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A Pan-Cancer Study of Somatic *TERT* Promoter Mutations and Amplification in 30,773 Tumors Profiled by Clinical Genomic Sequencing

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TERT gene promoter mutations are known in multiple cancer types. Other *TERT* alterations remain poorly characterized. Sequencing data from 30,773 tumors analyzed by a hybridization capture next-generation sequencing assay (Memorial Sloan Kettering Cancer Center Integrated Mutation Profiling of Actionable Cancer Targets) were analyzed for the presence of *TERT* alterations. Promoter rearrangements (500 bases upstream of the transcriptional start site), hypermethylation ($n = 57$), and gene expression ($n = 155$) were evaluated for a subset of cases. Mutually exclusive and recurrent promoter mutations were identified at three hot spots upstream of the transcriptional start site in 11.3% of cases (−124: 74%; −146: 24%; and −138: <2%). Mutually exclusive amplification events were identified in another 2.3% of cases, whereas mutually exclusive rearrangements proximal to the *TERT* gene were seen in 24 cases. The highest incidence of *TERT* promoter mutations was seen in cutaneous melanoma (82%), whereas amplification events significantly outnumbered promoter mutations in well-differentiated/dedifferentiated liposarcoma (14.1% versus 2.4%) and adrenocortical carcinoma (13.6% versus 4.5%). Gene expression analysis suggests that the highest levels of gene expression are seen in cases with amplifications and rearrangements. Hypermethylation events upstream of the *TERT* coding sequence were not mutually exclusive with known pathogenic alterations. Studies aimed at defining the prevalence and prognostic impact of *TERT* alterations should incorporate other pathogenic *TERT* alterations as these may impact telomerase function. *(J Mol Diagn 2021, 23: 253–263; https://doi.org/10.1016/j.jmoldx.2020.11.003)*

Human telomeres are composed of telomeric TTAGGG DNA repeats that are protected by the shelterin complex and the telomerase complex.[1,2] Unlike stem cells, in most differentiated cells, silencing of telomerase reverse transcriptase (*TERT*) occurs over time with consequent telomeric shortening.[1] This leads to activation of DNA damage response signaling and replicative arrest.[1] An early event in many neoplasms involves bypassing this replicative arrest through telomerase reactivation, thereby increasing both cancer cell viability and genomic instability.[1] The most common mechanism of telomerase reactivation across several cancer types involves *TERT* reactivation through hot spot promoter mutations, which generates a *de novo* binding site for activating erythroblast transformation-specific (ETS) family transcription factors.[2–4] This leads to increased recruitment of multimeric GA-binding protein transcription factors to the mutant *TERT* promoter sequence and transcriptional up-regulation of *TERT*.[2,3,5]

In addition to promoter mutations, the spectrum of activating *TERT* alterations includes genomic amplifications as

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well as structural alterations at the *TERT* locus or hypermethylation events upstream of the *TERT* transcriptional start site.[6–10] The Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) assay used in this study is designed to detect *TERT* promoter mutations and genomic amplifications. Although a few structural variants were detected within a short 500-bp sequence immediately upstream of the transcriptional start site, the vast majority of these rearrangements (which have primarily been reported in neuroblastomas) occur over a 50-kb region proximal to the *TERT* gene.[7–9] This assay is therefore not designed to detect these alterations when they occur further upstream of the transcription start site. Finally, hypermethylation at specific CpG islands upstream of the transcriptional start site has been correlated with increased *TERT* gene expression, and this assay does not document methylation status at these sites.[6,10,11] Methylation status was determined for a smaller subset of cases using an alternate assay.

Relatively few large-scale pan-cancer studies have studied *TERT* alterations across tumor types.[2,12,13] Herein, we present pan-cancer data pertaining to both promoter mutations and amplification events across 30,773 specimens that were profiled as part of an institutional clinical sequencing cohort. This data set is unique as *TERT* promoter mutations are often not evaluated in whole exome sequencing assays, whereas *TERT* amplification events are rarely documented in large pan-cancer data sets.[14] In addition, a limited number of cases in this study have been profiled for rarer alterations, such as structural variants and hypermethylation events upstream of the *TERT* transcription start site, which have been shown to up-regulate *TERT* expression, as well as relative gene expression status.

Materials and Methods

Case Selection

This study was approved by the institutional review board and involved analysis of molecular profiling data of multiple solid tumors profiled by a next-generation sequencing–based assay (MSK-IMPACT), as part of an institutional clinical cancer genomics initiative.[14–16] DNA sequencing results for 30,773 tumor samples obtained from formalin-fixed, paraffin-embedded tissue, performed in a Clinical Laboratory Improvement Amendments–approved setting, were analyzed for *TERT* alterations.

Next-Generation Sequencing: MSK-IMPACT

Details of the MSK-IMPACT assay have been previously reported.[14–16] In brief, this assay involves paired analysis of tumor and normal specimens to filter out germ-line variants. Specifically, hybridization capture-based library preparation is followed by deep sequencing of select noncoding regions and 6614 protein-coding exons of 468 genes. Noncoding sequences include the *TERT* promoter (extending to 500 bp upstream of the transcriptional start site), intronic sequences of commonly rearranged genes, microsatellite sites, and several single-nucleotide polymorphisms. In all, the capture probes target approximately 1.5 megabases of the human genome. Accurate genome-wide copy number assessment is facilitated by homogeneous distribution of single-nucleotide polymorphism tiling probes across the genome. On the basis of previously reported criteria, amplifications were defined as a fold change $\geq$2.0.[17–20] Rearrangements involving the *TERT* promoter (500-bp sequence immediately upstream of the transcriptional start site) were identified on the basis of previously published criteria, which included the following: five paired or split reads, a mapping quality of 20, and a length >500-bp sequence.[15] This assay is currently approved by the US Food and Drug Administration as a class II *in vitro* diagnostic test.

TERT Gene Expression Analysis

Details pertaining to RNA extraction and the MSK-Fusion assay have been previously reported.[21,22] Eight gene-specific primers were designed by ArcherDx (Boulder, CO) and added to the MSK-Fusion panel to specifically detect *TERT* expression levels. The total number of unique RNA reads for *TERT*, obtained using all eight gene-specific primers, were normalized to the total number of unique RNA reads for five separate housekeeping genes interrogated as part of the assay. The relative *TERT* gene expression status was evaluated for 155 cases that were profiled using MSK-IMPACT from available archived formalin-fixed, paraffin-embedded tissue. Broad categories of tumor types evaluated included colorectal adenocarcinoma (n = 35), lung adenocarcinoma (n = 25), sarcoma (n = 20), gliomas (n = 19), breast carcinoma (n = 14), melanoma (n = 12), pancreaticobiliary cancer (n = 10), salivary cancer (n = 4), thyroid carcinoma (n = 4), carcinoma of unknown primary (n = 4), and miscellaneous tumor types (n = 8).

TERT Promoter Methylation Analysis

TERT promoter hypermethylation status was detected via bisulfite conversion followed by methylation array for 57 cases profiled by MSK-IMPACT, as previously described.[23] Herein, methylation status of three CpG sites upstream of the *TERT* transcription start site was evaluated. This included the cg11625005 CpG site, where hypermethylation has been previously reported to be associated with high-grade tumors and increased *TERT* gene expression.[6,10,11]

Statistical Analysis

All statistical tests were two sided, and $P < 0.05$ was considered statistically significant.

Results

TERT Promoter Mutation and Amplification

A hybrid capture library preparation strategy was used to sequence the *TERT* promoter region, including 500 bases upstream of the transcriptional start site. The mean depth of coverage for the promoter region across all 30,773 specimens that were profiled was 425×, with an interquartile range of 240× to 551× (Figure 1A). In all, 4205 of these specimens (13.6%) harbored either a *TERT* promoter mutation or an amplification. Of note, *TERT* promoter mutations (11.3%) and amplifications (2.3%) were mutually exclusive, with the promoter mutations occurring 4.9 times as frequently compared with amplification events (Figure 1B).

Although *TERT* promoter mutations showed a wide spatial distribution, consistent with our prior studies, three recurring hot spots that were mutually exclusive were identified.[14] Relative to the transcription start site, hot spot promoter alterations at position −124 were the most

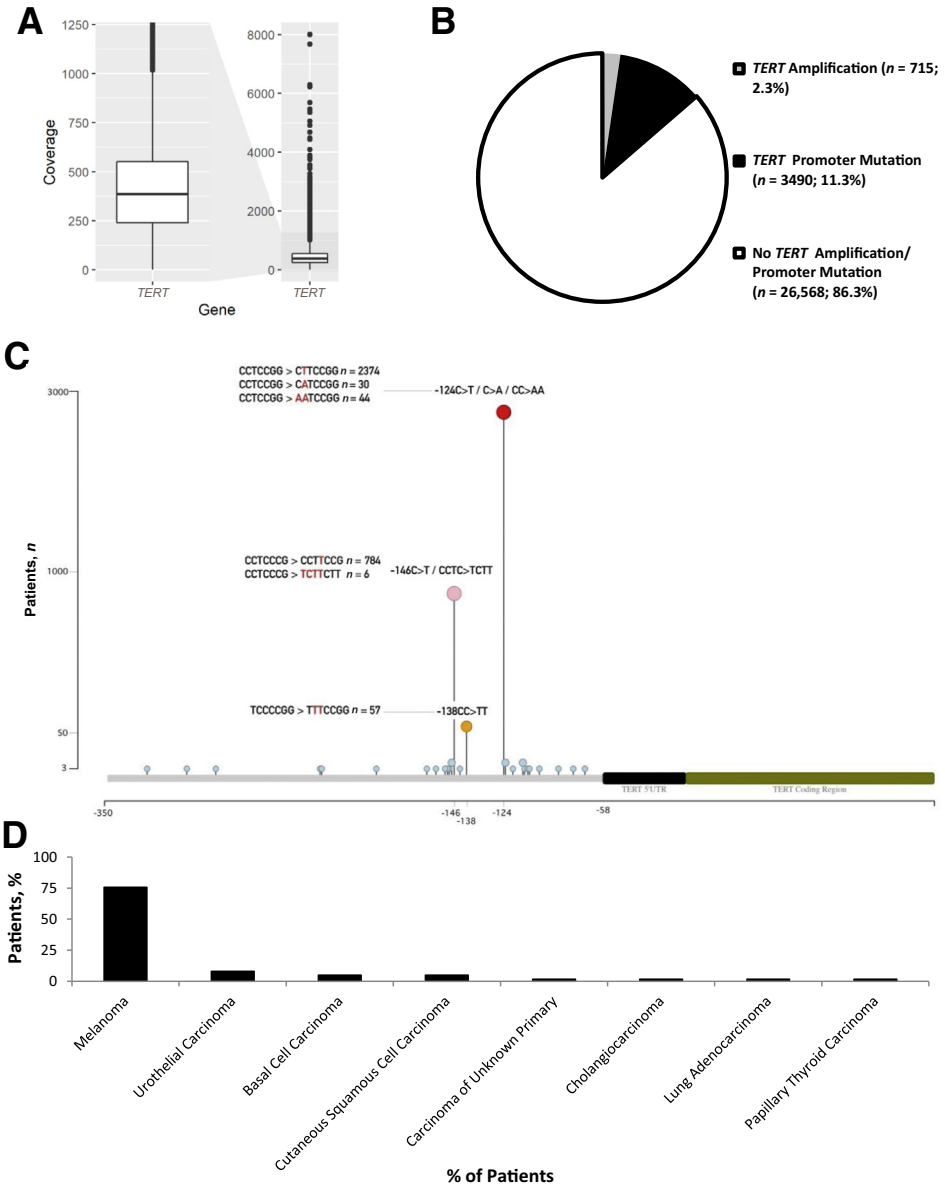

Figure 1 Next-generation sequencing (MSK-IMPACT) for *TERT* alterations. **A:** A box plot depicting the mean depth of coverage for the *TERT* promoter region (500 bp upstream of the transcription start site) for 30,773 specimens is depicted. The *y* axis demonstrates the depth of coverage. The range of coverage across 30,773 specimens is depicted on the **right side** of the figure, whereas the **left side** of the figure shows a magnified area of the box plot that highlights the interquartile range. **B:** The frequency of *TERT* genomic amplifications at the 5p15.33 locus and promoter mutations, both mutually exclusive events, for all cases that were profiled is shown. **C:** The spatial distribution of *TERT* promoter mutations relative to the transcription start site is depicted. Specific nucleotide changes that contribute to presumptive ETS transcription factor binding sites are highlighted for three mutational hot spots (−124, −146, and −138 bases upstream of the transcriptional start site). Variant nucleotide sequences are highlighted in red. **D:** Furthermore, tumor types that exhibit *TERT* promoter mutations at the −138 position are shown.

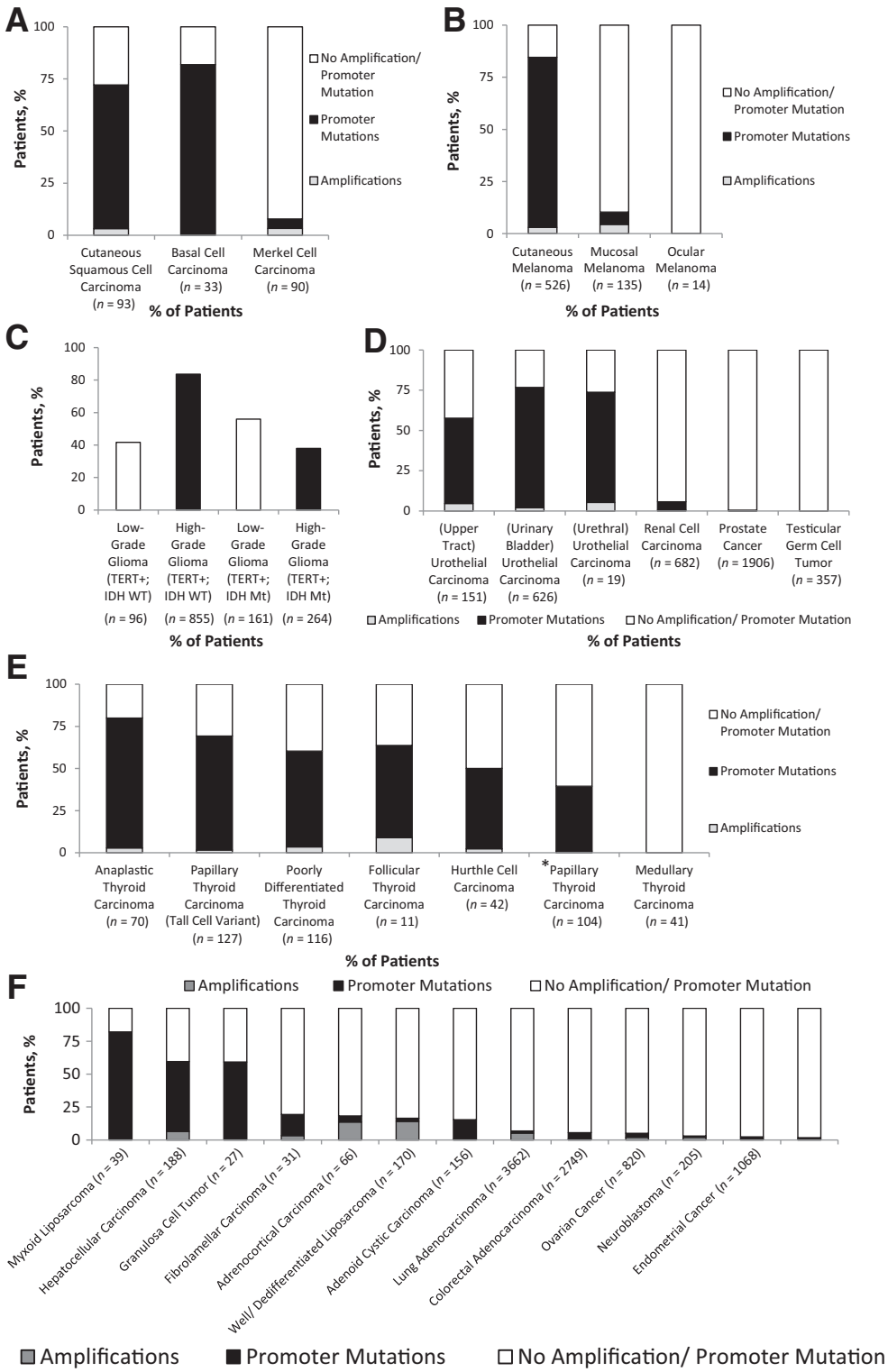

Figure 2 Relative frequency of *TERT* alterations in multiple tumor types. **A**–**F:** The relative frequency of *TERT* promoter mutations and amplifications in nonmelanoma skin cancer (**A**), melanoma (**B**), gliomas (**C**), genitourinary neoplasia, including urothelial carcinoma (**D**), thyroid carcinoma (**E**), and miscellaneous tumor types (**F**) is depicted. **C:** *TERT* alterations in high- and low-grade gliomas are further stratified on the basis of *IDH1* mutation status. The papillary thyroid carcinoma category includes all well-differentiated subtypes and excludes the tall cell variant. The **asterisk** indicates papillary thyroid carcinoma excluding tall cell variant. IDH Mt, *IDH1* mutant; IDH WT, *IDH1* wild type; $TERT^{+}$, *TERT* alteration present.

common ($n = 2448$; 74%), followed by those at positions −146 ($n = 790$; 24%) and −138 ($n = 57$; <2%) (Figure 1C). Although *TERT* promoter mutations at the −138 position were seen in varied tumor types, they predominantly occurred in melanoma (75.8% of cases) (Figure 1D). Consistent with prior reports, G-to-A substitutions at these sites led to the formation of presumptive ETS transcription factor binding sites, including at position −138.[14]

Relative Frequency of *TERT* Alterations in Multiple Tumor Types

Solid tumor types with high rates of *TERT* alterations (in combined primary and metastatic tumors) have been highlighted (Figure 2). Cutaneous neoplasia, such as squamous cell carcinoma, basal cell carcinoma, and cutaneous melanoma, primarily harbored *TERT* hot spot promoter mutations in association with a UV-induced mutational signature (ie, predominant G-to-A and C-to-T mutations), whereas amplifications were relatively rare (Figure 2, A and B). In contrast, similar alterations were infrequently identified in Merkel cell carcinoma and mucosal/ocular melanoma. Consistent with prior reports, *TERT* hot spot mutations in central nervous system glial neoplasia predominantly occurred in an *IDH1* wild-type setting in high-grade gliomas (84% of *IDH1* wild-type cases) compared with low-grade gliomas, where they tended to occur in association with *IDH1* mutations (56% of *IDH1* mutant cases) (Figure 2C).[24–26] Among genitourinary malignancies, the highest incidence was seen for urothelial carcinoma, with a relatively lower frequency observed for upper tract disease (53% versus 74%) (Figure 2D). As previously reported, *TERT* promoter mutations showed a strong association with the underlying subtype in thyroid carcinoma (Figure 2E).[13,27–33] No alterations were identified in medullary thyroid carcinoma, and up to 77% of anaplastic thyroid carcinomas harbored these alterations. Interestingly, although a lower frequency was seen for papillary thyroid carcinoma (including classic and follicular variants; 38%), the aggressive tall cell variant showed a much higher frequency (68%). As expected, other tumor types with a high frequency of *TERT* promoter mutations included myxoid liposarcoma, hepatocellular carcinoma, and granulosa cell tumors (Figure 2F).[34–36] In contrast, tumor types where *TERT* amplification was the prevalent alteration included well-differentiated/dedifferentiated liposarcoma (14.1%) and adrenocortical carcinoma (13.6%) (Figure 2F).[37,38]

Of note, high rates of *TERT* alterations were documented in the primary tumors themselves for aggressive variants of thyroid cancer, such as poorly differentiated ($n = 73$; 52%), anaplastic ($n = 55$; 69%), and tall cell variant of papillary thyroid cancer ($n = 43$; 74%), as is depicted in Figure 3. In contrast, these alterations were predominantly documented in metastatic/recurrent tumors for classic well-differentiated papillary thyroid cancer ($n = 58$; 52%) and Hurthle cell carcinoma ($n = 26$; 54%).

TERT Promoter Rearrangements

Prior studies have identified recurrent genomic rearrangements at the *TERT* locus, upstream of the *TERT* coding sequence, primarily in high-risk neuroblastoma.[7,8] These cases showed substantial induction of *TERT* gene expression and downstream increases in telomerase activity, likely secondary to the juxtaposition of strong enhancer elements adjacent to the *TERT* coding sequence.[7,8] Although subsequent studies have confirmed the presence of *TERT* promoter rearrangements, these remain challenging to identify as these rearrangements often occur over a 50-kb region proximal of the *TERT* gene.[9,11,39,40] Although the MSK-IMPACT assay only tiles for 500 bases of this 50-kb region, 24 cases occurring in multiple tumor types were found to harbor similar rearrangements upstream of the *TERT* coding sequence (Figure 4). No recurrent break point or partner gene was identified for these cases. In addition, these events were mutually exclusive with *TERT* hot spot promoter mutations/genomic amplifications.

Relative *TERT* Gene Expression

Herein, 155 specimens of archived formalin-fixed, paraffin-embedded specimens that were initially evaluated using

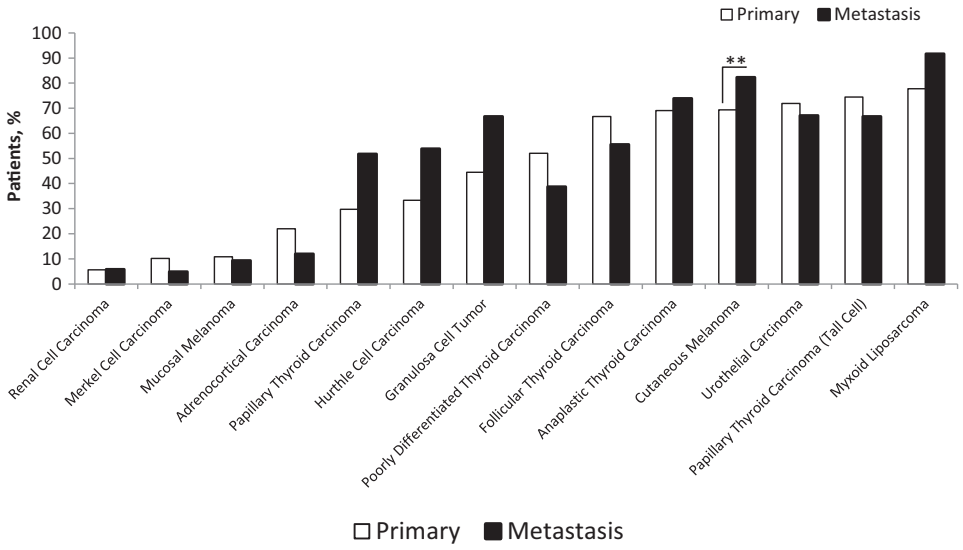

Figure 3 Relative distribution of *TERT* alterations in primary compared with metastatic/recurrent tumors. *TERT* promoter mutations and amplifications combined, for specific tumor types, are depicted for primary compared with metastatic/recurrent tumors. $^{**}P < 5 \times 10^{-3}$.

MSK-IMPACT were retrieved and further interrogated for *TERT* mRNA expression using the MSK-Fusion assay. Unique *TERT*-specific RNA reads were normalized to the expression of multiple housekeeping genes and quantified. This included cases that lacked *TERT* alterations or harbored amplifications, hot spot promoter mutations, or rearrangements. Interestingly, cases with genomic amplifications and promoter rearrangements showed statistically significant increases in *TERT* gene expression, in contrast to cases with hot spot promoter mutations (Figure 5).

Although multiple studies have attempted to demonstrate TERT protein expression using immunohistochemistry, this has historically been controversial. Such efforts have been hampered by poor reproducibility, unexpected patterns of subcellular localization, as well as documented cross-reactivity with other proteins.[41,42] Validation of such an immunohistochemical assay would require establishing a baseline status for all cases that was inclusive of not only promoter mutations, but also amplifications, upstream rearrangements, and methylation events to correlate protein expression with underlying tumor biology. Although multiple antibodies were to be validated, the validation failed because of a lack of consistency with the underlying prediction of *TERT* expression status and unexpected patterns of protein localization (data not shown).

TERT Promoter Hypermethylation Status

Recent studies have suggested that hypermethylation upstream of the *TERT* transcriptional start site is required for *TERT* gene expression and corresponding telomerase activity in neoplastic cells compared with nonneoplastic cells.[6,9–11] At least one study has suggested that these hypermethylation events are not mutually exclusive with either TERT promoter mutations or rearrangements.[11]

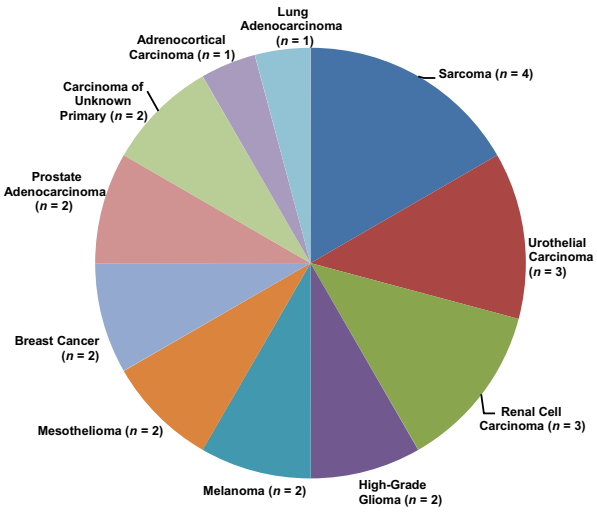

Figure 4 *TERT* promoter rearrangements. Diverse tumor types for which *TERT* promoter rearrangements were identified within 500 bp immediately upstream of the transcriptional start site are shown.

Although cases with hypermethylation were not evaluated for relative gene expression, 57 cases of high-grade glioma profiled by MSK-IMPACT were further evaluated for methylation status upstream of the *TERT* gene. This was performed to determine if hypermethylation events were mutually exclusive with activating alterations, such as hot spot promoter mutations and amplifications. Our results suggest that these are not mutually exclusive events as 37 of 42 (88%) of cases with promoter mutations also exhibited hypermethylation upstream of the transcriptional start site (Figure 6).

Discussion

Telomerase reactivation in cancer is often driven by *TERT* expression, and the predominant underlying alteration involves promoter mutations that generate a *de novo* binding site for activating ETS family transcription factors that lead to transcriptional up-regulation.[1–5] Other alterations that can up-regulate *TERT* expression include genomic amplification and rearrangements or hypermethylation events upstream of the *TERT* transcriptional start site.[6–10]

The mean coverage for the *TERT* promoter region across all 30,773 specimens evaluated by MSK-IMPACT was 425× and revealed the presence of *TERT* alterations in 13.7% of all cases. Promoter mutations were found to be 4.9 times more prevalent compared with amplification events. Although promoter mutations were distributed along the length of the entire promoter region that was sequenced, consistent with our prior report, three recurrent and mutually exclusive hot spots were identified.[14] Among the hot spot mutations, the canonical alterations at positions −124 and −146 bases relative to the transcription start site accounted for 74% and 24% of cases, respectively. An additional noncanonical hot spot at position −138 accounted for <2% of cases, and all these alterations are predicted to generate *de novo* ETS transcription factor binding sites.[14] Therefore, this latter category of promoter mutations (−138) might be missed in a small subset of cases in targeted assays that interrogate/report only the canonical hot spot alterations (−124 and −146). Similarly, amplification events can be missed in a much larger subset of cases. This is particularly relevant for tumor types where amplification events significantly outnumber promoter mutations, such as in well-differentiated/dedifferentiated liposarcoma (14.1% versus 2.4%) and adrenocortical carcinoma (13.6% versus 4.5%).[37,38,43]

TERT alterations were found to be enriched in specific tumor types, and a subset of these has been highlighted with the caveat that the institutional (tertiary referral center) clinical sequencing cohort presented herein has a bias toward patients who present with high stage and aggressive disease.

Some relevant associations include cutaneous melanomas, which showed a high rate of *TERT* alterations (85%)

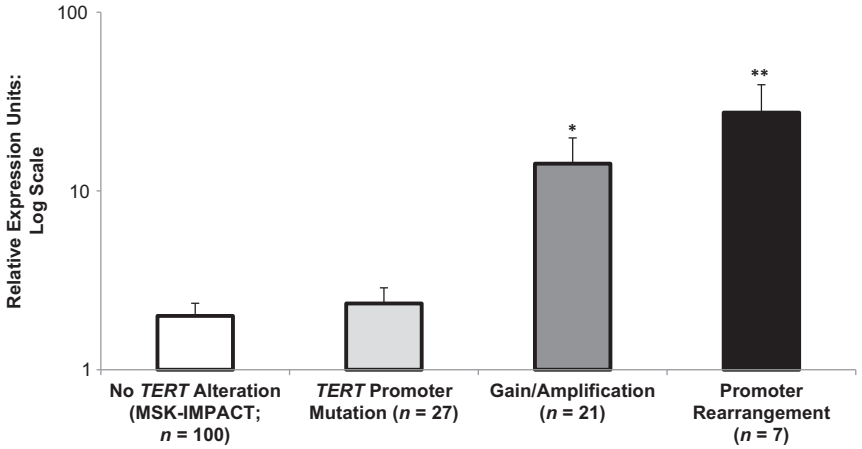

Figure 5 Relative *TERT* gene expression. Relative *TERT* gene expression from archived formalin-fixed, paraffin-embedded material for 155 cases is depicted. $n = 100$ for no *TERT* alteration on the basis of Memorial Sloan Kettering Cancer Center Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT); $n = 27$ for *TERT* promoter mutation; $n = 21$ for *TERT* genomic amplification; $n = 7$ for *TERT* promoter rearrangement. $^{*}P < 5 \times 10^{-5}$, $^{**}P < 5 \times 10^{-9}$.

in a background of a UV-induced mutational signature, as opposed to both mucosal (10%) and ocular melanoma (0%), and these trends and frequencies are consistent with what has been previously reported in the literature.[2,13,44] A similar high incidence of *TERT* alterations was seen in sun-exposed cutaneous squamous cell (72%) and basal cell carcinomas (82%).[3]

Prior studies have shown that *TERT* promoter mutations in high-grade gliomas occur infrequently in the background of *IDH1* mutations (<3%) as opposed to low-grade gliomas (34%).[26] Our results support this trend as the vast majority of *TERT* alterations in high-grade gliomas occurred in the *IDH1* wild-type setting ($n = 856$; 84%) and, similarly, a large number of *TERT* alterations in low-grade gliomas occurred in the *IDH1* mutant setting ($n = 161$; 56%).

Among genitourinary cancers, *TERT* alterations had an exceedingly low prevalence in prostatic adenocarcinoma and testicular germ cell tumors (<0.5%), followed by a low incidence in renal cell carcinoma (5.7%), as has been previously reported.[2,14,45] The highest incidence was seen in urothelial carcinoma (overall: 73%). The overall frequency is consistent with published reports that suggest that on the basis of a high incidence, and the observation that *TERT* alterations may be an early event in urothelial carcinoma, the detection of these alterations may be exploited as a potential urothelial cancer screening tool, particularly using urine/cytology specimens.[2,12,13,46–48] On the basis of our results, some variables that may confound the results of urothelial cancer screening assays that rely on the detection of *TERT* promoter mutations include the presence of these alterations in a subset of renal cell carcinoma (false positives), whereas the absence of *TERT* alterations in a subset of cases or the presence of amplification events (2.6% of cases) may contribute to false negatives.[45,46,48]

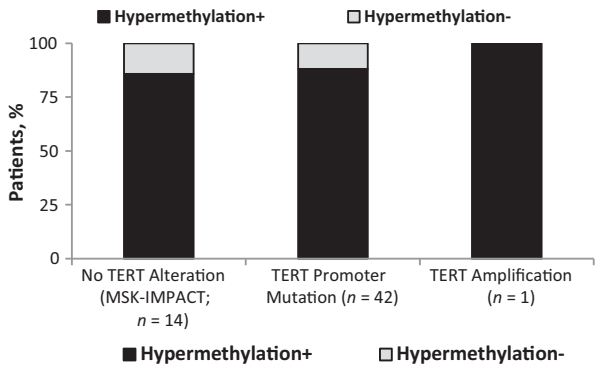

Figure 6 *TERT* promoter hypermethylation status. *TERT* promoter hypermethylation upstream of the transcriptional start site, including at the cg11625005 CpG site, was evaluated for 57 cases profiled by Memorial Sloan Kettering Cancer Center Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) to assess for mutual exclusivity of hypermethylation with cases harboring promoter mutations and amplification. $n = 42$ cases harboring promoter mutations; $n = 1$ case harboring amplification.

With regard to thyroid cancer, prior studies have shown absence of *TERT* promoter mutations in medullary thyroid carcinoma, a relatively low incidence in papillary carcinoma (7% to 25.5%), Hurthle cell carcinoma (13% to 33%), and follicular thyroid carcinoma (14% to 36%), and the highest incidence in poorly differentiated carcinoma (21% to 60%) and anaplastic thyroid carcinoma (13% to 73%); these studies suggest that these alterations are enriched in thyroid carcinomas that exhibit more aggressive behavior.[2,13,27–33] In our series of 511 thyroid carcinomas, with many patients presenting with advanced disease, an absence of *TERT* promoter mutations in medullary thyroid carcinoma was confirmed, followed by progressively increasing incidence in papillary, Hurthle cell, and follicular thyroid carcinomas (39%, 48%, and 55%, respectively) compared with 57% in poorly differentiated and 77% in anaplastic thyroid carcinomas. *TERT* amplifications were detected in 2% of cases, which is close to the previously reported incidence of 4.9%.[27] Furthermore, consistent with prior reports, our results underline the higher incidence of *TERT* alterations in the tall cell variant compared with classic well-differentiated

papillary thyroid carcinoma (69% versus 39%).[2] Among poorly differentiated, anaplastic and tall cell variants of papillary thyroid carcinoma, there was no significant difference in the frequency of *TERT* promoter mutations when comparisons were made between primary and metastatic tumors. This finding is consistent with their (more) aggressive biology.[30,33] However, among classic well-differentiated papillary thyroid carcinoma and Hurthle cell carcinoma, *TERT* was more commonly mutated in metastatic/recurrent disease than in primary tumors (classic well-differentiated papillary thyroid carcinoma: 52% versus 30%; and Hurthle cell carcinoma: 54% versus 33%), suggesting that these genetic alterations may identify a relatively more aggressive subset of well-differentiated thyroid carcinomas with a tendency to metastasize/recur. However, these results must be interpreted with caution because of sampling bias (ie, the fact that the vast majority of thyroid cases subjected for testing were from patients with relatively more aggressive disease).

Other tumor types that have high reported incidences of *TERT* alterations include hepatocellular carcinoma (amplifications: 10%; and promoter mutations: 44%) and ovarian granulosa cell tumors (22%), whereas myxoid liposarcomas (74%) have the highest reported incidence among soft tissue tumors.[34–36] Our results revealed a similar trend, revealing an overall frequency of promoter mutations/amplifications of 59%, 60%, and 82% for hepatocellular carcinoma, granulosa cell tumors, and myxoid liposarcoma, respectively. Of note, the frequency of these alterations in ovarian granulosa cell tumors has been reported to be significantly higher among recurrences, and furthermore, their presence has been correlated with poor outcomes.[34] In hepatocellular carcinoma, up to 6.4% of cases harbored amplifications of *TERT* and are likely to be missed if they are evaluated using assays that do not evaluate copy number changes.

With regard to *TERT* gene expression, although a large number of studies have demonstrated the impact of *TERT* hot spot promoter mutations on transcriptional up-regulation *in vitro*, similar studies that have directly evaluated the effect of *TERT* promoter mutations on gene expression in tumor-derived biospecimens are limited.[3] Furthermore, several studies have documented only modest increases in *TERT* gene expression in both *in vitro* studies and in tumor specimens.[11,46,49] Our results confirm that cases with genomic amplifications and promoter rearrangements showed statistically significant increases in *TERT* gene expression and highlight the importance of identifying such cases.[7–9] In contrast, relative gene expression in cases with promoter mutations did not show a statistically significant increase. This could be secondary to the age and type (archived formalin fixed and paraffin embedded) of specimen evaluated, potentially modest increases in gene expression, and/or potentially low sensitivity of the assay used. A limitation of our study involves a lack of correlation of *TERT* expression with downstream gene expression signatures, particularly related to ETS transcription factors.[50,51] This approach was not utilized in the current study as gene expression–specific primers to perform such an analysis were not included in the MSK-Fusion assay. However, future studies may utilize such a strategy when interrogating *TERT* gene expression status.

Perhaps the most intriguing category of *TERT* alterations involves structural variants that have been previously reported to be present in neuroblastomas.[7–9] Specifically, these have been reported in 23% to 31% of high-risk neuroblastomas, have exhibited a large structural diversity of *TERT* promoter rearrangement events, and were frequently clustered approximately 50 kb upstream of the *TERT* transcriptional start site.[7,8] These events were reported to lead to a juxtapositioning of superenhancer elements and were associated with a massive transcriptional up-regulation of the *TERT* gene.[7,8] Although reports of such alterations are predominantly restricted to neuroblastomas that show aggressive clinical behavior, 24 such events were identified across diverse tumor types and significantly higher *TERT* gene expression was reported in seven such cases where archived material was available for downstream analysis, to validate these findings.[7–9] In summary, prior studies pertaining to neuroblastoma suggest that *TERT* promoter rearrangements are associated with high levels of *TERT* gene expression, and our results confirm these findings.[7,8] However, future studies are needed to define the true prevalence of these alterations and their impact on tumor biology.[7–9]

Recent studies have shown that hypermethylation at specific CpG islands upstream of the *TERT* transcriptional start site has been associated with increased *TERT* gene expression, particularly in pediatric brain tumors and in melanoma.[6,10,11] As there is a paucity of studies that have evaluated large data sets for such hypermethylation events in the context of other pathogenic alterations, 57 cases with known promoter mutation and copy number status on the basis of profiling by MSK-IMPACT were evaluated. Consistent with the results of a prior study that evaluated a limited number of cases in a similar manner, these events were not mutually exclusive with either amplification events or promoter mutations.[11] However, it is possible that a subset of cases that are considered to be wild type for *TERT* may in fact harbor epigenetic alterations that have a significant impact on gene expression.[11]

Given the high prevalence of *TERT* alterations in cancer, there have been ongoing efforts to target components of the telomerase holoenzyme.[14,52–54] Although such approaches are currently not in clinical use, they represent exciting future cancer therapy strategies. Herein, we provide a snapshot of the landscape of somatic *TERT* alterations across multiple cancer types. Although canonical hot spot promoter mutations (positions −124 and −146, relative to the transcription start site) are the prevalent pathogenic alteration, our data support the presence of an additional, mutually exclusive, hot spot alteration at position −138.[14] Other alterations of note include genomic amplification events and rearrangements proximal to the *TERT* gene,

which may have a significant impact on gene expression but are likely missed by targeted assays that interrogate only the conventional promoter hot spot mutations. In addition, these data suggest that upstream hypermethylation events, which have been reported to be associated with increased gene expression, are not mutually exclusive with known activating *TERT* alterations, and additional studies are needed to further define their role across varied cancer types. In summary, studies aimed at defining the prognostic impact of *TERT* alterations should attempt to incorporate other pathogenic alterations, including amplifications, upstream rearrangements, and hypermethylation events, as these might significantly impact telomerase function.

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