 100 with stage II/III colorectal carcinoma receiving curative surgery and the other 109 with metastatic diseases.

Results: An excellent correlation in codon 497 statuses examined by patients' WBCs and tumor tissues was found but no significant between-group difference in patients with or without colorectal carcinoma ($P = 0.97$). A marked decrease on EGFR phosphorylation ($P < 0.01$) and c-Myc activation ($P = 0.02$) was observed in patients with R497K polymorphism, which is associated with decreased invasion ($P = 0.01$), lower nodal involvement ($P = 0.02$), reduced subsequent metastasis ($P < 0.01$), and longer disease-free ($P < 0.01$) as well as overall ($P < 0.01$) survival in stage II/III colorectal carcinoma patients who had received curative surgery. For patients with metastatic colorectal carcinoma, this polymorphism was associated with a higher response to 5-fluorouracil/oxaliplatin treatment ($P = 0.02$) and a longer survival ($P < 0.01$). By multivariate analysis, this polymorphism was also identified as an independent prognostic factor ($P = 0.03$).

Conclusions: These data suggest that the R497K polymorphism of the EGFR, by reducing its activation and a consequential down-regulation of its target genes, could be a key determinant for reduced tumor recurrence of stage II/III colorectal carcinoma patients receiving curative surgery and a longer survival of patients with stage II/III as well as metastatic colorectal carcinoma.

Colorectal carcinoma is one of the leading causes of cancer-related mortality worldwide, and its incidence has increased steadily over the last few decades. Traditionally, the anatomic tumor-node-metastasis (TNM) staging system, including the extent of tumor invasion, involvement of regional lymph nodes, and distant metastasis, is commonly used for prognostic prediction and treatment selection for colorectal carcinoma patients (1). In the meantime, the quantification of enzymes that involve in the targeting and metabolism of specific chemotherapeutic drugs, as well as DNA repair, may also effectively predict the sensitivity and clinical outcome to treatment in patients with advanced colorectal carcinoma (2). Additionally, the analysis of genomic polymorphisms for predicting the efficacy and toxicity of treatment may also be helpful in identifying those who may benefit from chemotherapy (2). For example, the functional polymorphisms of genes involved in the targeting (3) and metabolism (4, 5) of 5-fluorouracil (5-FU), as well as DNA repair during platinum-based treatment (6, 7), can effectively predict the response and prognosis of advanced colorectal carcinoma patients receiving 5-FU and oxaliplatin.

The epidermal growth factor receptor (EGFR), also known as HER-1 or erbB-1, is a transmembrane glycoprotein with tyrosine kinase activity (8). EGFR plays a central role in a wide variety of cellular functions, including cell proliferation, migration, adhesion, differentiation, and survival; therefore, dysregulation of its signaling pathway often occurs in malignant diseases (9, 10). For example, up-regulation of EGFR has been found in several epithelial tumors, including non–small cell lung cancer, head and neck carcinoma, and colorectal carcinoma (11–13). In fact, EGFR expression was detected in ~80% of colorectal carcinoma tumor tissues and up-regulation

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Received 10/27/06; revised 3/18/07; accepted 3/30/07.

Grant support: Taipei Veterans General Hospital grant V95S5-005 and National Science Council of the Republic of China grant NSC 94-2320-B-010-050.

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doi:10.1158/1078-0432.CCR-06-2601

of its signaling seemed to be associated with an unfavorable prognosis and increased risk of metastasis (13). Concordantly, a better survival of colorectal carcinoma patients was observed in those with a down-regulated EGFR signaling (14). Moreover, the susceptibility of tumors in colorectal carcinoma patients to chemotherapy was effectively enhanced by a monoclonal antibody (e.g., cetuximab) that interferes with the ligand binding of EGFR (15, 16).

Genomic profiling of the EGFR signaling is helpful in identifying rectal cancer patients who are at risk of tumor recurrence and those who are more likely to benefit from chemoradiation therapy. For example, a functional polymorphism (-216 G/T) of the Sp1-binding site in the *EGFR* promoter correlates with an altered promoter activity and a higher response to chemoradiation in locally advanced rectal cancer patients (17, 18). Cells with a lower number of CA dinucleotide repeats in an intron 1 region of the *EGFR* have a higher expression level (19) and may predict a poor efficacy to oxaliplatin treatment in patients with advanced colorectal carcinoma (20). The A61G polymorphism of *EGFR* has also been shown as a useful marker for predicting clinical outcome in colorectal carcinoma patients treated with a monoclonal anti-EGFR antibody cetuximab (21). A polymorphic variant EGFR arising from a single nucleotide change (G→A) leading to an arginine (Arg) to lysine (Lys) substitution in codon 497 (R497K) in the extracellular domain of EGFR has been identified, which has attenuated functions in ligand binding, growth stimulation, tyrosine kinase activation, and induction of myc, fos, and jun in comparison with the wild-type one (22). This polymorphism alone or in combination with another polymorphism in the same gene (i.e., more than 20 CA repeats in an intron 1 region) is associated with a lower recurrence of tumor in rectal cancer patients treated with chemoradiation (23).

Based on these earlier findings, we proposed that R497K polymorphism of the EGFR might associate with a reduced likelihood of tumor recurrence in stage II/III colorectal carcinoma patients who have received curative surgery and a favorable prognosis for patients with metastatic diseases. We thereby examined the correlations between this polymorphism and clinicopathologic features of colorectal carcinoma patients in this study.

Materials and Methods

Patients. From January 2002 to December 2003, a total of 153 patients with stage II or III colorectal carcinoma who had received curative surgery was examined retrospectively. One hundred of them were enrolled in this study (patients' characteristics were shown in Table 1). The remainders were excluded because they either received oxaliplatin-based adjuvant chemotherapy (n = 35), died before blood sampling (n = 8), lost in follow-up (n = 6), or were unwilling to participate (n = 4). Patients' staging was based on the international TNM staging system for colorectal carcinoma. For patients with stage III colorectal carcinoma and stage II diseases with tumor perforation, obstruction, lymphovascular invasion, poorly histologic differentiation, or inadequate (<10) lymph node sampling, adjuvant chemotherapy with weekly bolus injection of 5-FU plus folinic acid was given (20 mg/m^2 folinic acid followed immediately by a rapid infusion of 425 mg/m^2 5-FU) for a total of 6 months. For patients with rectal cancer, adjuvant pelvic irradiation is indicated. All patients were followed up every 3 months for 2 years and then every 6 months until disease progression, death, or lost in follow-up at a similar intensity regardless of the EGFR polymorphism status. Follow-up examinations included physical examination, serum carcinoembryonic antigen (CEA) levels, chest X-rays, abdominal ultrasonography, or thoracoabdominal computed tomography when pathologic findings were suspected in previous tests. The median follow-ups for disease-free and overall survivals were 43.0 and 46.8 months, respectively.

Table 1. Clinicopathologic influences of the EGFR codon 497 polymorphism in stage II/III colorectal cancer patients receiving curative surgery

Characteristics	G/G (wild-type), *n* (%)	G/A or A/A, *n* (%)	*P*
All patients	27 (100)	73 (100)	
Age (y)			
<50	13 (48.1)	39 (53.4)	0.81
≥50	14 (51.9)	34 (46.6)	
Gender			
Male	17 (63.0)	50 (68.5)	0.78
Female	10 (37.0)	23 (31.5)	
Performance status			
0	18 (66.7)	52 (71.2)	0.84
1, 2	9 (33.3)	21 (28.8)	
Primary tumor			
Colon	16 (59.3)	46 (63.0)	0.91
Rectum	11 (40.7)	27 (37.0)	
Histologic differentiation			
Well/moderate	20 (74.1)	58 (79.5)	0.76
Poorly/unknown	7 (25.9)	15 (20.5)	
Invasive extent			
T_1-T_2	9 (33.3)	47 (64.4)	0.01
T_3-T_4	18 (66.7)	26 (35.6)	
Lymph node status			
N_0	11 (40.7)	51 (69.9)	0.02
N_1-N_3	16 (59.3)	22 (30.1)	
Subsequent distant metastasis			
No	6 (22.2)	63 (86.3)	<0.01
Yes	21 (77.8)	10 (13.7)	
Preoperative CEA level (ng/mL)			
≤6	13 (48.1)	42 (57.5)	0.54
>6	14 (51.9)	31 (42.5)	

NOTE: According to international TNM staging system for colorectal carcinoma.
Abbreviations: G, guanine; A, adenine.

For examining the effect of R497K polymorphism on the response to chemotherapy and the prognosis of patients with metastatic colorectal carcinoma, a total of 146 patients who had received systemic chemotherapy from January 2004 to December 2005 was considered eligible for this study. Among them, 109 (including 13 patients did not have their primary tumor removed to know the accurate T and N stages) who had received oxaliplatin (85 mg/m^2 every 2 weeks) plus weekly bolus 5-FU (500 mg/m^2) and folinic acid (20 mg/m^2) as first-line treatments were enrolled. In the meantime, patients who were under irinotecan-based (n = 20) or fluoropyrimidine-only (n = 8) regimens, were unwilling to participate (n = 5), or lost in follow-up (n = 4) were excluded. During treatment, all patients visited our outpatient clinic regularly for physical examination and checkup of complete blood count, liver and renal functions, and serum CEA levels. Chest X-ray, ultrasonography of the abdomen, or computed tomography scan were conducted every 2 months, and colonoscopy was done on an annual basis. Patients with or without R497K polymorphism of EGFR were followed up at a similar intensity with a median duration of 27.8 months. The responses to chemotherapy and treatment-related

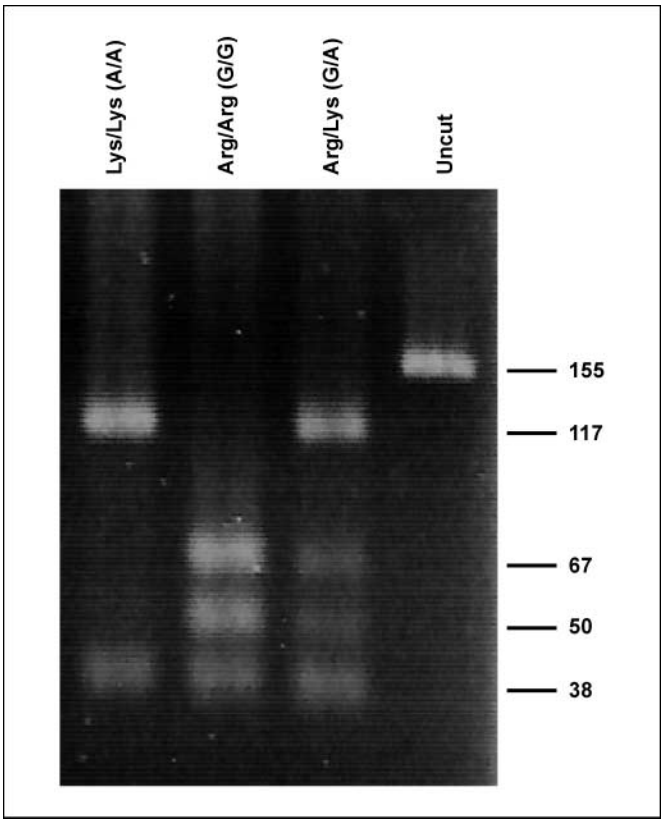


Fig. 1. Representative PCR-RFLP patterns of different EGFR codon 497 status examined by patients' blood samples. Genomic DNA obtained from patients' WBC was subjected to PCR amplification using 5′-TGCTGTGACCCACTCTGTCT-3′ and 5′-CCAGAAGGTTGCACTTGTCC-3′ as forward and reverse primers, respectively. PCR products after being digested by *Bst*N1 were separated by agarose gel electrophoresis.

toxicities were evaluated based on standard WHO criteria. Patients with complete response, partial response, or stable disease remained in the protocol until progressive disease or unacceptable toxicity was documented. For patients who failed on front-line treatment, irinotecan-based or fluoropyrimidine-only regimens were subsequently administered according to physicians' decision. None of them have been treated with EGFR antagonists (either cetuximab or tyrosine kinase inhibitors). For examining the between-group difference of codon 497 polymorphism of the *EGFR* gene in patients with or without colorectal carcinoma, a total of 63 patients with nonmalignant diseases, including hypertension (n = 23), diabetes mellitus (n = 22), chronic obstructive pulmonary diseases (n = 10), upper respiratory tract infection (n = 6), and urinary tract infection (n = 2), was also enrolled. An institutional review board has approved this study and all patients who participated have signed informed consents.

Examination of the R497K polymorphism of EGFR. Genomic DNA was extracted from patients' WBCs obtained via 0.5 mL whole blood using standard phenol-chloroform procedures and subjected to EGFR R497K testing. For assessing the similarity of this genetic polymorphism in patients' WBC and their colorectal tumor tissues, genomic DNA extracted from colorectal tumor tissues of 36 patients was analyzed using a similar protocol (described below) and the statistical difference was determined by χ^2 test. The R497K (G→A) polymorphism of the *EGFR* gene was examined by PCR-RFLP method as described previously (23). Briefly, 0.1 μg genomic DNA (forward primer, 5′-TGCTGTGACCCACTCTGTCT-3′ and reverse primer, 5′-CCAGAAGGTTGCACTTGTCC-3′) was used for PCR amplification. After initial denaturation at 95°C for 3 min, the reaction was carried out at 94°C denaturation for 1 min, 59°C annealing for 1 min, and 72°C extension for 1 min for a total of 35 cycles. PCR products after being digested by *Bst*N1 restriction enzyme (New England Biolabs) at 60°C for 16 h were separated on 4% Nusieve ethidium bromide–stained agarose gels. In some cases, reverse transcription-PCR was carried out using RNA extracted form patients' WBC with the same primers to assess the influence of this genetic polymorphism on EGFR mRNA levels.

Immunohistochemical staining. For examining the influence of R497K polymorphism of EGFR on its phosphorylation and the activation of its downstream effectors, paraffin-embedded primary colorectal tumor tissues obtained from 36 colorectal carcinoma patients who agreed to release their tumor tissues for examination were subjected to immunohistochemical staining. Tumor tissue sections were stained with an anti–phosphorylated EGFR (Tyr^{1068}) antibody (Cell Signaling Technology) and an anti-c-Myc antibody (Santa Cruz Biotechnology), respectively, using a streptavidin-biotin immunoperoxidase kit (BioGenex) according to the manufacturer's instructions. An experienced pathologist who served at Taipei Veterans General Hospital examined these slides microscopically, and both the intensity and distribution of immunohistochemical staining signals were analyzed. Positive phosphorylated EGFR and c-Myc stainings were defined, respectively, as diffused strong membranous and intense nuclear signals. The statistical difference of this correlation was determined by χ^2 test.

Statistical analysis and survival curve plotting. Patients were divided into two groups according to codon 497 statuses of the *EGFR* gene for survival analysis. The cause-specific survival curves were plotted using the Kaplan-Meier product limit method, and the statistical differences in survival among subgroups were compared by log-rank test. The correlations of age, gender, performance status, primary anatomic site, histologic grade, TNM classification, serum CEA levels, and response to 5-FU plus oxaliplatin treatment (for patients with metastatic diseases) were analyzed separately according to the codon 497 status of the *EGFR* gene. The statistical differences of these correlations were determined by χ^2 test. To assess the independent prognostic values of this polymorphism, we used Cox's proportional hazards regression analysis (multivariate) that included *EGFR* codon 497 status and other clinicopathologic variables. All statistical analyses were done using the Statistical Package for the Social Sciences software system (version 10.0).

Results

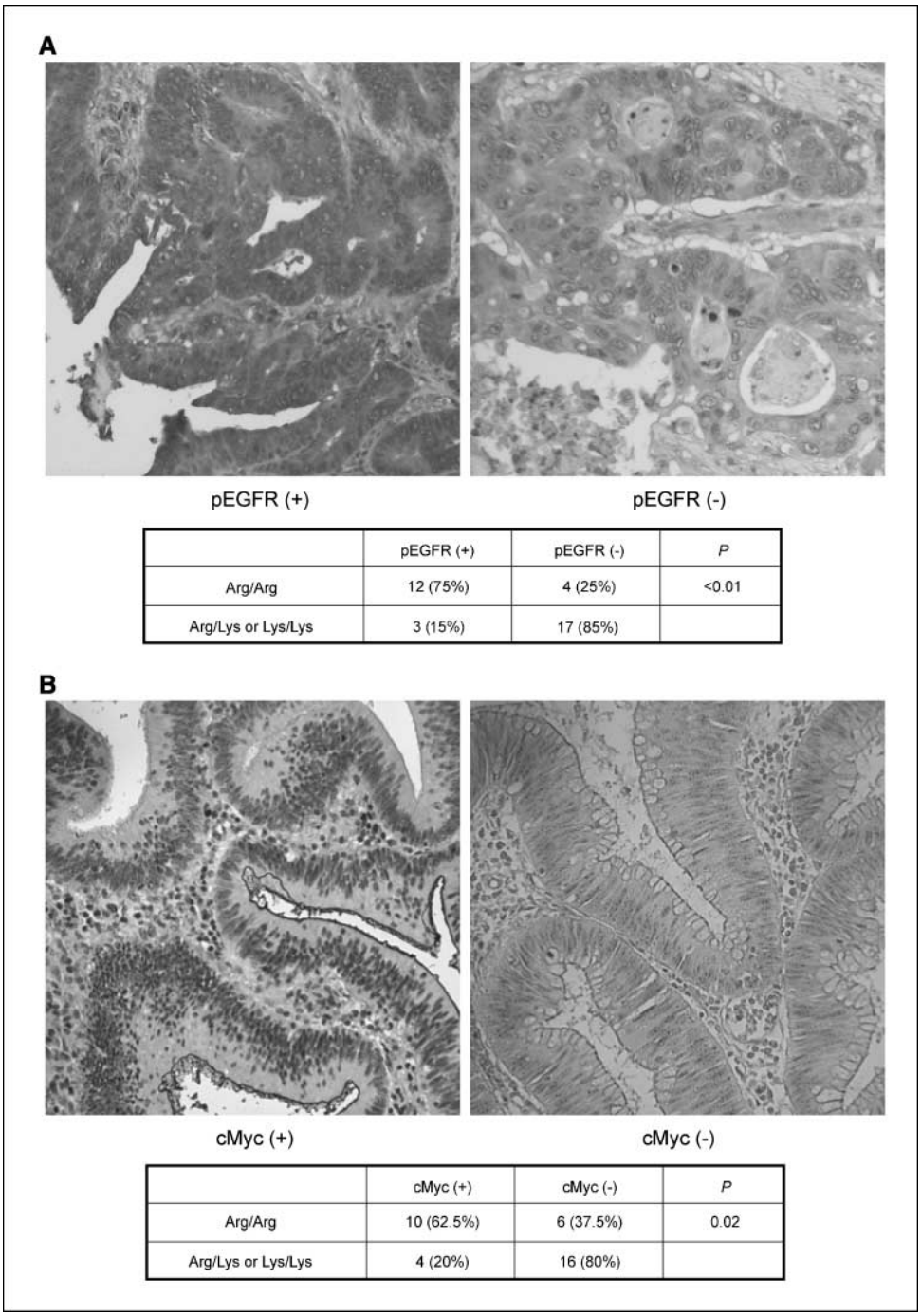
Similar codon 497 polymorphism of the* EGFR *gene is present in patients' WBC and primary colorectal tumor tissues and no significant difference in this polymorphism between patients with or without colorectal carcinoma. At first, we assessed the feasibility of using patients' WBC as a surrogate for tumor tissue to examine the R497K polymorphism of EGFR because blood samples are much easier to obtain. In this regard, it is necessary to show that the results obtained using patients' WBC and their corresponding tumor tissues are similar. Example of

Table 2. The correlation of *EGFR* codon 497 polymorphism between patients' WBCs and colorectal tumor tissues (n = 36)

WBC	Primary colorectal tumor tissues		
	G/G (Arg/Arg)	G/A (Arg/Lys)	A/A (Lys/Lys)
G/G (Arg/Arg)	9	0	0
G/A (Arg/Lys)	0	17	0
A/A (Lys/Lys)	0	0	10

different allele patterns of codon 497 polymorphism of the *EGFR* gene analyzed by PCR-RFLP method was shown in Fig. 1. Indeed, similar codon 497 polymorphism of *EGFR* was found in both tissues isolated from 36 colorectal carcinoma patients who agreed to release their fresh tumor tissues for examination (Table 2), showing the feasibility of using patients' blood samples, instead of tumor tissues, for analyzing this polymorphism in colorectal carcinoma patients. Because the 497K polymorphism of this receptor attenuates its ligand binding as well as subsequent activation of its downstream effectors in comparison with the wild-type one (22), we wondered whether a higher percentage of wild-type 497R is associated and consequentially contribute to the malignant progression of colorectal epithelial cells in colorectal carcinoma patients. A total of 63 patients free of malignant diseases was enrolled and the incidences of codon 497 G/G, G/A, and A/A are 31.1%, 47.4%, and 21.5%, respectively, in colorectal carcinoma patients, which was similar to 27.0%, 52.4%, and 20.6%, respectively, in patients without malignant diseases (P = 0.97), indicating no significant between-group difference.

The R497K polymorphism of EGFR is associated with a reduced phosphorylation and c-Myc activation. Because the R497K polymorphism of EGFR significantly attenuates its ligand binding and a consequential signaling (22), a reduced



	pEGFR (+)	pEGFR (-)	*P*
Arg/Arg	12 (75%)	4 (25%)	<0.01
Arg/Lys or Lys/Lys	3 (15%)	17 (85%)	

	cMyc (+)	cMyc (-)	*P*
Arg/Arg	10 (62.5%)	6 (37.5%)	0.02
Arg/Lys or Lys/Lys	4 (20%)	16 (80%)	

Fig. 2. Representative immunohistochemical staining patterns of the phosphorylated EGFR as well as activated c-Myc in patients' tumor tissues. Immunohistochemical staining of 36 colorectal carcinoma patients' tumor tissues was done using anti – phosphorylated EGFR (*pEGFR*; *A*) and anti-c-Myc (*B*) antibodies, respectively. Left, positive staining; right, negative staining. Correlations between EGFR phosphorylation as well as c-Myc activation and EGFR R497K polymorphism were analyzed by χ^2 test.

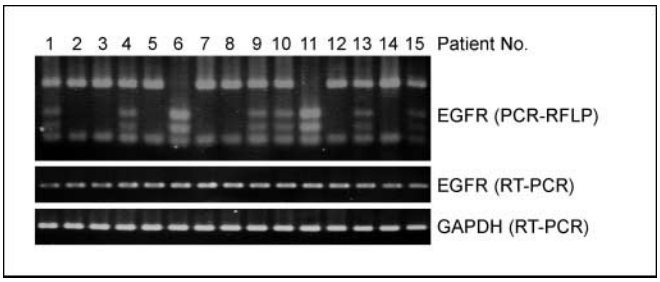


Fig. 3. The EGFR mRNA levels are not affected by the R497K polymorphism in its gene. PCR-RFLP analysis of 15 colorectal carcinoma patients was done as described in Fig. 1, whereas reverse transcription-PCR (*RT-PCR*) was carried out using total RNAs isolated from the same blood samples with EGFR-specific and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) – specific primer sets, respectively.

autophosphorylation of EGFR as well as a consequential activation of c-Myc, one important downstream effector of EGFR pathway, were proposed in patients with this polymorphism. Paraffin-embedded tumor tissue sections from 36 colorectal carcinoma patients were stained, respectively, with a phosphorylated-specific anti-EGFR and an anti-c-Myc antibody followed by a standard immunohistochemical staining protocol. As can be seen in Fig. 2, the diffused membranous/cytosolic and intensive nuclear signals represented positive stainings for EGFR phosphorylation and c-Myc, respectively. A marked decrease in EGFR phosphorylation was observed in patients with the R497K polymorphism, as the percentage of phosphorylated EGFR positive in patients with or without this polymorphism was 15% and 75%, respectively ($P < 0.01$; Fig. 2A). Concordantly, a dramatic decrease in c-Myc activation was also found in patients with this polymorphism ($P = 0.02$; Fig. 2B). To assess the effect of this polymorphism on *EGFR* transcription, reverse transcription-PCR analysis was carried out using total RNAs extracted from 15 patients' blood samples with the same primers. No significant difference in *EGFR* mRNA levels was detected in patients with or without R497K polymorphism (Fig. 3).

R497K genotype correlates with reduced tumor invasion, nodal involvement, and subsequent metastasis in stage II/III colorectal carcinoma patients receiving curative surgery. Because the R497K polymorphism of EGFR dramatically attenuates its activation and downstream signaling, alterations in clinicopathologic features and the prognosis of colorectal carcinoma patients carrying this polymorphism were postulated. We therefore examined the correlation between the R497K polymorphism of EGFR and the clinicopathologic variables of 100 patients with stage II or III colorectal carcinoma who have received curative surgery, although inverse correlations between this polymorphism and the extent of tumor invasion ($P = 0.01$), lymph node involvement ($P = 0.02$), and subsequent distant metastasis ($P < 0.01$) were clearly found in these patients (Table 1). Accordingly, a longer disease-free ($P < 0.01$) as well as overall ($P < 0.01$) survival of stage II/III colorectal carcinoma patients receiving curative surgery was also observed regardless of the types (homozygous or heterozygous) of this polymorphism (Fig. 4). By adjusted analysis, this polymorphism was further identified as an independent prognostic factor for stage II/III colorectal carcinoma patients receiving curative surgery ($P = 0.03$; Table 3).

R497K genotype is associated with a better response to oxaliplatin-based chemotherapy and a favorable prognosis of metastatic colorectal carcinoma patients. Because an up-regulated EGFR signaling increases the antiapoptotic ability of colorectal tumor cells, which may translate into an unfavorable prognosis of colorectal carcinoma patients, we postulated that

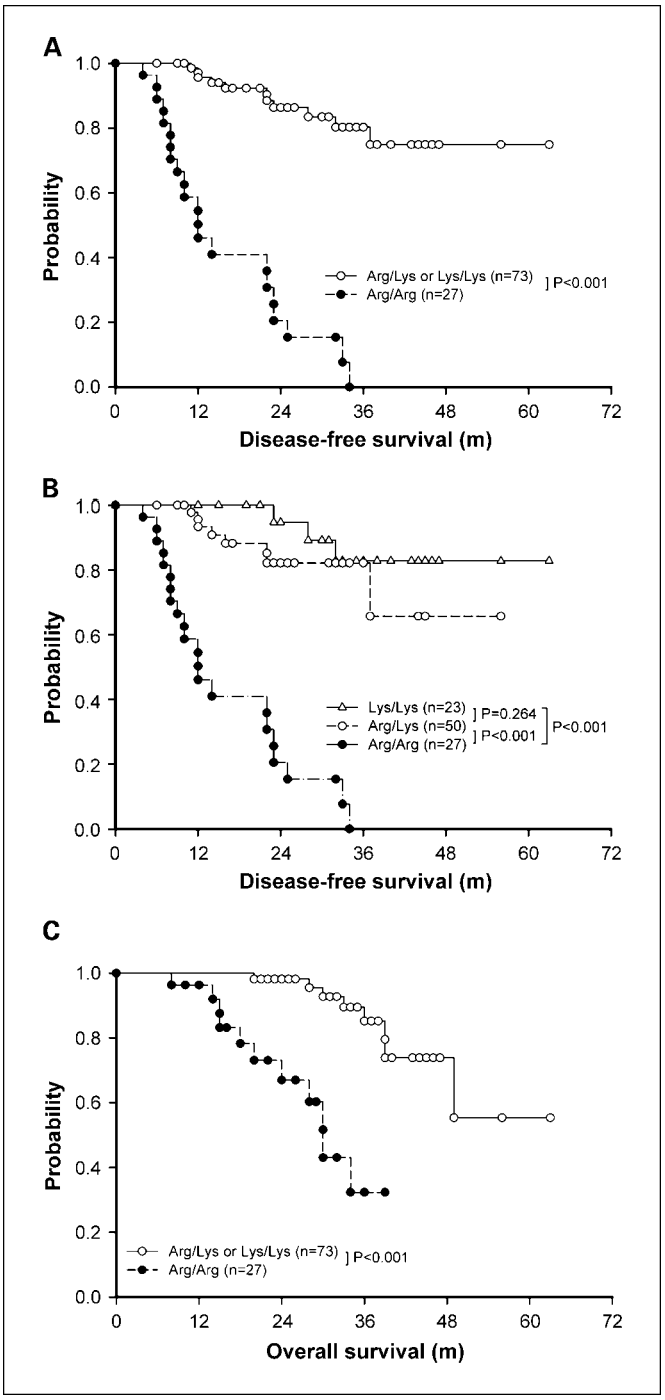


Fig. 4. Stage II/III colorectal carcinoma patients with the R497K polymorphism of EGFR are associated with a longer disease-free as well as overall survival. *A,* disease-free survival curves of 100 stage II/III colorectal carcinoma patients with EGFR 497R (●) or 497K (○) were plotted by Kaplan-Meier method ($P < 0.001$, log-rank test). *B,* similar method was used to plot disease-free survival curves of patients with wild (●), homozygous (△), or heterozygous (○) codon 497 genotypes ($P < 0.001$, log-rank test). *C,* overall survival curves of patients with EGFR 497R (●) or 497K (○) were plotted by similar method ($P < 0.01$, log-rank test).

Table 3. Analysis of factors that may affect the survival of patients with stage II/III colorectal carcinoma (n = 100)

Characteristics	Univariate, P	Multivariate, P
Age (y)		
<50 vs ≥50	0.77	0.82
Gender		
Male vs female	0.95	0.70
Performance status		
0 vs 1, 2	0.12	0.08
Primary tumor		
Colon vs rectum	0.56	0.91
Histologic differentiation		
Well-moderate vs poorly	0.22	0.49
Invasive extent		
T_1-T_2 vs T_3-T_4	0.03	0.05
Nodal status		
Negative vs positive	0.04	0.03
Subsequent distant metastasis		
No vs yes	0.01	<0.01
Preoperative CEA level (ng/mL)		
≤6 vs >6	0.48	0.65
EGFR codon 497 polymorphism		
G/G (wild-type) vs G/A or A/A	0.02	0.03

NOTE: According to international TNM staging system for colorectal carcinoma.

metastatic colorectal carcinoma patients with the R497K polymorphism may be more sensitive to chemotherapy and thereby lived longer (i.e., favorable prognosis). Hence, a total of 109 metastatic colorectal carcinoma patients, including 38 with wild-type and 71 with 497K, receiving first-line systemic chemotherapy with oxaliplatin plus 5-FU/folinic acid was enrolled and their responses to chemotherapy and survival were analyzed. Indeed, metastatic colorectal carcinoma patients with this EGFR polymorphism had a higher response rate to oxaliplatin-based chemotherapy (62.0% versus 34.2%; P = 0.02; Table 4) as well as a longer survival (P < 0.01; Fig. 5). In addition, this polymorphism was also identified as an independent prognostic factor for metastatic colorectal carcinoma patients receiving systemic chemotherapy by multivariate analysis (P = 0.03; Table 5).

Table 4. The response to 5-FU plus oxaliplatin treatment in metastatic colorectal cancer patients with different *EGFR* codon 497 status

Response	G/G (wild-type), n (%)	G/A or A/A, n (%)	P*
All patients enrolled	38 (100)	71 (100)	
OR (CR + PR)	13 (34.2)	44 (62.0)	0.02
CR	2 (5.3)	6 (8.5)	
PR	11 (28.9)	38 (53.5)	
SD	18 (47.4)	19 (26.8)	
PD	7 (18.4)	8 (11.3)	

Abbreviations: OR, overall response; CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease.
*Comparison of overall response rate between patients with different EGFR codon 497 polymorphisms.

Discussion

The application of pharmacogenomics in developing better therapeutics for cancer treatment will be largely dependent on the discovery of novel surrogate biomarkers as well as the identification of disease- and therapeutic-relevant polymorphisms. In this regard, the polymorphisms in *EGFR* gene have attracted a lot of attentions recently because they affect not only their expression and activation in several tumors but also their responses to radiotherapy, immunotherapy, and chemotherapy (19, 24–28).

In the present study, we showed that the R497K polymorphism of EGFR in stage II/III colorectal carcinoma patients who received curative surgery may account for a reduced tumor invasion, lymph node involvement, and subsequent distant metastasis (Table 1), hence a longer disease-free as well as overall survival (Fig. 4). Moreover, this polymorphism was shown to correlate with a better response to 5-FU and oxaliplatin treatment (Table 4) and a favorable prognosis for metastatic colorectal carcinoma patients (Table 5; Fig. 5). Although the underlying mechanisms remain unclear, an attenuated ligand interaction and consequential signal transduction might be the main reason for the suboptimal function of this receptor variant because a diminished growth response to EGF and transforming growth factor-α has been observed in Chinese hamster ovary cells overexpressing the corresponding gene (22). In good agreement, a marked reduction in EGFR phosphorylation as well as c-Myc activation was found for the first time in metastatic colorectal carcinoma patients with this polymorphism (Fig. 2), which may explain their enhanced sensitivity to chemotherapy and a favorable prognosis afterwards.

The quantification of certain intratumoral molecules (protein or mRNA) involved in the targeting or metabolism of specific chemotherapeutic agents may be valuable in predicting their efficacies or toxicities in cancer patients (2). For example, colorectal carcinoma patients with a higher intratumoral level of thymidylate synthase may have a lower response to 5-FU treatment and a shorter survival compared with those with a

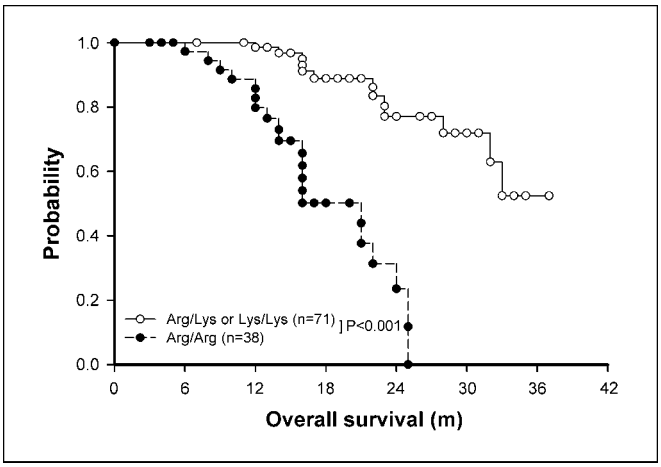


Fig. 5. Metastatic colorectal carcinoma patients with the R497K polymorphism of EGFR are associated with a longer survival. Survival curves of 109 metastatic colorectal carcinoma patients with EGFR 497R (homozygote; ●) and 497K (homozygote or heterozygote; ○) in their blood samples were plotted by Kaplan-Meier method (P < 0.001, log-rank test).

Table 5. Analysis of factors that may affect the survival of patients with metastatic colorectal carcinoma (n = 109)

Characteristics	Univariate, P	Multivariate, P
Age (y)		
<50 vs ≥50	0.64	0.72
Gender		
Male vs female	0.76	0.88
Performance status		
0 vs 1, 2	0.32	0.04
Primary tumor		
Colon vs rectum	0.81	0.84
Histologic differentiation		
Well-moderate vs poorly	0.53	0.68
Invasive extent		
T_1-T_2 vs T_3-T_4	0.08	0.03
Known vs unknown	0.56	0.68
Nodal status		
Negative vs positive	0.03	0.03
Known vs unknown	0.72	0.66
Metastasis at diagnosis		
No vs yes	0.01	<0.01
Serum CEA level (ng/mL)		
≤6 vs >6	0.59	0.42
EGFR codon 497 polymorphism		
G/G (wild-type) vs G/A or A/A	0.02	0.03

NOTE: According to international TNM staging system for colorectal carcinoma.

normal level (29, 30). Patients deficient in dihydropyrimidine dehydrogenase, a rate-limiting enzyme for the catabolism of fluoropyrimidines, may experience a profound (even fatal) toxicity when treated with 5-FU (31). Moreover, patients with a higher intratumoral mRNA level of ERCC1, an enzyme involved in nucleotide excision repair, may have a higher resistance to oxaliplatin and hence a poor prognosis when treated with oxaliplatin plus 5-FU (32). Although directly analyzing patients' tumor tissues may provide a more accurate prediction in the efficacy or toxicity of anticancer drugs, these samples are not always available or easily obtained during clinical practice. Fortunately, DNA extracted from patients' blood sample is suitable for polymorphism testing of certain genes (18, 21). In good agreement, a similar codon 497 status of *EGFR* was found in WBC and primary colorectal tumor tissues in 36 colorectal carcinoma patients (Table 2), indicating the feasibility of using the blood samples to analyze this polymorphism in colorectal carcinoma patients.

Subsequent distant metastasis remains the major cause of death for stage II and III colorectal carcinoma patients receiving curative surgery despite the improvements in adjuvant chemotherapy are continuously being made. Because the efficacy of chemotherapy may be severely compromised because of the inter-subject variation, it is of great importance to identify patients who will benefit from chemotherapy and who will develop metastasis even after extensive treatment. In this regard, the R497K polymorphism of EGFR has previously been shown to be associated with a lower tumor recurrence in advanced rectal cancer patients treated with chemoradiation (23); by interfering the ligand binding and self-activation, this polymorphism was also accompanied by a decreased probability of subsequent metastasis. To no surprise, patients with the "favorable" Lys/Lys or Arg/Lys genotype had a significantly decreased risk of subsequent distant metastasis than those with the Arg/Arg genotype (Table 1).

Although our results revealed an inverse correlation between the R497K polymorphism of EGFR and the extent of tumor invasion, lymph node involvement, and subsequent distant metastasis, respectively (Table 1), no significant differences in both the demographic and the clinicopathologic characteristics were found in 59 advanced rectal cancer patients with or without these polymorphisms (23). The discrepancy between the two studies might be explained by that both findings were based on retrospective analyses on a few patients treated at a single institution, which could lead to spurious associations between EGFR polymorphisms and clinical outcome. On the other hand, negative associations between EGFR polymorphism and established markers for tumor aggressiveness, such as invasive extent as well as nodal involvement, may possibly be neglected due to a small sample size. Although we felt confident that assessing the R497K polymorphism of EGFR should be helpful in predicting subsequent metastasis for stage II/III colorectal carcinoma patients as well as the resistance to chemotherapy in patients with metastatic colorectal carcinoma, larger cohorts of patients with advanced colorectal carcinoma enrolled in prospective study are required to solidify our findings.

In summary, we showed for the first time that the R497K (G→A) polymorphism of *EGFR* is associated with a decreased phosphorylation of its gene product as well as c-Myc activation in colorectal carcinoma patients' tumor tissues. By reducing its binding with ligand and a consequential down-regulated expression of the target genes, this EGFR polymorphism is likely to be one of the key determinants for a reduced tumor recurrence in stage II/III colorectal carcinoma patients receiving curative surgery as well as a longer survival of patients with stage II/III and metastatic colorectal carcinoma.

Acknowledgments

We thank Chih-Tung Ho for excellent technical assistance.

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Clin Cancer Res 2007;13:3597-3604.