Abstract

Activating mutations in the TERT promoter were recently identified in up to 71% of cutaneous melanoma. Subsequent studies found TERT promoter mutations in a wide array of other major human cancers. TERT promoter mutations lead to increased expression of telomerase, which maintains telomere length and genomic stability, thereby allowing cancer cells to continuously divide, avoiding senescence or apoptosis. TERT promoter mutations in cutaneous melanoma often show UV-signatures. Non-melanoma skin cancer, including basal cell carcinoma and squamous cell carcinoma, are very frequent malignancies in individuals of European descent. We investigated the presence of TERT promoter mutations in 32 basal cell carcinomas and 34 cutaneous squamous cell carcinomas using conventional Sanger sequencing. TERT promoter mutations were identified in 18 (56%) basal cell carcinomas and in 17 (50%) cutaneous squamous cell carcinomas. The recurrent mutations identified in our cohort were identical to those previously described in cutaneous melanoma, and showed a UV-signature (C>T or CC>TT) in line with a causative role for UV exposure in these common cutaneous malignancies. Our study shows that TERT promoter mutations with UV-signatures are frequent in non-melanoma skin cancer, being present in around 50% of basal and squamous cell carcinomas and suggests that increased expression of telomerase plays an important role in the pathogenesis of these tumors.
recent publication found TERT promoter mutations in 78% of BCC and 50% of SCC [26].

In our study we investigate the presence of TERT promoter mutations in BCCs and SCCs, and their associations with clinical and pathologic features.

**Materials and Methods**

**Sample selection**

Samples of primary BCCs and SCCs were obtained from 66 patients treated in the Department of Dermatology, University Hospital Essen, Germany. The study was approved by the Institutional Review Board of the University of Duisburg-Essen (Ethikkommission der Universität Duisburg-Essen) under the IRB protocol number 12-4961-BO. All patients provided written informed consent.

**DNA isolation**

10 µm-thick sections were cut from formalin-fixed, paraffin-embedded tumor tissues. The sections were deparaffinized and tumor tissue was manually macrodissected. Genomic DNA was isolated using the QiAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions.

**Direct (Sanger) sequencing**

PCR amplification of the TERT promoter region was performed using primers hTERT_F ACGAAGTGGC-CAGGGGCA and hTERT_R CTGCGCTGACCCCTCACTC and primers hTERT_short_F CAGGGCTGAAACCCTC and hTERT_short_R GTCTGTGGCCCTTCACCTTT (163bp product) as previously described [20]. PCR products were used as templates for sequencing after purification with the QiAquick PCR Purification Kit (Qiagen). Sequencing chromatogram files were examined using Chromas (version 2.01, University of Sussex, Brighton, United Kingdom) or Sequencher (demo version 5.1, Gene Codes Corporation, Ann Arbor, MI, USA) software.

**Results**

**Study cohort**

The study cohort consisted of 32 BCC and 34 SCC samples, from 43 males and 23 females. The median age was 72.6 years for SCC and 73.0 years for BCC. Histopathologic analysis was performed on all samples to assess histologic subtype, tumor thickness, cystic component, ulceration status, and presence of pigmentation for BCC, as well as tumor thickness, Clark level, acantholysis, lymphovascular involvement (LVI), perineural involvement (PNI), and presence of ulceration in SCC. Clinico-pathologic characteristics are listed in Table 1.

**TERT promoter mutation analysis**

Recurrent TERT promoter mutation analysis was performed using clinical and pathologic features.

| Mutations | BCC | SCC |
|-----------|-----|-----|
| Wild-type | 14 (44%) | 17 (50%) |
| Mutant | 18 (56%) | 17 (50%) |
| c.-146C>T | 10 (31%) | 5 (15%) |
| c.-124C>T | 4 (13%) | 5 (15%) |
| c.-126C>T | 2 (6%) | 4 (12%) |
| c.-138,139CC>TT | 1 (3%) | 2 (6%) |
| c.-124C>T, c.-126C>T | 0 | 1 (3%) |
| c.-126,127CC>TT, c.-146C>T | 1 (3%) | 0 |

**Associations of clinical and pathologic parameters with TERT promoter mutation status**

Apart from a small, statistically significant (p = 0.046) difference in age between patients with TERT promoter-mutant BCCs (median 75.5 years) and those with TERT promoter-wild type BCCs (median 71.0 years), there were no statistically significant associations of TERT mutation status with clinicopathologic parameters (Table 2 and 3).

**Discussion**

BCC and SCC harbor distinct patterns of genetic alterations. BCC have genetic alterations activating the hedgehog signaling pathway. In contrast, SCC show alterations leading to activation of the MAPK and AKT signaling pathway, such as overexpression or mutations of genes such as RAS, EGFR, or PI3KCA [17,18]. Losses of CDKN2A [17] and inactivation of NOTCH1 [18] are also frequent in SCC, but not in BCC. The only previously recognized common genetic event in both tumors is TP53 mutations. We found TERT promoter mutations in a substantial proportion of both BCC and SCC. The frequency of these mutations in BCC, SCC, melanoma [19,20] and other cancers [21] suggests that increased expression of the holoenzyme telomerase is an important event in a wide range of human malignancies.

The role of UV-mediated tumorigenesis in BCC and SCC is supported by epidemiologic data and by the presence of UV-signature mutations in TP53 (BCC and SCC), PTCH1 (BCC) or RAS (SCC) [2,3,5,11,16]. The mutations we identified in the TERT promoter have a UV-signature with C>T or CC>TT changes, consistent with an etiologic role for UV exposure. However, c.-124C>T and c.-146C>T mutations have also been identified in cancer types such as hepatocellular cancer, bladder cancer, thyroid cancer and gliomas, in which UV-induced mutations are unlikely [21–23]. CC>TT alterations are considered virtually pathognomonic of UV-induction [16,27] and were rare or not described in the aforementioned tumors, however were shown in Figure 1. One c.-146C>T mutant tumor also harbored a c.-126,127CC>TT mutation. Seventeen (50%) SCCs harbored TERT promoter mutations (Table 1), which included c.-124C>T (n = 6, 18%), c.-124,125CC>TT (n = 2, 6%), c.-138,139CC>TT (n = 4, 12%), and c.-146C>T (n = 5, 15%). One c.-124C>T case had a concomitant c.-126C>T mutation. All identified mutations showed a UV-signature (C>T and CC>TT) [27].
frequent in cutaneous melanoma and other cutaneous tumors occurring on sun-damaged skin [20,25]. There is a rare C>T SNP (rs35550267) at position c.-139. To our knowledge no known SNP has been reported at c.-125 (dbSNP). Thus, although we cannot exclude that of the ten CC>TT alterations detected (six c.-138_139CC>TT, three c.-124_125CC>TT and one c.-

Figure 1. Recurrent mutations identified in the TERT promoter of BCC and SCC. Representative sequencing chromatograms showing the wild type sequence (on top) and representative examples of the mutations identified in both basal and squamous cell carcinoma samples – c.-124C>T, c.-124_125CC>TT, c.-138_139CC>TT or c.-146C>T (alternatively annotated according to the chromosome location as Chr.5. 1295228C>T, Chr.5. 1295228_1295229CC>TT, Chr.5. 1295242_1295243CC>TT or Chr.5.1295250C>T, respectively). All presented mutations were found in both tumor cohorts however the presented chromatograms are from a BCC, SCC, BCC, and BCC, respectively.

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Table 2. Associations of clinical and pathologic parameters in BCC with TERT promoter mutation status.

| Parameter                | Level          | All cases | TERT\textsuperscript{wt} | TERT\textsuperscript{mut} | P value** |
|--------------------------|----------------|-----------|---------------------------|---------------------------|-----------|
|                          | N = 32 | N = 14 | N = 18                     |                           |           |
| Age at diagnosis (years) | Median 73 | 71.0 | 75.5 | 0.046                     |           |
| Sex                      | Female 10 | 4 | 6 | 1.00                      |           |
| Tumor location           | Head & neck 23 | 10 | 13 | 0.36                      |           |
| Tumor thickness          | Median 1.2 mm | 1.1 mm | 1.35 mm | 0.16                     |           |
| Histologic type          | Nodular 15 | 7 | 8 | 0.20                      |           |
|                          | Micronodular 5 | 1 | 4 |                           |           |
|                          | Superficial 8 | 5 | 3 |                           |           |
|                          | Infiltrative 3 | 0 | 3 |                           |           |
| Cystic component         | No 24 | 10 | 14 | 0.70                      |           |
| Ulceration               | No 17 | 10 | 7 | 0.07                      |           |
| Pigment                  | No 31 | 14 | 17 | 1.00                      |           |
|                          | Yes 1 | 0 | 1 |                           |           |

TERT\textsuperscript{wt} = TERT promoter wild-type; TERT\textsuperscript{mut} = TERT promoter mutant.

**Based on chi-squared or Fisher exact tests for categorical variables, and on Mann-Whitney test for continuous variables. Cases with missing data were excluded from statistical analyses.

Histologic parameters analyzed were based on the World Health Organization’s classification and histologic criteria[31]. Tumor thickness was measured as for cutaneous melanomas[32].

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some, or potentially even all, represent a preexisting C>T variation with an additional C>T mutation, we do believe these alterations, found in 13% of BCC and 18% of SCC, most likely primarily represent UV-exposure tandem mutations, arguing for UV-exposure inducing TERT promoter mutations in these tumors. Future larger studies with paired tumor and constitutional DNA should be able to definitively address the role of dipyrimidine mutations in the TERT promoter of BCC and SCC.

Matched blood samples of the tumors analyzed in our study were not available, precluding us from directly excluding the presence of germ-line mutations at the mutation hotspots c.-124 or c.-146. However, germ-line mutations at these hotspots have not been observed in various TERT promoter mutation studies which compared paired tumor and normal (blood) tissue isolated DNA [19–21,24,26], nor were they present in the 1000 Genomes database [28]. This makes it almost certain that the mutations detected at these loci in our tumor cohort were somatically acquired.

Table 3. Associations of clinical and pathologic parameters in SCC with TERT mutation status.

| Parameter                  | Level     | All cases | TERT<sup>wt</sup> | TERT<sup>mut</sup> | P value** |
|----------------------------|-----------|-----------|-------------------|-------------------|-----------|
| Age at diagnosis           | Median    | 72.6      | 76.8              | 72.5              | 0.69      |
| (years)                    | Range     | 46–95     | 46–90             | 52–95             |           |
| Sex                        | Female    | 13        | 7                 | 6                 | 0.72      |
|                            | Male      | 21        | 10                | 11                |           |
| Tumor location             | Head & neck | 25       | 11                | 14                | 0.80      |
|                            | Extremities | 5         | 3                 | 2                 |           |
|                            | Trunk     | 2         | 1                 | 1                 |           |
|                            | Missing data | 2       | 2                 | 0                 |           |
| Tumor thickness            | Median    | 3.95 mm   | 4.7 mm            | 3.9 mm            | 0.95      |
|                            | Range     | 2.8–10.0 mm | 2.8–10.0 mm       | 3.0–10.0 mm       |           |
| Clark level                | III       | 6         | 5                 | 1                 | 0.13      |
|                            | IV        | 17        | 6                 | 11                |           |
|                            | V         | 10        | 5                 | 5                 |           |
| Grade                      | 1 (well differentiated) | 8       | 4                 | 4                 | 0.72      |
|                            | 2 (moderately differentiated) | 16     | 7                 | 9                 |           |
|                            | 3 (poorly differentiated) | 10    | 6                 | 4                 |           |
| Acantholysis               | No        | 29        | 15                | 14                | 1.00      |
|                            | Yes       | 5         | 2                 | 3                 |           |
| Ulceration                 | No        | 19        | 8                 | 11                | 0.30      |
|                            | Yes       | 15        | 9                 | 6                 |           |
| Desmoplasia                | No        | 30        | 14                | 16                | 0.48      |
|                            | Yes       | 3         | 2                 | 1                 |           |
| Perineural invasion        | No        | 28        | 15                | 13                | 0.66      |
|                            | Yes       | 6         | 2                 | 4                 |           |
| Lymphovascular invasion    | No        | 33        | 16                | 17                | 1.00      |
|                            | Yes       | 1         | 1                 | 0                 |           |
| Mean survival (months)*    |           | 42.5      | 68.6              | (33.7–51.4)       | (57.5–79.7) |

TERT<sup>wt</sup> = TERT promoter wild-type; TERT<sup>mut</sup> = TERT promoter mutant.

*Estimates of mean survival from Kaplan Meier method (median survival not reached); p value estimated using log-rank test.

**Based on chi-squared or Fisher exact tests for categorical variables, and on Mann-Whitney test for continuous variables. Cases with missing data were excluded from statistical analyses.

Histologic parameters analyzed were based on the World Health Organization’s classification and histologic criteria[33]. Clark level of invasion and tumor thickness were measured as for cutaneous melanomas[32].

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The only significant correlation with clinical parameters observed in our cohort, was a slight difference in age between TERT promoter mutant and non-mutant BCC. Our cohort is small and larger more detailed follow up studies will be required to verify this finding and to determine if additional clinicopathologic correlations with TERT promoter mutation status can be identified.

In summary, our study identifies TERT promoter mutations with a UV-signature as frequent events in BCC and SCC non-melanoma skin cancer. Similar results independently validating these findings were recently reported by Scott et al., who found recurrent TERT promoter mutations in 78% of BCC and 50% of SCC [26]. Future studies will be required to determine whether TERT promoter mutations have prognostic implications or may be targeted therapeutically. This would be especially valuable in patients with metastatic SCC for whom prognosis is poor and effective therapies are lacking.

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Author Contributions

Conceived and designed the experiments: KGG RM UH TS. Performed the experiments: I. Moller I. Moll MS AS KGG. Analyzed the data: KGG BS TS LZ DS UH. Contributed reagents/materials/analysis tools: KGG UH TS. Wrote the paper: KGG RM BS TS I. Moller I. Moll MS AS LZ DS UH.

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