TERT promoter mutations are rare in parathyroid tumors

Dear Editor,

The majority of parathyroid tumors are benign, and parathyroid carcinomas represent a diagnostic challenge with limited treatment options. Multiple endocrine neoplasia type 1 gene and cell division cycle 73 (*CDC73*) are major genes in parathyroid adenomas and carcinomas respectively. However, a large group of parathyroid tumors remain without defined genetic background.

Telomeres are chromosome-capping structures designed to protect DNA integrity. During mitosis, the telomere repeats are gradually shortened, which limits the number of possible cell divisions. In order for cancer cells to escape senescence, alterations in telomere maintenance are required. The telomerase reverse transcriptase (*TERT*) gene encodes the catalytic subunit of the telomerase complex and is largely responsible for maintaining the telomere length. Mutations in the promoter of the *TERT* gene may cause alterations in transcription factor binding sites, which leads to *TERT* overexpression and telomerase activation, as was first described for melanoma (Horn et al. 2013).

Recently, the common *TERT* promoter mutations -146C>T (also called C250T) and -124C>T (C228T) have been implicated in the development of several endocrine tumors. They have been identified in papillary, follicular, and anaplastic carcinomas of the thyroid, adrenocortical carcinomas, and paragangliomas. Interestingly, the mutations were found to be associated with telomerase activation, telomere length, malignant disease, patient age, and adverse prognosis (Liu et al. 2014). In addition to the potential prognostic value, the ongoing development of telomerase inhibitors for clinical use could provide additional treatment options for patients with aggressive endocrine cancer.

Previous studies have identified telomerase activity as well as *TERT* gene expression in parathyroid carcinomas but not in adenomas or normal parathyroid tissue (Falchetti et al. 1999, Kammori et al. 2003). Additionally, the telomerase inhibitor azidothymidine was able to

reduce cell proliferation in parathyroid carcinoma cells *in vitro* (Falchetti et al. 2005).

In the present study, we sought to investigate the *TERT* promoter status in parathyroid tumors by direct Sanger sequencing. A total of 120 parathyroid tumors from 119 patients were studied, including 88 adenomas, 22 carcinomas, and ten atypical adenomas (Table 1). The carcinomas were classified according to the World Health Organization 2004 guidelines. The tumors were collected and analyzed at the Karolinska University Hospital, Stockholm, Sweden (*n*=90) and the Yale University School of Medicine, New Haven, CA, USA (*n*=30). Samples were obtained with informed patient consent, and the study of the tissue material was approved by the local ethical committees. Subsets of the parathyroid tumors studied at the Karolinska University Hospital have been previously published.

Genomic DNA isolated from fresh frozen or paraffin-embedded tumor samples was used for amplification of the *TERT* promoter and sequencing as previously described (Liu et al. 2014). Sanger sequencing was carried out at the KIGene core facility at Karolinska Institutet for 90 cases and the Keck DNA Sequencing Facility, Yale University School of Medicine, for 30 cases. All samples displaying *TERT* promoter alterations or with ambiguous chromatograms were reanalyzed in the Clinical Molecular Pathology division at Karolinska University Hospital.

All 120 tumors exhibited the WT sequences at positions -124C and -146C (Table 1). A *TERT* promoter variant was identified in an adenoma (T1) and an atypical adenoma (T2) from a single patient. This heterozygous variant -145C>T was also detected in constitutional DNA from the patient (Fig. 1A). It has previously been reported in a pleomorphic xanthoastrocytoma (Koelsche et al. 2013). The patient, a 16-year-old male with unknown family history of the disease and lost to follow-up, carries a constitutional W45X mutation of *CDC73* together with a second W30X mutation of *CDC73* in T1 (Sulaiman et al. 2012).

Table 1 *TERT* promoter sequence and expression in parathyroid tumors

Parameter	Series		Total (n = 120)
	Karolinska (n = 90)	Yale (n = 30)	
Diagnosis			
<i>TERT</i> mutation -124C>T or -146C>T			
Adenomas	0/62	0/26	0/88 (0%)
Atypical adenomas	0/10	–	0/10 (0%)
Carcinomas	0/18	0/4	0/22 (0%)
<i>TERT</i> variant -145C>T			
Adenomas	1/62 ^a	0/26	1/88 (1%)
Atypical adenomas	1/10 ^a	–	1/10 (10%)
Carcinomas	0/18	0/4	0/22 (0%)
<i>TERT</i> mRNA expression ^b			
Adenomas	0/34	–	0/34 (0%)
Atypical adenomas	1/6	–	1/6 (17%)
Carcinomas	–	–	–

–, no data; n, number of tumors analysed.

^aBoth tumors with -145C>T were from the same patient.^brelative to HeLa-cells.

The potential effect of the -145C>T variant on transcription factor motifs in the promoter was analyzed *in silico* using PROMO (Transfac database v8.3, dissimilarity margin $\leq 15\%$) together with the -146C>T mutation as a control. As a validation of the method, PROMO identified the putative Ets-1 and Elk-1 binding sites induced by the -146C>T mutation (Horn *et al.* 2013). Furthermore, PROMO predicted loss of the MAZ and TFII-I binding sites (Fig. 1B) for the -145C>T variant. This specific MAZ binding site has previously been identified in the *TERT* promoter, and the MAZ transcription factor has been suggested to suppress *TERT* expression (Su *et al.* 2007).

TERT expression analysis was undertaken using previously described methodology based on quantitative real-time PCR (Liu *et al.* 2014). In short, sample cDNA were run in quadruplicate for the Taqman gene expression assays for *TERT* (Hs00972656_m1, catalogue no. 4331182) and the endogenous control *RPLP0* (Hs99999902_m1, catalogue no. 4331182). A HeLa cell line was used as a positive control and an arbitrary relative reference. Reactions with *TERT* Ct values of <35 in all replicates were classified as having detectable expression (as per the manufacturer's recommendations).

TERT expression was only identified in one of the six atypical adenomas and in none of the 34 adenomas (Table 1). In the *TERT* positive atypical adenoma (T2 from the patient carrying -145C>T), the *TERT* expression level was 220-fold higher than that in HeLa cells. The adenoma (T1) from the same patient showed no detectable expression.

The present study suggests that *TERT* promoter mutations are rare or absent in parathyroid tumors. The role of -145C>T in telomerase activation is ambiguous, and it is uncertain whether this represents an unusual single nucleotide variant or a mutation.

Previous studies have reported telomerase activity using telomeric repeat amplification protocol (TRAP) analysis in all analyzed parathyroid carcinomas (6/6) as well as in *TERT* mRNA expression (4/4) by RNA *in situ* (Falchetti *et al.* 1999, Kammori *et al.* 2003, Onoda *et al.* 2004). By contrast, telomerase activity was virtually absent in adenomas (0/47), hyperplasias (0/11), and normal parathyroid tissues (0/23) (Falchetti *et al.* 1999, Velin *et al.* 2001, Kammori *et al.* 2003, Onoda *et al.* 2004).

Figure 1

Analysis of the *TERT* promoter. (A) Chromatograms showing the WT sequence and the heterozygous -145C>T variant. (B) Predicted transcription factor binding sites for -146C>T and -145C>T. The -146C>T mutation

is predicted to lose the MAZ and gain the STAT4, c-ETS1, and Elk-1 motifs. The -145C>T variant is predicted to lose the MAZ and TFII-I motifs.

These observations suggest that telomerase activation is necessary for parathyroid malignancy but not for the proliferation of benign lesions.

Given the absence of -124C>T and -146C>T, *TERT* promoter mutations should be considered a rare event in parathyroid tumors. The previously reported telomerase activation is therefore expected to be caused by other mechanisms, which could include copy number alterations, DNA methylation, or other genetic and regulatory events.

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Declaration of interest

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