CLINICAL STUDY

Telomerase is not activated in human hyperplastic and adenomatous parathyroid tissue

Åsa K Velin^{1,2}, Anders Herder³, Kenth J M Johansson⁴, Lena M Trulsson^{1,2} and Staffan Smeds^{1,2}

¹Department of Biomedicine and Surgery, ²Clinical Research Center, ³Department of Pathology, Faculty of Health Sciences, University Hospital, Linköping and ⁴Department of Surgery, Västerviks sjukhus, Västervik, Sweden

(Correspondence should be addressed to S Smeds, Department of Biomedicine and Surgery, Faculty of Health Sciences, SE-581 85 Linköping, Sweden; Email: staffan.smeds@lio.se)

Abstract

Background: Telomerase is a specific enzyme that appears to have a key role in cellular senescence and the progression of neoplastic tissue. High telomerase activity has been found in several cancers, but not in most normal and benign tissue. Little is known about the influence of telomerase on the abnormal growth associated with hyperparathyroidism.

Objective: To analyse telomerase activity in parathyroid tissue obtained from 29 patients undergoing surgery for primary hyperparathyroidism.

Design: Tissue for telomerase activity measurements was collected from six hyperplastic, 20 adenomatous and 22 normal parathyroid glands.

Methods: The highly sensitive PCR-based telomeric repeat amplification protocol, TRAP, combined with ELISA, was used to detect telomerase activity in tissue extracts containing 3.0 µg protein.

Result: Telomerase was not activated in any of the analysed tissue by 3 µg protein. Reassay of 12 samples containing 6.0 µg protein verified these negative TRAP results.

Conclusion: Our findings indicate that telomerase is not a part of the mechanism promoting parathyroid proliferation and the underlying conditions remain to be determined.

European Journal of Endocrinology 145 161–164

Introduction

Primary hyperparathyroidism is the syndrome caused by increased secretion of parathyroid hormone as a result of parathyroid hyperplasia, adenoma or, more rarely, carcinoma; adenomas account for 80–90% of all cases. The diagnosis is based on histopathological parameters; however, the distinction between the lesions can be problematic (1). To enhance the understanding of parathyroid pathogenesis, attention has been focused on factors that might influence the progression. Oncogene overexpression or loss of suppressor genes seem to be crucial, but the exact genes involved remain to be identified. Recent studies have investigated the roles of telomeres and telomerase in benign and malignant tumours (2–4) and it has been stated that telomerase might be a useful complement to microscopic cytopathology in the detection of malignant cells in small tissue biopsies (5). Telomeres are specialized segments of DNA found at the ends of chromosomes, which have been proposed to have a key role in cellular senescence. They shorten continuously during cell replication, and when they decline to a critical length the cell will stop dividing and eventually

die. Telomerase is a specific enzyme that synthesizes telomeric DNA onto the ends of chromosomes, thereby preventing telomere shortening. Previous research shows that most normal and benign cells lack telomerase activity, but in a majority of malignant tumours telomerase is activated (2–4). Recently, a highly sensitive PCR-based assay called the telomeric repeat amplification protocol (TRAP) has been developed to measure telomerase activity (6).

To our knowledge, there is only one study to date that has measured telomerase activity in parathyroid tissue. Results from this study (7) showed that telomerase was activated in carcinomatous tissue, but not in hyperplastic, adenomatous and normal parathyroid tissue. In the present study we wanted to analyse telomerase activity in a larger material. Using the TRAP assay, followed by ELISA, we measured telomerase activity in adenomatous, hyperplastic and macroscopically normal parathyroid specimens from a total of 29 consecutive patients. Because less than 1% of primary hyperparathyroidism is due to a carcinoma, no malignant tissue was available for this study. Results showed that all tissues were negative for telomerase activity.

Material and methods

Ethics

This study was approved by the ethics committee for human research in Linköping, Sweden. Written informed consent was obtained from each patient before their entry to the study.

Tissue samples

Biopsies of parathyroid hyperplasias ($n = 6$) and adenomas ($n = 20$) were collected from patients undergoing parathyroidectomy for hyperparathyroidism. Normal tissue was obtained either at the same time as pathological tissue ($n = 19$), or from patients in whom no pathological gland was available for telomerase analysis ($n = 3$). The distribution of patients and tissues is detailed in Table 1. After peroperative histopathological diagnosis, the pathological gland(s) were resected and transported on ice to the laboratory, where 10–20 mg samples were embedded in cryomount (Histolab, Gothenburg, Sweden) and snap-frozen in liquid nitrogen. Ten consecutive 10 µm

Table 1 Distribution of parathyroid tissue obtained for telomerase analysis. Both pathological and macroscopically normal tissue were obtained from patients undergoing surgery for hyperparathyroidism.

Patient	Sex	Age (years)	Diagnosis
1	M	25	H
2	F	53	N, A
3	F	41	N
4	F	73	N
5	F	74	H
6	F	64	N, A
7	F	88	N, H
8	F	80	N, A
9	F	72	A
10	F	79	N, A
11	F	79	N, A
12	F	77	N, H
13	F	80	H
14	M	39	N, A
15	F	78	N, A
16	M	80	N
17	F	78	N, A
18	F	69	N, A
19	F	82	N, A
20†	F	34	—
21	F	57	H
22	M	69	N, A
23	F	84	A
24	F	38	N, A
25	F	51	A
26	F	79	N, A
27	F	54	N, A
28	M	81	N, A
29	F	53	N, A
30	M	54	N, A

†Patient 20 was excluded because the tissue obtained proved to be a lymph node. A, adenoma; H, hyperplasia; N, normal.

sections were transferred to a sterile tube, snap-frozen in liquid nitrogen and stored at -70°C . Normal tissue was sectioned from the frozen blocks prepared for peroperative examination. To verify the peroperative diagnoses, one part of each specimen was fixed in formalin and stained with haematoxylin and eosin for a definitive histopathological examination.

Telomerase assay

Telomerase activity was measured using the Telomerase PCR ELISA kit (Roche Diagnostics, Mannheim, Germany). The frozen sections were added to 100 µl lysis buffer and incubated on ice. After centrifugation, the supernatants were collected and protein concentrations were determined by the Lowry assay (8). The extracts were snap-frozen and stored at -70°C before telomerase analysis.

Cell extracts containing 3.0 µg protein were used for PCR amplification and all samples were run in duplicate. A positive control (enclosed with the kit) and a negative control (consisting of RNase-free water added to lysis buffer) were included in each analysis. In the first step, telomeric repeats (TTAGGG) were added to a biotin-labelled primer by telomerase in the sample. In the second step these elongation products were amplified by PCR. The PCR product was denatured and hybridized to a digoxigenin (DIG)-labelled telomeric repeat-specific detection probe and bound to a strept-avidin-coated microtitre plate via the biotin-labelled primer. The immobilized PCR product was detected with a peroxidase-conjugated antibody against DIG and finally visualized by incubation with tetramethyl benzidine. The absorbance of the coloured reaction product was measured at 450 nm, with a reference wavelength of 690 nm, using an ELISA microtitre plate reader (Anthos htIII, Anthos, Labtec Instruments, Saltzburg, Germany). The mean of the readings of the negative controls was subtracted from those of the samples. Samples were regarded as telomerase-positive if the difference in absorbance, $A_{450\text{ nm}} - A_{690\text{ nm}}$, was greater than 0.20 units. Samples with TRAP results between 0.10 and 0.19 units were further analysed with 6.0 µg protein.

Results

A total of 48 frozen samples of adenomatous, hyperplastic and morphologically normal parathyroid tissue were analysed for telomerase activity. All specimens stained with haematoxylin and eosin were morphologically judged and the histopathological diagnoses are presented in Table 1. All tissues were negative for telomerase activity (Table 2), whereas the positive control exhibited high telomerase activity. Repeated analysis of 12 samples with 6.0 µg protein gave no change in TRAP results. In one case (no. 20) a lymph node was sent for peroperative cryopreservation

Table 2 Telomerase activity (TA) measured by TRAP assay in normal, hyperplastic and adenomatous parathyroid tissue.

Diagnosis	TA–	TA+
Normal (<i>n</i> = 22)	22	0
Hyperplasia (<i>n</i> = 6)	6	0
Adenoma (<i>n</i> = 20)	20	0

TA– = < 0.20 $A_{450\text{ nm}} - A_{690\text{ nm}}$; TA+ = > 0.20 $A_{450\text{ nm}} - A_{690\text{ nm}}$.

and histopathological examination. This gland was telomerase-positive.

Discussion

Primary hyperparathyroidism is a syndrome caused by excessive secretion of parathyroid hormone as a result of parathyroid hyperplasia, adenoma or, in rare cases, carcinoma. The classical features of adenoma are expansile growth, reduced or nil parenchymatous fat, cellular pleomorphism and presence of normal parathyroid tissue outside the capsule (1). By definition, parathyroid hyperplasia affects more than one gland and is most often a diffuse growth, although it can be nodular. When nodular, the only criterion which discriminates hyperplasia from adenoma is multiple-gland vs single-gland disease, and as well-defined morphological criteria for benign neoplasia are lacking, classical terminology can be disputed. The terms single-gland and multiple-gland disease have recently been proposed for nomenclature, which is in good concordance with clinical, therapeutic and prognostic knowledge. It has been suggested that parathyroid adenomas might arise from proliferating micronodules in hyperplasias (9). It has also been questioned whether adenomas progress to carcinomas or if the two entities are independent lesions with no pathogenetic relation.

The underlying molecular basis of parathyroid pathogenesis is only partially understood. A disturbed balance between cell proliferation and apoptosis might play an important part, and several molecules have been assessed for their contribution. To date, mutations in the oncogenes *cyclin D1/PRAD1* and *MEN1* have been implicated to alter the growth regulation of adenomatous parathyroid cells (10). Loss of calcium sensitivity is another event discussed, however, mutations of the calcium-sensing receptor gene have not yet been found (10). Complete inactivation of the retinoblastoma (*RB*) tumour suppressor gene (11) and abnormalities in the genes *bcl-2*, *p53* and *MIB-1* (12, 13) have also been reported to influence the proliferation. However, most of these markers are not easily applicable and clear criteria to differentiate the three entities are not yet available.

Telomerase activation appears to be an important step in the progression of an aggressive phenotype of

epithelial parathyroid cells and seems to be required for the growth of at least some immortal cell types. Several investigators have detected telomerase activity in different malignant tissues (2–4), but not in benign or normal tissue, except for haematopoietic stem cells (14), basal cells of the epidermis (15), intestinal crypt cells (16) and activated lymphocytes (14), and telomerase expression is now widely accepted as a marker for malignant disease. However, because lymphocytes have detectable telomerase activity, lymphocytic infiltrates in the analysed tissue can lead to false-positive results. Therefore, it is of major importance to judge the telomerase result together with a careful morphological review for evidence of inflammatory infiltrates. In the present study, one specimen sent for analysis had high telomerase activity; when examined morphologically, it appeared to be a lymph node rather than parathyroid tissue, and was therefore excluded from the material.

A recent study (7) reported one case in which carcinomatous parathyroid tissue showed high telomerase activity, whereas normal, hyperplastic and adenomatous tissue were telomerase-negative. In the present study, we have confirmed this result in a larger number of patients, by showing that normal, hyperplastic and adenomatous parathyroid tissue were not telomerase-positive. In the perspective of the above cited difficulties in mapping parathyroid pathogenesis, these results might have great theoretical significance.

In conclusion, telomerase is an enzyme known to enhance cell proliferation and progression of neoplastic tissue. Our findings indicate that telomerase activity is not involved in the abnormal growth associated with hyperparathyroidism. This observation contributes to the understanding of parathyroid pathogenesis, although the underlying mechanisms remain to be determined.

Acknowledgements

This work was supported by a grant from FORSS (the Health Research Council in the South-East of Sweden).

References

- 1 Grimelius L & Johansson H. Pathology of parathyroid tumors. *Seminars in Surgical Oncology* 1997 **13** 142–154.
- 2 Greider CW & Blackburn EH. Telomeres, telomerase and cancer. *Scientific American* 1996 **274** 80–85.
- 3 Holt SE & Shay JW. Role of telomerase in cellular proliferation and cancer. *Journal of Cellular Physiology* 1999 **180** 10–18.
- 4 Vasef MA, Ross JS & Cohen MB. Telomerase activity in human solid tumors. Diagnostic utility and clinical applications. *American Journal of Clinical Pathology* 1999 **112** 68–75.
- 5 Aogi K, Kitahara K, Buley I, Backdahl M, Tahara H, Sugino T *et al*. Telomerase activity in lesions of the thyroid: application to diagnosis of clinical samples including fine-needle aspirates. *Clinical Cancer Research* 1998 **4** 1965–1970.
- 6 Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL *et al*. Specific association of human telomerase activity with immortal cells and cancer. *Science* 1994 **266** 2011–2015.

- 7 Falchetti A, Becherini L, Martinetti V, Morelli A, Benvenuti S, Picariello L *et al.* Telomerase repeat amplification protocol (TRAP): a new molecular marker for parathyroid carcinoma. *Biochemical and Biophysical Research Communications* 1999 **265** 252–255.
- 8 Lowry OH, Rosenbrough NJ, Farr AL & Randall RJ. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* 1951 **39** 265–275.
- 9 Backdahl M, Howe JR, Lairmore TC & Wells SA Jr. The molecular biology of parathyroid disease. *World Journal of Surgery* 1991 **15** 756–762.
- 10 Mallya SM & Arnold A. Cyclin D1 in parathyroid disease. *Frontiers in Bioscience* 2000 **5** 367–371.
- 11 Dotzenrath C, Teh BT, Farnebo F, Cupisti K, Svensson A, Toell A *et al.* Allelic loss of the retinoblastoma tumor suppressor gene: a marker for aggressive parathyroid tumors? *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 3194–3196.
- 12 Naccarato AG, Marcocci C, Miccoli P, Bonadio AG, Cianferotti L, Vignali E *et al.* Bcl-2, p53 and MIB-1 expression in normal and neoplastic parathyroid tissues. *Journal of Endocrinological Investigation* 1998 **21** 136–141.
- 13 Vargas MP, Vargas HI, Kleiner DE & Merino MJ. The role of prognostic markers (MiB-1, RB, and bcl-2) in the diagnosis of parathyroid tumors. *Modern Pathology* 1997 **10** 12–17.
- 14 Hiyama K, Hirai Y, Kyoizumi S, Akiyama M, Hiyama E, Piatyszek MA *et al.* Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. *Journal of Immunology* 1995 **155** 3711–3715.
- 15 Harle-Bachor C & Boukamp P. Telomerase activity in the regenerative basal layer of the epidermis in human skin and in immortal and carcinoma-derived skin keratinocytes. *PNAS* 1996 **93** 6476–6481.
- 16 Tahara H, Yasui W, Tahara E, Fujimoto J, Ito K, Tamai K *et al.* Immuno-histochemical detection of human telomerase catalytic component, hTERT, in human colorectal tumor and non-tumor tissue sections. *Oncogene* 1999 **18** 1561–1567.

Received 4 December 2000

Accepted 18 April 2001