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Original Article

Telomerase reverse transcriptase (TERT) promoter mutations in Korean melanoma patients

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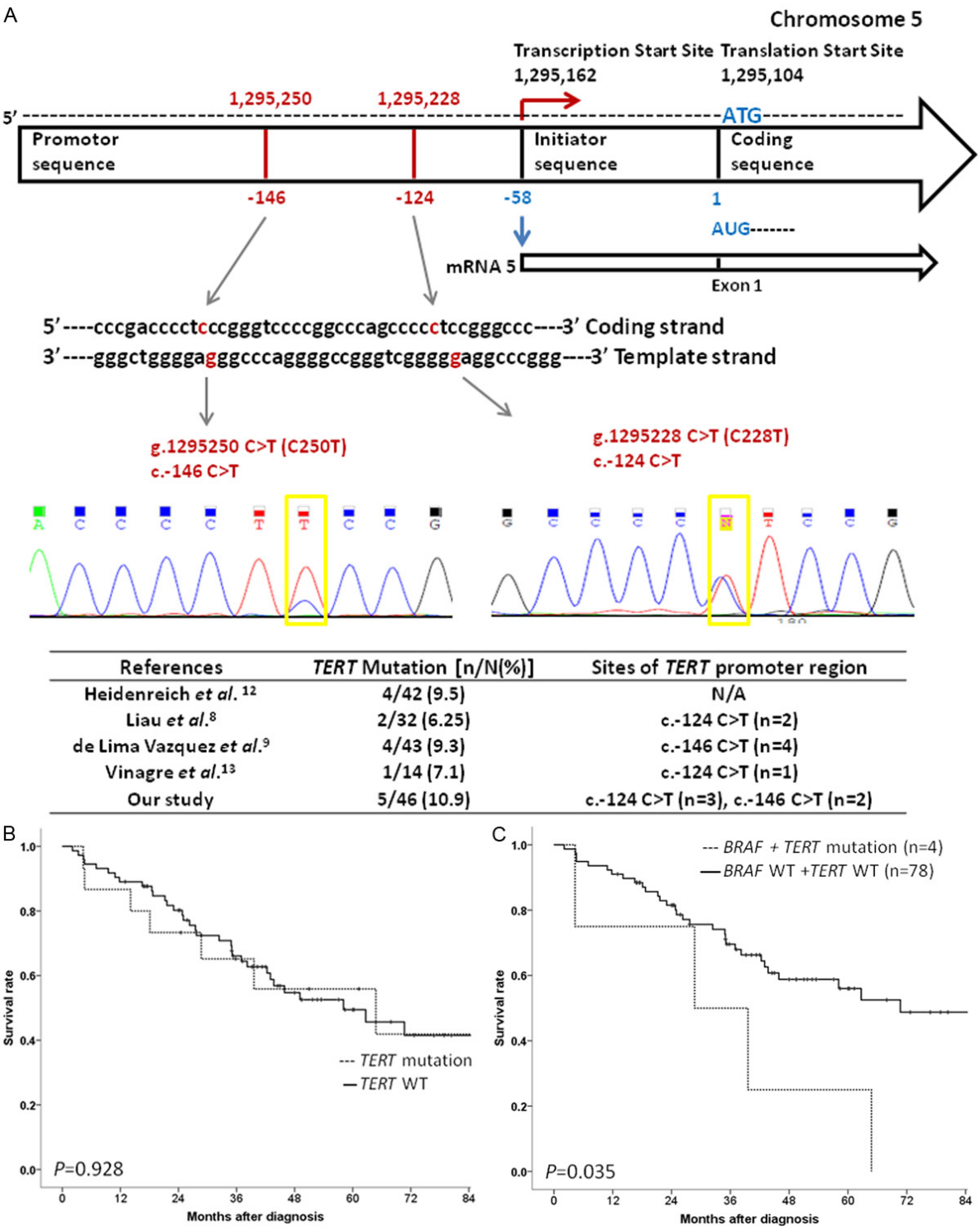
Abstract: Telomerase reverse transcriptase (TERT) is the reverse transcriptase component of the telomeric complex, which synthesizes terminal DNA to protect chromosomal ends and to maintain genomic integrity. In melanoma, mutation in *TERT* promoter region is a common event and theses promoter variants have been shown to be associated with increased gene expression, decreased telomere length and poorer outcome. In this study, we determined the frequency of *TERT* promoter mutation in 88 Korean primary melanoma patients and aimed to see the association of *TERT* promoter mutation status to other major molecular features, such as *BRAF*, *NRAS*, *KIT* mutations and correlate with clinicopathological features. In our study, acral melanoma (n=46, 52.3%) was the most common type. Overall, *TERT* promoter mutation was observed in 15 cases (17%) with ten c. -124C>T altertions and five c. -146C>T alterations. None of our samples showed CC>TT mutation which is considered pathognomonic of UV induction. Among the 46 acral melanoma patients, 5 patients (10.9%) harbored *TERT* promoter mutation. Tumors with *TERT* promoter mutation showed significantly greater Breslow thickness compared to WT tumors (*P*=0.039). A combined analysis for the presence of *TERT* promoter and *BRAF* mutations showed that patients with both *TERT* promoter and *BRAF* mutation showed decreased survival compared with those with only *TERT* promoter mutation, only *BRAF* mutation, or without mutations in either *TERT* promoter or *BRAF* (*P*=0.035). Our data provides additional evidence that UV-induced *TERT* promoter mutation frequencies vary depending on melanoma subtype, but preserves its prognostic value.

Keywords: *TERT* mutation, Korean, melanoma, survival, prognosis

Telomerase reverse transcriptase (TERT) is the reverse transcriptase component of the telomeric complex, which synthesizes terminal DNA to protect chromosomal ends and to maintain genomic integrity. Its upregulation has been demonstrated in several human cancers, and the promoter region of the gene is considered the critical regulatory element for telomerase expression. Mutually exclusive -124C>T and -146C>T mutations in *TERT* promoter region have been detected in more than 65% of melanomas [1]. These promoter variants have been shown to be associated with increased gene expression, decreased telomere length and poorer outcome [2, 3].

Here we intend to determine the frequency of *TERT* promoter mutation in 88 Korean primary melanoma patients who were followed at Severance hospital in Seoul, Korea during the period from 2005 to 2012 (Supplementary Table 1). Furthermore, we aimed to see the association of *TERT* promoter mutation status to other major molecular features, such as *BRAF*, *NRAS*, *KIT* mutations and correlate with clinicopathological features. The study was approved by the Institutional Review Board of Yonsei University College of Medicine. Written informed consent was obtained from all participants or their legal guardians. DNA was extracted from formalin-fixed, paraffin-embedded tumor tissues. Genomic DNA was isolated using proteinase K digestion and boiling method. Polymerase chain reaction (PCR) amplification of the *TERT* promoter region was performed using primers 5'-CCCACGTGCGCAGCAGGAC-3'



References	*TERT* Mutation [n/N(%)]	Sites of *TERT* promoter region
Heidenreich *et al.* [12]	4/42 (9.5)	N/A
Liau *et al.*[8]	2/32 (6.25)	c.-124 C>T (n=2)
de Lima Vazquez *et al.*[9]	4/43 (9.3)	c.-146 C>T (n=4)
Vinagre *et al.*[13]	1/14 (7.1)	c.-124 C>T (n=1)
Our study	5/46 (10.9)	c.-124 C>T (n=3), c.-146 C>T (n=2)

Figure 1. A. *TERT* promoter mutation variants identified in acral melanoma. B. Kaplan-Meier curves for overall survival according to *TERT* promoter mutation status. Kaplan-Meier survival analysis showed no association of *TERT* promoter mutation status with patient survival (P=0.928). C. Kaplan-Meier curves for overall survival in patients with both *TERT* promoter and *BRAF* mutation. Patients with both *TERT* promoter and *BRAF* mutation showed decreased survival compared with those with only *TERT* promoter mutation , only BRAF mutation, or without mutations in either *TERT* promoter or BRAF (P=0.035).

Table 1. Associations of *TERT* mutation with clinical and pathological variables in 88 melanoma patients

Clinicopathologic features	*TERT* genotype		*p*-value
	Wild type (n=73)	Mutation (n=15)	
Age (year)			
Mean	60.2	57	0.415
Gender			
Male	34 (46.6)	10 (66.7)	0.156
Female	39 (53.4)	5 (33.3)	
Stage at diagnosis (%)*			
0/I/II	43 (58.9)	9 (60)	0.937
III/IV	30 (41.1)	6 (40)	
Subtype (%)			
Acral	41 (89.1)	5 (10.9)	0.389
Mucosal	7 (87.5)	1 (12.5)	
CSD	8 (66.7)	4 (33.3)	
Non-CSD	14 (77.8)	4 (22.2)	
UP	3 (75)	1 (25)	
Mutant Oncogene			
BRAF V600E	10/67 (14.9)	4/14 (28.5)	0.219
NRAS	6/68 (8.8)	1/14 (7.1)	0.526
KIT	6/50 (12)	3/11 (27.3)	0.39
Breslow thickness (mm)			
Median	2.35	5	0.039
Range	0.6-15	0.4-27	
0.01-1.00	11 (19.6)	2 (18.2)	
1.01-2.00	12 (21.4)	2 (18.2)	
2.01-4.00	11 (19.6)	0 (0)	
>4.00	22 (39.3)	7 (63.6)	
Anatomic sites of tumors			
Trunk	5 (83.3)	1 (16.7)	0.477
Extremities	51 (85)	9 (15)	
Head and neck	8 (66.7)	4 (33.3)	
Other	7 (87. 5)	1 (12.5)	

*Staging according to the American Joint Committee on Cancer (AJCC) Melanoma Staging System 2009.

(forward), 5'-Biotin-CTCCCAGTGGATTCGCGGGC-3' (reverse) and 5'-AGGGGCTGGGAGGGC (sequencing). PCR products were used as templates for pyrosequencing with PyroMark Gold Q24 reagent (Qiagen, Germantown, MD, USA) according to the manufacture's protocol. Sequencing analysis was performed using PyroMark Q24 version 1.0.10 software in the allele quantification analysis mode. For statistical analysis, categorical data are described using frequencies and percentages, and continuous data are described using means ± standard deviations or median (range) for normally distributed data. Chi-squared (χ^2) test or Fisher's exact test was used to differentiate the rates of different groups, and differences in measurement data of 2 groups were evaluated by unpaired *t*-test or Mann-Whitney test. We used univariate logistic regression analyses to explore associations of *TERT* promoter mutation status with available clinical and pathologic variables, including age, sex, stage, oncogene mutation status, anatomical distribution of primary tumor, Breslow's thickness, and ulceration.

We investigated association between clinico-pathologic factors, *TERT* promoter mutation status, and oncogene mutation status with overall survival, defined as the interval from time of diagnosis of primary melanoma to death. Cases in which the endpoint was not reached at the time of the last follow-up were censored. Univariate results were displayed by the Kaplan-Meier method and hazard ratio estimates and *p*-values were derived from Cox proportional hazard model. Multivariable analyses were performed on variables with a *p*-value of 0.20 or less in univariate analyses. Confidence intervals (CI) were calculated with coverage of 95%. All reported *p*-value are nominal and two-sided. We applied a significance level of 5%. All statistical analyses were performed using SPSS Statistics software (version 18.0; SPSS Chicago, IL) or R 3.1.1.

The median age at diagnosis was 59 years (range 28-87 years) with equal male and female patients (M:F=44:44). Extremities (n=60) was the most common location of the primary melanoma, followed by head and neck area (n=12), other including mucosa (n=8), and trunk (n=6). Acral melanoma (n=46) was the most common type, followed by non-CSD (chronic sun-damage) melanoma (n=18), CSD melanoma (n=12), mucosal melanoma (n=8), and melanoma of unknown primary (n=4). Breslow tumor thickness ranged from 0.3 to 27 mm, with a median of 2.5 mm. *BRAF* mutations were detected in 14 tumors (15.9%), *NRAS* mutations in 10 cases (11.4%), and *KIT* mutations in 9 specimens (10.2%). *TERT* promoter variants were identified in 15 cases (17%) with ten c. -124C>T

altertions and five c. -146C>T alterations. Among ther 46 acral melanoma patients, 5 patients (10.9%) harbored *TERT* promoter mutation. Three cases were c. -124C>T altertions and two cases were c. -146C>T alterations (**Figure 1A**). The clinical and pathologic characteristics of tumors with regard to *TERT* promoter mutation status are detailed in **Table 1**. There were no significant differences in age, gender, stage at diagnosis, subtype, oncogene mutation status, or anatomic site of tumor. However, tumors with *TERT* promoter mutation showed significantly greater Breslow thickness compared to WT tumors (P=0.039). *TERT* promoter mutations were observed in 33.3% of CSD melanoma, 22.2% of non-CSD melanoma, 12.5% of mucosal melanoma and 10.9% of acral melanoma. Four patients were identified to have both *BRAF* and *TERT* promoter mutation. Survival analyses were performed for all patients (Supplementary Table 2). Univariate predictors of survival were gender (P=0.019), Breslow thickness (P=0.003), stage at diagnosis (P<0.001), and *BRAF* mutation (P=0.001). Kaplan-Meier survival analysis showed no association of *TERT* promoter mutation status with patient survival (P=0.928, **Figure 1B**). Multivariable analysis indicated that stage at diagnosis (HR=4.328, 95% CI: 2.5-7.5; P<0.001) was the only independent factor associated with survival in our cohort. A combined data analysis for the presence of *TERT* promoter and *BRAF* mutations showed that the patients with both *TERT* promoter and *BRAF* mutation showed decreased survival compared with those with only *TERT* promoter mutation , only *BRAF* mutation, or without mutations in either *TERT* promoter or *BRAF* (P=0.035, **Figure 1C**).

In this study carried out on patients with primary melanoma, the simultaneous occurrence of *TERT* promoter and *BRAF* mutations was associated with decreased survival in Korean melanoma patients. *TERT* promoter mutation has been reported to be associated with older patients, increased Breslow thickness, and worse prognosis in simultaneous occurrence with *BRAF* mutation which was in line with our study [4]. Overall, *TERT* promoter mutation was observed in 17% of the patients, which is much lower than previous reports showing up to 65% of the patients [1, 3, 4]. Huang *et al*. screened whole-genome sequencing data of melanoma and found that, apart from mutations in *BRAF* and *NRAS*, recurrent *TERT* promoter mutations were the most frequent genomic alteration [2]. The possible explanation of low incidence of *TERT* promoter mutation in our study may be due to the difference in the subtypes of melanoma in our cohort. It is well-known that acral melanoma is the most frequent type of melanoma in Asian patients [5]. In our study, acral and mucosal melanoma consisted of 61.4% of our patients. In acral melanoma, *TERT* promoter mutation was present in only 10.9% which is in line with previous studies reporting that *TERT* promoter mutation is uncommon in acral melanoma compared to non-acral melanoma [6]. Second possible reason is that our cohort of samples was all primary melanomas. Horn *et al*. detected *TERT* promoter mutations in 33% of primary melanomas and at considerably higher frequencies in melanoma cell lines (74%) and corresponding tissue from metastasis (85%) [2]. Dipyrimidine CC>TT mutations are considered pathognomonic of UV induction [7]. In our study, none of our samples showed CC>TT mutation, and *TERT* promoter mutations were considerably more frequent in nonacral cutaneous melanomas than acral melanomas. In the literature, there were only 16 cases (including 5 cases in our study) [6, 8-10] of acral melanoma which harbor *TERT* promoter mutations and none of the cases had shown CC>TT mutation (**Figure 1A**). This finding is consistent with a role for UV-induction in the pathogenesis, as acral sites are rarely sun-exposed and further supported by the absence of UV signature mutations, particularly CC>TT. Our data provides additional evidence that UV-induced *TERT* promoter mutation frequencies vary depending on melanoma subtype, but preserves its prognostic value.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. Clinical characteristics of enrolled melanoma patients

Characteristics	N (%)
Patient No. (%)	88 (100)
Age (year)	
Median (Range)	59 (28-87)
Gender	
Male:Female	44:44
Stage at diagnosis (%)*	
0/I/II	52 (59.1)
III/IV	36 (0.934.5)
Subtype (%)	
Acral	46 (52.3)
Mucosal	8 (9.1)
CSD	12 (13.6)
Non-CSD	18 (20.5)
Unknown primary	4 (4.5)
Mutant Oncogene	
BRAF	14 (15.9)
NRAS	10 (11.4)
KIT	9 (10.2)
TERT promoter mutations†	
All mutations	15/88 (17)
124C>T	10/88 (11.4)
138_139CC>TT	0/88 (0)
146C>T	5/88 (5.6)
Breslow thickness (mm)	
Median (Range)	2.5 (0.3-27)
0.01-1.00	13 (14.8)
1.01-2.00	14 (15.9)
2.01-4.00	11 (12.5)
>4.00	29 (33)
Anatomic sites of tumors	
Trunk	6 (6.8)
Extremities	60 (68.2)
Head and neck	12 (13.6)
Other	8 (9.1)
Sample type sequenced	
Primary	84 (1800)
Metastasis	0 (0)

*Staging according to the American Joint Committee on Cancer (AJCC) Melanoma Staging System 2009. †Mutations are annotated applying the last three digits of the first nucleotide mutated in the chromosome location according to hg19: Chr.5: 1295xxx (where xxx is a place holder for the mutation number).

Supplementary Table 2. Univariate and Multivariate Cox-Regression Analyses for Overall survival

	Risk factors	Univariable			Multivariable		
		HR	95% CI	*P* value	HR	95% CI	*P* value
Definite event	Age *(by 1 yr increment)*	1.014	0.997-1.032	0.116	1.012	0.992-1.032	0.230
	Sex (vs female)	1.783	1.100-2.889	0.019			
	Thickness	1.091	1.031-1.155	0.003			
	Stage III or IV (vs. I or II)	4.597	2.802-7.544	0.000	4.328	2.492-7.516	0.000
	Tumor site			0.484			
	Extremities vs others	0.498	0.208-1.189	0.116			
	BRAF	2.619	1.481-4.634	0.001	1.493	0.815-2.737	0.195
	NRAS	0.768	0.359-1.689	0.511			
	TERT	0.963	0.425-2.181	0.928			

For multivariable analyses, variables with a *P* value of 0.20 or less in univariable analyses were candidates for the multivariable Cox models. Independent predictors from baseline demographic and clinical variables were determined by multivariable Cox-regression analyses. Influences of “Genetic aberration” on overall survival were analyzed by adjustment for independent predictors determined by multivariable analyses.