High frequency of *TERT* promoter mutations in human cancers

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Abstract Reactivation of telomerase has been implicated in human tumourigenesis, but the underlying mechanisms remain poorly understood. Here, we report the presence of recurrent somatic mutations in *TERT* promoter in cancers of the central nervous system, (43%), bladder (59%), thyroid (follicular cell-derived) (10%) and skin (melanoma, 29%). We also show that, in central nervous system and thyroid cancers, the presence of *TERT* promoter mutations is associated with increased telomerase and *TERT* mRNA expression, respectively, and older age of the patients. Our results show that *TERT* promoter mutations are frequently found in specific types of human cancers, where they lead to enhanced expression of telomerase/*TERT*.

Introduction

Reactivation or re-expression of telomerase is referred to be a widespread feature in human cancers, although the genetic basis remains poorly understood in many cancer types. Somatic mutations in the coding region of *TERT* (human telomerase reverse transcriptase) are infrequent in human tumours, but germline and somatic mutations in *TERT* promoter were recently found in a high percentage of human melanomas and human cancer cell lines[1,2]. Such mutations occurred in two hotspot positions, located -124bp and -146bp upstream from the ATG start site (-124G>A and -146G>A, C>T on opposite strand) and conferred enhanced *TERT* promoter activity[1,2], by putatively generating a consensus binding site (GGAA) for ETS transcription factors within the *TERT* promoter region[1,2].

Our aim was to investigate whether the aforementioned *TERT* promoter mutations were present in cancer types other than melanoma, having for that matterscreened 741 primary tumours from the thyroid, kidney, bladder, gastrointestinal stroma (GISTs), adrenal medulla (phaeochromocytomas) and central nervous system (CNS). Besides skin melanoma, we have also included ocular melanoma (not studied in the previous reports), benign lesions of the thyroid and skin and 58 human cancer-derived cell lines. Our results highlight *TERT* promoter mutations as frequent events in specific types of human cancers.

Results

Overall, *TERT* promoter mutations were found in 142 (19%) human tumour samples (Table 1, Fig. 1A) and in 14 (24%) human cell lines (Supplementary Fig.1, Supplementary Table 1 and 2). The -124bp mutation was the most frequent, being present in 99 cases, whereas the -146bp was present in 43 cases. The -124G>A and -146G>A mutations were found in a mutually exclusive fashion. Two tandem GG>AA mutations at positions -124/-125bp and -138/-139bp were observed in one cell line each (Fig. 1, bottom, Supplementary Table 1). *TERT* promoter mutations were not detected in normal thyroid tissue or in benign lesions (nevi, thyroiditis, goitres and adenomas). No mutations were detected in 26 kidney cancers, 17 phaeochromocytomas, and 36 GISTs.

We have analysed both skin and ocular melanomas, a type of melanoma that was not previously studied[1,2]. *TERT* mutations were present in 16 out of 56 (29%) skin melanomas, but absent in the 25 ocular melanomas (Table 1). All the 4 skin melanoma-derived cell lines but none of the 6 ocular melanoma-derived cell lines harboured *TERT* mutations (Supplementary Table 1 and Supplementary Fig. 1). Ten out 16 (63%) skin melanomas with *TERT* mutation also harboured the *BRAFV600E* mutation whereas the *BRAF* mutation was present in 9 out 38 (24%) of the melanomas without *TERT* mutation (p=0.01) (Supplementary Fig. 2).

In CNS tumours *TERT* promoter mutations were found in 43% (51/118) of all cases with an equal prevalence of both mutations (Table 1, and Supplementary Table 4). The frequency of *TERT* mutations was different according to the tumour histology and grade (Fig 1C): while pilocytic astrocytomas (WHO grade 1) and diffuse astrocytoma (WHO grade 2) showed a lower frequency of mutations (8% and 15% respectively), the most aggressive form, glioblastoma multiforme (GBM; WHO grade 4) present the highest frequency of *TERT* mutations (62%). Oligodendrogliomas (WHO grade 2) and anaplastic oligodendrogliomas (WHO grade 3) also harboured a high frequency of *TERT* mutations (45% and 54% respectively).

The presence of *TERT* mutations was significantly associated with a higher mean age at diagnosis (Supplementary Table 5) in CNS patients as a whole

(p<0.0001) and also in GBM patients only (p=0.0247); in oligodendroglioma patients, we found the same trend, although not statistically significant (p=0.0709). Additionally, we observed that, in a subset of 14 GBM (8 TERT wt tumours and 6 TERT mutated tumours), the tumours with TERT mutations showed increased expression of telomerase (Fig. 2).

We analysed a large series of follicular cell-derived thyroid samples including normal thyroid (n=27), benign (n=81) and malignant lesions (n=263) (Supplementary Table 6). *TERT* mutations were only detected in malignant tumours (10%) (Fig. 1B), namely in 11% of papillary thyroid carcinomas (PTC), 14% of follicular thyroid carcinomas (FTC), 21% of poorly differentiated thyroid carcinoma (PDTC) and 13% of the anaplastic carcinomas. Within the group of PTC, *TERT* mutations were detected associated to the in the so-called conventional PTC (cPTC) (11%).The majority of *TERT*-mutated thyroid cancers harboured the -124G>A mutation (22 out of 27 cases) (Figure 1B, Supplementary Table 7). In thyroid cancer patients, *TERT* mutations were significantly associated with guarded prognosis features such as larger tumours (p=0.008), older patients (p<0.0001) and male gender (p=0.03) (Supplementary Table 5). After histotype stratification, such correlations were only kept in the group of cPTC where we also found significant associations with lymph node metastasis (p=0.03) and *BRAFV600E* mutation (p=0.001) (Supplementary Table 8). Accordingly, we verified by quantitative RT-PCR that *TERT* mRNA is expressed at variable amounts in thyroid tumours, and the ones with coexistent *TERT* and BRAF mutations showed the highest levels of *TERT* mRNA expression (p<0.005; Figure 3).

In bladder cancer, *TERT* mutations were frequently detected (48/82; 59%) both in low grade tumours (14/21; 67%) and in high grade tumours (34/61; 56%) (Table 1 and Fig. 1D; Supplementary Table 9). The -124G>A mutation was present in 30 cases while the -146 G>A was found in 18 cases. No association was found between the presence of the mutation and the age of the patients. No mutations were detected in 26 kidney cancers, 17 non-metastatic phaeochromocytoma, and 36 non-metastatic GISTs (Supplementary Table 10, 11 and 12, respectively).

Discussion

From recent findings TERT promoter mutations arise as a novel mechanism of telomerase reactivation/expression in human cancers[1,2]. In this work we report for the first time the presence of these mutations in different histotypes of thyroid cancer and our results validate the findings reported by others in melanoma, bladder and gliomas[1-3]. Furthermore we present the first evidence that TERT promoter mutations are associated with increased telomerase expression in gliomas and with increased *TERT* mRNA expression in thyroid cancers. In the latter, TERT mRNA was particularly high in cases harbouring both TERT and BRAF mutations.

The location of these mutations in the TERT promoter, rather than the coding region of the gene, creating additional binding sites for transcription factors, represents also a novel mechanism of genetic activation in cancer.

The frequency of *TERT* mutations we detected in skin melanomas is similar to the one reported by Horn et al. in primary melanomas (33%)[1], but lower than the frequency found by the same authors in metastatic melanomas (85%) and by Huang et al.[2] in a series of 70 melanomas and short term cultures[1,2]. In our series, we have analysed few metastatic melanomas that did not show a significantly higher percentage of *TERT* mutations. Since our series is too small to allow a conclusion, it remains to be verified if there is an increased frequency of TERT mutations in metastatic melanomas, as suggested by previous reports[1,2].

We have confirmed, in skin melanomas, the significant association between TERT mutations and BRAF mutations, as previously advanced by Horn et al.[1] and that we now also demonstrate in thyroid cancers (see below). Interestingly, in benign nevi, that frequently harbour *BRAF* mutations, we did not detect *TERT* mutations. This finding, together with the high frequency of TERT mutations in metastatic melanomas[1] suggests that, while *BRAF* mutations are thought to be an early event in melanoma genesis, *TERT* mutations may occur at a later stage.

No TERT promoter mutations were found in ocular melanomas, which, at variance with skin melanomas, harbour *GNAQ* mutations instead of *BRAF* mutations[4,5]. Our results thus support the assumption that ocular melanomas result from different etiopathogenic mechanism than skin melanomas[5,6].

Our data on CNS tumours indicate that TERT promoter mutations are frequent events in gliomas, particularly in GBM where the highest mutation frequency was found. While others[1,2] had shown that such mutations conferred enhanced TERT promoter activity *in vitro*, we present evidence that GBM with TERT promoter mutations have increased telomerase expression. Our results also fit with those of Lotsch et al.[7] who reported that 60% of GBM were positive for *TERT* mRNA and telomerase activity[7]. It will be interesting to assess whether the GBM positive for telomerase activity described by Lotsch et al.[7], also harbour *TERT* promoter mutations. Our findings also indicate that TERT mutations are associated with older patients, in accordance with a recent report[3]. The high frequency of *TERT* mutations in CNS tumours, particularly in intermediate and high grade tumours, opens a window for new approaches in the therapy of CNS tumours.

In thyroid cancer *TERT* mutations were only found in follicular cell-derived cancers (PTC, FTC, PDTC and ATC). No mutations were detected in medullary thyroid carcinomas, as advanced by Killela et al.[3], or in normal thyroid, and benign lesions, such as goitre, adenomas or thyroiditis; this finding fits with previous studies that reported telomerase expression in malignant lesions and not in normal tissue or hyperplastic lesions[8,9]. *TERT* mutations were associated with clinico-pathological features (older age, increased tumour size and male gender) but, after histotype stratification, these associations were only maintained in cPTC. Studies in larger series will be necessary to clarify these associations. In cPTC *TERT* mutations were associated with *BRAF* mutation, highlighting the coexistence of activation of *BRAF* and *TERT*, previously reported in melanoma[1] (Supplementary Fig. 2). Our results reinforce the hypothesis of a link between *BRAF* activation and telomerase expression, which can be mediated by transcription factors binding to the newly created consensus binding sites, as previouslyadvanced by Horn et al[1]. Both in thyroid carcinoma and in melanoma it seems that a “background” status of activated

BRAF enhances the effects of *TERT* promoter mutation. Our results in TERT mRNA expression strengthen this assumption, showing, for the first time, an increased TERT expression in tumours harbouring BRAF and TERT mutation (Fig 3). Further observational and mechanistic studies are needed to clarify these points.

TERT alterations seem to constitute an early and frequent event in bladder cancer. These findings fit with the high prevalence of telomerase activity previously described in bladder tumours[10]. The recurrent hotspot mutations in *TERT* were advanced to be, in melanomas, induced by UV radiation[2]. Bladder is a target for several chemical carcinogens; it remains to be found whether *TERT* mutations can also result from the action of such agents. The identification of *TERT* mutations in urine may provide a biomarker for early diagnosis and monitoring of bladder cancer.

No TERT mutations were detected in kidney cancers, nor in 17 phaeochromocytoma, and 36 GISTs. It remains to be clarified the underlying reasons for the tissue specificity of *TERT* mutations and to be confirmed the association of their presence with malignancy. We can hypothesized that TERT mutations can be present in two settings. As advanced by Killela et al.[3], TERT mutations can be relevant in tissues with relative low rates of self-renewal, which fits with our findings in follicular cell-derived thyroid cancer and gliomas where they are associated with the older age of the patients. On the other hand, these mutations can also result from environmental factors such as UV radiation and chemical carcinogens as suggested by their high frequency in melanoma, bladder and tongue[1-3]. In summary, our data identifies *TERT* mutations as common events in human cancers and support the assumption[2] that *TERT* promoter mutations may be one of the mechanisms that underlies telomerase reactivation in several types of human tumours.

Material and methods

All the procedures described in this study were in accordance with national and institutional ethical standards.

Human cancer samples

Nevi and melanoma samples - Formalin-fixed, paraffin-embedded tissues from 9 cases of sporadic nevi, 56 cases of sporadic skin melanomas and 25 cases of ocular melanoma were retrieved from the files of the Hospital S. João (HSJ)/Medical Faculty of Porto (FMUP), Porto, Portugal, the Department of Pathology of HSJ and the Department of Pathology of Hospital S. Marcos (HSM), Braga, Portugal. Clinico-pathological and follow-up data were retrieved from the files of the Department of Pathology, Department of Dermatology and Oncology Registry of HSJ, Department of Pathology of HSM, and from RORENO (Oncology Registry of North Region). All skin melanoma cases were re-evaluated and staged according to the seventh edition of the AJCC[11]. The overall female to male ratio was 1.2:1. The mean age of the patients was 58 years for females (SD±17.6), with a range from 9 to 94 years, and 62 years for males (SD±13.1), with a range from 33 to 79 years.

Follow-up data were available for 53 patients, including the diagnosis of metastases, through the evaluation of the patients or direct interview with their relatives and by review of in-hospital patient files/RORENO. The mean follow-up time of patients was 41 months (range 1 - 170). During follow-up, 5 (9%) patients developed (lymph node, liver or brain) metastases and 8 (15%) patients died due to malignant melanoma. The remaining patients were alive and without evidence of melanoma recurrence at the last follow-up. Additional information regarding the melanoma subtype, age, gender, thickness and molecular analysis of *TERT*, *BRAF* and *NRAS* is compiled in supplementary Table 3.

Thyroid samples - Formalin-fixed, paraffin-embedded tissues from 372 tumours and tumour-like lesions of the thyroid and 27 normal thyroids were collected from the files of IPATIMUP, HSJ/FMUP and the Portuguese Institute of Oncology (IPO-C), Coimbra, Portugal. The histology of all tumour samples was revised and the final classification was made according to the WHO criteria[12]. In supplementary Tables 6 and 8 we have summarized the information regarding histological classification of the lesions, gender, mean age of the patients, molecular data and the size of the tumours.

Central Nervous System samples – Representative formalin-fixed paraffin-embedded samples from 118 gliomas were retrieved from pathology archives of the Department of Pathology of HSJ/FMUP and the Department of Pathology of Hospital S. Marcos (HSM), Braga, Portugal. The tumours were reviewed and classified according to the WHO classification of CNS tumors[13,14] (, 2007). This cohort includes lesions that were classified as pilocytic astrocytoma (n=13), astrocytoma grade 2 (n=20), oligodendroglioma (n=22), anaplastic oligodendroglioma (n=24) and glioblastoma (n=39). Further information regarding the CNS cases is available in supplementary Table 4.

Bladder - Formalin-fixed, paraffin-embedded tissues were collected from 82 patients with non-muscle invasive bladder cancer who underwent transurethral resection of the bladder malignant tumours in the Portuguese Institute of Oncology (IPO-P)-Porto. Haematoxylin-eosin (H&E)-stained sections were reviewed according to standard histopathological examination by two independent pathologists. Staging and grading

were conducted according to the American Joint Committee on Cancer[11] and to the 2004 WHO classification systems[15]. Supplementary Table 9 summarizes the clinic-pathological parameters.**Kidney** - Formalin-fixed, paraffin-embedded tissues from 26 kidney cancers were collected from the HSJ/FMUP. Cases were classified as clear cell renal cell carcinoma (n=12), chromophobe renal cell carcinoma (n=4) and papillary renal cell carcinoma (n=10). Information addressing the diagnosis, age, gender, nuclear grade and staging is obtainable in Supplementary table 10.

Adrenal – Formalin-fixed, paraffin-embedded tissues from 17 pheochromocytomas were collected from the Hospital de S. João/Medical Faculty of Porto, Porto, Portugal; IPATIMUP, Porto, Portugal. Supplementary Table 11 summarizes the clinic-pathological parameters.

GIST - Formalin-fixed, paraffin-embedded tissues from 36 GIST were collected from the HSJ/FMUP and IPATIMUP, Porto, Portugal. Tumours were classified according to WHO pathological classification[16] and the parameters analysed in each case included: age, gender, and tumour size. These data and the molecular characterization of these tumors can be observed in supplementary Table 12.

Cell lines - DNA from 58 cell lines deposited in IPATIMUP cell line bank was retrieved. All the cell lines were authenticated using DNA profile analysis, obtained with the PowerPlex 16 system (Promega, Madison, USA), according to ATCC and HSRRB available DNA profiles.

DNA extraction

DNA from formalin-fixed, paraffin-embedded tissues was retrieved from 10 micrometres cuts after careful micro dissection. DNA extraction was performed using the Ultraprep Tissue DNA Kit (AHN Biotechnologie, Nordhausen, Germany) following the manufacturer's instructions.

RNA Extraction

Total RNA was extracted from 27 frozen thyroid tumours (n=24) and normal tissue specimens (n=3) using a Trizol commercial kit (Life Technologies, GIBCO BRL, Carlsbad, CA) according to the manufacturer's protocol. RNA was quantified spectrophotometrically, and its quality was checked by analysis of 260ηm/280ηm and 260ηm/230ηm ratios.

PCR and Sanger sequencing

Coding regions of *BRAF, GNAQ HRAS, NRAS, cKIT* and *PDGFR* were screened for mutations in DNA extracted from paraffin blocks using PCR and Sanger sequencing. The genetic characterization of part of the tumours had already been previously reported. Refer to these works for primers and PCR conditions information[5,17,18]. To screen for *TERT* promoter mutations, we analysed the hotspots previously identified by PCR followed by Sanger sequencing. *TERT* promoter mutation analysis was performed with the pair of primers FwTERT: CAGCGCTGCCTGAAACTC; and RwTERT: GTCCTGCCCCTTCACCTT. Amplification of genomic DNA (25–100 ng) was performed by PCR using the Qiagen Multiplex PCR kit (Qiagen, Hilden, Germany) following the manufacturer instructions. Sequencing reaction was performed with the ABI Prism BigDye Terminator Kit (Perkin-Elmer, Foster City, California) and the fragments were run in an ABI prism 3100 Genetic Analyser (Perkin-Elmer). The sequencing reaction was performed in a forward direction, and an independent PCR

amplification/sequencing, both in a forward and reverse direction, was performed in positive samples or samples that were inconclusive.

Quantitative RT-PCR

q RT-PCR for human TERT was performed in 28 thyroid samples, 24 tumours and 3 normal tissue specimens. We also included a normal reference that was produced by pooling RNAs from 9 samples of normal thyroid tissue[19].

For cDNA preparation, 1 µg of total RNA was reverse transcribed using the RevertAid® first strand cDNA synthesis kit (Fermentas, Burlington, ON, Canada). Reverse transcription products were amplified for the hTERT by real-time quantitative PCR (IDT, #HS.PT.56a.40988589) using TaqMan® PCR Master Mix (Applied Biosystems, Foster City, CA, USA) with TBP gene (TATA-binding protein) as endogenous control (Applied Biosystems, #4326322E-0705006). The ABI PRISM 7500 Fast Sequence Detection System (Applied Biosystems) was used to detect the amplification level and was programmed to an initial step of 2 minutes at 50°C, 10 minutes at 95°C, followed by 45 cycles of 95°C for 15 seconds and 60°C for 1 minute. huTBP and hTERT amplification were done in triplicate using 1µl of cDNA (~50ng) for each sample. The relative quantification of target genes was determined using the ΔΔCT method witch was previously validated by Livak's Linear Regression Method (Slope=0.0696) (Sequence Detector User Bulletin 2, Applied Biosystems). Primers used for qPCR are available at the manufacturer's website

Immunohistochemistry (IHC)

Immunohistochemistry (IHC) for telomerase was performed in representative tumor tissue sections of 14 glioblastomas. Briefly, deparaffinised and rehydrated sections were subjected to microwave treatment in 10 mM sodium citrate buffer, pH 6.0 for antigen retrieval. The sections were incubated overnight at 4ºC in a humidified chamber with the primary antibody hTERT (polyclonal, rabbit, 1:500) from Rockland Immunochemicals Inc., Gilbertsville, PA. The detection was performed with a labeled streptavidin-biotin immunoperoxidase detection system (Thermo Scientific/Lab Vision, Fremont, USA) and the immunohistochemical staining was developed with DAB (3,3'-diaminobenzidine) substrate. Omission of the primary antibody incubation was used as negative control. Previously tested liver cancer case was used as positive control. IHC evaluation was performed independently by two observers (VM and JL). An IHC score was established, which corresponded to the product of the intensity of expression (absent=0, faint=1, moderate=2 strong=3) with the tumour extent of protein expression (0-25%=0, 26%-50%=1, 51%-75%=2, >75%=3).

Statistical analysis

Statistical analysis was conducted with StatView for Windows, version 5.0 (SAS Institute, Cary, NC). The results are expressed as a percentage or mean±SD. Statistical analysis was performed both on the whole series and considering the different groups of lesions. For the analysis of the relationship between patients' age and *TERT* status of the tumours, we used the unpaired t-test and ANOVA. Fisher's exact test was used in the statistical analysis of the other parameters. Graphs and figures were done in GraphPad v6.0. Results were considered statistically significant whenever $P<0.05$.

Legend to Figures:

Figure 1. Frequency and schematic illustration of TERT promoter mutations in human cancers. Graphics (A) depicts the overall frequency of TERT mutations in the four tumour types where TERT mutations were detected. The frequency and type of TERT mutations in different histological subtypes of thyroid (B), CNS (C) and bladder (D) tumours is also shown.
Abbreviations: PTC-papillary thyroid carcinoma; FTC-follicular thyroid carcinoma; PDTC-poorly differentiated thyroid carcinoma; ATC-anaplastic thyroid carcinoma; PA-pilocytic astrocytoma; A-diffuse astrocytoma; O-oligodendroglioma; AO-anaplastic oligodendroglioma; GB-glioblastoma.

Figure 2 - Immunohistochemistry for TERT in two glioblastomas. Panel A represents a tumour without TERT expression and without TERT mutation, whereas panel B represents a tumour with positive staining both in nucleus and cytoplasm that also harboured a TERT mutation (magnification 40x). The graph (C) displays quantification of TERT nuclear expression level (immunohistochemistry) in glioblastomas with and without TERT mutation. We have measured the extent (less than 25%, 25-50%, 50-75% and over 75%) and the intensity (absent, faint, moderate or strong) of TERT nuclear staining in 14 glioblastomas (8 TERT wt and 6 TERT mutant). The scoring was performed by two independent observers and is the product of extent and intensity of the staining (see Material and Methods).

Figure 3 - Quantification of TERT mRNA expression level in thyroid tumours. We have performed Q-PCR in normal thyroid tissue (N), a pool of mRNA of 9 normal thyroids (pool N) and in thyroid tumours with different genetic backgrounds: without known genetic alterations (WT), with BRAF mutation (B), with RET rearrangement (RET), with RAS mutation (R), with TERT mutation (T), with TERT and BRAF mutations (BT) or with RAS and TERT mutations (RT). In panel A each bar represents TERT mRNA expression in an individual tumour measured in triplicate. TERT mRNA levels were normalized against TBP mRNA levels. In panel B quantification of TERT mRNA mean expression

level in thyroid tumours with and without TERT mutation. The difference is statistically significant ($p<0.0001$, unpaired T test).

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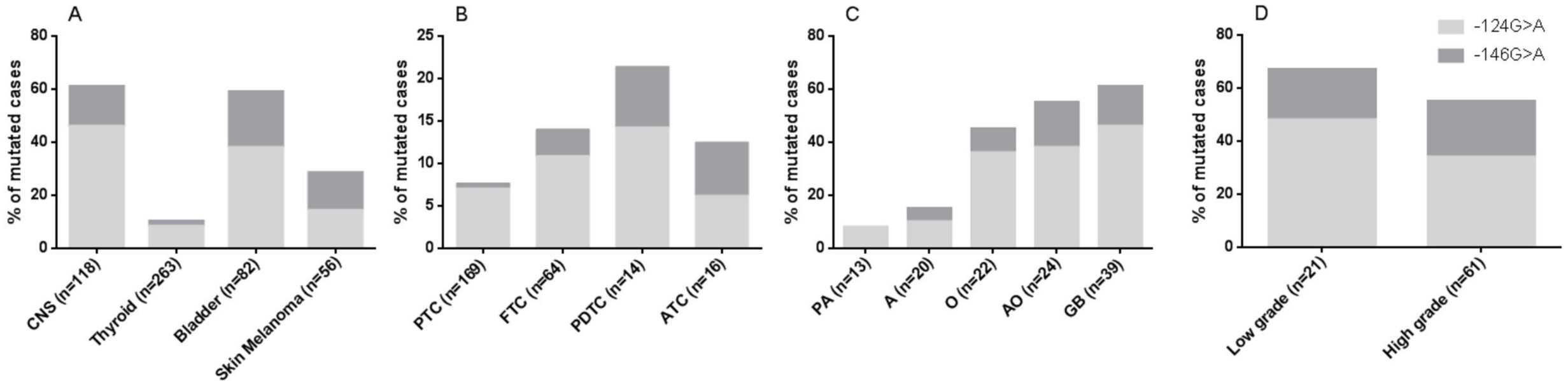
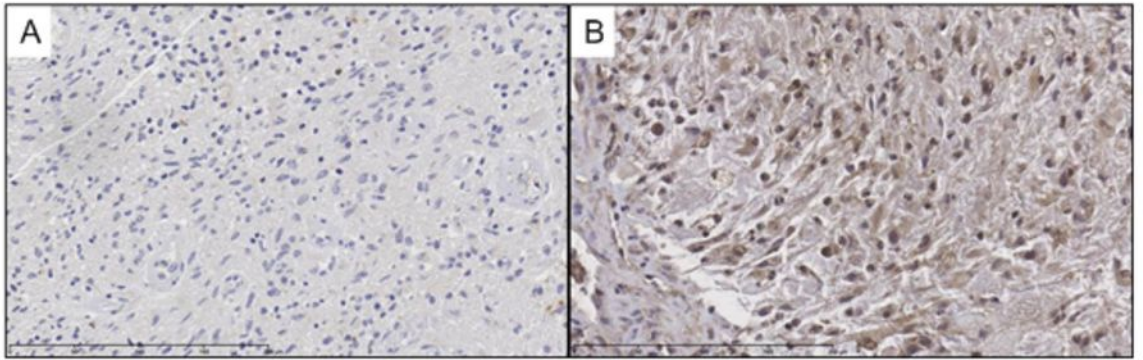
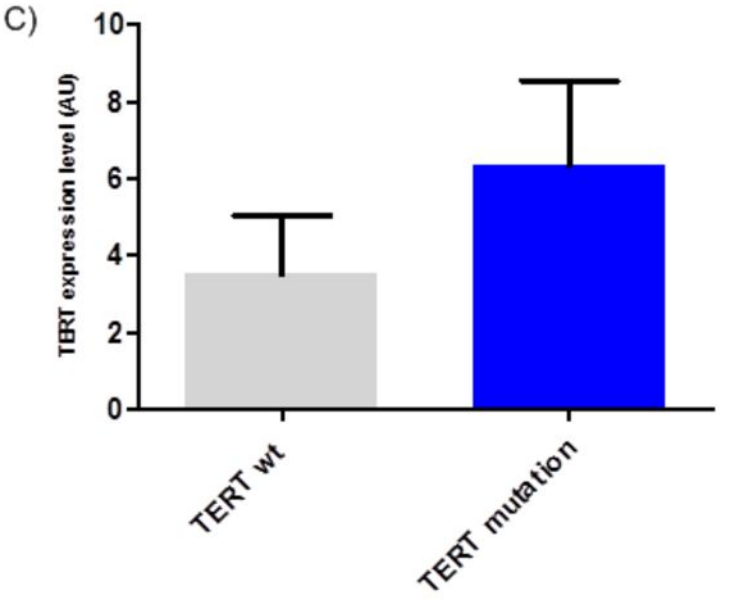

A
B
C
D
% of mutated cases
-124G>A
-146G>A
CNS (n=118)
Thyroid (n=263)
Bladder (n=82)
Skin Melanoma (n=56)
PTC (n=169)
FTC (n=64)
PDTC (n=14)
ATC (n=16)
PA (n=13)
A (n=20)
O (n=22)
AO (n=24)
GB (n=39)
Low grade (n=21)
High grade (n=61)


A
B


C)
TERT expression level (AU)
0
2
4
6
8
10
TERT wt
TERT mutation

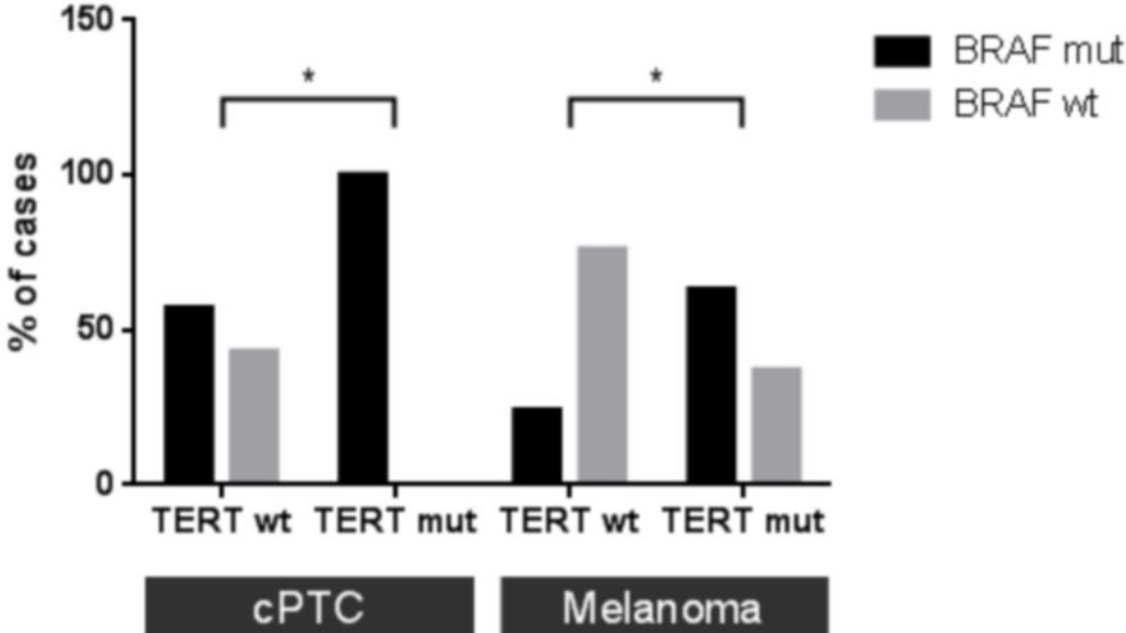

150
100
50
0
% of cases
*
*
BRAF mut
BRAF wt
TERT wt
TERT mut
TERT wt
TERT mut
cPTC
Melanoma

Table 1 – Prevalence of *TERT* promoter recurrent mutations in human cancers

Organ/tissue		Number	*TERT* mutation N(%)
Melanocytes			
	Nevi	9	0
	Skin Melanoma	56	16 (29%)
	Ocular melanoma	25	0
CNS			
	Pilocytic Astrocytoma	13	1 (8%)
	Diffuse Astrocytoma	20	3 (15%)
	Oligodendroglioma	22	10 (45%)
	Anaplastic Oligodendroglioma	24	13 (54%)
	Glioblastoma	39	24 (62%)
Thyroid			
	Benign	81	0
	PTC	169	13 (8%)
	FTC	64	9 (14%)
	PDTC	14	3 (21%)
	ATC	16	2 (13%)
	MTC	28	0
Bladder			
	Low grade	21	14 (67%)
	High grade	61	34 (56%)
Kidney			
	CCRCC	12	0
	CromRCC	4	0
	PRCC	10	0
Adrenal			
	Pheochromocytoma	17	0
GI			
	GIST	36	0
TOTAL		741	142 (19%)

PTC – papillary thyroid carcinoma; FTC - follicular thyroid carcinoma; PDTC – poorly differentiated thyroid carcinoma; ATC – anaplastic thyroid carcinoma; MTC – medullary thyroid carcinoma; CCRCC - clear cell renal cell carcinoma; CromRCC - chromophobe renal cell carcinoma; PRCC - papillary renal cell carcinoma; GIST – gastrointestinal stromal tumor.