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Association of Fibroblast Growth Factor Receptor 4 Genetic Polymorphisms With the Development of Uterine Cervical Cancer and Patient Prognosis

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Abstract

This is the first study to investigate the relationships among fibroblast growth factor receptor 4 (FGFR4) genetic polymorphisms, development of uterine cervical cancer, clinicopathological variables, and patient prognosis in Taiwanese women. Real-time polymerase chain reaction and genotyping were used to detect the genotype frequencies of 4 FGFR4 single-nucleotide polymorphisms (SNPs), rs351855 (C/T, Gly388Arg), rs2011077 (G/A), rs7708357 (G/A), and rs1966265 (Ile10Val), in 138 patients with invasive cancer, 89 with precancerous lesions of uterine cervix, and 335 normal controls. The results showed that there is no significant difference in the frequencies of FGFR4 SNPs rs351855, rs2011077, rs7708357, and 1966265 between women with cervical invasive cancer and normal controls even after controlling for age. However, significant differences existed in the distributions of the FGFR4 genetic polymorphism rs2011077, when mutant homozygotes (AA) were compared using other genotypes (GG/GA) as a reference, as well as rs1966265, when mutant homozygotes (AA) were compared using GG/GA as a reference, between women with cervical precancerous lesions and normal women even after controlling for age. In multivariate analysis, lymph node metastasis was associated with cancer recurrence, and lymph node metastasis and FGFR4 rs351855 were associated with patient survival. In conclusion, our study demonstrated that FGFR4 rs2011077 and rs1966265 are associated with the progression of cervical normal tissues to precancerous lesions in Taiwanese women. Moreover, rs351855 (Gly388Arg) is the only FGFR4 genetic polymorphism that is associated with patient survival.

Keywords

fibroblast growth factor receptor 4, genetic polymorphisms, uterine cervical cancer, patient survival

Introduction

The fibroblast growth factors (FGFs) are multifunctional proteins that signal to receptor tyrosine kinases and modulate the mitogenesis, proliferation, differentiation, and migration of various cells, the angiogenesis, and repair of tissue injury.[1-3] Phylogenetic analysis showed that 22 FGF genes can be categorized into 7 subfamilies in humans.[4] The fibroblast growth factor receptors (FGFRs) act as transmembrane tyrosine kinase receptors, consisting of 4 family members FGFR1 to 4, concerned with signal transduction via interaction with members of the FGF family.[5] The FGFR5, which lacks the FGF signaling tyrosine kinase domain, is proposed to effect as decoy receptor that binds FGF ligands and sequesters them away from the conventional FGFRs.[6] Tiong et al revealed that the presence of FGFR4/FGF19 autocrine signaling mediates the survival of a subset of basal-like breast cancer cells.[7] The expression of FGFR4 was suggested to be higher in cancer tissues than in normal tissues of the uterine cervix.[8]

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Jiang et al revealed that the minor alleles of rs1966265 and rs351855 in FGFR4 were strongly associated with breast cancer in Chinese women of Heilongjiang province.[9] Koole et al found that the FGFR4 Gly388Arg polymorphism was detected in 62% of oropharyngeal squamous cell carcinoma.[10] Fibroblast growth factor receptor 4 gene polymorphisms, including rs351855 (C/T, Gly388 to Arg388), rs2011077 (G/A), rs7708357 (G/A), and rs1966265 (Ile10Val), have been reported to probably influence the protein expression.[11-13] Therefore, FGFR4 gene polymorphisms are associated with the development of cancer via the impact on the protein expression.

Our previous study found that FGFR4 genetic polymorphism rs351855 is associated with the risk of hepatocellular carcinoma accompanied with liver cirrhosis.[14] Uterine cervical cancer is highly prevalent in Taiwan and known as the fifth most common type of cancer in women in this country. Cervical carcinogenesis is a multistep process, from normal cervix to precancerous lesions and finally to invasive cancer of the uterine cervix. Precancerous lesions are composed of cervical intraepithelial neoplasia 1 (CIN1; low-grade CIN or low-grade dysplasia), in which immature and mitoses cells occupy and are limited to the basal one-third of the epithelium, as well as CIN2, in which immature and mitoses cells occupy and are limited to the basal two-third of the epithelium, and CIN3, in which immature and mitoses cells occupy more than the basal two-third. The CIN 2 and CIN3 are collectively referred to as high-grade CIN or high-grade dysplasia. However, few studies investigated the associations among FGFR4 genetic polymorphisms, development of uterine cervical cancer, and patient prognosis in Taiwanese women. Therefore, we conducted this study to associate the distributions of FGFR4 genetic polymorphisms with the cervical tumorigenesis, cancer recurrence, and patient survival.

Materials and Methods

Study Population

Five hundred sixty-two women, including 138 with invasive cancer and 89 with precancerous lesions of the uterine cervix, as well as 335 normal controls were consecutively recruited into this study. All 335 normal control individuals were recruited at the same hospital and these control groups had neither self-reported history of cancer of any sites. The ages of women with cervical invasive cancer, precancerous lesions, and normal controls were 55.0 (12.5), 42.7 (12.0), and 44.2 (10.2), mean (SD), years, respectively. One hundred thirty-eight patients with invasive cervical cancer were staged according to the 2009 International Federation of Gynecology and Obstetrics Classification and received standard treatment protocols at the Department of Obstetrics and Gynecology in Chung Shan Medical University Hospital, Taiwan, from August 1993 to August 2014. Eighty-nine patients with precancerous lesions received colposcopy-directed cervical punch biopsy, large loop excision of the transformation zone, and total abdominal or vaginal hysterectomy. The diagnosis of all patients with cervical invasive cancer or precancerous lesions was confirmed according to the pathologic report before treatment initiation. All study population were Taiwanese women who resided in central Taiwan. The institutional review board of Chung Shan Medical University Hospital approved this study (CSMUH IRB: CS12219 and CS14014), and informed written consents were obtained from all patients.

Blood Sample Collection and Genomic DNA Extraction

Blood specimens were obtained from all individuals and placed into Vacutainer tubes containing EDTA and then stored at 4°C immediately. DNA was extracted from buffy coats (white blood cells) using QIAamp DNA blood mini kits (Qiagen, Valencia, California) as described in detail previously.[15] DNA was used as the template in polymerase chain reactions (PCRs).

Selection of FGFR4 Gene Polymorphisms

In this study, we selected 4 FGFR4 genetic polymorphisms based on the International HapMap Project data and previous studies.[11-13] These genetic polymorphisms included the nonsynonymous single-nucleotide polymorphisms (SNPs) rs351855 (Gly388Arg) and rs1966265 (Ile10Val) in the coding sequences and rs2011077 (G/A) in intron 11 and rs7708357 (G/A in 3′-flanking region).

Single-Nucleotide Polymorphisms by Real-Time PCR and Genotyping

Allelic discrimination of the FGFR4 genetic polymorphisms rs351855 (C/T, Gly388 to Arg388), rs2011077 (G/A), rs7708357 (G/A), and rs1966265 (Ile10Val) was evaluated using an ABI StepOne Real-Time PCR System (Applied Biosystems, Foster City, California) and analyzed by SDS version 3.0 software (Applied Biosystems) using the TaqMan assay as described previously.[16,17]

Statistical Analysis

We used analysis of variance method to analyze the age distributions of patients with invasive cancer and precancerous lesions of the uterine cervix and control women. Then, Bonferroni test was performed for post hoc analysis. Hardy-Weinberg equilibrium was used to analyze the genotype distributions of SNPs rs351855 (Gly388Arg), rs1966265 (Ile10Val), rs2011077, and rs7708357 in the normal controls (degree of freedom = 2). χ^2 or Fisher exact test was used to examine the relationships between genotype frequencies of FGFR4 SNPs and the incidence of cervical neoplasias (including invasive cancer). The adjusted odds ratios with their 95% confidence intervals (CIs) were used to examine the relationships among distributions of FGFR4 SNPs, haplotypes, and the incidence of cervical neoplasias by the logistic and multiple logistic regression models after controlling for age. χ^2 or Fisher

Table 1. Genotypic Distributions of the Single-Nucleotide Polymorphisms of the Fibroblast Growth Factor Receptor 4 Gene in Taiwanese Patients With Neoplasias of the Uterine Cervix and Normal Controls.[a]

Variables	Normal Controls (n = 335)	Cervical Neoplasias[b] (n = 227)	*P* Values[c]	Odds Ratio (95% CI)	Adjusted *P* Values[c]	Adjusted Odds Ratio (95% CI)[d]
rs351855						
CC[e]	96	69	.556	1.00	.670	1.00
CT	165	101		0.95 (0.60-1.51)		0.84 (0.51-1.39)
TT	74	56		1.05 (0.97-4.02)		1.01 (0.64-1.61)
CC[e]	96	69	.633	1.00	.371	1.00
CT/TT	239	157		0.91 (0.62-1.35)		0.84 (0.56-1.24)
CC/CT[e]	261	170	.459	1.00	.783	1.00
TT	74	56		1.16 (0.76-1.76)		1.06 (0.69-1.64)
rs2011077						
GG[e]	94	63	.526	1.00	.270	1.00
GA	163	102		0.93 (0.62-1.40)		1.11 (1.72-1.73)
AA	78	62		1.19 (0.75-1.88)		1.48 (0.90-2.45)
GG[e]	94	63	.937	1.00	.319	1.00
GA/AA	241	164		1.02 (0.69-1.51)		1.23 (0.82-1.86)
GG/GA[e]	257	165	.279	1.00	.122	1.00
AA	78	62		1.24 (0.82-1.85)		1.18 (0.96-1.45)
rs7708357						
GG[e]	321	222	.343	1.00	.504	1.00
GA	13	4		0.45 (0.14-1.38)		0.56 (0.18-1.78)
AA	1	1		1.45 (0.09-23.20)		2.44 (0.15-39.52)
GG[e]	321	222	.203	1.00	.462	1.00
GA/AA	14	5		0.52 (0.14-1.55)		0.82 (0.49-1.39)
GG/GA[e]	334	226	.781	1.00	.096	1.00
AA	1	1		1.48 (0.02-116.35)		1.42 (0.94-1.06)
rs1966265						
GG[e]	91	61	.497	1.00	.216	1.00
GA	168	105		0.93 (0.62-1.40)		1.13 (0.73-1.76)
AA	76	61		1.20 (0.75-1.91)		1.54 (0.93-1.56)
GG[e]	91	61	.939	1.00	.282	1.00
GA/AA	244	166		1.01 (0.68-1.51)		1.26 (0.83-1.90)
GG/GA[e]	259	166	.257	1.00	.096	1.00
AA	76	61		1.25 (0.83-1.88)		1.42 (0.94-2.16)

Abbreviation: 95% CI, 95% confidence interval.
[a]Statistical analysis: logistic regression model or χ^2 square test.
[b]Cervical neoplasias included precancerous lesions and invasive cancer of the uterine cervix.
[c]$P < .05$.
[d]The adjusted odds ratios and their 95% CIs were estimated by logistic regression model after controlling for age.
[e]Used as a reference for comparison to evaluate the odds ratio of other genotypes.

test and the logistic regression model were separately used for univariate and multivariate analyses among FGFR4 SNPs, haplotypes, clinicopathological variables, cancer recurrence, and patient survival (follow-up until October 31, 2015).

Results

The age distributions of study population are significantly different between patients with cervical cancer and those with precancerous lesion (55.0 $\pm$ 12.5 vs 42.7 $\pm$ 12.0, $P < .001$) as well as between those with cervical cancer and control women (55.0 $\pm$ 12.5 vs 44.2 $\pm$ 10.2, $P < .001$) but not significantly different between those with precancerous lesions and control women (42.7 $\pm$ 12.0 vs 44.2 $\pm$ 10.2, $P = .841$). The genotype distributions of FGFR4 SNPs rs351855, rs2011077, and rs1966265 met the Hardy-Weinberg equilibrium in the normal controls ($P = .845$, χ^2 value: 0.038; $P = .625$, χ^2 value: .203; and $P = .927$, χ^2 value: .008, respectively).

Association of FGFR4 Gene Polymorphisms With Uterine Cervical Neoplasias in Taiwanese Women

There were no significant differences in the frequencies of FGFR4 SNPs rs351855, rs1966265, rs2011077, and rs7708357 between women with cervical neoplasias and normal controls (Table 1). Further controlling for age, no significant differences still existed. After the cervical neoplasias group was subdivided into subgroups of invasive cancer and precancerous lesions, significant differences existed in the distributions of the FGFR4 gene SNP rs2011077, when mutant homozygotes (AA) were compared using other genotypes (GG/GA) as a reference, as well as SNP rs1966265, when mutant homozygotes (AA) were

Table 2. Genotypic Distributions of the Single-Nucleotide Polymorphisms of Fibroblast Growth Factor Receptor 4 Gene in Patients With Invasive Cancer and Precancerous Lesions of the Uterine Cervix and Normal Controls.[a]

Variables	Normal Controls (n = 335)	Precancerous Lesions (n = 89)	Invasive Cancer (n = 138)	Adjusted *P* Values[b]	AOR (95% CI)[c]	Adjusted *P* Values[b]	AOR (95% CI)[d]
rs351855							
CC[e]	96	29	40		1.00		1.00
CT	165	39	62	.284	0.74 (0.43-1.29)	.806	0.94 (0.55-1.60)
TT	74	21	35	.584	0.83 (0.43-1.62)	.826	0.93 (0.49-1.76)
CC[e]	96	29	40		1.00		1.00
CT/TT	239	60	97	.082	0.77 (0.46-1.28)	.787	0.93 (0.56-1.54)
CC/CT[e]	261	68	102		1.00		1.00
TT	74	21	35	.988	1.00 (0.55-1.79)	.916	0.97 (0.56-1.67)
rs2011077							
GG[e]	94	22	41		1.00		1.00
GA	163	38	64	.844	1.06 (0.58-3.50)	.662	1.13 (0.66-1.95)
AA	78	29	33	.077	1.81 (0.94-3.50)	.658	1.16 (0.61-2.18)
GG[e]	94	22	41		1.00		1.00
GA/AA	241	67	97	.365	1.30 (0.74-2.29)	.623	1.14 (0.68-1.89)
GG/GA[e]	257	60	105		1.00		1.00
AA	78	29	33	.038[b]	1.74 (1.03-2.94)	.805	1.07 (0.63-1.83)
rs7708357							
GG[e]	321	87	135		1.00		1.00
GA	13	2	2	.501	0.60 (0.13-2.70)	.485	0.58 (0.12-2.72)
AA	1	0	1	UA	UA	.153	7.77 (0.47-129.48)
GG[e]	321	87	135		1.00		1.00
GA/AA	14	2	3	0.431	0.55 (0.12-2.46)	.837	0.87 (0.23-3.25)
GG/GA[e]	334	89	137		1.00		1.00
AA	1	0	1	UA	UA	.149	7.93 (0.48-132.11)
rs1966265							
GG[e]	91	22	39		1.00		1.00
GA	168	38	67	.999	1.00 (0.54-1.85)	.446	1.24 (0.71-2.16)
AA	76	29	32	.082	1.80 (0.93-3.48)	.488	1.26 (0.66-2.42)
GG[e]	91	22	39		1.00		1.00
GA/AA	244	67	99	.455	1.24 (0.70-2.19)	.411	1.25 (0.74-2.10)
GG/GA[e]	259	60	106		1.00		1.00
AA	76	29	32	.028[b]	1.80 (1.06-3.04)	.739	1.10 (0.64-1.88)

Abbreviations: AOR, adjusted odds ratio; 95% CI, 95% confidence interval; UA, unavailable.
[a]Statistical analysis: multiple logistic regression model. The AORs with their 95% CIs were estimated by the multiple logistic regression model after controlling for age.
[b]$P < .05$.
[c]Comparison between patients with cervical precancerous lesions and normal controls after adjusting for age.
[d]Comparison between patients with cervical invasive cancer and normal controls after adjusting for age.
[e]Used as a reference for comparisons to evaluate the odds ratio of other genotypes.

compared using GG/GA as a reference, between women with cervical precancerous lesions and normal women after controlling for age ($P = .038$ and $P = .028$, respectively; Table 2), but no such difference was found between women with invasive cancer and normal women ($P = .805$ and $P = .739$, respectively; Table 2). However, no such finding was observed in FGFR4 gene SNPs rs351855 and rs7708357.

Association of FGFR4 Haplotypes With Uterine Cervical Neoplasias

Based on the locations of the analyzed variants (FGFR4 SNPs rs351855, rs1966265, rs2011077, and rs7708357), the FGFR4 haplotypes, rs351855, rs2011077, rs7708357, and rs1966265 in order, were established. Because the mutant homozygous AA and AA in SNP rs2011077 and SNP rs1966265 increased the risk of developing cervical precancerous lesions, haplotypes containing them (CAGA and TAGA) were regarded as a risk subgroup, while other haplotypes (TGGG, CGGG, TGAG, CGAG, CAGG, and CGGA) were regarded as a control subgroup. However, no significant difference was found in the distributions of FGFR4 haplotypes among women with invasive cancer or precancerous lesions and normal controls (Table 3).

Univariate and Multivariate Analyses of Clinicopathological Variables and FGFR4 Genetic Polymorphisms for Cancer Recurrence and Patient Survival in Taiwanese Women With Uterine Cervical Cancer

We found that some clinicopathological variables may increase the risk of cancer recurrence and present poor survival in

Table 3. Haplotype Distributions of the Fibroblast Growth Factor Receptor 4 (FGFR4) Gene in Patients With Invasive Cancer and Precancerous Lesions of the Uterine Cervix and Normal Controls.[a]

FGFR4 Haplotypes[b]	Control Women	Patients With Precancerous Lesions	Patients With Invasive Cancer	Adjust *P* Value; OR (95% CI)[c]	Adjust *P* Value; OR (95% CI)[d]
CAGA and TAGA	316	96	128	.057; 1.40 (0.99-1.97)	.645; 1.08 (0.78-1.49)
Others	352	82	148	1.00 (Reference)	1.00 (Reference)

Abbreviations: 95% CI, 95% confidence interval; OR, odds ratio.
[a]Statistical analysis: multiple logistic regression model. The AORs with their 95% CIs were estimated by the multiple logistic regression model after controlling for age.
[b]Haplotype distributions of the FGFR4 genes in order: rs351855, rs2011077, rs7708357, and rs1966265. Haplotypes containing CAGA and TAGA were regarded as a risk subgroup; other haplotypes (TGGG, CGGG, TGAG, CGAG, CAGG, and CGGA) were regarded as a control subgroup.
[c]Comparison between patients with precancerous lesions and normal controls after adjusting for age.
[d]Comparison between patients with cervical cancer and normal controls after adjusting for age.

univariate analysis (Table 4). More advanced cancer stages (stages II-IV) increased the risk of cancer recurrence (risk: 4.27, 95% CI: 1.48-12.84, $P = .002$; Table 4) and patient death (risk: 4.60, 95% CI: 1.45-15.97, $P = .003$; Table 4). Other risk factors for cancer recurrence included poor and moderate cell difference, deep cancer stromal invasion, large tumor size, and lymph node metastasis (Table 4). The risk factors for poor patient survival included more advanced cancer stages (stages II-IV), deep cancer stromal invasion, large tumor size, and lymph node metastasis (Table 4). Considering the FGFR4 genetic polymorphisms, we found that only rs351855 mutant homozygote TT tends to increase the risk of cancer recurrence (risk: 2.57, 95% CI: 0.84-7.50, $P = 0.053$; Table 4) and poor patient survival (risk: 3.10, 95% CI: 0.93-9.77, $P = .039$; Table 4), using genotypes CC/CT as a reference.

Using multivariate analysis, we found that only lymph node metastasis could carry the significant risk of cancer recurrence (risk: 4.41, 95% CI: 1.01-19.23, $P = .048$; Table 5) among clinicopathological variables. However, lymph node metastasis and more advanced cancer stages (stages II-IV) could increase the risk of poor patient survival (risk: 8.26, 95% CI: 1.63-41.67, $P = .011$ and risk: 6.13, 95% CI: 1.14-33.33, $P = .035$, respectively; Table 5). In addition, rs351855 mutant homozygote was the only FGFR4 genetic polymorphism that increased the risk of poor patient survival (risk: 4.07, 95% CI: 1.09-15.13, $P = .036$; Table 5).

Analysis of FGFR4 Haplotypes for Cancer Recurrence and Patient Survival in Taiwanese Women With Uterine Cervical Cancer

We further investigated the association of FGFR4 haplotypes with cancer recurrence and patient survival. We found that there is no association of FGFR4 haplotypes with cancer recurrence when risk haplotype subgroup (CAGA and TAGA) is compared, using control haplotype subgroup (TGGG, CGGG, TGAG, CGAG, CAGG, and CGGA) as a reference (risk: 0.70, 95% CI: 0.35-1.42, $P = .289$; Table 6). There was also no association of FGFR4 haplotypes with patient survival when risk haplotype subgroup was compared, using control haplotype subgroup as a reference (risk: 0.56, 95% CI: 0.25-1.21, $P = .109$; Table 6).

Discussion

Our study found that 4 FGFR4 gene polymorphisms, rs351855 (C/T, Gly388 to Arg388), rs2011077 (G/A), rs7708357 (G/A), and rs1966265 (Ile10Val), are not associated with the development of invasive cancer of the uterine cervix, that is, progression from normal cervix to invasive cancer in Taiwanese women. However, FGFR4 SNPs rs2011077 and rs1966265, but not rs351855 and rs7708357, were associated with the progression from normal cervix to precancerous lesions of the uterine cervix. Some studies reported that FGFR4 gene polymorphisms, rs351855, rs2011077, rs7708357, and rs1966265, may exert a pathophysiological influence on tumor or dysplasia development in various cancers and diseases.[11-13,18,19] Rezvani et al demonstrated that FGFR4 SNP rs1966265 (Ile10Val) is a possible key genetic variant in alveolar dysplasia diseases of preterm newborns.[11] Ma et al found that FGFR4 SNP rs2011077 significantly affects the development of prostate cancer and benign prostate hyperplasia and the progression of prostate cancer in Japanese population.[12] FitzGerald et al suggested no association of the FGFR4 SNPs rs351855, rs7708357, and rs1966265 as well as haplotypes with the risk of prostate cancer in Caucasian or African American individuals.[18] In contrast to our findings, the meta-analysis of Xu et al revealed that the FGFR4 SNP rs351855 (Gly388Arg) most likely contributes to susceptibility to cancer, especially in Asians.[13] In addition, the Arg (388) allele might be associated with an increased risk of breast and prostate cancers.

In order to evaluate the impact of FGFR4 genetic polymorphisms on patient prognosis, we used univariate and multivariate analyses to investigate the relationships among FGFR4 genetic polymorphisms, clinicopathological variables, cancer recurrence, and patient survival in Taiwanese women. In univariate analysis for the association of clinicopathological variables with patient prognosis, more advanced cancer stages (stages II-IV), stromal invasion, tumor diameter, and lymph node metastasis were associated with cancer recurrence and patient survival. However, in multivariate analysis, lymph node

Table 4. Univariate Analysis of Clinicopathological Variables and Fibroblast Growth Factor Receptor 4 (FGFR4) Genetic Polymorphisms for Cancer Recurrence or Patient Survival in Taiwanese Women With Uterine Cervical Cancer.[a,b]

	Recurrence[c]			Survival[d]		
	+	−	*P* Value and Risk of Recurrence	+	−	*P* Value and Risk of Death
Stage			.002[e]			.003[e]
II-IV	15	29	4.27 (1.48-12.84)	32	13	4.60 (1.45-15.97)
I[f]	8	66	1.00	68	6	1.00
Pathology type			.526			.982
Adenocarcinoma	5	14	1.61 (0.40-5.52)	16	3	0.98 (0.16-4.06)
SCC[f]	18	81	1.00	84	16	1.00
Cell differentiation			.015[e]			.041[e]
Poor and moderate	22	68	8.74 (1.26-373.64)	73	18	6.66 (0.95-287.49)
Well[f]	1	27	1.00	27	1	1.00
Stromal invasion			<.001[e]			.003[e]
>1/2 depth	18	32	7.09 (2.23-26.24)	37	14	4.77 (1.45-18.07)
≤1/2 depth[f]	5	63	1.00	63	5	1.00
Tumor diameter (cm)			<.001[e]			.001[e]
>4	16	24	6.76 (2.26-21.52)	28	13	5.57 (1.74-19.42)
≤4[f]	7	71	1.00	72	6	1.00
Parametrium			.027[e]			.021[e]
Invasion	10	20	2.88 (0.97-8.30)	22	9	3.19 (1.00-9.90)
No invasion[f]	13	75	1.00	78	10	1.00
Vagina			.027[e]			.209
Invasion	9	17	2.95 (0.95-8.73)	20	6	2.00 (0.54-6.62)
No invasion[f]	14	78	1.00	80	12	1.00
Lymph node			.001[e]			<.001[e]
Metastasis	12	17	5.01 (1.68-14.74)	19	11	5.86 (1.83-19.02)
No metastasis[f]	11	78	1.00	81	8	1.00
rs351855			.053			.039[e]
TT	9	19	2.57 (0.84-7.50)	19	8	3.10 (0.93-9.77)
CC/CT[f]	14	76	1.00	80	11	1.00
rs2011077			.996			.777
AA	6	25	1.00 (0.29-3.06)	27	4	0.72 (0.16-2.55)
GG/GA[f]	17	71	1.00	73	15	1.00
rs7708357			1.00			1.00
AA	0	1	0.00 (0.00-3744.00)	1	0	0.00 (0.00-205.26)
GG/GA[f]	1	95	1.00	99	19	1.00
rs1966265			.914			.778
AA	6	24	1.06 (0.31-3.24)	26	4	0.76 (0.17-2.69)
GG/GA[f]	17	72	1.00	74	15	1.00

Abbreviation: SCC, Squamous cell carcinoma.
[a]Statistical analysis: χ^2 square or Fisher test for univariate analysis.
[b]Some clinicopathological data could not be collected from patients with cervical cancer due to incomplete medical charts or records.
[c]Recurrence: +, recurrence; −, no recurrence.
[d]Survival: +, survival; −, dead.
[e]$P < .05$.
[f]Used as a reference for comparisons to evaluate the odds ratio of other subgroups.

metastasis was the only clinicopathological variable that was associated with cancer recurrence. More advanced cancer stages (stages II-IV) and lymph node metastasis were associated with patient survival. Using Kaplan-Meier curves, our previous study demonstrated that lymph node metastasis but not more advanced cancer stage (stage II) was associated with cancer recurrence.[20] Moreover, more advanced cancer stage (stage II) and lymph node metastasis were associated with patient survival. Focused on the main objective of this study, we found that FGFR4 SNP rs351855 is the only gene polymorphism that tends to increase the risk of cancer recurrence and poor patient survival in univariate analysis. However, FGFR4 SNP rs351855 significantly increased the risk of poor patient survival but not cancer recurrence in multivariate analysis.

The FGRF4 Gly388Arg activates the FGFR4, and human cancers characteristic of this SNP were demonstrated to be highly aggressive and metastatic.[21] In addition, Sugiyama et al found that the FGFR4-R388 can stabilize the endosomal MMP14, which then promotes collagen degradation, and is associated with cancer invasion and metastasis.[22] In contrast to the effects of the Arg allele, the Gly allele of rs351855

Table 5. Multivariate Analysis of Clinicopathological Variables and Fibroblast Growth Factor Receptor 4 (FGFR 4) Genetic Polymorphisms for Cancer Recurrence or Patient Survival in Taiwanese Women With Uterine Cervical Cancer.[a,b]

	Recurrence[c]				Survival[d]		
	+	−	*P* Value and Risk of Recurrence		+	−	*P* Value and Risk of Death
Lymph node			.048[e]	Lymph node			.011[e]
Metastasis	12	17	4.41 (1.01-19.23)	Metastasis	19	11	8.26 (1.63-41.67)
No metastasis[f]	11	78	1.00	No metastasis[f]	81	8	1.00
				Stage			.035[e]
				II-IV	32	13	6.13 (1.14-33.33)
				I[f]	68	6	1.00
				rs351855			.036[e]
				TT	19	8	4.07 (1.09-15.13)
				CC/CT[f]	80	11	1.00

[a]Statistical analysis: logistic regression model for multivariate analysis; only significant data are shown.
[b]Some clinicopathological data could not be collected from patients with cervical cancer due to incomplete medical charts or records.
[c]Recurrence: +, recurrence; −, no recurrence.
[d]Survival: +, survival; −, dead.
[e]$P < .05$.
[f]Used as a reference for comparisons to evaluate the odds ratio of other subgroups.

Table 6. The Analysis of Fibroblast Growth Factor Receptor 4 (FGFR 4) Haplotypes for Cancer Recurrence or Patient Survival in Taiwanese Women With Uterine Cervical Cancer.[a,b]

	Recurrence[c]			Survival[d]		
	+	−	*P* Value and Risk of Recurrence	+	−	*P* Value and Risk of Death
FGFR4 haplotypes[e]			.289			.109
CAGA and TAGA	19	96	0.70 (0.35-1.42)	102	14	0.56 (0.25-1.21)
Others[f]	27	96	1.00	98	24	1.00

[a]Statistical analysis: χ^2 square or Fisher test.
[b]Some clinicopathological data could not be collected from patients with cervical cancer due to incomplete medical charts or records.
[c]Recurrence: +, recurrence; −, no recurrence.
[d]Survival: +, survival; −, dead.
[e]Haplotype distributions of the FGFR4 genes in order: rs351855, rs2011077, rs7708357, and rs1966265. Haplotypes containing CAGA and TAGA were regarded as a risk subgroup; other haplotypes (TGGG, CGGG, TGAG, CGAG, CAGG, and CGGA) were regarded as a control subgroup.
[f]Used as a reference for comparisons to evaluate the odds ratio of other subgroups.

seems to function as a tumor suppressor in breast cancer culture assays, inhibiting the cell motility of invasive breast cancer cells.[23] Shim et al showed that patients with lymph node–positive early-stage esophageal squamous cell carcinoma, who carried the Gly388 allele, had improved survival compared to those carrying Arg388 when they received concurrent chemoradiotherapy.[24] It implies that the impact of the FGFR4 nonsynonymous SNPs rs351855 (Gly388Arg) on patient survival may act via the influence of protein expression. Although FitzGerald et al demonstrated an association between FGFR4 SNP rs351855 and prostate cancer risk in radical prostatectomy cases, they suggest that the association of FGFR4 SNP rs351855 with prostate cancer–specific mortality was weak.[18]

Because Shifman et al revealed that haplotypes consisted of each genetic polymorphism lead to the susceptibility to disease, although not obviously, haplotype study exerts a greater statistical power than individual SNP analysis for the detection of an association of the alleles with a disease phenotype,[25] we used the FGFR4 haplotypes to relate FGFR4 genetic polymorphisms to the development of cervical cancer and patient prognosis. Unfortunately, we could not find an association between the FGFR4 haplotypes and the progression of normal tissues to invasive cancer of the uterine cervix in Taiwanese women. Moreover, we could not associate the FGFR4 haplotypes with cancer recurrence and patient survival.

In conclusion, our study demonstrated that FGFR4 SNPs rs2011077 and rs1966265 are associated with the progression of normal tissues to precancerous lesions of the uterine cervix in Taiwanese women. However, rs351855 (Gly388Arg) is the only FGFR4 genetic polymorphism that is associated with patient survival. FGFR4 haplotypes are not associated with the development of cervical cancer and patient prognosis.

Declaration of Conflicting Interests

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