dermato

MDPI

Article

Rare *TERT* Promoter Mutations Present in Benign and Malignant Cutaneous Vascular Tumors

Philipp Jansen [1,*], Georg Christian Lodde [1], Anne Zaremba [1], Carl Maximilian Thielmann [1], Johanna Matull [1], Hansgeorg Müller [2], Inga Möller [1], Antje Sucker [1], Stefan Esser [1], Jörg Schaller [3], Dirk Schadendorf [1], Thomas Mentzel [4], Eva Hadaschik [1] and Klaus Georg Griewank [1,5,*]

1 Department of Dermatology, University Hospital Essen, University Duisburg-Essen and German Cancer Consortium (DKTK), 45147 Essen, Germany; georg.lodde@uk-essen.de (G.C.L.); anne.zaremba@uk-essen.de (A.Z.); carlmaximilian.thielmann@uk-essen.de (C.M.T.); johanna.matull@uk-essen.de (J.M.); inga.moeller@uk-essen.de (I.M.); antje.sucker@uk-essen.de (A.S.); stefan.esser@uk-essen.de (S.E.); dirk.schadendorf@uk-essen.de (D.S.); eva.hadaschik@uk-essen.de (E.H.)
2 Dermatohistologie am Stachus, 80331 München, Germany; mueller@dermatohistologie.bayern
3 Dermatopathologie Duisburg, 47166 Duisburg, Germany; drjoergschaller@gmail.com
4 MVZ Dermatopathologie Friedrichshafen, Bodensee, 88048 Friedrichshafen, Germany; mentzel@dermpath.de
5 Dermatopathologie bei Mainz, 55268 Nieder-Olm, Germany
* Correspondence: philipp.jansen@uk-essen.de (P.J.); klaus.griewank@uk-essen.de (K.G.G.); Tel.: +49-201-723-4700 (P.J.); +49-201-723-4700 (K.G.G.)

Abstract: Mutations in the promoter of the telomerase reverse transcriptase (*TERT*) gene have been described as the most common hot-spot mutations in different solid tumors. High frequencies of *TERT* promoter mutations have been reported to occur in tumors arising in tissues with low rates of self-renewal. For cutaneous vascular tumors, the prevalence of *TERT* promoter mutations has not yet been investigated in larger mixed cohorts. With targeted next-generation sequencing (NGS), we screened for different known recurrent *TERT* promoter mutations in various cutaneous vascular proliferations. In our cohort of 104 representative cutaneous vascular proliferations, we identified 7 *TERT* promoter mutations. We could show that 4 of 64 (6.3%) hemangiomas and vascular malformations harbored *TERT* promoter mutations (1 Chr.5:1295228 C > T mutations, 1 Chr.5:1295228_9 CC > TT mutation, and 2 Chr.5:1295250 C > T mutations), 1 of 19 (5.3%) angiosarcomas harbored a Chr.5:1295250 C > T *TERT* promoter mutation, and 2 of 21 (9.5%) Kaposi's sarcomas harbored *TERT* promoter mutations (2 Chr.5:1295250 C > T mutations). To our knowledge, this is the first general description of the distribution of *TERT* promoter mutations in a mixed cohort of cutaneous vascular tumors, revealing that *TERT* promoter mutations seem to occur with low prevalence in both benign and malignant cutaneous vascular proliferations.

Keywords: *TERT* promoter mutation; vascular tumors; genetics; dermatology; next-generation sequencing

Citation: Jansen, P.; Lodde, G.C.; Zaremba, A.; Thielmann, C.M.; Matull, J.; Müller, H.; Möller, I.; Sucker, A.; Esser, S.; Schaller, J.; et al. Rare *TERT* Promoter Mutations Present in Benign and Malignant Cutaneous Vascular Tumors. *Dermato* **2021**, *1*, 18–25. https://doi.org/10.3390/dermato1010003

Academic Editors: Armand Bensussan and Jose Manuel Lopes

Received: 10 May 2021
Accepted: 21 June 2021
Published: 25 June 2021

1. Introduction

Mutations in the promoter of the telomerase reverse transcriptase (TERT) gene have been described as the most common hot-spot mutations in various solid tumors [1]. These mutations induce *TERT* promoter activity and subsequent *TERT* gene transcription [2,3]. The recurrent hotspot point mutations, primarily affecting two sites, were first identified in melanomas [2,3] and consequently described in more than 50 distinct cancer types [1]. Based on their hg 19 genomic coordinates, the mutations are referred to as Chr.5:1295228 C > T (228 C > T) and Chr.5:1295250 C > T (250 C > T), being located 124 and 146 bp upstream of the translation start. For different solid tumors, both the sole occurrence of *TERT* promoter mutations and the frequent occurrence with other activating oncogenes, e.g., *BRAF* in thyroid cancer [4,5] or *FGFR3* in bladder cancer [6], have been described to promote aggressiveness [7–9] and to be associated with poorer prognosis [10,11]. *TERT* promoter mutation status has been described as an independent prognostic marker in

melanoma [12,13] and head and neck cancer [14]. For many entities, it remains unclear whether *TERT* promoter mutations are mandatory at an early stage of tumorigenesis or for sustained tumor growth [1,15,16]. High frequencies of *TERT* promoter mutations have been reported to occur in tumors arising in tissues with low rates of self-renewal [17].

Comparative genetic analysis of *TERT* promoter mutation status may aid to understand the role of these mutations in tumorigenesis. In melanocytic tumors, *TERT* promoter mutations are rarely found in benign common nevi; however, they are more frequent in dysplastic or atypical nevi and most frequently present in melanomas [18]. *TERT* promoter mutations with a UV mutation signature are also frequently present in the most common cutaneous neoplasm (basal cell carcinomas and cutaneous squamous cell carcinomas) [19,20].

We hypothesized that *TERT* promoter mutations may also be present in cutaneous vascular neoplasms. To determine the presence and frequency of *TERT* promoter mutations in cutaneous vascular entities and observe if the mutation frequency is enhanced in malignant tumor entities, we analyzed a larger cohort of hemangiomas, vascular malformations, Kaposi's sarcomas, and angiosarcomas by next-generation sequencing (NGS).

2. Materials and Methods

2.1. Sample Selection

Vascular tumor samples were obtained from the Department of Dermatology University Hospital Essen (n = 38), Dermatopathologie bei Mainz (n = 43), Dermatopathologie Duisburg (n = 8), and Dermatopathologie Friedrichshafen (n = 15), Germany. All tumor samples included in the study were primary cutaneous proliferations (no metastases). All cases were screened by at least one experienced board-certified dermatopathologist (KGG, EH, JS, TM, HM). The study was performed in accordance with the approval of the ethics committee of the University of Duisburg-Essen (IRB-number 20–9688-BO). The cohort has been described previously [21,22]. Hemangiomas with characteristic morphology were sub-classified as cherry hemangioma, a term not applied in the current ISSVA classification [23].

2.2. DNA Isolation

DNA was isolated from 10-µm-thick sections, cut from formalin-fixed, paraffin-embedded tumor tissues. The sections were de-paraffinized and the whole tissue was manually macrodissected. DNA isolation was performed with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

2.3. Targeted Sequencing

PCR amplification of the *TERT* promoter region was performed using primers: hTERT_F ACGAACGTGGCCAGCGGCAG and hTERT_R CTGGCGTCCCTGCACCCTGG (474 bp product), or primers hTERT_short_F CAGCGCTGCCTGAAACTC and hTERT_short_R GTCCTGCCCCTTCACCTT (163 bp product) as previously described [2]. PCR products were used as templates for sequencing after purification with the QIAquick PCR Purification Kit (Qiagen (Hilden, Germany)). Adapter ligation and barcoding of individual samples were done by applying the NEBNext Ultra DNA Library Prep Mastermix Set and NEBNext Multiplex Oligos for Illumina from New England Biolabs. Up to 100 samples were sequenced in parallel on an Illumina MiSeq next-generation sequencer. Sequencing analysis was performed applying the CLC Cancer Research Workbench from QIAGEN®. In brief, the following steps were applied: The workflow in CLC included adapter trimming and read pair merging before mapping to the human reference genome (hg19). Insertions and deletions as well as single nucleotide variant detection, local realignment, and primer trimming followed. Additional information was then obtained regarding potential mutation type, known single nucleotide polymorphisms, and conservation scores by cross-referencing varying databases (COSMIC, ClinVar, dbSNP, 1000 Genomes Project, HAPMAP, and PhastCons-Conservation_scores_hg19). The resulting csv files were analyzed manually and in all cases, the location of the recurrent *TERT* promoter mutations

were assessed (Chr.5:1295–228, −242, and −250). Protein coding gene mutations were identified as previously described [2]. The mean coverage achieved of the *TERT* promoter region in all samples was 865 reads. Mutations were reported if the overall coverage of the mutation site was ≥30 reads, ≥5 reads reported the mutated variant, and the frequency of mutated reads was ≥2%.

3. Results

3.1. Mutation Analysis for TERT Promoter Mutations

In our cohort of 104 vascular tumors from 102 patients, the *TERT* promoter region showed wild-type reads in 97 tumors (93.3%) and harbored one mutation in 7 cases (6.7%) (Table 1). Mutations identified were located at the previously described hotspots: Chr.5:1295228 C > T, Chr.5:1295228_1295229 CC > TT, or Chr.5:1295250 C > T. All annotations are reported according to human genome assembly 19 (hg19). For simplicity, the mutations will further be referred to using solely the last three digits of the chromosome location nomenclature representing the first nucleotide altered as 228 C > T, 228 CC > TT, and 250 C > T, respectively.

Table 1. Clinical variables of vascular proliferations with oncogene and *TERT* promoter mutations.

Hemangioma/Vascular Malformation		**All**	**WT**	***GNA Mutant***	***RAS Mutant***	***TERT-P-Mutant***	***p-Value ****
		***n* = 64**	***n* = 43**	***n* = 16 $**	***n* = 2**	***n* = 4 $**	
Mean age (years)		53	54	48	60	57	0.4
Sex	Female	28	20	5 $	0	3 $	0.36
	Male	35 #	22	10 #	2	1	
Sites of involvement	head/neck	21	16	3 $	1	2 $	0.06
	ventral trunk	16	8	7	1	0	
	dorsal trunk	13	7	6	0	0	
	upper extremity	5	4	0	0	1	
	lower extremity	7	7	0	0	0	
	LND	2	1	0	0	1	
Angiosarcoma		**All**	**WT**	***GNA Mutant***	***RAS Mutant***	***TERT-P-Mutant***	***p-Value ****
		***n* = 19**	***n* = 15**	***n* = 0**	***n* = 3**	***n* = 1**	
Mean age (years)		67	65	-	75	74	0.4
Sex	Female	15	11	-	3	1	1.0
	Male	4	4		0	0	
Sites of involvement	head/neck	8	6	-	2	0	1.0
	ventral trunk	7	5	-	1	1	
	dorsal trunk	0	0	-	0	0	
	upper extremity	0	0	-	0	0	
	lower extremity	1	1	-	0	0	
	LND	3	3	-	0	0	
Kaposi's sarcoma		**All**	**WT**	***GNA Mutant***	***RAS Mutant***	***TERT-P-Mutant***	***p-Value ****
		***n* = 21**	***n* = 19**	***n* = 0**	***n* = 0**	***n* = 2**	
Mean age (years)		56	58	-	-	34	0.1
Sex	Female	4	4	-	-	0	1.0
	Male	16 !	14 !			2	
Sites of involvement	head/neck	2	2	-	-	0	0.13
	ventral trunk	2	1	-	-	1	
	dorsal trunk	0	0	-	-	0	
	upper extremity	2	1	-	-	1	
	lower extremity	13	13	-	-	0	
	LND	2	2	-	-	0	

LND—localization not determined, *TERT-P*-mutant = *TERT*-promoter mutation (228 C > T, 228 CC > TT or 250 C > T), WT = wild-type for *GNA14*, *GNA11*, *GNAQ*, *HRAS*, *NRAS* and *TERT*-promoter-mutation, # one male had two hemangiomas both showing a *GNA14* mutation, $ one female patient showed a *GNAQ* and a *TERT*-promoter (228 C > T)-mutation, ! one male had two Kaposi's sarcomas, * Age—Kruskal–Wallis test; all others—Fisher exact test.

In our cohort of benign hemangiomas and vascular malformations, 4 *TERT* promoter mutations could be detected in 64 samples (6.3%) (Table 1). Targeted amplicon next-generation sequencing identified 1228 C > T, 1228 CC > TT, and 2250 C > T mutations (Figure 1). One hemangioma harbored a 228 C > T *TERT* promoter and a *GNAQ* c.627 A > C, Q209H mutation. No hemangiomas or vascular malformations harbored more than one *TERT* promoter mutation (Tables 1 and 2).

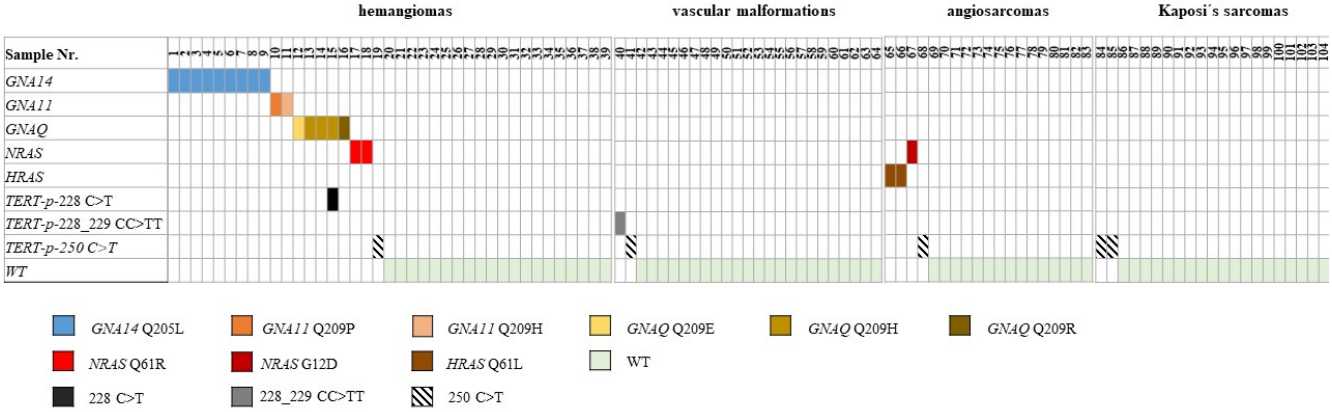

Figure 1. Distribution of TERT promoter mutations and activating mutations identified in vascular tumors.

Table 2. Oncogene and *TERT* promoter mutations in histological subtypes of hemangiomas and vascular malformations.

Hemangioma/Vascular Malformation	All	WT	*GNA* Mutant	*RAS* Mutant	*TERT-P-* Mutant
	***n* = 64**	***n* = 43**	***n* = 16** [$]	***n* = 2**	***n* = 4** [$]
lobular capillary/pyogenic granuloma	7	3	3	1	0
microvenular	6	6	0	0	0
cherry/senile [#]	25	10	13 [$]	1	2 [$]
tufted	1	1	0	0	0
Angiokeratoma [+]	3	3	0	0	0
Arteriovenous [+]	4	4	0	0	0
superficial hemosiderotic lymphovascular [+x]	2	2	0	0	0
venous/cavernous [+]	16	14	0	0	2

[#] not listed as an independent entity according to ISSVA (23), would alternatively be seen as a form of lobular capillary hemangioma (or pyogenic granuloma); [+] classified as malformations according to ISSVA; [x] previously referred to as targetoid hemosiderotic; [$] one tumor harbored a *GNAQ* and *TERT*-promoter (228 C > T)-mutations.

In the cohort of 19 angiosarcomas, one 250 C > T *TERT* promoter mutation was identified (5.3%). In those angiosarcomas with a known activating oncogene mutation (2 *HRAS* c.182 A > T, Q61L and 1 *NRAS* c.35 G > A, G12D), no *TERT* promoter mutations were identified (Table 1 and Figure 1).

In the cohort of Kaposi's sarcomas, two *TERT* promoter mutations were identified in two tumors, respectively (9.5%) (Table 1 and Figure 1).

The resulting amino acid changes are color-coded according to the scheme underneath the illustration. WT = wild-type for *GNA14*, *GNA11*, *GNAQ*, *HRAS*, *NRAS*, and *TERT*-promoter-mutation

3.2. Associations of Clinical and Pathological Parameters with TERT Promoter Status

An analysis with available clinicopathological data was performed. In the three different cohorts, statistically significant associations could not be detected between *TERT* promoter status, oncogene mutation status and patient age, sex, and sites of involvement, respectively (Table 1). We could not identify differences in histomorphological patterns between vascular proliferations harboring a *TERT* promoter mutation, an oncogene mutation, or no mutation ("wild-type") [2] (Figure 2). Occurrence of *TERT* promoter mutations

was not statistically significantly associated with prior radiotherapy (angiosarcomas), identification of human herpes virus 8 (HHV8), or HIV infection (Kaposi's sarcomas).

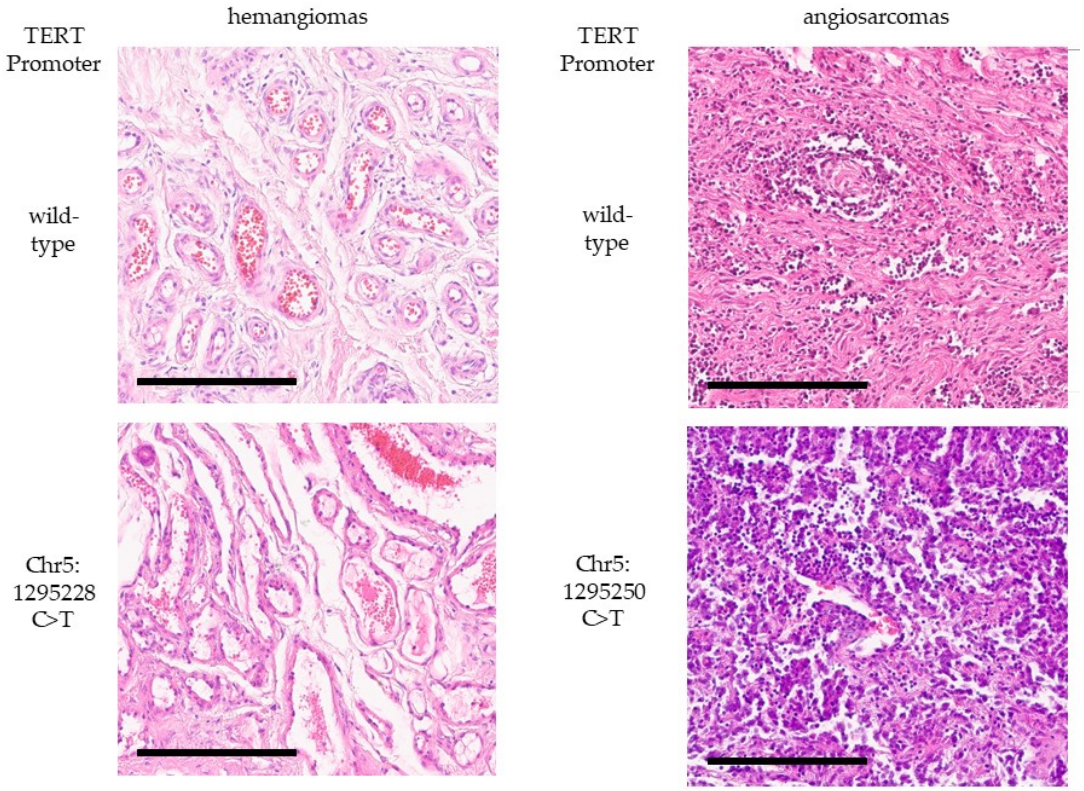

Figure 2. Representative histologic images of cherry hemangiomas * and two angiosarcomas, with and without a *TERT* promoter mutation (The bars represent a distance of 200 µm. * cherry hemangiomas are grouped as lobular capillary hemangiomas according to the current ISSVA classification [23]).

4. Discussion

We analyzed the presence of *TERT* promoter mutations in a previously characterized cohort of vascular tumors comprising benign hemangiomas, vascular malformations, angiosarcomas, and Kaposi's sarcomas by targeted next-generation sequencing [2]. All proliferations showed a low frequency of *TERT* promoter mutations.

In other neoplasms, *TERT* promoter mutations can be present in both benign and malignant tumor entities with the same origin but are often elevated in the malignant entity [24–27]. In our cohort, we could show that both benign and malignant cutaneous vascular proliferations harbor a low frequency of different *TERT* promoter mutations (6.3% in hemangiomas and vascular malformations, 5.3% in angiosarcomas, and 9.5% in Kaposi's sarcomas).

In the literature, the frequency and types of *TERT* promoter mutation vary greatly depending on the type of tumor [4–9]. The recurrent mutations we found in the *TERT* promoter were at previously reported hotspots [2,3] and had a UV signature represented by [28] C > T or CC > TT changes [29,30]. C > T mutations can be identified in tumors developing independent of UV exposure, e.g., bladder cancer [6]. However, UV light is still considered to play a major role in the induction of both C > T and CC > TT in cutaneous tumors [13,29,30]. CC >> TT alterations are considered pathognomonic for UV induction [29,30] and could be identified in one benign venous malformation localized on the head/neck of a 70-year-old man. As a UV-mutation profile has been described for angiosarcomas arising in sun-exposed regions [31], we analyzed the association of *TERT* promoter mutation occurrence and localization. We identified a 250 C > T *TERT* promoter mutation in 1 of 15 angiosarcomas on the ventral trunk after prior radiotherapy due to

breast cancer. In the other six angiosarcomas known to have arisen following radiotherapy due to breast cancer, no *TERT* promoter mutation could be identified.

Of the seven cutaneous vascular proliferations harboring one *TERT* promoter mutation, two were located in the head/neck region, two on the ventral trunk, and two on the lower extremity (one with unknown localization). Although proliferations with *TERT* promoter mutations were identified in areas favoring sun exposure, a statistically significant association between the occurrence of a *TERT* promoter mutation and localization could not be calculated in any of our cohorts.

The prevalence of *TERT* promoter mutations detected (hemangiomas/vascular malformations (6.3%), angiosarcomas (5.3%), and Kaposi's sarcomas (9.5%)) was low in both benign and malignant cutaneous vascular proliferations. Rare *TERT* promoter mutations in angiosarcomas fit previous studies [28,32]. *GNA14*, *GNA11*, and *GNAQ* mutations are frequent in congenital and cherry hemangiomas [33–35] (cherry or senile hemangiomas is still a widely used term, although not recognized as an individual entity according to the ISSVA classification 2018. This classification would group these lesions as a form of pyogenic granuloma or lobular capillary hemangioma). A single hemangioma harbored a 228 C > T *TERT* promotor and a *GNAQ* c.627 A > C, Q209H mutation. Otherwise, no co-occurrence with known *GNA* or *RAS* mutations was observed. In melanoma, *TERT* promoter mutations frequently co-occur with activating *BRAF* mutations [36,37]. In our study, a similar co-occurrence of mutations could not be observed.

The role for the *TERT* (telomerase reverse transcriptase) protein in cutaneous vascular tumors remains intriguing. Our finding suggests that it promotes tumor proliferation in a few cutaneous vascular proliferations. However, based on the frequency of mutations, a mutation of the promoter to increase protein activity appears to be non-mandatory in most tumors. Potentially, gains of the region do occur in vascular tumors lacking *TERT* promoter mutations. Additionally, alternative lengthening of telomeres (ALT), e.g., mutations in *ATRX* or *DAXX*, may contribute to the progression of cutaneous vascular proliferations. These mechanisms, both generally occurring much rarer than *TERT* promoter mutations [38], could not be assessed in the assay we applied and will need to be examined in future studies.

The overall frequency (<10%) and its variation between tumor groups was low. This suggests determining *TERT* promoter mutation status is of no diagnostic aid in terms of classifying tumor entities or predicting prognosis.

A limitation of our study is the lack of detailed clinical data, including therapy and follow-up information. Strengths of our study are the considerable number of vascular tumors included in the analysis (n = 104), making it the largest cohort of vascular tumors analyzed for the presence of *TERT* promoter mutations.

5. Conclusions

In conclusion, we could show that *TERT* promoter mutations are present in both benign and malignant cutaneous vascular tumors and malformations. The prevalence was 6.3% in hemangiomas and vascular malformations, 5.3% in angiosarcomas, and 9.5% in Kaposi's sarcomas. In our comparative analysis, we could not confirm the tendency observed in other cutaneous tumors of *TERT* promoter mutations being more prevalent in malignant entities. While *TERT* promoter mutations appear to be relevant in a small percentage of tumors, the majority of these tumors arise independent of these mutations.

Author Contributions: Conceptualization, methodology and software, K.G.G. and P.J.; validation, P.J., D.S., E.H., K.G.G.; histological analysis, H.M., J.S., T.M., E.H., K.G.G.; investigation all authors.; data curation, P.J. and K.G.G.; writing—original draft preparation, P.J., H.M., T.M., E.H., K.G.G.; writing—review and editing, all authors; visualization and supervision, P.J. and K.G.G.; project administration, P.J. and K.G.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the University of Duisburg-Essen (IRB-number 20–9688-BO, date of approval: 26 November 2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/genbank/, PRJNA717731 (accessed on 27 March 2021).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bell, R.J.; Rube, H.T.; Xavier-Magalhaes, A.; Costa, B.M.; Mancini, A.; Song, J.S.; Costello, J.F. Understanding TERT Promoter Mutations: A Common Path to Immortality. *Mol. Cancer Res.* **2016**, *14*, 315–323. [CrossRef]
2. Horn, S.; Figl, A.; Rachakonda, P.S.; Fischer, C.; Sucker, A.; Gast, A.; Kadel, S.; Moll, I.; Nagore, E.; Hemminki, K.; et al. TERT promoter mutations in familial and sporadic melanoma. *Science* **2013**, *339*, 959–961. [CrossRef]
3. Huang, F.W.; Hodis, E.; Xu, M.J.; Kryukov, G.V.; Chin, L.; Garraway, L.A. Highly recurrent TERT promoter mutations in human melanoma. *Science* **2013**, *339*, 957–959. [CrossRef] [PubMed]
4. Liu, X.; Qu, S.; Liu, R.; Sheng, C.; Shi, X.; Zhu, G.; Murugan, A.K.; Guan, H.; Yu, H.; Wang, Y.; et al. TERT promoter mutations and their association with BRAF V600E mutation and aggressive clinicopathological characteristics of thyroid cancer. *J. Clin. Endocrinol. Metab.* **2014**, *99*, E1130–E1136. [CrossRef]
5. Rusinek, D.; Pfeifer, A.; Krajewska, J.; Oczko-Wojciechowska, M.; Handkiewicz-Junak, D.; Pawlaczek, A.; Zebracka-Gala, J.; Kowalska, M.; Cyplinska, R.; Zembala-Nozynska, E.; et al. Coexistence of TERT Promoter Mutations and the BRAF V600E Alteration and Its Impact on Histopathological Features of Papillary Thyroid Carcinoma in a Selected Series of Polish Patients. *Int. J. Mol. Sci.* **2018**, *19*, 2647. [CrossRef]
6. Hosen, I.; Rachakonda, P.S.; Heidenreich, B.; de Verdier, P.J.; Ryk, C.; Steineck, G.; Hemminki, K.; Kumar, R. Mutations in TERT promoter and FGFR3 and telomere length in bladder cancer. *Int. J. Cancer* **2015**, *137*, 1621–1629. [CrossRef] [PubMed]
7. Spiegl-Kreinecker, S.; Lotsch, D.; Neumayer, K.; Kastler, L.; Gojo, J.; Pirker, C.; Pichler, J.; Weis, S.; Kumar, R.; Webersinke, G.; et al. TERT promoter mutations are associated with poor prognosis and cell immortalization in meningioma. *Neuro. Oncol.* **2018**, *20*, 1584–1593. [CrossRef] [PubMed]
8. Ren, H.; Shen, Y.; Hu, D.; He, W.; Zhou, J.; Cao, Y.; Mao, Y.; Dou, Y.; Xiong, W.; Xiao, Q.; et al. Co-existence of BRAF(V600E) and TERT promoter mutations in papillary thyroid carcinoma is associated with tumor aggressiveness, but not with lymph node metastasis. *Cancer Manag. Res.* **2018**, *10*, 1005–1013. [CrossRef]
9. Jeong, D.E.; Woo, S.R.; Nam, H.; Nam, D.H.; Lee, J.H.; Joo, K.M. Preclinical and clinical implications of TERT promoter mutation in glioblastoma multiforme. *Oncol. Lett.* **2017**, *14*, 8213–8219. [CrossRef]
10. Campos, M.A.; Macedo, S.; Fernandes, M.; Pestana, A.; Pardal, J.; Batista, R.; Vinagre, J.; Sanches, A.; Baptista, A.; Lopes, J.M.; et al. TERT promoter mutations are associated with poor prognosis in cutaneous squamous cell carcinoma. *J. Am. Acad. Dermatol.* **2019**, *80*, 660–669 e666. [CrossRef] [PubMed]
11. Malkki, H. Neuro-oncology: TERT promoter mutations could indicate poor prognosis in glioblastoma. *Nat. Rev. Neurol.* **2014**, *10*, 546. [CrossRef] [PubMed]
12. Blateau, P.; Coyaud, E.; Laurent, E.; Beganton, B.; Ducros, V.; Chauchard, G.; Vendrell, J.A.; Solassol, J. TERT Promoter Mutation as an Independent Prognostic Marker for Poor Prognosis MAPK Inhibitors-Treated Melanoma. *Cancers* **2020**, *12*, 2224. [CrossRef] [PubMed]
13. Griewank, K.G.; Murali, R.; Puig-Butille, J.A.; Schilling, B.; Livingstone, E.; Potrony, M.; Carrera, C.; Schimming, T.; Moller, I.; Schwamborn, M.; et al. TERT promoter mutation status as an independent prognostic factor in cutaneous melanoma. *J. Natl. Cancer Inst.* **2014**, *106*. [CrossRef] [PubMed]
14. Arantes, L.; Cruvinel-Carloni, A.; de Carvalho, A.C.; Sorroche, B.P.; Carvalho, A.L.; Scapulatempo-Neto, C.; Reis, R.M. TERT Promoter Mutation C228T Increases Risk for Tumor Recurrence and Death in Head and Neck Cancer Patients. *Front. Oncol.* **2020**, *10*, 1275. [CrossRef] [PubMed]
15. Borah, S.; Xi, L.; Zaug, A.J.; Powell, N.M.; Dancik, G.M.; Cohen, S.B.; Costello, J.C.; Theodorescu, D.; Cech, T.R. Cancer. TERT promoter mutations and telomerase reactivation in urothelial cancer. *Science* **2015**, *347*, 1006–1010. [CrossRef] [PubMed]
16. Chiba, K.; Lorbeer, F.K.; Shain, A.H.; McSwiggen, D.T.; Schruf, E.; Oh, A.; Ryu, J.; Darzacq, X.; Bastian, B.C.; Hockemeyer, D. Mutations in the promoter of the telomerase gene TERT contribute to tumorigenesis by a two-step mechanism. *Science* **2017**, *357*, 1416–1420. [CrossRef]
17. Killela, P.J.; Reitman, Z.J.; Jiao, Y.; Bettegowda, C.; Agrawal, N.; Diaz, L.A., Jr.; Friedman, A.H.; Friedman, H.; Gallia, G.L.; Giovanella, B.C.; et al. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 6021–6026. [CrossRef]
18. Shain, A.H.; Yeh, I.; Kovalyshyn, I.; Sriharan, A.; Talevich, E.; Gagnon, A.; Dummer, R.; North, J.; Pincus, L.; Ruben, B.; et al. The Genetic Evolution of Melanoma from Precursor Lesions. *N. Engl. J. Med.* **2015**, *373*, 1926–1936. [CrossRef]

19. Griewank, K.G.; Murali, R.; Schilling, B.; Schimming, T.; Moller, I.; Moll, I.; Schwamborn, M.; Sucker, A.; Zimmer, L.; Schadendorf, D.; et al. TERT promoter mutations are frequent in cutaneous basal cell carcinoma and squamous cell carcinoma. *PLoS ONE* **2013**, *8*, e80354. [CrossRef]
20. Scott, G.A.; Laughlin, T.S.; Rothberg, P.G. Mutations of the TERT promoter are common in basal cell carcinoma and squamous cell carcinoma. *Mod. Pathol.* **2014**, *27*, 516–523. [CrossRef] [PubMed]
21. Murali, R.; Chandramohan, R.; Moller, I.; Scholz, S.L.; Berger, M.; Huberman, K.; Viale, A.; Pirun, M.; Socci, N.D.; Bouvier, N.; et al. Targeted massively parallel sequencing of angiosarcomas reveals frequent activation of the mitogen activated protein kinase pathway. *Oncotarget* **2015**, *6*, 36041–36052. [CrossRef]
22. Jansen, P.; Müller, H.; Lodde, G.C.; Zaremba, A.; Möller, I.; Sucker, A.; Paschen, A.; Esser, S.; Schaller, J.; Gunzer, M.; et al. GNA14, GNA11, and GNAQ Mutations Are Frequent in Benign but Not Malignant Cutaneous Vascular Tumors. *Front. Genet.* **2021**, *12*, 656. [CrossRef] [PubMed]
23. ISSVA Classification for Vascular Anomalies. 2018. Available online: https://www.issva.org/UserFiles/file/ISSVA-Classification-2018.pdf (accessed on 23 June 2021).
24. Koopmans, A.E.; Ober, K.; Dubbink, H.J.; Paridaens, D.; Naus, N.C.; Belunek, S.; Krist, B.; Post, E.; Zwarthoff, E.C.; de Klein, A.; et al. Prevalence and implications of TERT promoter mutation in uveal and conjunctival melanoma and in benign and premalignant conjunctival melanocytic lesions. *Investig. Ophthalmol. Visual Sci.* **2014**, *55*, 6024–6030. [CrossRef] [PubMed]
25. Munoz-Jimenez, M.T.; Blanco, L.; Ruano, Y.; Carrillo, R.; Santos-Briz, A.; Riveiro-Falkenbach, E.; Requena, L.; Kutzner, H.; Garrido, M.C.; Rodriguez-Peralto, J.L. TERT promoter mutation in sebaceous neoplasms. *Virchows Arch.* **2021**. [CrossRef]
26. Jansen, P.; Cosgarea, I.; Murali, R.; Moller, I.; Sucker, A.; Franklin, C.; Paschen, A.; Zaremba, A.; Brinker, T.J.; Stoffels, I.; et al. Frequent Occurrence of NRAS and BRAF Mutations in Human Acral Naevi. *Cancers* **2019**, *11*, 546. [CrossRef]
27. Zaremba, A.; Murali, R.; Jansen, P.; Moller, I.; Sucker, A.; Paschen, A.; Zimmer, L.; Livingstone, E.; Brinker, T.J.; Hadaschik, E.; et al. Clinical and genetic analysis of melanomas arising in acral sites. *Eur. J. Cancer* **2019**, *119*, 66–76. [CrossRef] [PubMed]
28. Painter, C.A.; Jain, E.; Tomson, B.N.; Dunphy, M.; Stoddard, R.E.; Thomas, B.S.; Damon, A.L.; Shah, S.; Kim, D.; Gomez Tejeda Zanudo, J.; et al. The Angiosarcoma Project: Enabling genomic and clinical discoveries in a rare cancer through patient-partnered research. *Nat. Med.* **2020**, *26*, 181–187. [CrossRef]
29. Pleasance, E.D.; Cheetham, R.K.; Stephens, P.J.; McBride, D.J.; Humphray, S.J.; Greenman, C.D.; Varela, I.; Lin, M.L.; Ordonez, G.R.; Bignell, G.R.; et al. A comprehensive catalogue of somatic mutations from a human cancer genome. *Nature* **2010**, *463*, 191–196. [CrossRef] [PubMed]
30. Brash, D.E.; Rudolph, J.A.; Simon, J.A.; Lin, A.; McKenna, G.J.; Baden, H.P.; Halperin, A.J.; Ponten, J. A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 10124–10128. [CrossRef]
31. Chan, J.Y.; Lim, J.Q.; Yeong, J.; Ravi, V.; Guan, P.; Boot, A.; Tay, T.K.Y.; Selvarajan, S.; Md Nasir, N.D.; Loh, J.H.; et al. Multiomic analysis and immunoprofiling reveal distinct subtypes of human angiosarcoma. *J. Clin. Investig.* **2020**, *130*, 5833–5846. [CrossRef]
32. Koelsche, C.; Renner, M.; Hartmann, W.; Brandt, R.; Lehner, B.; Waldburger, N.; Alldinger, I.; Schmitt, T.; Egerer, G.; Penzel, R.; et al. TERT promoter hotspot mutations are recurrent in myxoid liposarcomas but rare in other soft tissue sarcoma entities. *J. Exp. Clin. Cancer Res.* **2014**, *33*, 33. [CrossRef] [PubMed]
33. Liau, J.Y.; Lee, J.C.; Tsai, J.H.; Chen, C.C.; Chung, Y.C.; Wang, Y.H. High frequency of GNA14, GNAQ, and GNA11 mutations in cherry hemangioma: A histopathological and molecular study of 85 cases indicating GNA14 as the most commonly mutated gene in vascular neoplasms. *Mod. Pathol.* **2019**, *32*, 1657–1665. [CrossRef] [PubMed]
34. Liau, J.Y.; Lee, J.C.; Tsai, J.H.; Chen, C.C.; Wang, Y.H.; Chung, Y.C. Thrombotic Hemangioma With Organizing/Anastomosing Features: Expanding the Spectrum of GNA-mutated Hemangiomas With a Predilection for the Skin of the Lower Abdominal Regions. *Am. J. Surg. Pathol.* **2020**, *44*, 255–262. [CrossRef]
35. Ayturk, U.M.; Couto, J.A.; Hann, S.; Mulliken, J.B.; Williams, K.L.; Huang, A.Y.; Fishman, S.J.; Boyd, T.K.; Kozakewich, H.P.; Bischoff, J.; et al. Somatic Activating Mutations in GNAQ and GNA11 Are Associated with Congenital Hemangioma. *Am. J. Hum. Genet.* **2016**, *98*, 789–795. [CrossRef] [PubMed]
36. Zehir, A.; Benayed, R.; Shah, R.H.; Syed, A.; Middha, S.; Kim, H.R.; Srinivasan, P.; Gao, J.; Chakravarty, D.; Devlin, S.M.; et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat. Med.* **2017**, *23*, 703–713. [CrossRef] [PubMed]
37. Macerola, E.; Loggini, B.; Giannini, R.; Garavello, G.; Giordano, M.; Proietti, A.; Niccoli, C.; Basolo, F.; Fontanini, G. Coexistence of TERT promoter and BRAF mutations in cutaneous melanoma is associated with more clinicopathological features of aggressiveness. *Virchows Arch.* **2015**, *467*, 177–184. [CrossRef]
38. Shay, J.W.; Wright, W.E. Telomeres and telomerase: Three decades of progress. *Nat. Rev. Genet.* **2019**, *20*, 299–309. [CrossRef]