The Solo Play of TERT Promoter Mutations

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Abstract: The reactivation of telomerase reverse transcriptase (TERT) protein is the principal mechanism of telomere maintenance in cancer cells. Mutations in the TERT promoter (TERTp) are a common mechanism of TERT reactivation in many solid cancers, particularly those originating from slow-replicating tissues. They are associated with increased TERT levels, telomere stabilization, and cell immortalization and proliferation. Much effort has been invested in recent years in characterizing their prevalence in different cancers and their potential as biomarkers for tumor stratification, as well as assessing their molecular mechanism of action, but much remains to be understood. Notably, they appear late in cell transformation and are mutually exclusive with each other as well as with other telomere maintenance mechanisms, indicative of overlapping selective advantages and of a strict regulation of TERT expression levels. In this review, we summarized the latest literature on the role and prevalence of TERTp mutations across different cancer types, highlighting their biased distribution. We then discussed the need to maintain TERT levels at sufficient levels to immortalize cells and promote proliferation while remaining within cell sustainability levels. A better understanding of TERT regulation is crucial when considering its use as a possible target in antitumor strategies.

Keywords: TERT promoter mutations; telomerase; cell immortalization; GBM/glioma; melanoma; thyroid cancer; APOBEC mutations; UV mutations

1. Introduction

Telomeres and their associated shelterin complex are located at chromosomal ends. Telomeres are tandem repeats of TTAGGG up to 15 kb long in humans. Together, telomeres and the shelterin complex protect chromosomal ends and preserve genomic DNA integrity [1–4]. Telomeres are shortened with each cell division. When telomere length falls below a critical threshold, cells become replicatively senescent and undergo apoptosis [5]. Cancer cells circumvent replicative telomere shortening by stabilizing them [6] through one of two mechanisms: reactivation of telomerase, the enzyme that extends telomeres (85–90% of cancers) [7–10], or homologous recombination between sister chromatids, a phenomenon termed alternative lengthening of telomeres (ALT) (3–10% of cancers) [10–12]. Telomerase is a ribonuclease holoenzyme composed of an RNA template (TERC) and a reverse transcriptase catalytic subunit (TERT) [1–4,13]. TERT is silent in most somatic cells, and is reactivated in cancer cells, endowing them with unrestricted proliferation capacity [6,14–16].

Although TERT activity is regulated principally at the transcriptional level (reviewed in References [3,4,9,17–22]), it may also be regulated through splicing [23,24], post-translational modifications, or intracellular trafficking [25–28]. The TERT promoter (TERTp) contains binding sites for numerous transcriptional activators including Sp-1, c-Myc, Hypoxia Induced Factor (HIF), AP-2, β-catenin, NF-κB, E-twenty-six (Ets)/ternary complex factors (TCF) family members, and transcriptional repressors (Wilms’ tumor (WT1), TP53, Nuclear Transcription Factor, X-Box Binding (NFX-1), Mad-1 and CCCTC binding factor (CTCF)) [3,4,9,17–21,29]. TERT expression can be reactivated.
by copy number variants (CNV), TERT or TERTp structural variants, chromosomal rearrangements juxtaposing TERTp to enhancer elements, cellular and viral oncogenes such as Hepatitis B virus (HBV) X protein (HBx) or high-risk Human papillomavirus (HPV)16 and HPV18 E6 oncoprotein, and, last but not least, mutations within TERTp (31% of TERT-expressing cancers) (Figure 1A) [10,30–38] (reviewed in [3,4,9,18–20,39]). Increased TERTp methylation is typically recorded in >50% of TERT-expressing tumors and cell lines [10,40–47]. Epigenetic regulation of TERTp is based on altered methylation patterns of specific regions. Hypermethylation of the region between −200 and −100 from the Translational Start site (TSS), encompassing the core promoter, enables binding of c-Myc and Sp-1, thus reactivating transcription. In contrast, the region spanning exon 1 (positions +1 to ±100 from the TSS) contains a binding site for the DNA insulator CTCF. Hypermethylation of this region disrupts binding of CTCF and therefore allows TERT transcription [41–44]. Similarly, the region between −600 and −200 from the TSS contains a second CTCF binding site and is partially hypermethylated in TERT-expressing cells [41–44]. The transcriptional control of TERT has been comprehensively reviewed recently [3,4,9,18–22,29,48] and, as such, is beyond the scope of this review. In this review, we focused on the distribution and exclusiveness of TERTp mutations.

![Figure 1](image-url)  
**Figure 1.** Mechanisms of telomerase reverse transcriptase (TERT) reactivation in cancer and TERT promoter (TERTp) mutations. (A) Different mechanisms of TERT reactivation in cancer according to Reference [10]. (B) Localization of TERTp mutations on Chromosome 5.

# 2. Telomerase Reverse Transcriptase Promoter (TERTp) Mutations

TERTp mutations were first described in congenital and sporadic melanoma in 2013 [49,50]. Subsequent large-scale cohort studies together with seminal mechanistic studies both ascertained the TERTp mutation prevalence in many other forms of cancer and characterized their mode of action.

The two main TERTp mutations are located at positions 1,295,228 and 1,295,250 on Chromosome 5, and generate C to T transitions. They are located 124 and 146 base pairs upstream from the TERTp TSS (Figure 1B). Less frequent tandem mutations −125/−124 CC>TT and −139/−138 CC>TT have been reported in cutaneous tumors (Table 1) [49,51]. While these are somatic mutations, a germline mutation at position −57A>C from the TSS has been identified in familial melanomas and showed similar effects [49]. All of these mutations have similar effects, increasing TERT expression ~2–6 fold as measured through qRT-PCR, immunohistochemistry, TRAP, or reporter vectors in numerous cancer types, as outlined in Table 1 [37,50,52–65]. This increased TERT expression maintains self-renewal potential and telomeres in both stem cells and terminally differentiated bladder cells, indicating that these mutations are sufficient to immortalize cells [66,67].

All of these TERTp mutations (at positions −146, −124, −57, and −139/−138) create novel Ets/TCF transcription factor binding sites. The Ets/TCF transcription factors bind to GGAA motifs (or TTCC on the opposite strand). The 30 members of the Ets/TCF-family transcription factors are important contributors to oncogenesis and include Ets-1, Ets-2, and GA binding protein (GABP) [68]. So far, GABP has been reported to selectively bind the −124 C>T and −146 C>T mutations in GBM, melanoma,
and urothelial bladder cancer cell lines [69–71]. Unlike the other Ets/TCF family transcription factors, GABP is an obligate dimer of GABPA and GABPB dimers. It binds two nearby in-phase GGAA sites [68,72–74] positioned 1, 2, or \( n \) helical turns away from each other [69], or brought close together by DNA looping [70]. TERTp mutations are associated with epigenetically active chromatin [54,69,75,76]. Intriguingly, whereas methylation of wild-type (wt) TERT promoter is associated with TERT expression [10,43,44], TERTp mutations are associated with decreased TERTp methylation [76]. The −146 C>T mutation was also shown to bind the non-canonical NF-κB-p52 and Ets-1 [59].

TERTp mutations have been recorded in a wide range of solid cancers. They are present in primary gliomas and glioblastoma multiforme (GBM), oligodendrogliomas and astrocytomas [10,40,52–54,57,58,60,64,65,77–86], melanomas, cutaneous basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) [49–52,55,87–91], myxoid liposarcomas [77], urothelial bladder carcinoma [50,57,78,92–94], hepatocellular carcinoma (HCC) [50,57,62,95–97], and thyroid cancers [98–106], as well as oral and cervical SCC [36,37,57] (Table 1). Furthermore, they were consistently found in tumor cell lines derived from these malignancies [37,50,52,54,58,77,100,107,108]. TERTp mutations often arise in tissues with low rates of self-renewal (brain, thyroid) [77], where they provide an immediate competitive advantage to cells that acquire them. Conversely, they appear to be infrequent (<15%) in hematopoietic, lymphoid, or gastrointestinal malignancies. These are from compartments with high cellular turnover and intrinsic telomerase activity. Here, the endogenously elevated TERT levels likely render TERTp mutations neutral [3,38,57,77,109].

3. Cancer Distribution of TERTp Mutations

The clinicopathological association of TERTp mutations is cancer-dependent. It is a consideration for fine tumor stratification and orientation of patients towards personalized treatments, and provides insight into the process of cellular transformation.

3.1. Gliomas and Glioblastoma (GBM)

GBM are WHO Grade IV tumors of the central nervous system (CNS). Primary GBM evolve rapidly without prior low-grade lesions, while secondary GBM progress slowly from diffuse or anaplastic astrocytoma and oligodendroglioma (WHO Grade II and III). Primary and secondary GBM differ genetically more than histologically. The 2016 WHO classification of CNS tumors is based on “integrated diagnosis” including histology and isocitrate dehydrogenase (IDH)-1/2 mutations (a biomarker for secondary GBM), and the presence of the 1p/19q codeletion (a marker for oligodendroglioma) [110]. TERTp mutations are relatively rare in diffuse (17.7%, range 10–19%) and anaplastic astrocytomas (24.7%, range 10–62.5%), as well as in IDH-mutated gliomas and secondary GBM (~28%). Their prevalence is highest (64.7%, range 45–88.6%) in oligodendrogliomas (where they coexist with the 1p/19q full deletion [53]) and in primary GBM (68%, range 44–100%) (Table 1). They tend to be found mainly in samples with epidermal growth factor receptor (EGFR) amplification, an early feature of primary GBM, [64,77,111]. Conversely, they appear to be mutually exclusive with mutations in α-thalassemia/mental retardation syndrome X-linked (ATRX) and Death Domain Associated Protein (DAXX) [38,65,77,80,81,84,85,111], two telomere-binding proteins mutated in ALT [11,12].

TERTp mutations are independently associated with older age, late clinical stage, poor prognosis, and shorter overall survival (OS) in GBM/glioma and IDH-wt astrocytoma patients. The presence of TERTp mutations alone is associated with a worse prognosis than TERTp mutations together with IDH-mutations [4,60,64,65,77,79–81,84,85,112]. Conversely, GBM patients with ALT and no TERTp mutations have longer OS than patients with TERTp mutations only [77,112,113]. In terms of treatment, Grade II and III IDH-wt CNS tumors generally respond to adjuvant radiation and chemotherapy with temozolomide (TMZ). However, the presence of TERTp mutations decreases sensitivity to genotoxic therapies. It has therefore been proposed to use TERTp mutations to further stratify IDH-wt Grade II and III gliomas into subgroups to orient treatment [60,81,114].
3.2. Melanoma and Non-Melanoma Skin Carcinoma

In patients with primary melanoma, TERTp mutations have been reported in 39.2% (range 22–71%) of tumors. They arise progressively in sun-exposed sites and have been attributed to UV radiation. They are associated with increased patient age, distal metastases, poor outcome, and compromised OS and disease-free survival (DFS) [49–52,88,89,115]. In ~50% of cases, they are associated with mutations in BRAF/NRAS [49,52,88,89,91,116], influencing OS in the following order: TERTp<sub>mut</sub> + BRAF/NRAS<sub>mut</sub> < TERTp<sub>mut</sub> - BRAF/NRAS<sub>mut</sub> < TERTp-wt + BRAF/NRAS-wt [56].

Consistent with their UV-induced origin in skin cancers, TERTp mutations are also highly prevalent at sun-exposed sites in non-melanoma squamous cell (50%) and basal cell carcinomas (46.2%, range 38–74%), the most common skin tumor [55,89,90]. TERTp mutations display unique features in melanoma and non-melanoma cancers. First, −146 C>T and −124 C>T occur with similar frequencies in contrast to all other cancers, where −124 C>T is by far the most prevalent mutation (Table 1). Second, −139/−138 CC>TT and −125/−124 CC>TT tandem mutations are often reported. Third, TERTp mutations were detected in 9/10 melanomas with ALT in one study [117] and together (−124 C>T + −146 C>T) in two patients with BCC in another study [89], indicating that more than one telomere maintenance mechanism can, unusually, coexist in skin cancers.

3.3. Urothelial Bladder Cancer

TERTp mutations have been detected in 64.6% (range 29.5–100%) of urothelial bladder and upper urinary tract cancers. They are the most common somatic lesions in this cancer type [52,57,61,77,92,94,118,119]. They have been associated with reduced survival, disease recurrence, and distal metastases [61,118,119], although there appears to be no difference between early- and late-stage patients [52,94].

3.4. Thyroid

Among thyroid cancers, TERTp mutations have been reported mainly in follicular-cell-derived thyroid malignancies (papillary thyroid carcinoma (PTC): 13.4%, range 4.1–37.7%; follicular thyroid carcinoma (FTC): 13.9%, range 5.9–66.7%; poorly differentiated thyroid carcinoma (PDTC): 43.7%, range 21–51.7%; and anaplastic thyroid carcinomas (ATC): 39.7%, range 13–50%). The presence of TERTp mutations is significantly associated with increased age, tumor size and stage, distal metastases, tumor recurrence, and shorter OS and DFS in PTC and FTC. Their prevalence increases from differentiated PTC and FTC to the more aggressive poorly differentiated ATC (Table 1) [98–106]. The association of TERTp mutations with the common BRAF-V600E mutation is a powerful predictor of poor OS and DFS [52,98,99,104–106,108]. As in glioma, TERTp mutations compromise the outcome of radioiodine therapy [101,105].

3.5. Hepatocellular Carcinoma (HCC)

TERTp mutations are an early event in hepatocellular tumorigenesis [57,62,77,95]. They are not only seen in established HCC (47.1%, range 29.3–65.4%). As hepatocellular adenomas transform into HCC, TERTp mutations are the first gene recurrently mutated after β-catenin (CTNNB1) in preneoplastic cirrhotic lesions [62,95]. Together with the CTNNB1 mutation, TERTp mutations are considered critical effectors of malignant transformation. As such, they have been proposed as early biomarkers for hepatocellular transformation [62,77,95,96,120,121].

TERTp mutations appear to be more frequent in HCV-associated HCC [62,77,95,96,122] and less frequent or excluded from HBV-associated HCC [62,96,121,122], although this remains controversial [63,77,95]. HBV DNA insertion in the TERTp is a recurrent mechanism of TERT transcriptional reactivation in HBV-associated HCC [34,123,124], and a genetic screen of TERT in HCC found TERTp mutations to be mutually exclusive with HBV integration, TERT CNVs, and ATRX mutations [121].
3.6. Cervical and Oral Head and Neck Squamous Cell Carcinoma (HNSCC)

*TERT* mutations were detected in cervical SCC (20.1%, range 4.5–21.8%) and HNSCC (22.5%, range 17–31.7%) [36,37]. These malignancies are often associated with high-risk-HPV16/18 E6 and E7 viral oncoproteins and with APOBEC mutations [125–127]. High-risk HPV–E6 transactivates *TERT* [30,32,33,128,129]. *TERT* mutations have a notably higher prevalence in HPV-negative cervical and oral SCC. This gives distinct patterns of *TERT* reactivation through mutually exclusive mechanisms [36,37]. In cervical SCC, they are associated with higher TERT levels than HPV16/18-E6-positive tumors and with advanced cervical cancer [36,37]. Broader studies are needed to evaluate the added value of screening for the molecular mechanism underlying *TERT* reactivation in cervical and oral SCC.

3.7. The rs2853669 Polymorphism

Among *TERT* polymorphisms, a common polymorphism (rs2853669 A>G) which disrupts a pre-existing Ets/TCF binding site located 245 bp upstream of the *TERT* TSS has been reported to modify the effect of *TERT* mutations. It decreases *TERT* transcription in vitro and reverses *TERT* upregulation by *TERT* mutations [56,61,81,85,130]. Controversial clinical impacts have been reported, from a beneficial effect on OS and limited tumor recurrence in *TERT* -mutated urothelial bladder cancer, renal clear cell carcinoma, melanoma, and GBM [56,61,81,85,116,131], to unchanged or worsened clinical outcome in GBM, melanoma, or differentiated thyroid carcinomas [64,65,84,91,102,103]. In HCC, the rs2853669 polymorphism in combination with *TERT* mutations has been associated with decreased OS and DFS, and increased *TERT* methylation and expression [47]. Possible reasons for these conflicting reports could be homozygosity versus heterozygosity of the variant, or its occurrence on the same allele as *TERT* mutations. Further studies are needed to assess the relevance of screening for this polymorphism for prognostic and treatment purposes.
Table 1. Prevalence and distribution of telomerase reverse transcriptase promoter (TERTp) mutations in cancer genomes. The prevalence of TERTp mutations is given as percentage and as total number of cases.

| Cancer Type          | Stage | Prevalence of Mutations | −146 C>T | −124 C>T | Tert Uprgulation | Methods                          | Sample Origin       | Remarks                                                                 | Ref. |
|---------------------|-------|-------------------------|----------|----------|------------------|----------------------------------|---------------------|-------------------------------------------------------------------------|------|
| Central nervous system (CNS) |       |                         |          |          |                  |                                  |                     |                                                                         |      |
| GBM                 | IV    | 83.9%                   | 34%      | 65.9%    | 31/47            | Yes DNA sequencing, qRT-PCR, IHC, DNA sequencing, qRT-PCR, TRAP, reporter assays | Patients (China)    | Associated with shorter OS, IDH-wt, ATRX-wt, exclusively in EGFRmut samples. | [77] |
| GBM (Primary)       | IV    | 83%                     | 24.6%    | 75.4%    | N/A              | DNA sequencing                  | Patients (US American) | Associated with late-stage disease and patient age. Only in gliomas, not in pituitary adenocarcinomas, meningiomas or secondary metastases. | [60] |
| GBM                 | I–IV  | 44.6%                   | 26.7%    | 73.3%    | Yes DNA sequencing, qRT-PCR, reporter assays | Patients (China) | Associated with shorter OS in patients without rs2853669 TERT−245 A>G polymorphism. | [111]|
| GBM (primary & secondary) | IV    | 80.3%                   | *        | *        | N/A DNA sequencing | Patients | Associated with shorter OS and with EGFRmut, more frequent in primary (58%) than in secondary GBM (28%). | [81] |
| GBM                 | IV    | 66.9%                   | 25.5%    | 74.5%    | N/A DNA sequencing | Patients (Portugal & Brazil) | Associated with older age, poor prognosis, and shorter survival. Reversed by rs2853669 TERT−245 A>G polymorphism. Not associated with OS or DFS. | [85] |
| GBM                 |       | 60.4%                   | 24.1%    | 75.8%    | Yes DNA sequencing, qRT-PCR | Patients (Korea) | Associated with MGMT methylation and EGFR amplification. Associated with rs2853669 TERT−245 A>G polymorphism reversed TERT upregulation by TERTp mutations. Mutually exclusive with IDH-1 mutations. Associated with shorter telomeres. | [64] |
| GBM                 |       | 73%                     | 28%      | 82%      | Yes DNA sequencing, qRT-PCR, TRAP, qPCR | Patients | Associated with lower OS in IDH-1wt patients. rs2853669 TERT−245 A>G polymorphism associated with improved OS in patients without TERTp mutations, and with worse OS in patients with TERTp mutations. | [65] |
| Cancer Type          | Stage   | Prevalence of Mutations | −146 C>T | −124 C>T | Tert Upregulation | Methods                          | Sample Origin | Remarks                                                                 | Ref. |
|---------------------|---------|-------------------------|----------|----------|------------------|----------------------------------|---------------|------------------------------------------------------------------------|------|
| GBM (primary)       | 86%     | 79/92                   | 25%      | 75%      | N/A              | DNA sequencing                   | Patients      | Associated with older age and shorter OS. Homozygous rs2853669 TERT −245 A-G polymorphism associated with worse OS in patients without and with TERTp mutations. | [84] |
| GBM and gliomas     | 100%    | 10/10                   | 10%      | 90%      | N/A              | DNA sequencing                   | Patients      | In primary GBM, characterized by 10q deletion EFGR amplification.        | [58] |
|                     | 94%     | 33/35                   | 36%      | 64%      | N/A              | DNA sequencing                   | Cell lines    |                                                                                        | [58] |
| Total GBM           | 905/1331| 206/762                 | 567/762  | 68%      |                  |                                  |               |                                                                                        |      |
| OligodendrogliomaII | 45%     | 10/22                   | 20%      | 80%      | 8/10             | DNA sequencing, qRT-PCR, IHC     | Patients      | Associated with older age.                                                | [52] |
|                     | 70%     | 7/10                    | 14.3%    | 85.7%    | 1/7              | DNA sequencing                   | Patients      | Associated with older age at diagnosis. Not associated with lower survival.  | [57] |
|                     | 46.3%   | 25/54                   | 24%      | 76%      | 6/25             | DNA sequencing                   | Patients (Portugal & Brazil) | Associated with total 1p19q loss and IDH-1/2 mutations (98%) but exclusive with IDH-1mut if not total loss of 1p19q. | [85] |
|                     | 73.5%   | 25/34                   | 20%      | 80%      | 5/25             | DNA sequencing                   | Patients (Japan) | Associated with shorter OS. Can be associated with ATRX mutations or IDHmut/1p19q loss. | [80] |
|                     | 66.81%  | 151/226                 | *        | *        | N/A              | DNA sequencing                   | Patients (US American) | IDH-wt only. Associated with worse prognosis in IDH-wt. Associated with older age. Mutually exclusive with ATRX mutations. | [77] |
|                     | 63.2%   | 12/19                   | 41.7%    | 58.3%    | 5/12             | DNA sequencing                   | Patients (US American) | Associated with older age.                                                | [52] |
| Anaplastic          | 54%     | 13/24                   | 30.8%    | 69.2%    | 4/13             | DNA sequencing                   | Patients (Portugal) | Associated with older age.                                                | [52] |
| oligodendroglioma   | III     | 46.3%                   | 24%      | 76%      | 6/25             | DNA sequencing                   | Patients (Portugal) | Associated with older age.                                                | [52] |
| Anaplastic          | 74.2%   | 23/31                   | 30.4%    | 69.6%    | 7/23             | DNA sequencing                   | Patients (Japan) | Associated with total 1p19q loss and IDH-1/2 mutations (98%) but exclusive with IDH-1 if not total loss of 1p19q. | [53] |
| Cancer Type                   | Stage | Prevalence of Mutations | −146 C>T | −124 C>T | Tert Upregulation | Methods                     | Sample Origin | Remarks                                                                                     | Ref.   |
|------------------------------|-------|-------------------------|----------|----------|------------------|----------------------------|----------------|---------------------------------------------------------------------------------------------|--------|
| Anaplastic oligodendroglioma III | 23/26 | 88.5%                   | 43.5%    | 56.5%    | 10/23           | DNA sequencing            | Patients (US American) | Associated with older age. IDH-wt only. Associated with worse prognosis in IDH-wt. Mutually exclusive with ATRX mutations. | [77]   |
| Diffuse astrocytoma I        | 19.2% | 10/52                   | 20%      | 80%      | Yes              | DNA sequencing, qRT-PCR   | Patients (Japan) | Associated with total 1p19q loss and IDH-1/2 mutations (98%) but exclusive with IDH-1 if not total loss of 1p19q. | [53]   |
| Diffuse astrocytoma II       | 15%   | 3/20                    | 33.3%    | 66.6%    | Yes              | DNA sequencing, qRT-PCR, IHC DNA sequencing, qRT-PCR, TRAP, reporter assays | Patients (Portugal) | Associated with older age.                                                                 | [52]   |
| Diffuse astrocytoma II       | 20%   | 8/40                    | 25%      | 62.5%    | Yes              | DNA sequencing, qRT-PCR, TRAP, reporter assays | Patients (China) | Associated with age.                                                                      | [57]   |
| Diffuse astrocytoma II       | 15.2% | 7/46                    | 16.7%    | 83.3%    | N/A              | DNA sequencing            | Patients (Portugal & Brazil) | Frequency increased with grade.                                                          | [85]   |
| Total Diffuse Astrocytoma I  | 62.5% | 416/665                 | N/A      | N/A      | N/A              | DNA sequencing            | Patients (US American) | Associated with shorter OS. Can be associated with ATRX mutations or IDHmut/1p1q loss. | [80]   |
| Anaplastic Astrocytomas III  | 10%   | 1/10                    | 0%       | 100%     | N/A              | DNA sequencing            | Patients (Portugal & Brazil) | Frequency increased with grade.                                                      | [85]   |
| Anaplastic Astrocytomas III  | 33.3% | 4/12                    | 0%       | 100%     | Yes              | DNA sequencing, qRT-PCR, IHC DNA sequencing, qRT-PCR, TRAP, reporter assays | Patients (China) | Correlation with age.                                                               | [57]   |
| Anaplastic Astrocytomas III  | 25.3% | 20/79                   | 20%      | 80%      | Yes              | DNA sequencing, qRT-PCR   | Patients (Japan) | Associated with total 1p19q loss and IDH-1/2 mutations (98%) but exclusive with IDH-1 if not total loss of 1p19q. | [53]   |
| Total Anaplastic Astrocytomas| 25/101| 4/25                    | 20/79    | 20%      | Yes              | DNA sequencing, qRT-PCR   | Patients (Japan) | Associated with total 1p19q loss and IDH-1/2 mutations (98%) but exclusive with IDH-1 if not total loss of 1p19q. | [53]   |
| Cancer Type       | Stage     | Prevalence of Mutations | −146 C>T | −124 C>T | Text Upregulation | Methods                          | Sample Origin                  | Remarks                                                                 | Ref. |
|-------------------|-----------|-------------------------|----------|----------|-------------------|---------------------------------|--------------------------------|-------------------------------------------------------------------------|------|
| Mixed Oligoastocytoma | II–IV     | 32.3% 63/195           | *        | *        | N/A               | DNA sequencing                  | Patients (US American)        | Associated with shorter OS. Can be associated with ATRX mutations or IDH<sup>mut</sup>/1p19q loss. | [80] |
| Oligoastrocytoma  |           | 40% 14/35               | 28.6%    | 71.4%    | Yes               | DNA sequencing, qRT-PCR         | Patients (Japan)               | Associated with total 1p19q loss and IDH-1/2 mutations (98%) but exclusive with IDH-1 if not total loss of 1p19q. | [53] |
| OligoastrocytomaII–III | 4.0% 4/10  | 50% 2/4                | 50%      | 2/4      | N/A               | DNA sequencing                  | Patients (Portugal & Brazil)  | Not associated with lower survival.                                     | [85] |
| Anaplastic Oligoastrocytoma |        | 48.9% 22/45             | 27.3%    | 72.7%    | Yes               | DNA sequencing, qRT-PCR         | Patients (Japan)               | Associated with total 1p19q loss and IDH-1/2 mutations (98%) but exclusive with IDH-1 if not total loss of 1p19q. | [53] |
| Total Oligoastrocytoma |           | 103/285 (36.1%) 12/40 (30%) 28/40 (70%) |          |          |                   |                                 |                                |                                                                         |      |
| Medulloblastoma   | 33.3% 2/6 | 50% 1/2                | 50%      | 1/2      | N/A               | DNA sequencing                  | Patients (China)               | Associated with age.                                                     | [57] |
| Medulloblastoma   | 20.9% 19/91 | 0%0/19               | 100%     | 19/19    | N/A               | DNA sequencing                  | Patients (US American)        | IDH<sup>-wt</sup> and ATRX<sup>-wt</sup> only. Associated with worse prognosis in IDH-1<sup>-wt</sup>. Associated with older age. Mutually exclusive with ALT. | [77] |
| Total Medulloblastoma |       | 21/97 (21.6%) 1/21 (4.7%) 20/21 (95.3%) |          |          |                   |                                 |                                |                                                                         |      |
| Skin              |           |                        |          |          |                   |                                 |                                |                                                                         |      |
| Melanoma          | 71% 50/70 | 46% 23/30              | 54%      | 27/50    | Yes               | DNA sequencing, reporter vectors | Patients & cell lines          | −57 C>T germline mutation in family with history of melanoma.           | [50] |
| Melanoma          | 32.5% 25/77 | 20% 5/25               | 28%      | 7/25     | N/A               | DNA sequencing                  | Patients                        | High prevalence in metastatic cell lines (85%) compared to primary melanoma (32.5%). CC>TT −139/−138 tandem mutation in 10.4% patients. Concomitant with BRAF mutations in 47% of cases. | [49] |
| Melanoma          | 29% 16/56 | 50% 8/16               | 50%      | 8/16     | N/A               | DNA sequencing                  | Patients (Portugal)            | Associated with BRAF mutations.                                         | [52] |
| Melanoma          | 34% 97/287 | 52.5% 51/97            | 36%      | 35/97    | Yes               | DNA sequencing, qRT-PCR         | Patients (Spain)               | CC>TT −139/−138 tandem mutations in 4/97 (4.1%) patients. Associated with BRAF mutations in 50% cases. | [88] |
| Cancer Type          | Stage | Prevalence of Mutations | −146 C>T | −124 C>T | Telomere Upregulation | Methods          | Sample Origin | Remarks                                                                                     | Ref. |
|---------------------|-------|-------------------------|----------|----------|-----------------------|------------------|---------------|---------------------------------------------------------------------------------------------|------|
| Melanoma            | 41.6% | 121/291                 | *        | *        | N/A                   | DNA sequencing    | Patients (Spain) | Associated with shorter telomeres in tumor and with accelerated telomere shortening rate.  | [115]|
| Melanoma            | 22%   | 26/116                  | 35%      | 46%      | Yes                   | DNA sequencing,   | Patients (Portugal)| Telomere shortening rate:  
BRAF/NRASmut>TERTpmut>BRAF/NRASmut  
Associated with reduced OS & DFS.  
More prevalent in sun-exposed regions.  
Associated with increased mitotic rates.  
−138/−138 CC>TT tandem mutation in 2/26 (7.7%) patients.  
−125/−124 CC>TT tandem mutation in 3/26 (11.5%) patients.  
Associated with BRAF-V600E mutation (58% of cases).  
Associated with shorter OS and DFS.  
−139/−138 CC>TT & −125/−124 CC>TT tandem mutations in 16/116 cases (13.8%).  | [89] |
| Melanoma            | 38.6% | 116/300                 | 50%      | 32.8%    | N/A                   | DNA sequencing    | Patients (Spain) | Associated with BRAF/NRAS mutations in 126/283 (44.5%) cases.  
Reversed by rs2853669 TERT-245 A>G polymorphism.  
−139/−138 CC>TT tandem mutations in 4/63 (6.3%) patients.  
−125/−124 CC>TT tandem mutation in 1/63 (1.6%) patient.  
Associated with BRAF/NRAS mutation in 75/243 cases.  
Associated with rs2853669 TERT-245 A>G polymorphism.  | [116]|
| Melanoma            | 54.8% | 63/115                  | 61.9%    | 30.2%    | N/A                   | DNA sequencing    | Patients (Austria)| Most homzygous.  
−139/−138 CC>TT tandem mutation in 7/31 (22.6%) patients.  
−125/−124 CC>TT tandem mutation in 5/31 (16.1%) patients.  
1 patient with −139/−138 CC>TT + −125/−124 CC>TT tandem mutations.  
Mutations more frequent in basal cell carcinoma than in squamous cell carcinoma.  
No correlation with clinical parameters.  
Higher prevalence in patients not exposed to X-irradiation:  
48/94 (51%) vs. 28/102 (27%) in X-irradiated patients.  
−124 C>T more frequent than −146 C>T in non-X-irradiated patients;  
−146 C>T more frequent in X-irradiated patients.  
−139/138 CC>TT tandem mutation in 2/76 (2.6%) patients.  
2 patients with −146 C>T + −124 C>T mutations.  | [90] |
| Basal cell carcinoma| 55.6% | 18/32                   | 55.6%    | 22.2%    | N/A                   | DNA sequencing    | Patients (Germany)| Mostly homzygous.  
−139/−138 CC>TT tandem mutation in 7/31 (22.6%) patients.  
−125/−124 CC>TT tandem mutation in 5/31 (16.1%) patients.  
1 patient with −139/−138 CC>TT + −125/−124 CC>TT tandem mutations.  
Mutations more frequent in basal cell carcinoma than in squamous cell carcinoma.  
No correlation with clinical parameters.  
Higher prevalence in patients not exposed to X-irradiation:  
48/94 (51%) vs. 28/102 (27%) in X-irradiated patients.  
−124 C>T more frequent than −146 C>T in non-X-irradiated patients;  
−146 C>T more frequent in X-irradiated patients.  
−139/138 CC>TT tandem mutation in 2/76 (2.6%) patients.  
2 patients with −146 C>T + −124 C>T mutations.  | [55]|
| Basal cell carcinoma| 74%   | 31/42                   | 35.5%    | 45.1%    | N/A                   | DNA sequencing    | Patients         | Mostly homzygous.  
−139/−138 CC>TT tandem mutation in 7/31 (22.6%) patients.  
−125/−124 CC>TT tandem mutation in 5/31 (16.1%) patients.  
1 patient with −139/−138 CC>TT + −125/−124 CC>TT tandem mutations.  
Mutations more frequent in basal cell carcinoma than in squamous cell carcinoma.  
No correlation with clinical parameters.  
Higher prevalence in patients not exposed to X-irradiation:  
48/94 (51%) vs. 28/102 (27%) in X-irradiated patients.  
−124 C>T more frequent than −146 C>T in non-X-irradiated patients;  
−146 C>T more frequent in X-irradiated patients.  
−139/138 CC>TT tandem mutation in 2/76 (2.6%) patients.  
2 patients with −146 C>T + −124 C>T mutations.  | [90]|
| Basal cell carcinoma| 38.7% | 76/196                  | 43%      | 49%      | no                    | DNA sequencing,   | Patients (Portugal)| Mostly homzygous.  
−139/−138 CC>TT tandem mutation in 7/31 (22.6%) patients.  
−125/−124 CC>TT tandem mutation in 5/31 (16.1%) patients.  
1 patient with −139/−138 CC>TT + −125/−124 CC>TT tandem mutations.  
Mutations more frequent in basal cell carcinoma than in squamous cell carcinoma.  
No correlation with clinical parameters.  
Higher prevalence in patients not exposed to X-irradiation:  
48/94 (51%) vs. 28/102 (27%) in X-irradiated patients.  
−124 C>T more frequent than −146 C>T in non-X-irradiated patients;  
−146 C>T more frequent in X-irradiated patients.  
−139/138 CC>TT tandem mutation in 2/76 (2.6%) patients.  
2 patients with −146 C>T + −124 C>T mutations.  | [89]|

**Total**  
Melanoma 514/1312 (39.2%) 193/398 (48.5%) 140/398 (35.1%)
| Cancer Type | Stage | Prevalence of Mutations | −146 C>T | −124 C>T | Tert Upregulation | Methods | Sample Origin | Remarks |
|-------------|-------|-------------------------|----------|----------|------------------|---------|---------------|---------|
| Total Basal cell carcinoma | 125/270 (46.2%) | 54/125 (43.2%) | 55/125 (44%) | DNA sequencing | Patients (Germany) | Mostly homozygous. |
| Cutaneous SCC | 50% | 29.4% | 29.4% | N/A | DNA sequencing | Patients | −139/−138 CC>TT tandem mutation in 2/13 (15.4) patients. Mutations more frequent in basal cell carcinoma than in squamous cell carcinoma. |
| Cutaneous SCC | 50% | 54% | 31% | N/A | DNA sequencing | Patients | |
| Total Cutaneous SCC | 30/60 (50%) | 12/30 (40%) | 9/30 (30%) | DNA sequencing | Patients | |
| Bladder/urinary tract cancers | | | | | | | |
| Bladder Cancer | | | | | | | |
| Urothelial bladder carcinoma | III | | | | | | |
| Urothelial bladder carcinoma | 80% | 17% | 83% | N/A | DNA sequencing | Patients (US American) | |
| Urothelial bladder carcinoma | 66.7% | 28.6% | 71.4% | N/A | DNA sequencing | Patients (US American) | |
| Urothelial bladder carcinoma | 61.7% | 25% | 58.8% | N/A | DNA sequencing | Patients (China) | Not associated with age. |
| Urothelial bladder carcinoma | 59% | 37.5% | 62.5% | N/A | DNA sequencing, qRT-PCR | Patients (Portugal) | Not associated with age. |
| Urothelial bladder carcinoma | 65.4% | 17.8% | 81.8% | N/A | DNA sequencing, relative telomere length | Patients (Sweden) | Associated with shorter telomeres and worse OS. FGFR3 mutations found in low-grade tumors, TERTp mutations in low-grade and high-grade tumors. Reversed by rs2853669 TERT −245 A>G polymorphism. |
| Urothelial bladder carcinoma | 77.1% | 17% | 83% | Not increased | DNA sequencing, qRT-PCR | Patients | Not associated with OS, DFS, or clinical outcome. Associated with FGFR3<sup>mut</sup>. |
| Urothelial bladder carcinoma | 100% | 12% | 85% | N/A | DNA sequencing | Patients | Pure micropapillary carcinoma and urothelial cancer with focal micropapillary features. |
| Urothelial upper tract urinary carcinoma | 76.9% | 12.5% | 72.5% | N/A | DNA sequencing | Patients (China) | Not associated with age. |
| Cancer Type                        | Stage                | Prevalence of Mutations | −146 C>T | −124 C>T | Tert Upregulation | Methods                  | Sample Origin | Remarks                                                                 | Ref.    |
|-----------------------------------|----------------------|-------------------------|----------|----------|-------------------|--------------------------|---------------|--------------------------------------------------------------------------|---------|
| Urothelial upper tract urinary carcinoma | 47.4%               | 11.1%                   | 88.9%    | 8/9      | N/A               | DNA sequencing            | Patients (US American)      |                                                          | [77]    |
| Urothelial upper tract urinary carcinoma | 29.5%               | 18.5%                   | 81.5%    | 53/65    | N/A               | DNA sequencing, Detection in urine | Patients (China)             | Associated with distant metastases.                        | [118]   |
| Total Urothelial bladder & upper tract urinary carcinoma | 988/1529 (64.6%) | 186/988 (18.8%)         | 771/988 (78%) |          |                    |                           |               |                                                          |         |
| Thyroid                           |                      |                         |          |          |                   |                           |               |                                                          |         |
| Differentiated thyroid cancer     | 12.2%               | 4.9%                    | 95.1%    |          | N/A               | DNA sequencing            | Patients (Portugal)          | Only in malignant lesions.                                | [108]   |
| Papillary thyroid cancer          | 8%                  | 7.7%                    | 84.6%    |          | Yes               | DNA sequencing, qRTP-PCR, IHC | Patients (Portugal)          | Associated with older age, larger tumor size, extrathyroid invasion, advanced clinical stage. | [99]    |
| Papillary thyroid cancer          | 11.3%               | 15.2%                   | 85.8%    |          | N/A               | DNA sequencing            | Patients (China)             | Associated with BRAF-V600E mutation.                      | [98]    |
| Papillary thyroid cancer          | 27%                 | 7.7%                    | 92.3%    |          | N/A               | DNA sequencing            | Patients (Sweden)            | Only in patients >45. Correlated with shorter telomeres and distal metastases. PTC: 27% (25/332); FTC: 22% (12/70); ATC: 50% (12/36). Associated with BRAF/RAS mutations. | [106]   |
| Papillary thyroid cancer          | 4.1%                | *                      | *        |          | N/A               | DNA sequencing            | Patients (Korea)             | Associated with tumor size, stage III-IV, recurrence, decreased OS and DFS with BRAF/RAS mutations: RAS/BRAF > TERTp > RAS/BRAF + TERTp. | [100]   |
| Papillary thyroid cancer          | 11.7%               | 0%                      | 100%     |          | N/A               | DNA sequencing            | Patients (Korea)             | Only in malignant lesions.                                | [108]   |
| Papillary thyroid cancer          | 30/257              | 0/30                    | 30/30    |          | N/A               | DNA sequencing            | Patients (Korea)             | No TERTp mutation found in 192 well differentiated cancers without distant metastasis. | [105]   |
| Papillary thyroid cancer          | 37.7%               | 10%                     | 90%      |          | N/A               | DNA sequencing            | Patients (Korea)             |                                                          |         |
| Papillary thyroid cancer          | 10/27               | 1/10                    | 9/10     |          | N/A               | DNA sequencing            | Patients (US & Japan)         | More frequent in BRAF-wt patients than in BRAFmut.        | [103]   |
| Papillary thyroid cancer          | 22%                 | 44%                     | 66%      |          | N/A               | DNA sequencing            | Patients (US)                | Associated with older age (>45 years), larger tumor size, stage III-IV, distant metastases, decreased OS and DFS, rs2853669 TERT −245 A>G polymorphism (46.7% (113/242) of patients) increases OS & DFS in patients without TERTp mutations and with BRAF-V600E. | [103]   |
| Cancer Type                  | Stage | Prevalence of Mutations | -146 C>T | -124 C>T | Tert Upregulation | Methods                                                                 | Sample Origin | Remarks                                                                 | Ref.     |
|-----------------------------|-------|-------------------------|----------|----------|------------------|--------------------------------------------------------------------------|---------------|------------------------------------------------------------------------|---------|
| Papillary thyroid cancer    | 12%   | 14.6%                   | 86.4%    | Yes      | DNA sequencing   | Patients (Italy)                                                         | Associated with older age and poor prognosis. Increased cytoplasmic localization of TERT. No impact of rs2853669 TERT -245 A>G polymorphism on outcome. | [102]   |
| Total Papillary thyroid cancer | 247/1848 (13.4%) | 21/229 (9.2%) | 207/229 (90.4%) |          |                  |                                                                          |               |                                                                        |         |
| Follicular Thyroid Cancer   | 13.9% | 18.2%                   | 81.8%    | N/A      | DNA sequencing   | Patients                                                                | Only in malignant lesions. |                                                                        | [108]   |
| Follicular Thyroid Cancer   | 66.7% | 50%                     | 50%      | N/A      | DNA sequencing   | Patients (Korea)                                                        | No TERTp mutation found in 192 well-differentiated cancers without distant metastasis. | [105]   |
| Follicular thyroid Cancer   | 14%   | 22.2%                   | 77.8%    | Yes      | DNA sequencing, qRT-PCR, IHC | Patients (Portugal)                                                    | Increased prevalence in ATC: PTC: 27% (25/332); FTC: 22% (12/70); ATC: 30% (12/36). | [98]    |
| Follicular thyroid cancer   | 22%   | 12.5%                   | 87.5%    | N/A      | DNA sequencing   | Patients (Sweden)                                                       | Associated with older age, larger tumor size, extrathyroid invasion, advanced clinical stage. | [99]    |
| Follicular thyroid cancer   | 36.4% | 12.5%                   | 87.5%    | N/A      | DNA sequencing   | Patients (China)                                                        | Associated with BRAF-V600E mutation. Associated with BRAF/RAS mutations. | [106]   |
| Follicular thyroid cancer   | 14%   | 38.5%                   | 62.5%    | Yes      | DNA sequencing, WB, and IHC | Patients (Italy)                                                       | Associated with tumor size, stage III-IV, recurrence, decreased OS and DFS with BRAF/RAS mutations: RAS/BRAF>TERTp > RAS/BRAF+TERTp. | [102]   |
| Total Follicular thyroid cancer | 53/381 (13.9%) | 10/46 (21.7%) | 36/46 (78.2%) |          |                  |                                                                          | No impact of rs2853669 TERT -245 A>G polymorphism on outcome. |         |
| Poorly differentiated thyroid cancer | 21%   | 33.3%                   | 66.7%    | Yes      | DNA sequencing, qRT-PCR, IHC | Patients (Portugal)                                                    | Only in malignant lesions. |                                                                        | [52]    |
| Poorly differentiated thyroid cancer | 37.5% | 0%                      | 100%     | N/A      | DNA sequencing   | Patients                                                                | No TERTp mutation found in 192 well-differentiated cancers without distant metastasis. | [108]   |
| Poorly differentiated thyroid cancer | 29%   | 50%                     | 50%      | N/A      | DNA sequencing   | Patients (Korea)                                                        |                                                                         | [105]   |
| Cancer Type                             | Stage | Prevalence of Mutations | −146 C>T | −124 C>T | Tert Upregulation | Methods          | Sample Origin | Remarks                                                                 | Ref.       |
|----------------------------------------|-------|-------------------------|----------|----------|------------------|------------------|---------------|------------------------------------------------------------------------|------------|
| Poorly differentiated thyroid cancer   | 51.7% | 40%                     | 60%      |          | N/A              | DNA sequencing   | Patients (US & Japan) | More prevalent in advanced cancer patients with BRAF/RASmut.            | [100]      |
| Total Poorly differentiated thyroid cancer | 38/87 (43.7%) | 14/38 (36.8%)           | 24/38    |          | N/A              | DNA sequencing   | Patients       | Only in malignant lesions.                                              | [108]      |
| Anaplastic thyroid cancer              | 46.3% | 8%                      | 92%      |          | N/A              | DNA sequencing   | Patients (Portugal) | More prevalent in advanced cancer patients with BRAF/RASmut.            | [52]       |
| Anaplastic thyroid cancer              | 13%   | 50%                     | 50%      |          | Yes              | DNA sequencing   | Patients (US & Japan) | Associated with older age, larger tumor size, distant metastases and disease-related death in FTC. | [100]      |
| Anaplastic thyroid cancer              | 50%   | 0%                      | 100%     |          | N/A              | DNA sequencing   | Patients (Sweden) | PTC: 7.5% (25/332); FTC: 17.1% (12/70); ATC: 33.4% (12/36).               | [101]      |
| Anaplastic thyroid cancer              | 50%   | 20%                     | 80%      |          | N/A              | DNA sequencing   | Patients (US & China) | PTC associated with BRAF-V600E mutation in 60.3% of cases. Associated with older age and distal metastases. | [104]      |
| Anaplastic thyroid cancer              | 33.3% | 12/36                   | *        |          | N/A              | DNA sequencing   | Patients (Portugal & Spain) | −124 C>T found in 56.3% of BRAF-V600E mutated cases.                      |            |
| Anaplastic thyroid cancer              | 38.7% | 10%                     | 90%      |          | N/A              | DNA sequencing   | Patients (US & China) |                                                                 |            |
| Total anaplastic thyroid cancer        | 100/252 (39.7%) | 9/88 (10.2%)            | 79/88    |          | N/A              | DNA sequencing   | Cell lines                                                                 | [108]      |
| Thyroid Cancer cell lines              | 91.7% | 27.3%                   | 72.7%    |          | N/A              | DNA sequencing   | Cell lines                                                                 | [108]      |
| Thyroid Cancer cell lines              | 75%   | 17.7%                   | 83.3%    |          | N/A              | DNA sequencing   | ATC cell lines                                                               | [98]       |
| Liver-Hepatocellular Carcinoma (HCC)  |       |                         |          |          |                  |                  |                                                                          |            |
| HCC                                    | 31.4% | 18.2%                   | 81.8%    |          | N/A              | DNA sequencing   | Patients (China)                                                          | [57]       |
| HCC                                    | 34%   | 33.3%                   | 66.7%    |          | N/A              | DNA sequencing   | Patients (Africa, Asia, Europe)                                            | [97]       |
| HCC                                    | 44.3% | 3.7%                    | 96.3%    |          | N/A              | DNA sequencing   | Detected in both HBV-associated and HBV-independent HCC                   | [77]       |

Cells 2020, 9, 749

[76x532]Cells 2020, 9, 749

[110x532]Cells 2020, 9, 749

[84x493]Cancer Type Stage Prevalence of Mutations −146 C>T −124 C>T Tert Upregulation Methods Sample Origin Remarks Ref.
| Cancer Type | Stage | Prevalence of Mutations | 146 C>T | 124 C>T | Tert Upregulation | Methods | Sample Origin | Remarks |
|-------------|-------|-------------------------|---------|---------|------------------|---------|---------------|---------|
| HCC         |       | 48.5% 65/131            | 3.1%    | 96.9%   | N/A              | DNA sequencing | Patients (Italy) | 41% of mutations in HBV-associated HCC. 53.6% mutations in HCV-associated HCC. All heterozygous. No −57 A>C. HBV-associated HCC. Correlated with age, not with HBV status. Found in 4/7 preneoplastic lesions (HBV-associated HCC). Associated with older age. |
| HCC         |       | 31% 85/275              | 1.1%    | 98.9%   | Yes              | DNA sequencing, IHC | Patients (China) | Associated with shorter OS and DFS. Associated with HCV infection and excluded from HBV+ HCC. |
| HCC         |       | 65.4% 68/104            | 3%      | 97%     | Yes              | DNA sequencing | Patients (Japan) | Detected in cirrhotic preneoplastic macronodules (25%) and cirrhotic adenomas (44%), at last step of malignant transformation into HCC. Absent from HBV-associated tumors 2/179 (1%) −146 C>T. Associated with older age. No impact on overall survival. Excluded from HBV-associated HCC. Higher frequency in HCV-associated HCC. −57 A>C mutation detected in 1.6%. |
| HCC         |       | 58.6% 179/305           | 6.1%    | 92.7%   | Yes              | DNA sequencing, qRT-PCR | Patients (French) | Present in 37% HBV-associated HCC but mutually exclusive with HBV sequence integration. Mutually exclusive with TERT CNV and ATRX mutations. Associated with HCV infection (64% or TERTp mutations). Associated with Wnt pathway mutations. |
| HCC         |       | 29.3% 57/195            | 5.3%    | 94.7%   | No               | DNA sequencing, qRT-PCR | Patients (Japan, US-European ancestry) | |
| HCC         |       | 54% 254/469             | 4.3%    | 93%     | N/A              | DNA sequencing | Patients (Japan, US-European ancestry) | |
| Total HCC   |       | 770/1634 (47.1%)        | 39/770  (5%) | 722/770 (93.7%) | DNA sequencing | Cell lines | |

Cervical

| Cancer Type | Stage | Prevalence of Mutations | 146 C>T | 124 C>T | Tert Upregulation | Methods | Sample Origin | Remarks |
|-------------|-------|-------------------------|---------|---------|------------------|---------|---------------|---------|
| Cervical SCC |      | 21.8% 22/101            | 31.8%   | 45.5%   | Yes              | qRT-PCR | Patients (Italian women) | 75% TERTp mutations in HPV-negative samples. −124 C>T 6/22 were TT homozygous. −146 C>T 2/8 were TT homozygous. |
| Cervical SCC |      | 21.4% 30/140            | 26.7%   | 73.3%   | N/A              | DNA sequencing, Association with clinical status | Patients (Indian women) | |
| Cancer Type                   | Stage          | Prevalence of Mutations | $\sim 146 \text{ C>T}$ | $\sim 124 \text{ C>T}$ | TERT Upregulation | Methods                          | Sample Origin       | Remarks                                                                 | Ref. |
|------------------------------|----------------|-------------------------|-------------------------|-------------------------|-------------------|----------------------------------|---------------------|--------------------------------------------------------------------------|------|
| Cervical SCC                 |                |                         |                         |                         | N/A               | DNA sequencing                   | Patients (US American) | 1 patient with $\sim 125 \text{ C>A}$ mutation.                     |      |
| Total Cervical SCC           | 53/263 (20.1%) | 16/53 (30.2%)           | 32/53 (60.4%)           |                         |                   |                                  |                     |                                                                          | [77] |
| Head and Neck Squamous Cell Carcinoma (HNSCC) |      |                         |                         |                         |                   |                                  |                     |                                                                          |      |
| HNSCC                        | 31.7%          | 30.8%                   | 69.2%                   | N/A                     | DNA sequencing    | Patients (Indian women)           | Association with clinical status.                                   |      |
|                              | 13/41          | 4/13                    | 12/13                   |                         |                   |                                  |                     |                                                                          | [36] |
| HNSCC                        | 17%            | 16.7%                   | 83.3%                   | N/A                     | DNA sequencing    | Patients (US American)           | 11/12 HNSCC with $TERTp$ mutations were in the oral tongue, and 11/23 (47.8%) of HNSCC of the oral tongue harbored $TERTp$ mutations. |      |
|                              | 12/70          | 2/12                    | 10/12                   |                         |                   |                                  |                     |                                                                          | [77] |
| Total HNSCC                  | 25/111 (22.5%) | 6/25 (24%)              | 19/25 (76%)             |                         |                   |                                  |                     |                                                                          |      |
| Ovarian cancer               |                |                         |                         |                         |                   |                                  |                     |                                                                          |      |
| Ovarian clear cell carcinoma | 15%            | 0%                      | 10%                     | N/A                     | DNA sequencing    | Patients (US American)           | 1 patient with $\sim 124 \text{ C>A}$ mutation.                     | [77] |
|                              | 3/20           | 0/3                     | 2/3                     |                         |                   |                                  |                     |                                                                          |      |
| Ovarian clear cell carcinoma | 16.5%          | 8.1%                    | 91.9%                   | N/A                     | DNA sequencing, IHC, telomere length evaluation | Patients $TERTp$ mutations tended to be mutually exclusive with loss of ARID1A protein expression and PIK3CA mutation. | [132] |
|                              | 37/233         | 3/37                    | 34/37                   |                         |                   |                                  |                     |                                                                          |      |
| Ovarian clear cell carcinoma | 30%            | 0%                      | 100%                    | Yes                     | qRT-PCR           | Cell lines                       | No link with survival or age.                                      | [132] |
|                              | 3/10           | 0/3                     | 3/3                     |                         |                   |                                  |                     |                                                                          |      |
| Total ovarian clear cell carcinoma | 43/263 (16.3%) | 3/43 (6.9%)             | 39/43 (90.7%)           |                         |                   |                                  |                     |                                                                          | [132] |

N/A: not assessed; *: data not available. TERT: telomerase reverse transcriptase; GBM: glioblastoma multiforme; SCC: squamous cell carcinoma; HNSCC: head and neck squamous cell carcinoma; HCC: hepatocellular carcinoma; GI: gastrointestinal; UC: urothelial cancer; MPC: micropapillary carcinoma; HPV: Human papilloma virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus; PTC: papillary thyroid cancer; FTC: follicular thyroid cancer; ATC: anaplastic thyroid cancer.
4. Cancer Bias of TERTp Mutations

TERTp mutations have been recorded in individuals of Caucasian, African, and Asian descent, with no race-related bias. The −124 C>T mutation has an overwhelmingly higher prevalence than the −146 C>T mutation in all cancers, with the exception of skin cancers, where both hotspots are mutated with comparable frequencies (Figure 2 and Table 1). Although both −124C>T and −146C>T mutations generate identical sequences, enable binding of GABPA, and are equally efficient in increasing TERT transcription in vitro [57,69], in vivo, the −124 C>T mutation was associated with higher TERT mRNA in GBM [57,112]. This would suggest that the Ets/TCF binding site at position −124 provides a more favorable or accessible hotspot for the transcriptional machinery [109]. The overrepresentation of the −146 C>T mutation in skin cancers hints at different etiologies of TERTp mutations. TERTp mutations in melanoma and non-melanoma skin cancers have been attributed to UV damage [49,51,55,88–91,116], which triggers C→T transitions at CC dinucleotides [55,127]. Nevertheless, C→T transitions where C is preceded by C also conform to the preferred target of Apolipoprotein B mRNA Editing Catalytic Polypeptide-like (APOBEC)3A/B de-aminations and to aging mutations [127,133]. APOBEC3 mutations are highly prevalent in ovarian and HPV-associated cervical and oral SCC [125–127], as well as in HCC and in cirrhotic lesions [121,134]. A role for APOBEC and aging-associated de-aminations is consistent with potentially increased accessibility of the −124 position to DNA binding proteins and with the association of TERTp mutations with older age at diagnosis in GBM, melanoma, and PTC [52,57,60,63,64,77,79,80,82,86,88,98,100–102]. These observations therefore raise the possibility that UV-driven lesions account for TERTp mutations in skin cancers, while APOBEC and age-driven de-aminations account for the −124 C>T mutation in other cancers. Further epidemiological and mechanistic studies are needed to shed light on this point.

**Figure 2.** Distribution of TERT promoter mutations in different cancers.
The −139/−138 CC>T tandem mutation is very infrequent, limited to skin cancers, and has been associated with lower DFS. This tandem mutation has been suggested to favor chromosomal instability [51].

5. Exclusiveness of TERTp Mutations

Aside from non-melanoma skin cancers [90], TERTp mutations are mostly monoallelic. This suggests that TERT reactivation on one allele is probably sufficient to ensure telomere maintenance or elongation in cancer cells [54]. In line with this observation, TERTp mutations appear to be mutually exclusive [50]. Likewise, TERTp mutations are generally absent from cancers where telomere elongation is ensured by ALT [77,79,80,98] or TERT copy-number duplications [38,121]. TERTp mutations are also less frequent in cancers where viral transformation or viral oncogenes reactivate TERT transcription, such as HBV-DNA or high-risk HPV16/18 E6 [30,32,33,36,37,62,95,96,121,122]. These observations reinforce the concept that, despite some exceptions [38,89,111,117], tumors generally rely on one mechanism for telomere maintenance. The reasons for such selectivity remain speculative to date. One possible explanation is that there is a threshold for TERT expression, above which the biological advantage is lost.

Consistent with this view, Phosphatidyl Inositol Kinase 3 (PIK3) CA and PIK3 Receptor 1 (PIK3R1) mutations are recorded in 50% of GBM with wt TERTp and tend to be mutually exclusive with TERTp mutations in ovarian clear cell carcinoma [79,86,132]. The PIK3CA/Akt signaling pathway is involved in cellular self-renewal in embryonic stem cells and cancer stem cells [135], as well as in TERT Ser227 and Ser824 phosphorylation, subsequent nuclear translocation, and cellular transformation [25–28]. Mutual exclusion of PIK3CA and TERTp mutations suggests that activation of the PIK3CA/Akt pathway or of TERT confer cells a similar growth and proliferative advantage. In the absence of TERT reactivation, other telomere maintenance mechanisms, such as ALT, can achieve immortalization [27]. Indeed, TERT also contributes to cell survival and proliferation through telomere-independent mechanisms; it facilitates Wnt/β-catenin-dependent [136,137], c-myc-dependent [138,139], and NF-κB-dependent gene transcription [140,141], and DNA damage responses [144,145], and protects cells from Endoplasmic Reticulum (ER) stress and apoptosis by buffering Reactive Oxygen Species (ROS) and modulating mitochondrial function [145–151]. It is highly likely that TERT homeostasis is also tuned by these functions within a given tumor type and microenvironment, and by related metabolic alterations that need to be preserved.

6. Discussion

Hints for a model come from the observation that overall, TERTp mutations are associated with late-stage disease in GBM, melanoma, urothelial, and thyroid carcinoma [49,52,60,61,66,85,98,100,101,103–105,112,118] and with the last steps of hepatocellular transformation [62,95]. They often occur with or after mutations in pathways associated with cell growth and proliferation. In GBM, TERTp mutations coexist with EGFR amplification [64,77,111], and in urothelial bladder carcinoma, they are associated with FGFR3 (Fibroblast Growth Factor Receptor 3) mutations [61,94]. In ~50% of melanoma, urothelial, and thyroid cancers, TERTp mutations coexist with the common BRAF-V600E mutation [52,88,89,105,106,108,116,152]. GFR and BRAF/RAS kinases control the MAPK and PI3K-Akt pathways that lead to cell growth, survival, and angiogenesis. Constitutive activation of the GFR/FGFR-BRAF/RAS pathway leads to constitutive cell growth and division [153]. Mutations in these oncogenes are often detectable in low-grade tumors and probably precede TERTp mutations [22,61,77,112]. The picture is even more clear-cut in HCC, where mutations in β-catenin (CTNNB1) neatly precede TERTp mutations during the process of malignant transformation [62,95,120]. β-catenin is involved in cell adhesion and interacts with Wnt, promoting cell growth and division. The proliferative advantage conferred by driver mutations in these pathways leads to accelerated telomere erosion. Accordingly, most tumors display telomere dysfunction and shortened telomeres,
which leads to chromosome instability [10,22,61,66,98,112,115]. In this scenario, TERT reactivation regenerates telomeres sufficiently to maintain them above the critical threshold and to stabilize the tumor genome [3,18,145]. This interpretation is consistent with the association of TERTp mutations with shortened telomeres and with age as in PTC, melanoma, and GBM/glioma, since cells from younger patients or with sufficiently long telomeres do not need to rely on telomerase reactivation to overcome telomeric crisis [10,29,57,77,85,98,101,115]. Partial telomere healing is coherent with a modest increase in TERT expression (2- to 4-fold) and with a single genetic mechanism of telomere elongation. It likely reflects an exquisite balance between escape from apoptosis resulting from telomere attrition and genomic instability, and cell sustainability in terms of oxygen and nutrient supplies.

Intriguingly, it was recently reported that GABPA controls the cell cycle and induces cell differentiation, thus acting as a tumor suppressor regulating cell proliferation, stemness, and adhesion. It decreased tumor invasiveness and distal metastases in PTC, HCC, and bladder carcinoma [154–156]. GABPA levels were decreased and even negatively associated with TERT expression in PTC [154–156]. One possible explanation is that other Ets/TCF family transcription factors bind TERTp mutations. Alternatively, the decrease in GABPA expression may follow rather than precede TERTp mutations. In this case, it would be a cellular adaptation which confers a selective advantage to TERTp-mutated (and GFR/BRAF/RAS-mutated) cells by containing TERT reactivation within sustainable limits. Decreased GABPA could also be an adaptation to the TERT-induced proliferation, stemness, and invasion to avoid contradictory signals. Further studies establishing the order of emergence of these mutations would be needed to shed light on this matter.

Taken together, these observations point to a fine tuning of TERT homeostasis and suggest that there is a narrow kinetic and quantitative window for TERT expression. Below that window, cells succumb to telomere crisis and DNA damage. Above that window, cells succumb to overwhelming genetic alterations or metabolic needs. This frailty could be exploited through strategies aiming to push cells either way beyond the threshold of TERT tolerability.

7. Concluding Remarks

TERTp mutations have only been described recently; however, they have prompted an impressive number of studies which draw a comprehensive picture of their prevalence across cancers, as well as providing clues on their mechanisms of action and their associated constraints. They have been proposed as potential biomarkers with predictive and treatment-orienting value. However, more structured studies are needed to validate their clinical potential, particularly since they appear at different stages in different malignancies, ranging from preneoplastic cirrhotic lesions to late stage GBM or melanoma with distal metastases. Cancer cells only require one mechanism of telomere maintenance. This underscores the key role of telomere stabilization in the process of transformation, as well as the necessity of maintaining an exquisitely balanced TERT homeostasis to achieve tumor cell selection, adaptation, and sustainability. TERT is a target of choice in antitumor strategies due to its reactivation in numerous cancers. A better understanding of TERT regulation, homeostasis, and functions could help to overcome the shortcomings of prior genetic and immunotherapy-based approaches targeting TERT.

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Abbreviations

ALT  Alternative lengthening of telomeres
ATC  Anaplastic thyroid carcinoma
ATRX α-Thalassemia/mental retardation syndrome X-linked
BCC  Basal cell carcinoma
CNS  Central nervous system
CNV  Copy number variant
DAXX Death-domain-associated protein
DFS  Disease-free survival
DTC  Differentiated thyroid carcinoma
EGFR  Epidermal growth factor receptor
FTC  Follicular thyroid carcinoma
GBM  Glioblastoma multiforme
HBV  Hepatitis B virus
HBx  Hepatitis B X protein
HCC  Hepatocellular carcinoma
HCV  Hepatitis C virus
HNSCC  Head and neck squamous cell carcinoma
HPV  Human papillomavirus
IDH  Isocytrate dehydrogenase
OS  Overall survival
PDTC  Poorly differentiated thyroid carcinoma
PTC  Papillary thyroid carcinoma
ROS  Reactive oxygen species
SCC  Squamous cell carcinoma
TERT  Telomerase reverse transcriptase
TERTp  TERT promoter
TF  Transcription factor
TMZ  Temozolomide
TSS  Translational start site

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