Frequent *TERT* Promoter Mutations in Ocular Surface Squamous Neoplasia

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Purpose. Ocular surface squamous neoplasia, including intraepithelial neoplasia (CIN) and invasive squamous cell carcinoma (SCC), are one of the most common malignant tumors of the conjunctiva. Little is known of the genetic alterations involved in their pathogenesis. Promoter mutations in telomerase reverse transcriptase (*TERT*) have been identified in various cancers, including many associated with ultraviolet (UV) exposure. Our study analyzes the mutation rate and clinicopathological associations of *TERT* promoter mutations in ocular surface squamous neoplasia.

Methods. DNA was isolated and the region of the *TERT* promoter where hotspot mutations can occur analyzed by Sanger-sequencing in 48 ocular surface squamous neoplasia tumor samples (6 CIN and 42 SCC). An analysis of associations between *TERT* promoter mutation status and various clinicopathological parameters was performed.

Results. We identified *TERT* promoter mutations in 21 of 48 ocular surface squamous neoplasia samples (43.8%), including 4 in CIN and 17 in SCC. The mutations consisted of 8 Chr.5:1295228C>T, 1 Chr.5:1295228_1295229CC>TT, 5 Chr.5:1295242_1295243CC>TT, and 12 Chr.5:1295250C>T mutations. All mutations were C>T or CC>TT alterations, demonstrating a UV-signature. *TERT* promoter mutations showed no statistically significant associations with clinicopathological parameters.

Conclusions. Telomerase reverse transcriptase promoter mutations are found in almost half of ocular surface squamous neoplasias and have a mutation profile supporting UV induction as the major source of mutagenesis. We conclude that UV induced *TERT* promoter mutations leading to aberrant overexpression of telomerase is a major pathogenetic factor in ocular surface squamous neoplasia.

Keywords: squamous cell carcinoma, conjunctiva, *TERT*, promoter mutation

One of the most common malignant tumors of the conjunctiva are epithelial-derived neoplasms, frequently termed ocular surface squamous neoplasias (OSSN), which include intraepithelial neoplasias (CIN) as well as fully invasive conjunctival squamous cell carcinoma (SCC).[1] The incidence varies depending on the geographical location and presumably is highly dependent on the level of sun exposure (Uganda: 1.2/100,000 vs. UK 0.02/100,000 cases/y).[2] The tumors have a high local recurrence rate[3] and the capability to metastasize.[4–6] Recurrence and metastasis rate vary depending on the TNM-stage at initial diagnosis and if complete excision of the tumor succeeds.[7,8]

The development of SCC is associated with different risk factors. Phenotypical manifestations, such as fair skin and light eye color, are involved. Predisposing infectious diseases, including human papillomavirus (HPV), human immunodeficiency virus (HIV), and hepatitis B and C also have been described.[9–11] Other factors include atopic dermatitis and immunosuppression. Environmental factors, in particular ultraviolet light (UV) exposure as well as cigarette smoking also have been implied.

To date, not much is known of the genetic alterations involved in the pathogenesis of OSSN. One of the key events reported, and frequently associated with typically UV-induced CC>TT alterations, are mutations affecting the tumor suppressor *TP53* in a high number of tumor samples (52.4%, 11 of 21 SCC cases).[12] Another potentially pathogenetically relevant factor reported is HPV infections, in particular serotype 16 and 18. Whereas some studies support a pathogenetic involvement,[13–15] others argue against a relevant role for HPV in OSSN pathogenesis.[16,17] A potential role of HPV appears to be

accepted by most groups. To our knowledge, other genetic events relevant in OSSN have not been described.

Promoter mutations in the telomerase reverse transcriptase (*TERT*) gene were first described in 2013 in cutaneous melanoma[18,19] and later identified in various additional major human neoplasias.[20] In many cancer types, including thyroid cancer, melanoma, and gliomas, they are associated frequently with poor prognosis and/or advanced disease.[21–25]

The *TERT* gene codes for the enzymatic subunit of the telomerase holoenzyme. Telomerase maintains telomere length by synthesizing telomeric TTAGGG repeats. In most somatic cells, telomerase is not expressed, resulting in telomere shortening upon each subsequent cell division. When the telomeres are consumed, cells become genetically instable, frequently leading to senescence or apoptosis.

Telomerase reverse transcriptase promoter mutations create novel ETS binding sites,[18,19] recruiting transcription factors such as nuclear respiratory factor 2 (GABP),[26] resulting in increased *TERT* gene expression. This increased telomerase expression enables tumors to maintain their telomere length and continuously proliferate without becoming apoptotic or senescent due to genetic instability.[27,28]

In previous studies, we and others have identified *TERT* promoter mutations in a number of different UV-exposed tumors,[29–32] including epithelial tumors of the skin (i.e., cutaneous SCC) and conjunctival melanomas.[33]

Considering the known effect of UV-exposure on the development of OSSN and the similarities to other tumors, in terms of location of origin (conjunctival melanoma) or tissue type (cutaneous epithelial tumors) where *TERT* promoter mutations have been shown to occur frequently, we decided to analyze the occurrence, distribution, and clinical implications of *TERT* promoter mutations in OSSN.

Materials and Methods

Sample Selection

In total, 48 OSSN formalin-fixed and paraffin-embedded (FFPE) tumor samples, including 6 CIN and 42 SCC, were obtained from the archives of the Institute of Pathology, University Hospital Essen of patients treated in the Department of Ophthalmology of the University Hospital Essen, Essen, Germany. The study was done with written patient informed consent in accordance with the tenets of the Declaration of Helsinki and the guidelines put forth by the ethics committee of the University of Duisburg-Essen.

DNA Isolation and Direct (Sanger-) Sequencing

Depending on the tumor size, between five and ten 5-μm thick sections were cut from FFPE tumor blocks and were deparaffinized. One additional 2-μm thick section of each tumor sample was sectioned and stained with hematoxylin and eosin (H&E) according to standard protocols. For genomic DNA isolation, we applied the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. From peripheral blood mononuclear cell (PBMC) samples, DNA was isolated as described previously.[34] Polymerase chain reaction was performed to amplify the *TERT* promoter region with the following primers: hTERT-F: CAGCGCTGCCTGAAACTC and hTERT-R GTCCTGCCCCTTCACCTT, resulting in a 163-bp fragment. As reported previously,[33] the PCR products were Sanger sequenced and Chromas software (version 2.01; University of Sussex, Brighton, UK) was applied for sequence-data analysis.

Clinical and Histopathological Variables

Patient records were screened with regard to various clinical parameters, including sex, age, tumor location, tumor size, clinical TNM (cTNM) stage,[35] treatment type, local recurrence, and metastasis rate.

Conventionally H&E stained slides of all tumor samples were reevaluated by experienced histopathologists (HR, KGG) blinded from outcome and all other clinical data. Where original slides were not available, new sections were prepared for evaluation. The following parameters were analyzed: tumor differentiation and grade, presence or absence of keratinization, ulceration, histological cell-type (epithelioid or spindle), nuclear-cytoplasmic ratio (nuclear-to-cytoplasm area ratio $\leq$ 1/3 defined as low, >1/3 as high), cell arrangement, as well as perineural or lymphovascular invasion.

For assessment of tumor grade/differentiation, grade 1 was defined as tumors with well-differentiated cells closely resembling nonneoplastic keratinocytes with minimal or minor nuclear anaplasia and enlargement, no or individual atypical mitosis, and often areas of well-defined keratinization. Grade 2 comprised tumors with features, such as atypical keratinocytes, exhibiting moderate nuclear anaplasia and enlargement often with coarse chromatin, nuclear membrane thickening, and visible nucleoli, single cell necrobioses or small zones of cellular necrosis, an increased frequency of (atypical) mitoses and diminished keratinization. Grade 3 was defined as tumors having atypical keratinocytes with prominent nuclear anaplasia sometimes with bizarre multinucleated and giant tumor cells, marked increase in (atypical) mitoses, single cell necrobioses and cellular necrosis, as well as often loss of keratinization or cohesiveness resulting in a nested or single cell growth pattern.

Statistical Analysis

Associations of *TERT* promoter mutation status and clinicopathological variables were explored using χ^2 or Fisher *exact* tests where appropriate. A P value < 0.05 was considered statistically significant. For all statistical analysis SPSS Statistics software (version 22.0; SPSS, Inc., Chicago, IL, USA) was applied.

Results

Tumors and Patients

The *TERT* promoter was sequenced successfully in 48 OSSN samples, including 6 CIN and 42 SCC samples. The samples were from 25 males and 22 females (1 case unknown) with a median age of 64.5 years (range, 29–91 years) and a mean follow-up of 24.2 months (range, 0.3–95 months). The clinicopathological information is summarized in Table 1.

TERT Promoter Mutation Analysis

We identified *TERT* promoter mutations in 21 of 48 tumor samples (43.8%), including 4 of 6 (66.6%) CIN tumor samples and 17 of 42 (40.4%) SCC samples (Table 2). Recurrent mutations were identified in the previously described mutation sites,[18] consisting of Chr.5.1295228C>T, Chr.5.1295228_1295229CC>TT, Chr.5.1295242_1295243CC>TT, or Chr.5.1295250C>T mutations (Figs. 1, 2). In the remaining manuscript, the mutations will

Table 1. Tumor Samples Characteristics in Regard to *TERT* Promoter Mutation Status ($n = 48$)

Parameter	Level	All Cases $N = 48$	%	$TERT^{wt}$ $N = 27$	%	$TERT^{mut}$ $N = 21$	%	*P* Value
Age at diagnosis	Median	64.5						0.29
	Range	29–91						
	<60 y	16	33	11	23	5	10	
	>60 y	31	65	16	33	15	31	
	Missing data	1	2	0	0	1	2	
Sex	Female	22	46	11	23	11	23	0.33
	Male	25	52	16	33	9	19	
	Missing data	1	2	0	0	1	2	
Eye	Right	29	60	15	31	14	29	0.31
	Left	18	382	12	25	6	13	
	Missing data	1		0	0	1	2	
Tumor	CIN	6	13	2	4	4	8	0.22
	SCC	42	88	25	52	17	35	
Stage at diagnosis*	CIN	6	13	2	4	4	8	0.62
	cT1	4	8	2	4	2	4	
	cT2	7	16	3	6	4	8	
	cT3	14	29	10	21	4	8	
	cT4	13	27	8	17	5	10	
	Missing data	4	8	2	4	2	4	
Tumor diameter	Median	1.2						0.8
	Range	0.1–3.0						
	0.1–0.5 cm	14	29	8	17	6	13	
	0.51–1.0 cm	7	16	3	6	4	8	
	1.1–1.5 cm	4	8	2	4	2	4	
	>1.51 cm	21	44	13	27	8	17	
	Missing data	2	4	1	2	1	2	
Treatment	Excision alone	5	10	2	4	3	6	0.3
	Local chemotherapy	4	8	0	0	4	8	
	Mouth mucosal transplant	16	34	12	25	4	8	
	Radiotherapy	4	8	1	2	3	6	
	Enucleation	1	1	1	2	0	0	
	Exenteration	14	29	9	19	5	10	
	Missing data	4	8	2	4	2	4	
Primary localization	Bulbar/limbal	16	33	8	17	8	17	0.91
	Fornix/tarsus	9	19	5	10	4	8	
	Eyelid	12	25	7	15	5	10	
	Missing data	11	23	7	15	4	8	
Local recurrence	Yes	7	15	3	6	4	8	0.44
	No	41	85	24	50	17	35	
Metastasis	Yes	2	4	0	0	2	4	0.26
	No	46	96	27	56	19	40	

* TNM Classification of Malignant Tumors, UICC, 7th ed.[35]

Table 2. *TERT* Promoter Mutations Identified in Ocular Surface Squamous Neoplasias

	CIN	SCC
Wild-type	2 (33.3%)	25 (59.5%)
Mutant	4 (66.6%)	17 (40.5%)
Mutations*		
228C>T	1 (2.1%)	4 (8.3%)
228CC>TT	0	1 (2.1%)
242CC>TT	1 (2.1%)	1 (2.1%)
250C>T	1 (2.1%)	7 (14.6%)
228C>T, 242CC>TT	0	1 (2.1%)
228C>T, 250C>T	0	2 (4.2%)
242CC>TT, 250C>T	1 (2.1%)	1 (2.1%)

* All mutations are annotated applying the last three digits of the chromosome location of the first nucleotide mutated: Chr.5:1295xxx (xxx is a place holder for the digits in the Table). All mutations identified were heterozygous.

be annotated applying the last three digits of their chromosome location nomenclature; that is, Chr.5.1295228C>T as 228C>T.

The *TERT* promoter mutations identified included 8 (16.7%) 228C>T, 1 (2.1%) 228CC>TT, 5 (10.4%) 242CC>TT, and 12 (25%) 250C>T mutations. Five samples were found to harbor more than one mutation in the *TERT* promoter (Table 2). All identified mutations were heterozygous.

Germ-Line *TERT* Promoter Analysis

For three patients having OSSN where *TERT* promoter mutations were identified, matching constitutional DNA from PBMCs was available. In all three cases, *TERT* promoter mutations detected in the tumor were not found present in the constitutional DNA, confirming the mutations were acquired somatically (Supplementary Fig. S1).

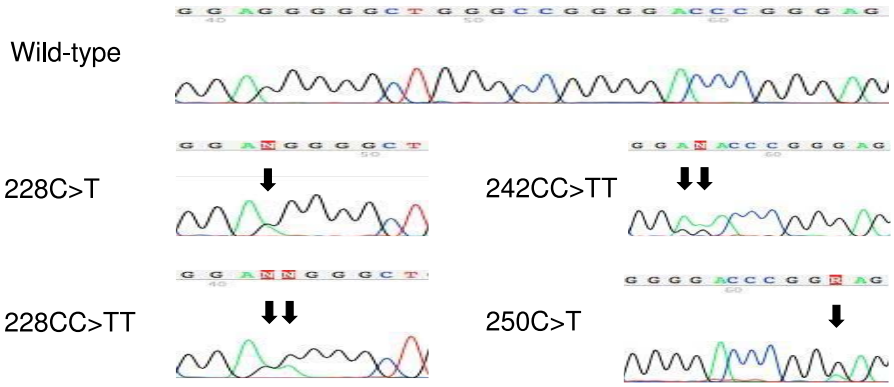

Figure 1. Recurrent *TERT* promoter mutations identified. Sanger sequencing chromatograms of the identified recurrent *TERT* promoter mutations located at Chr.5.1295228C>T, Chr.5.1295228_1295229CC>TT, Chr.5.1295242_1295243CC>TT, or Chr.5.1295250C>T (according to human genome assembly 19 [hg19]). Only the last three digits of the first mutated nucleotide are used to annotate the mutation site in the Figure. The *black arrows* highlight the mutations locations. A wild-type promoter sequence for comparison is shown on the top.

Clinical Parameters and Association With *TERT* Promoter Mutation Status

Telomerase reverse transcriptase promoter mutation occurred in 15 (31.3%) elderly patients, but only in 5 (10.4%) patients younger than 60 years. The median tumor diameter was 1.2 cm. In all cases, initial surgical tumor excision was performed. Additionally local chemotherapy, radiotherapy, or transplantation of mouth mucosal tissue was performed in 4 (8.3%), 4 (8.3%), and 16 (33.3%) cases, respectively. Enucleation was

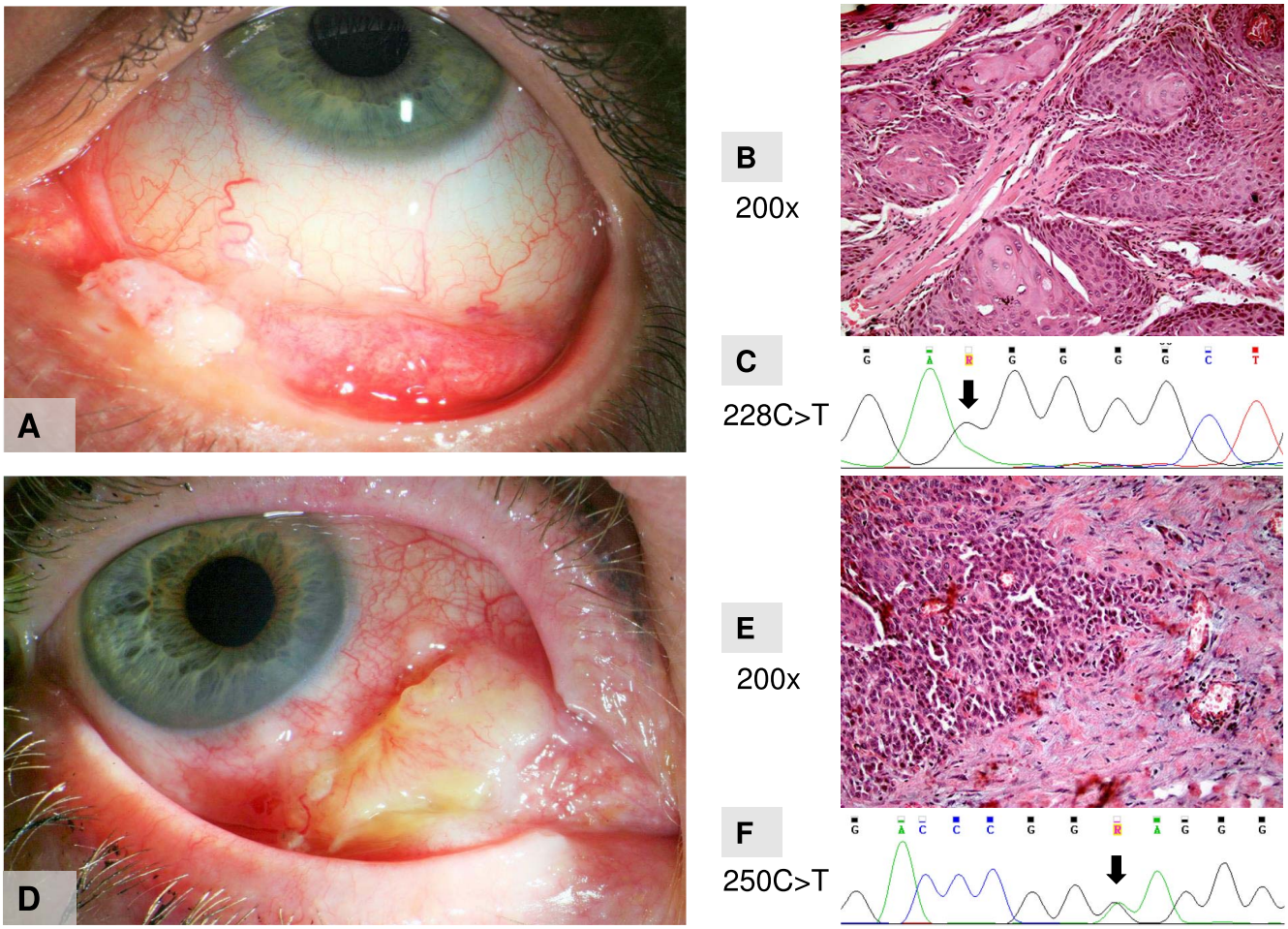

Figure 2. Clinical pictures of the tumor with corresponding histopathology and *TERT* promoter mutation chromatograms. (**A**, **D**) Clinical pictures of squamous cell carcinoma with (**B**, **E**) corresponding H&E slides ,and (**C**, **F**) chromatograms with mutations highlighted by *black arrows*. (**B**) shows a well-differentiated (classified as grade 1) SCC. Magnification: ×200. (**C**) corresponding chromatogram of the *TERT* promoter with Chr.5.1295228C>T mutation. (**E**) demonstrates a poorer differentiated (classified as grade 2) SCC. (**F**) Telomerase reverse transcription promoter chromatogram with Chr.5.1295250C>T mutation.

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Table 3. Tumor Sample Characteristics in Regard to *TERT* Promoter Mutation Status ($n = 48$)

Parameter	Level	All Cases $N = 48$	%	$TERT^{wt}$ $N = 27$	%	$TERT^{mut}$ $N = 21$	%	*P* Value
Grading/differentiation	G1 (high)	10	21	4	8	6	13	0.38
	G2 (moderate)	23	48	12	25	11	23	
	G3 (poor)	12	25	9	19	3	6	
	Missing data	3	6	2	4	1	2	
Keratinization	Yes	20	42	11	23	9	19	0.99
	No	21	44	12	25	9	19	
	Missing data	7	15	4	8	3	6	
Cell type	Epithelioid	22	46	11	23	11	23	0.63
	Spindle	15	31	8	17	7	15	
	Missing data	11	19	8	17	3	6	
Nuclear-cytoplasmic ratio	Low	12	25	8	17	4	8	0.31
	High	26	54	12	25	14	29	
	Missing data	10	23	7	15	3	6	
Cell arrangement	Nests	6	13	2	4	4	8	0.48
	Sheets	3	6	1	2	2	4	
	Lobules	17	33	9	19	8	17	
	Cords	13	27	9	19	4	8	
	Missing data	9	19	6	13	3	6	
Ulceration	Yes	14	29	8	17	6	13	0.83
	No	27	56	14	29	13	27	
	Missing data	7	15	5	10	2	4	
Perineural invasion	No	40	83	22	46	18	38	0.91
	Yes	3	6	2	4	1	2	
	Missing data	5	10	3	6	2	4	
Lymphovascular invasion	No	39	81	22	46	17	35	0.95
	Yes	4	8	2	4	2	4	
	Missing data	5	10	3	6	2	4	

necessary in 1 (2.1%) and exenteration in 14 (29.2%) cases. While the frequency of *TERT* promoter mutations varied (Table 1), no statistically significant associations between the assessed clinical variables and *TERT* promoter mutation status were observed.

Histological Parameters and Association With *TERT* Promoter Mutation Status

A detailed report of measured histopathological criteria analyzed, including grade/differentiation, keratinization, cell type (epithelioid or spindle), nuclear-cytoplasmic ratio, cell arrangement, ulceration, perineural invasion and lymphovascular invasion, is listed in Table 3. None of the histopathological variables analyzed showed statistically significant associations with *TERT* promoter mutation status.

Associations of *TERT* Promoter Mutation Status and Prognostic Factors

Telomerase reverse transcriptase promoter mutations were present in 4 of 7 (57.1%) patients with local recurrence of disease (including SCC and CIN III) and all patients with metastasis ($n = 2$, 100%, Table 4). There was no statistical significance in Kaplan-Meier survival analysis in regard to *TERT* promoter mutation status and local recurrence rate (Fig. 3).

Discussion

Overall recurrent *TERT* promoter mutations were identified in 43.8% of all OSSN cases analyzed. In addition to known frequent mutations in *TP53*, our results highlight *TERT* promoter mutations as a very common genetic alteration in OSSN, arguing that aberrant telomerase expression is a highly relevant pathogenetic event in OSSN.

All *TERT* promoter mutations we identified were in previously reported hotspots[18,19] and had a clear UV signature consisting solely of C>T or CC>TT changes.[36,37] However, although C>T alterations are typical for UV induction, 228C>T and 250C>T mutations also have been detected at high frequency in cancers of non-UV exposed internal organs, such as bladder cancer, hepatocellular cancer, thyroid cancer, and gliomas.[20,22,38] This means, that based solely on the occurrence of the 228C>T and 250C>T mutations, one cannot conclude the mutations are UV induced. In contrast, dipyrimidine CC>TT changes are considered virtually pathognomic of UV induction[36,37] and are only very rarely described in internal organ tumors.[22,24,25] In our study, in 6 of 21 (28.6%) cases, *TERT* promoter mutations with CC>TT substitutions were identified. This is higher than the general frequency in cutaneous melanoma, which is approximately 10%.[21,39] CC>TT substitution frequencies of approximately 30% have only been identified otherwise in cutaneous SCC and a few other highly sun exposed cutaneous tumors (i.e., atypical fibroxanthomas).[31,32] All six tumors identified in our study harboring CC>TT mutations were located at the palpebral fissure, a location with particularly high UV exposure. In contrast, C>T changes were identified in tumor samples primarily located at the fornix or tarsus. Overall, the mutation signature in the *TERT* promoter identified in our cohort, further underlines the already well-recognized pathogenetic role of UV exposure in the etiology of OSSN.[2,40,41]

In three cases, matched blood samples of tumors harboring *TERT* promoter mutations could be obtained and the presence of germ-line mutations excluded. In the other cases, the

Table 4. Associations of *TERT* Promoter Mutations and Clinicopathological Parameters

		WT		228C>T		228CC>TT		242CC>TT		250C>T		Total*
***TERT* Promoter Mutation**		*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*
	Total	27	51	8	15	1	2	5	9	12	23	53
Sex	Female	11	21	3	6	0	0	3	6	8	15	25
	Male	16	30	5	9	1	2	2	4	4	8	28
Tumor	CIN	2	4	1	2	0	0	2	4	2	4	7
	SCC	25	47	7	13	1	2	3	6	10	19	46
Stage at diagnosis	CIN	2	4	1	2	0	0	2	4	2	4	7
	cT1	2	4	1	2	0	0	1	2	1	2	5
	cT2	3	6	2	4	0	0	2	4	2	4	9
	cT3	10	19	2	4	0	0	0	0	3	6	15
	cT4	8	15	1	2	0	0	0	0	4	8	13
	Missing data	2	4	1	2	1	2	0	0	0	0	4
Tumor diameter	0.1–0.5 cm	8	15	3	6	0	0	1	2	4	8	16
	0.51–1.0 cm	3	6	2	4	0	0	1	2	3	6	9
	1.1–1.5 cm	2	4	1	2	0	0	1	2	0	0	4
	>1.51 cm	13	25	2	4	1	2	2	4	4	8	22
	Missing data	1	2	0	0	0	0	0	0	1	2	2
Treatment	Excision alone	3	6	4	8	0	0	0	0	0	0	7
	Local chemotherapy	0	0	1	2	0	0	1	2	4	8	6
	Mouth mucosal transplant	12	23	1	2	0	0	3	6	2	4	18
	Radiotherapy	1	2	1	2	0	0	1	2	2	4	5
	Enucleation	1	2	0	0	0	0	0	0	0	0	1
	Exenteration	9	17	1	2	1	2	0	0	3	6	14
	Missing data	1	2	0	0	0	0	0	0	1	2	2
Primary tumor localization	Bulbar/limbal	8	15	1	2	1	2	5	9	5	9	20
	Fornix/tarsus	5	9	2	4	0	0	0	0	2	4	9
	Eyelid	7	13	3	6	0	0	0	0	4	8	14
	Missing data	7	13	2	4	0	0	0	0	1	2	10
Local recurrence	Yes	3	6	2	4	0	0	2	4	3	6	10
	No	24	45	6	13	1	2	3	6	9	17	43
Metastasis	Yes	0	0	1	2	0	0	1	2	0	0	2
	No	27	51	7	13	1	2	4	8	12	23	51

* Five cases harbored more than one *TERT* promoter mutation.

presence of germ-line mutations in the *TERT* promoter could not be excluded. However, as germ-line mutations at the observed hotspots have not been observed in various *TERT* promoter mutation studies where germ-line DNA was available,[18–20,30,33] nor were mutations present in the 1000 Genomes database,[42] we believe one can assume all mutations detected in our tumor cohort were somatically acquired.

The mutations found in OSSN in our study exclusively affected the *TERT* promoter at the previously identified hotspots. This although C>T or CC>TT mutations also could have affected multiple adjacent nucleotides (Fig. 1). Functional studies have proven the identified recurrent *TERT* promoter mutations result in a 2- to 4-fold increase in gene expression.[18,19] Obviously, there is a high selection pressure for mutations occurring at these sites, leading to increased telomerase expression in the pathogenesis of OSSN tumors. Interestingly, although generally *TERT* promoter mutations have been reported as mutually exclusive,[19,21] our study did identify a few tumor samples (Table 2) harboring multiple hotspot mutations.

Telomerase reverse transcription promoter mutations have been associated with poor prognosis in a number of different major tumors, including melanoma,[21,29] bladder cancer,[24] thyroid cancer,[22,23] and glioblastomas.[25] In our study, no associations of *TERT* promoter mutation status with histopathological markers of poor prognosis in OSSN, such as differentiation, spindle cell type, high N/C ratio, and ulceration[43] were observed. Arguing against an association of *TERT* promoter mutations with advanced disease is that the mutation rate observed in CIN (66.6%) was higher than in SCC (40.5%). On the other hand, the slightly higher mutation rate in tumors that recurred (57.1%) and both tumors which metastasized harboring mutations would seem to fit with *TERT* promoter mutant tumors having a poorer prognosis. In essence, our study provides no convincing evidence suggesting *TERT* promoter mutations are of prognostic relevance in OSSN.

The limitation of our study is the small sample size. To further assess potential associations of *TERT* promoter mutations in OSSN with various clinicopathological parameters, including prognostic markers, such as recurrence and metastasis, considerably larger tumor cohorts would be required. If one considers that the OSSN cohort analyzed in our study is already one of the largest presented to date, it could prove difficult obtaining considerably higher sample numbers to allow such an analysis.

In summary, 43.8% of all OSSN tumor samples harbored *TERT* promoter mutations, which are likely UV-induced. These results fit well with other results showing *TERT* promoter mutations are frequent in cutaneous[31,44] and other ocular neoplasms,[33] where UV exposure has a highly relevant role in pathogenesis. Future studies will need to address if, similar to other tumor entities, *TERT* promoter status also is of prognostic relevance in OSSN.

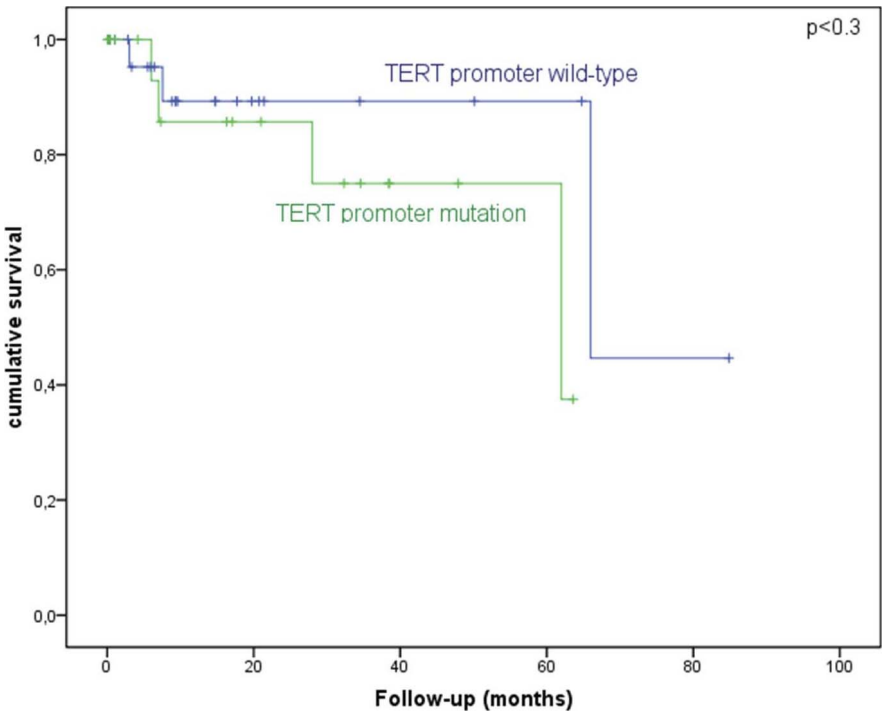

FIGURE 3. Telomerase reverse transcriptase promoter mutation status in regard to local recurrence rate. Kaplan-Meier curve of local recurrence rate in 48 patients with conjunctival SCC or intraepithelial neoplasia according to *TERT* promoter mutation status (mutant versus wild-type). The median time of local recurrence was 8 months overall, with the median in the *TERT* promoter wild-type group ($n = 3$) being 8 months, in the *TERT* promoter mutation group ($n = 4$) 18 months. (In all groups, the mean time of local recurrence was almost identical at 26 months.)

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