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***TERT* structural rearrangements in metastatic pheochromocytomas**

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Abstract

Pheochromocytomas (PC) and paragangliomas (PGL) are endocrine tumors for which the genetic and clinicopathological features of metastatic progression remain incompletely understood. As a result, the risk of metastasis from a primary tumor cannot be predicted. Early diagnosis of individuals at high risk of developing metastases is clinically important and the identification of new biomarkers that are predictive of metastatic potential is of high value. Activation of *TERT* has been associated with a number of malignant tumors, including PC/PGL. However, the mechanism of *TERT* activation in the majority of PC/PGL remains unclear. As *TERT* promoter mutations occur rarely in PC/PGL, we hypothesized that other mechanisms – such as structural variations – may underlie *TERT* activation in these tumors. From 35 PC and four PGL, we identified three primary PCs that developed metastases with elevated *TERT* expression, each of which lacked *TERT* promoter mutations and promoter DNA methylation. Using whole genome sequencing, we identified somatic structural alterations proximal to the *TERT* locus in two of these tumors. In both tumors, the genomic rearrangements led to the positioning of super-enhancers proximal to the *TERT* promoter, that are likely responsible for the activation of the normally tightly repressed *TERT* expression in chromaffin cells

Key Words

- ▶ *TERT*
- ▶ pheochromocytoma
- ▶ metastatic
- ▶ whole genome sequencing
- ▶ rearrangements

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Introduction

Pheochromocytomas (PC) and paragangliomas (PGLs) are neuroendocrine tumors derived from neural crest cells of the sympathetic and parasympathetic nervous system – PCs arising in the adrenal medulla and PGLs in paraganglia outside the adrenal medulla. Although rare, they often occur as part of hereditary endocrine tumor syndromes and their study has been richly rewarding for identifying genetic events that drive tumorigenesis (Castro-Vega *et al.* 2015a). Metastatic disease occurs in approximately 10% of PC and up to 40% of PGL (Castro-Vega *et al.* 2015a); however, it is now accepted that all PC/PGL have some metastatic potential; therefore, the concept of ‘benign’ and ‘malignant’ is no longer applicable (Tischler *et al.* 2017). Despite germline mutations in succinate dehydrogenase subunit B (*SDHB*) and fumarate hydratase (*FH*) being associated with metastatic disease (*SDHB* (Benn *et al.* 2015), *FH* (Castro-Vega *et al.* 2014)), genetic associations with most metastatic PC/PGL remain unknown. Identification of new genetic biomarkers of metastatic potential are of high priority, given there are no recommended molecular or histopathological tumor features that can be used to guide patient management (Tischler *et al.* 2017).

Activation of *TERT* as a result of promoter mutations (specifically, chr5:1295228C>T and chr5:1295250C>T) were initially found in melanoma (Horn *et al.* 2013) and have subsequently been shown to be one of the most frequent somatic mutations in cancer (reviewed in Ramlee *et al.* 2016). Although approximately 25% of all PC/PGLs overexpress *TERT* (Liu *et al.* 2014), promoter mutations are rare (1.7% (Liu *et al.* 2014); 1.2% (Papathomas *et al.* 2014)), suggestive of another mechanism of *TERT* activation. Recently, activation of *TERT* as a result of structural rearrangements, leading to the placement of enhancers proximal to the *TERT* promoter have been described in a number of cancer types (Davis *et al.* 2014, Peifer *et al.* 2015, Valentijn *et al.* 2015, Alaei-Mahabadi *et al.* 2016, Barthel *et al.* 2017). We therefore hypothesized that structural alterations involving the *TERT* promoter may also contribute to PC/PGL. We identified three metastatic PCs with elevated *TERT* expression and using whole genome sequencing (WGS) identified genomic rearrangements that led to super-enhancer positioning proximal to the *TERT* promoter in two tumors.

Materials and methods

Patients and samples

Informed and written patient consent was obtained for the collection and study of all samples, under approval of the Northern Sydney Local Health District (Protocol #1103-107M) and Peter MacCallum Cancer Centre (Protocol #10-119) Human Research Ethics Committees. The patient clinical data have been described previously (Flynn *et al.* 2015).

RNA sequencing analysis

RNA-seq data were available for 35 PCs and four PGLs described in a previous study (Flynn *et al.* 2015). RNA-seq reads mapping to *TERT* were converted to gene feature counts then normalized for sequencing depth. Visualization of exon-level read depth and *TERT* splice-isoform usage was done using the Sashimi function in Integrated Genomics Viewer (Robinson *et al.* 2011) (version 2.3.68).

Targeted amplicon sequencing for detection of *TERT* promoter mutations

PCR primers were designed across base pair positions at canonical *TERT* promoter mutations, chr5:1295228C>T and chr5:1295250C>T and contained universal 5-prime sequences compatible with Fluidigm CS1 and CS2 primers (Fluidigm, San Francisco, CA, USA). Following PCR amplification of genomic DNA, PCR products were pooled and then subject to secondary PCR using indexed CS1 and CS2 primers (Fluidigm) before sequencing on a MiSeq platform (2×150bp) (Illumina, San Diego, CA, USA). Primer design and methods for read alignment and variant calling have been previously described (Wong *et al.* 2015).

Methylation-sensitive high-resolution melting (MS-HRM)

100ng of tumor DNA was modified using the EZ DNA Methylation-Lightning kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. Primer locations covered regions previously analyzed by Lee *et al.* (2017a) (first assay) and Castelo-Branco *et al.* (2013) (second assay), respectively. Primers for the first

assay were 5'-CGGAGTTGGAAGGTGAAGGGGTA-3' and 5'-GCTAACNTCCCTACACCCTAAAAAC-3'. Primers for second assay were 5'-CGGGGAAGTGTGTAGGGAGG-3' and 5'-GAAAACCCAAAACCTCCAAATCC-3'.

All PCR and MS-HRM reactions were performed on a Rotorgene 6000 (Corbett, Sydney, Australia). PCR reactions (20 μ L) contained 200 nmol/L of each primer, 200 μ mol/L dNTPs (Fisher Biotec, Perth, Australia), 5 μ mol/L SYTO 9 (Molecular Probes), 2.5 mmol/L MgCl₂, 0.5 U HotStarTaq DNA Polymerase in its supplied buffer (1 $\times$) (Qiagen) and 0.5 $\times$ Q-Solution (supplied with polymerase) in the supplied buffer (1 $\times$) (Qiagen). Both MS-HRM-PCR conditions began with 95°C for 15 min, and then 55 cycles of 95°C for 30 s, 63°C for 30 s and 72°C for 30 s for the first primer set; and 95°C for 10 s, 62°C for 20 s and 72°C for 20 s for the second primer set. HRM immediately followed the PCR with a hold at 97°C for 1 min, 72°C for 1 min then an analysis step from 65°C to 95°C rising 0.2°C per second and holding for 1 s at each increment.

Whole genome sequencing (WGS)

WGS was performed on paired DNA from fresh frozen tumor and peripheral blood. Sequencing libraries were prepared (1 μ g of DNA) according to the manufacturer's instructions (TruSeq Nano DNA HT Sample Prep Kit, Illumina) and sequenced on the Illumina HiSeq X Ten platform (Kinghorn Centre for Clinical Genomics, Garvan Institute of Medical Research, Sydney, Australia). Sequencing reads were aligned to the UCSC hg19 reference genome using BWA-mem (Li 2013). Duplicate reads were marked using Picard (<http://broadinstitute.github.io/picard>). Mean sequence coverage in individual samples was 50–60 $\times$ (Supplementary Table 2, see section on supplementary data given at the end of this article).

Copy-number analysis

Copy-number analysis was done using Fraction and Allele-Specific Copy-Number Estimates from Tumor Sequencing (FACETS; version 0.5.6, segmentation c-value=500) (Shen & Seshan 2016)). Tumor purity estimates were based on subclonal model (Supplementary Table 2).

Detection of genomic rearrangements

Genomic rearrangements were identified using Genomic Rearrangement Identification Software Suite (GRIDSS, version 0.11.5) (Cameron *et al.* 2017). Breakpoints were filtered out based on occurrence within ENCODE DAC

blacklisted regions and presence within the germline. Reported breakpoints calls were supported by at least two read pairs, one split read and a breakend assembly contig at both respective breakpoint regions. They were also at least 2 Kb in size with less than 50 bp homology observed between the breakpoint ends and had GRIDSS confidence scores greater than 200.

Validation of genomic rearrangements by PCR and Sanger sequencing

PCR was carried out in the tumor and matched normal genomic DNA using primers spanning the predicted breakpoints (chr5:1295558 and chr5:5601933 (V-PH-03T); chr5:1302401 and chr22:33960879 (V-PH-04T)), with the products subsequently analyzed by Sanger sequencing.

Telomere length analysis

Telomere length was assessed using TelSeq (Ding *et al.* 2014), a bioinformatic tool known to correlate with the standard experimental methods of qPCR and terminal restriction fragment (TRF)-length analysis (Lee *et al.* 2017a). Reads containing the telomeric repeat (TTAGGG) $\times$ 3 or (CCCTAA) $\times$ 3 were counted and normalized to the average genomic coverage (the average base coverage of each genome).

Results

Overexpression of *TERT* in a subset of PC/PGL

A focused re-analysis of RNA-seq data generated from our prior study (Flynn *et al.* 2015) showed detectable *TERT* mRNA expression in 20 of 39 (51%) PC/PGL tumors (18 PC and two PGL), including five of six PCs that developed metastases (Fig. 1A, Supplementary Table 1). Markedly elevated *TERT* expression (4.66 (square root counts per million, SQRT-CPM), 5.07 SQRT-CPM) was observed in two metastatic PC (V-PH-01T, V-PH-04T, respectively) while a third metastatic PC (V-PH-03T) showed moderately elevated expression (0.98 SQRT-CPM). Analysis of splice-mapped sequence reads corresponding to *TERT* exons 6–9 showed that full-length *TERT*, which is required for *TERT* enzymatic activity, could be detected in the moderate-to-high expressing cases in addition to the common β -deletion (β -) isoform that skips exons 7 and 8 (Fig. 1B).

Targeted amplicon sequencing did not detect canonical *TERT* promoter mutations in any of the 39 PC/PGL tumors analyzed. As *TERT* promoter methylation

Figure 1

TERT expression in PC/PGL ($n=39$) taken from RNA-seq data described in Flynn et al. (2015). (A) Square root of RNA-seq counts per million aligned to the *TERT* locus in 39 PC/PGL tumor samples. (B) Sashimi plot depicting mapped RNA-seq reads spanning *TERT* exons 6–9 (supporting read number shown in exon links) and read depth (maximum range shown in parenthesis). Exons 7–8 are spliced out in β -deletion (β -) isoform.

has also shown to be positively correlated with *TERT* expression in other cancer types, such as pediatric brain cancers and neuroblastoma, we used high-resolution melting following DNA bisulfite conversion and PCR amplification (MS-HRM) to target two distinct regions of the promoter CpG island studied by other groups (Castelo-Branco et al. 2013, Lee et al. 2017b). The limit of detection using HRM-PCR is ~1% depending on the degree of methylation heterogeneity at the CpG sites. We did not detect any DNA methylation at target CpG regions in the 39 tumors analyzed (Supplementary Fig. 1). We therefore excluded canonical *TERT* promoter mutations and DNA methylation as possible mechanisms of *TERT* dysregulation in our tumor cohort.

Whole genome sequencing, telomere length analysis and identification of *TERT* promoter rearrangements in PC

We performed WGS on tumor and matching blood for the moderate-to-high *TERT*-expressing cases (V-PH-01T, V-PH-03T and V-PH-04T). An *in silico* analysis of telomere length utilizing the WGS data indicated that telomeres were shorter in tumors V-PH-01T and V-PH-04T compared to their matched normal blood controls, whereas

V-PH-03T had an approximately equal telomere length to the matched blood (Supplementary Table 3). Our observation of reduced telomere length in tumor compared to matched blood in two of our *TERT*-overexpressing tumors is consistent with a recent observation made across 31 tumor types (Barthel et al. 2017). Importantly, we note that case V-PH-03T had a relatively low tumor purity assessed by FACETS (~30%), and this may have reduced the accuracy of telomere length estimation and level of detected *TERT* expression in this case.

Structural variant analysis of the WGS data indicated that V-PH-03T and V-PH-04T had complex patterns of somatic rearrangement including regions of localized genomic instability (V-PH-03T: chr5p, chr7q; and V-PH-04T: chr3p, chr11p, chr22q; Fig. 2A). In contrast, V-PH-01T had no detectable structural rearrangements with the presence of whole chromosome or chromosome arm level loss events that are common to PC/PGL tumors (e.g. chr1, chr3q, chr17; Fig. 2A) (Castro-Vega et al. 2015b). We previously detected a *TP53* mutation in case V-PH-03T by whole exome sequencing (Flynn et al. 2015), and given the known functional role of mutant *TP53* in causing genomic instability in cancer (reviewed by Kastenhuber & Lowe 2017), this may explain the higher number of structural variants observed in this case. No deleterious

Figure 2

Structural alterations involving the *TERT* promoter identified in metastatic pheochromocytomas. (A) Circos plots displaying global somatic copy-number and breakpoints detected by WGS analysis across the three *TERT* overexpressing PC tumors. No somatic breakpoints were detected in V-PH-01T. Chromosome ideograms displayed as outer ring, somatic copy-number changes detected as inner ring. Green line indicates neutral (diploid) or copy-number gain from segmentation, and red line indicates copy-number loss. Inner lines show detected breakpoints. Red lines highlight the validated *TERT* promoter rearrangements. Data for chrX and chrY not shown. (B) Chromosome level view of somatic copy-number alterations and breakpoints in V-PH-03T highlighting an intra-chromosomal deletion detected proximal to *TERT* as indicated by red line. (C) Sanger sequencing validation of 4.3Mb deletion upstream of *TERT* in V-PH-03T. (D) Chromosome level view of somatic copy-number alterations and breakpoints in V-PH-04T involving chr5p and chr22q. Red line indicates *TERT* proximal breakpoint. (E) Sanger sequencing validation of non-reciprocal translocation (t(5;22)(p15.3;q12.3)) identified in V-PH-04T. Arrows in panels C and E indicate *TERT* proximal structural breakpoint previously described in aggressive neuroblastoma (Peifer et al. 2015).

somatic mutations in genes associated with genome maintenance could be identified in cases V-PH-01T or V-PH-04T.

Somatic breakpoints were detected in close proximity to *TERT* in V-PH-03T and V-PH-04T. In V-PH-03T, a 4.3Mb

intra-chromosomal deletion was identified (Fig. 2B and C) corresponding to breakpoints at chr5:1295558 (454bp upstream of the *TERT* transcription start site (TSS)) and at chr5:5601933 (a non-coding region of the genome, with the closest gene (*LINC02145*) located approximately

708Kb upstream). The 4.3Mb deletion in V-PH-03T was further supported by segmental hemizygous loss (as detected by copy-number analysis) and occurred in the context of localized genomic instability on chr5p involving several additional rearrangements and focal amplifications centromeric of *TERT*.

In V-PH-04T, a non-reciprocal inter-chromosomal translocation (t(5;22)(p15.3;q12.3)) was detected arising from a breakpoint at chr5:1302401 (approximately 7.3 Kb upstream of the *TERT* TSS) being juxtaposed to a break at chr22:33960879 ((c.742G, NM_004737) within exon 7 of *LARGE1* (a member of the N-acetylglucosaminyltransferase gene family)) (Fig. 2D and E). Again breakpoints on chr5 and chr22 in V-PH-04T arose in the context of marked genomic instability involving localized regions of copy-number loss and gain on both chromosomes.

TERT structural alterations involve enhancer elements

To interpret the functional effect of *TERT* rearrangements with respect to *cis* regulatory elements, we assessed adrenal specific super-enhancer proximity using annotation from dbSUPER (an integrative database of super-enhancers (Khan & Zhang 2016)) defined by histone H3K27 acetylation in normal adult adrenal tissue (GEO Accession: GSM1013168). The location of

super-enhancer regions was further supported by ENCODE data (v3, enabling visualization of enhancer-like regions) representing enrichment peaks for H3K27 acetylation and DNase hypersensitivity in fetal adrenal tissue (GEO: GSM1160190) (Rosenbloom *et al.* 2013). The 4.3Mb intra-chromosomal deletion (chr5:1295558-chr5:5601933) in V-PH-03T resulted in the positioning of a super-enhancer (SE_01397) within 1.1 Mb of the *TERT* TSS (Fig. 3A), while the non-reciprocal translocation (t(5;22)(p15.3;q12.3)) involving juxtaposition of *LARGE1* (chr22:33960879) to the *TERT* promoter (chr5:1302401) in V-PH-04T resulted in the positioning of a super-enhancer (SE_01144) within 1.9Mb of the *TERT* TSS (Fig. 3B). In contrast, the closest super-enhancer (SE_01397 (chr5: 6704085-6734741)) within the normal reference genome is positioned 5.4Mb upstream of the *TERT* TSS.

Interestingly, additional enhancer-like elements identified in fetal adrenal tissue were positioned approximately 3Kb and 18Kb upstream of the *TERT* TSS when assessing the 4.3Mb deletion (chr5:1295558-chr5:5601933) in V-PH-03T (Supplementary Fig. 2A). When assessing the non-reciprocal translocation (t(5;22)(p15.3;q12.3)) in V-PH-04T, enhancer-like regions in fetal adrenal tissue were observed 577Kb and 593Kb upstream of the *TERT* TSS (Supplementary Fig. 2B). In summary, somatic structural alterations were found in two of

Figure 3

Structural rearrangements juxtapose *TERT* proximal to active enhancers in metastatic PC. The potential for enhancer-driven activation of *TERT* was assessed using dbSUPER (an integrative database of super-enhancers; Khan & Zhang 2016). Candidate super-enhancers are based on H3K27ac signals from normal adult adrenal tissue (GEO accession: GSM1013168). ENCODE data for H3K27ac (blue track) and DNase hypersensitivity (green track) in fetal adrenal tissue supporting super-enhancer features was accessed through UCSC browser (build hg19) (Rosenbloom *et al.* 2013). (A) The 4.3Mb somatic deletion observed in V-PH-03T is predicted to result in placement of a super-enhancer 1.1Mb from the *TERT* TSS (SE_01397, dbSUPER). (B) The somatic non-reciprocal translocation, t(5;22)(p15.3;q12.3), identified in V-PH-04T is predicted to result in the placement of a super-enhancer 1.9Mb from the *TERT* TSS (SE_01144, dbSUPER).

the three *TERT* overexpressing PC tumors that resulted in juxtaposition of super-enhancer and enhancer-like elements close to the *TERT* promoter.

Discussion

Re-activation of *TERT* is common to many cancers, and it is mediated by genetic or epigenetic variation at the *TERT* locus (Barthel *et al.* 2017). In PC/PGL, *TERT* expression is typically low to undetectable in non-metastatic cases and increased in a subset of metastatic tumors (Liu *et al.* 2014). In this study, we examined the mechanism for increased *TERT* expression in three metastatic PCs, and by using WGS, somatic structural rearrangements involving the *TERT* promoter in two cases were identified for the first time in this disease; in each case, the structural variants resulted in super-enhancer positioning proximal to the *TERT* transcription start site (TSS), which are likely responsible for *TERT* activation.

In our PC/PGL cohort, *TERT* expression (as assessed by RNA-seq) was detectable in 20 of 39 (51%) cases, which included five of six metastatic cases; of these, two metastatic PCs had markedly elevated *TERT* expression and a third metastatic PC showed moderately elevated *TERT* expression. Our data closely resemble those of Liu and coworkers who found detectable *TERT* expression by RT-PCR in 18 of 75 (24%) PC/PGL (Liu *et al.* 2014). In that study, six (8%) tumors (including three metastatic cases) showed markedly elevated *TERT* expression and 12 (16%) had moderately elevated expression. Further analysis of *TERT* mRNA gene expression in 178 primary PC/PGL, assessed by The Cancer Genome Atlas (TCGA), showed 34 (19%) primary tumors with detectable *TERT* expression (Fishbein *et al.* 2017). Importantly, two of the top three *TERT*-expressing tumors in the TCGA dataset were metastatic (Supplementary Table 4). Taken together, moderate-to-high *TERT* expression appears in a minority of PC/PGL tumors, but we confirm this group is enriched in tumors with a metastatic phenotype.

None of our three *TERT*-overexpressing cases were associated with germline *SDHB* mutations, which are well known to be associated with increased risk of metastasis in PC/PGLs (Benn *et al.* 2015). Exome sequencing of V-PH-01T and V-PH-04T had identified somatic *NF1* (p.Thr467HisfsTer6 (ENSP00000351015.4)) and *VHL* (p.Ile109Ser (ENSP00000256474.2)) mutations, respectively, while no somatic or germline mutation involving a known PC/PGL gene was detected in V-PH-03T (Flynn *et al.* 2015). Similarly, of the top five

TERT-expressing tumors in the TCGA dataset, none harbored *SDHB* mutations, but included one hereditary *VHL*-associated tumor and four tumors of unknown gene driver. Telomere dysfunction through alternate lengthening of telomeres (ALT) has also been associated with metastatic PC/PGLs. Fishbein and coworkers identified somatic *ATRX* mutations in two *SDHB*-associated PC/PGLs and in addition, 13 of 103 (12.6%) of a validation cohort of PC/PGLs, many of which were associated with clinically aggressive behavior (Fishbein *et al.* 2015). However, in our prior study, only 2 of 39 (5.1%) PC/PGLs had somatic protein-truncating *ATRX* mutations, both of which were non-metastatic and neither associated with germline *SDHB* mutation (Flynn *et al.* 2015). Owing to the long latency of disease progression in PC/PGL, it is plausible that such non-metastatic cases may still develop metastases in time. We conclude that telomere dysfunction through somatic mutation or structural alterations affecting *TERT* or *ATRX* are likely to be predictors of metastatic risk in PC/PGL and can arise independently of *SDHB* driver mutations.

Given the shared neural crest origin of PC/PGL and neuroblastoma, there is a striking parallel between telomere dysfunction due either to *TERT* expression or *ATRX* loss of function in these two diseases (Peifer *et al.* 2015, Valentijn *et al.* 2015). Indeed, structural rearrangements involving the upstream region of the *TERT* promoter were observed in 21% (Peifer *et al.* 2015) and 23% (Valentijn *et al.* 2015) of neuroblastomas and occurred only in high-risk cases that did not have *MYCN* amplification or *ATRX* mutations (Peifer *et al.* 2015, Valentijn *et al.* 2015). Interestingly, the *TERT* breakpoints identified in PC tumors from our study, at chr5:1295558 (V-PH-03T) and chr5:1302401 (V-PH-04T) occur within 15 bp and 44 bp, respectively, of breakpoints observed in two neuroblastoma cases from a previous neuroblastoma study (Fig. 2C (NBL54: chr5:1295550) and Fig. 2E (NBL40: chr5:1302358), respectively (Peifer *et al.* 2015)). These correlative observations in neuroblastoma further validate the likely functional effect of *TERT* rearrangements in PC/PGL.

Our study emphasizes the value of using WGS to detect *TERT* rearrangements as a plausible cause of *TERT* overexpression in metastatic PC/PGL, which would have been missed by studies employing whole exome sequencing (Castro-Vega *et al.* 2015b, Flynn *et al.* 2015, Toledo *et al.* 2016, Fishbein *et al.* 2017). It was evident that the two *TERT*-rearranged tumors identified in our study displayed higher degrees of genomic instability, indicating that defects in genome maintenance are important mechanisms for

perturbation of PC/PGL driver genes. Indeed, we and others have previously identified chromothripsis in PC/PGL tumors and structural rearrangements involving known PC/PGL tumor suppressor genes, such as *NF1* (Castro-Vega *et al.* 2014, Flynn *et al.* 2015), while the recent TCGA study described putative oncogenic *MAML3* and *BRAF* gene fusions (Fishbein *et al.* 2017). Ongoing structural instability during PC/PGL evolution has also been described as a characteristic of metastatic PC/PGL (Crona *et al.* 2015). However, it is apparent that some metastatic PC/PGL can also have relatively stable genomes, as we observed in case V-PH-01T. It is curious that despite the use of WGS and a thorough targeted search for canonical mutations and CpG methylation events in the *TERT* promoter, we could not detect genetic or epigenetic events explaining *TERT* overexpression in tumor V-PH-01T. Manual inspection of the WGS data in V-PH-01T revealed an uneven and low read coverage in the *TERT* promoter that precluded variant detection outside of the two known *TERT* promoter mutation hotspots. Deeper sequencing of the *TERT* promoter for detection of non-canonical mutations may be warranted to provide a higher sensitivity of detection in future studies. It is also plausible that *TERT* can be dysregulated by another currently unknown mechanism in some PC/PGL tumors.

In summary, analysis of genomic data from a subset of PC/PGL has identified a role for genomic rearrangements as a cause of *TERT* overexpression in PC/PGL. Further analysis in larger sets of these tumors will be required to verify the frequency of *TERT* rearrangements in PC/PGL and to validate their association with aggressive disease. Furthermore, additional analysis is required to verify the canonical and non-canonical functions of *TERT* in PC/PGL tumors (Martinez & Blasco 2011). Finally, a number of therapeutic strategies (such as telomerase inhibition, *TERT* promoter-driven therapy and *TERT*-directed immunotherapy) are currently under investigation, which may lead to improved outcomes in individuals with *TERT*-overexpressing metastatic PC/PGL tumors (Xu & Goldkorn 2016).

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/ERC-17-0306>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

T D, R C B and R W T conceived the study and participated in its design and coordination. T D, A F, K A, R L, J L, D L C, S B, I L M C, S Q W, A T P and A D performed experiments and analyses. A J G performed pathology review. D E B, A H, B G R, R J H and R C B recruited patients and/or curated clinical data. T D, R C B and R W T wrote the manuscript, with critical review provided by A F, K A, D E B, R L, J L, D L C, A H, S B, I L M C, S Q W, B G R, A T P, A J G, A D, R J H. All authors have read and approved the final manuscript; take public responsibility for appropriate portions of content and are accountable in relation to the accuracy and integrity of the work.

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