Urol Res (1998) 26:29–32
© Springer-Verlag 1998

ORIGINAL PAPER

Hidefumi Kinoshita · Osamu Ogawa · Mutsuki Mishina
Hiroya Oka · Kazuhiro Okumura · Hirohiko Yamabe
Toshiro Terachi · Osamu Yoshida

Telomerase activity in adrenal cortical tumors and pheochromocytomas with reference to clinicopathologic features

Received: 8 August 1997 / Accepted: 31 July 1997

Abstract Telomeres consist of short repeated sequences that are shortened on continuous cell proliferations and synthesized by telomerase, an RNA-dependent DNA polymerase. Recent molecular studies have reported that telomerase is activated in most human cancers, whereas it is not detected in most somatic cells. These findings indicate that the positive telomerase activity is closely related to the malignant potential of human tumors. In several types of human tumors, including adrenal cortical tumors and pheochromocytomas, it is very difficult to predict the malignant potential using conventional histopathologic examination. To determine whether telomerase activity is useful as a diagnostic marker, we examined telomerase activity in adrenal cortical tumors and pheochromocytomas with special reference to their clinicopathologic features. Using a highly sensitive polymerase chain reaction (PCR)-based detection method, telomerase activity was demonstrated in one of 13 adrenal cortical tumors and two of seven pheochromocytomas, whereas all seven normal portions of adrenal gland failed to showed any telomerase activity. Although none of the tumors examined in this study was associated with metastasis, these three telomerase-positive tumors were accompanied by clinicopathologic features suggesting malignant potential. Telomerase activity might be a potential marker for estimating the biologic characteristics of adrenal cortical tumors and pheochromocytomas.

H. Kinoshita · O. Ogawa · M. Mishina · H. Oka
K. Okumura · T. Terachi · O. Yoshida (✉)
Department of Urology, Faculty of Medicine,
Kyoto University, Kyoto University Hospital,
54 Kawahara-cho, Shogoin, Sakyo-ku,
Kyoto 606, Japan
Tel: (+81) 075-751-3326, fax: (+81) 075-761-3441

H. Yamabe
Laboratory of Anatomic Pathology, Faculty of Medicine,
Kyoto University, Kyoto University Hospital,
54 Kawahara-cho, Shogoin, Sakyo-ku,
Kyoto 606, Japan

Key words Adrenal tumor · Pheochromocytoma · Telomerase activity · Telomere · TRAP assay · Malignant potential

Introduction

Telomerase is an enzyme that synthesizes telomeric DNA, shortened on continuous cell proliferations [4, 12]. Recently evidence has accumulated to suggest that activation of telomerase is crucial for acquisition of cellular immortality [2] and that telomerase is closely related to malignant phenotype. Indeed, more than 80% of various malignant tumors demonstrate positive telomerase activity, contrary to its absence in normal tissues [1, 3, 5, 7, 9].

It is generally possible to assess the biologic properties of neoplasms according to empirical criteria, based on the identification of histopathologic findings that are significantly correlated with the clinical behavior of certain tumors. However, there are several types of tumors, including adrenal cortical tumors and pheochromocytomas, in which the conventional histopathologic examination fails to predict the biologic characteristics of a given tumor [13]. To test the possibility that telomerase activity might be a marker for the malignant potential of such tumors, we analyzed the telomerase activity in 13 adrenal cortical tumors and seven pheochromocytomas with special reference to their clinicopathologic features.

Materials and methods

Samples and histopathologic examination

The specimens in this study consisted of 13 adrenal cortical tumors classified into 10 hormonally functional tumors and three nonfunctional tumors, and seven pheochromocytomas including two extra-adrenal tumors. These tumors were mainly obtained from laparoscopic surgeries. Seven normal portions of adrenal gland

adjacent to tumor tissue were also examined. All specimens were immediately frozen in liquid nitrogen after removal and then stored at −80°C. None of the patients examined showed apparent metastatic lesions in preoperative or postoperative routine clinical examinations. The diagnosis of these tumors was performed by a single pathologist (H.Y.), and at the same time a careful search was made for the following histopathologic features: diffuse growth pattern, vascular invasion, tumor cell necrosis, broad fibrous bands, capsular invasion, mitotic index and pleomorphism. These features have generally been considered to reflect the malignant potential of these tumors [8, 13].

TRAP assay

Protein extracts from surgically obtained specimens were prepared as described elsewhere [9]. The lysates were prepared using the Bio-Rad DC protein Assay (Bio-Rad, Calif). Telomerase activity was determined by the telomeric repeat amplification protocol (TRAP) as described elsewhere [9] with some modifications. Briefly, we used the 5′ biotin-labeled primer, instead of ^{32}P-dCTP and ^{32}P-dTTP, incorporated into polymerase chain reaction (PCR) products. After the amplified products had been separated on 10% non-denatured acrylamide gels, they were transferred onto nylon membrane (Hybond-N+, Amersham, Bucks, UK) overnight by semi-dry transfer methods. Signal detection was carried out by chemiluminescence methods (Imaging High; Toyobo, Osaka, Japan). The sensitivity was almost the same as that in the original protocol. Telomerase activity was considered to be positive when a six-base-ladder extension was detected. In every reaction, we used the lysate obtained from the fetal kidney cell line, 293, and lysis buffer without cell extract as, respectively, a positive and a negative control. TRAP was performed at least three times on all samples, and the replication of results was verified.

To exclude the inhibitory effects of relatively highly concentrated lysates for the TRAP assay, lysates containing serial 10-fold dilutions of protein from 6 μg to 0.006 μg were prepared in all cases. In the cases with positive telomerase activity, the activity was re-evaluated using multiple samples from different parts of the surgical specimens. Furthermore we confirmed that all the telomerase-positive lysates were sensitive to RNase pretreatment. When a negative result was obtained, the lysates were re-extracted and the TRAP assay repeated to confirm the replication of the negative result. To exclude false negative results due to inhibitors in the lysates of telomerase-negative samples, the TRAP assay was performed by mixing the lysates obtained from telomerase-negative materials with a telomerase-positive fetal kidney cell line, 239. Furthermore, we also tested the inhibitory effects of the PCR step as described by previously [14]. Two tubes containing only lysate (0.6 μg of protein) of the 239 cell line with reaction buffer were prepared, and the lysates (6 μg of protein) from telomerase-negative cases added at two different times: at the beginning of the TRAP assay and just before the PCR step. To confirm the integrity of the extracted proteins, we carried out the assay for alkaline phosphatase activity as described elsewhere and also tested the quality of extracted RNA by ribosomal signals on gel electrophoresis.

Results

Telomerase activity was detected in one aldosterone-producing tumor (case 12) of the 13 adrenal cortical tumors and in two (cases 17 and 19) of the seven pheochromocytomas (Table 1). In contrast, all seven normal samples of adrenal gland failed to showed any telomerase activity: one was obtained from a patient with a telomerase-positive pheochromocytoma (case 17) and the others were from patients with telomerase-negative tumors (cases 4, 5, 6, 7, 11 and 20). All the samples obtained from different parts of each telomerase-positive tumor showed a similar intensity of the telomerase ladder. Furthermore, we referred to the histopathology of all telomerase-positive samples, and confirmed that the contamination of blood cells was minimal. Although a recent report showed that weak telomerase signals were occasionally detected in mononuclear cells in blood [7], these results indicated that the telomerase signals in tumor samples were derived from telomerase activity of tumor cells. We also found some inhibitory effects when

Table 1 Telomerase activity in adrenal cortical tumors and pheochromocytomas (*NF* non-functioning, *PTH-RP* parathyroid hormone related protein, *Csol* cortisol, *Ald* aldosterone, *CA* catecholamines, *Rt* right, *Lt* left, *E* extra-adrenal gland, *TPF* typical pathologic findings, *Pleo* pleomorphism, *Nec* necrosis, *less Pleo* less pleomorphism, *ND* not done)

Case no.	Hormonal activity	Age (years)/ Sex	Site	Tumor weight	Pathologic findings	Telomerase activity	
						Tumor	Normal
Cortical tumor							
1	NF	75/M	Rt	< 5	TPF	−	ND
2	NF	46/F	Rt	< 5	TPF	−	ND
3	NF	49/M	Lt	< 5	TPF	−	ND
4	PTH-RP	29/F	Lt	63	Pleo	−	−
5	Csol	61/F	Lt	< 5	TPF	−	−
6	Csol	48/F	Lt	8	TPF	−	−
7	Csol	47/F	Rt	8	TPF	−	−
8	Csol	41/F	Rt	12	TPF	−	ND
9	Ald	57/M	Lt	< 5	TPF	−	ND
10	Ald	34/F	Rt	< 5	Pleo	−	ND
11	Ald	67/M	Lt	< 5	Pleo	−	−
12	Ald	28/M	Rt	10	Pleo/Nec	+	ND
13	Ald	41/F	Rt	11	TPF	−	ND
Pheochromocytoma							
14[a]	CA	27/M	Lt	10	TPF	−	ND
15[a]	CA	27/M	Rt	64	TPF	−	ND
16	CA	59/F	Lt	26	TPF	−	ND
17	CA	59/M	E	29	less Pleo	+	−
18	CA	52/M	Rt	68	TPF	−	ND
19	CA	18/F	E	42	Nec	+	ND
20	CA	56/M	Rt	180	less Pleo	−	−

[a] The tumors of cases 14 and 15 were obtained from the same patient

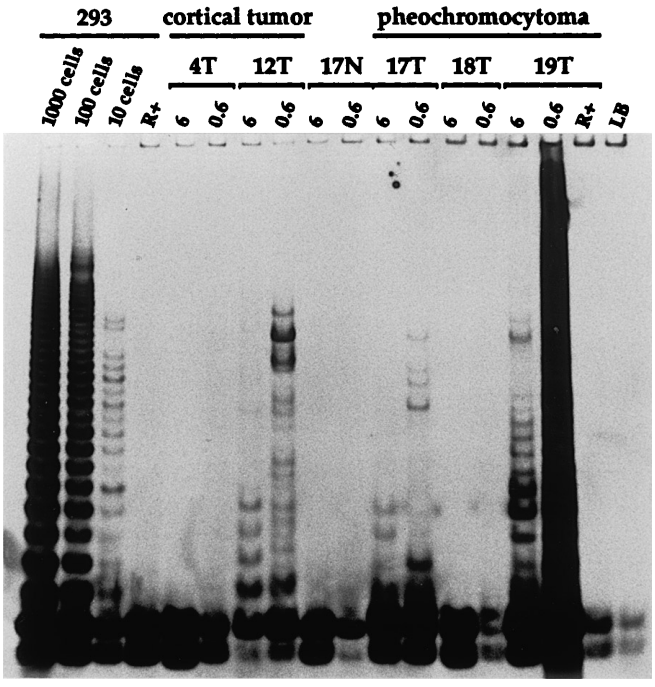

Fig. 1 Telomerase activity in adrenal cortical tumors and pheochromocytomas. Telomerase activity was positive in the tumors (T) in cases 12, 17 and 19, in contrast to the negative result in the normal adrenal gland (17 N). The fetal kidney cell line, 293, and lysis buffer without cell extract (LB), were used as a positive and a negative control, respectively. The assay using 0.06 and 0.006 μg of lysates, and the sensitivity test to RNase pretreatment (R +), were conducted in all cases (only representative data are shown)

the TRAP assay was conducted on a large amount (i.e., 6 μg) of tissue lysate as shown in Fig. 1. However, a significant inhibitory effect that might have caused false negative results was not demonstrated in any telomerase-negative samples by control analyses described in Materials and methods.

The aldosterone-producing tumor with unequivocal telomerase activity presented in spite of its small size, two distinctive histopathologic features: extensive necrosis and nuclear pleomorphism. Increased mitotic activity, diffuse growth pattern, broad fibrous band, and capsular or venous invasion were not observed in any adrenal cortical tumors examined. One telomerase-positive pheochromocytoma exhibited necrosis with hemorrhage, and both the telomerase-positive pheochromocytomas were considered to be derived from extra-adrenal tissues. The clinicopathologic features and telomerase activity status of each tumor are summarized in Table 1.

Discussion

We found one telomerase-positive adrenal cortical tumor that showed extensive necrosis and nuclear pleomorphism. There has been a series of reports discriminating adrenal cortical carcinomas from adenomas based on the clinical and histopathologic features. Hough et al. analyzed seven pathologic features in both metastasizing and non-metastasizing adrenal cortical tumors [8, 13]. These features were diffuse growth pattern, vascular invasion, tumor cell necrosis, broad fibrous band, capsular invasion, mitotic index and pleomorphism. In their study, tumor cell necrosis and pleomorphism were observed in 86% (12/14) and 93% (13/14) of the metastasizing tumors but only in 11% (3/27) and 33% (9/27) of non-metastasizing tumors. Although tumor cell necrosis, diffuse growth pattern, vascular invasion and broad fibrous band are the most significant features, no single factor is completely definitive for predicting metastasis. The authors concluded that the summation of statistically weighted positive parameters is useful for predicting metastasis. Weiss reported a similar analysis of nine pathologic features in 43 adrenal cortical tumors [17]. He proposed a simpler system: that is, when the number of positive pathologic criteria is four or more it is a reliable marker for metastasis.

In our present study there were three other adrenal cortical tumors with nuclear pleomorphism but without telomerase activity. Interestingly these three tumors did not show any other pathologic features suspicious of carcinoma, such as confluent tumor necrosis. Because of the absence of metastatic lesions and the lack of distinctive histologic criteria, all adrenal cortical tumors in our study were diagnosed as benign tumors. According to the recent hypothesis that the reactivation of telomerase is associated with the acquisition of immortality, the tumor with positive telomerase activity might have more aggressive biologic properties than the telomerase-negative tumors.

We noted that telomerase activity was also detected in two (29%) of the seven pheochromocytomas. One was accompanied by a large area of necrosis with extensive hemorrhage, and the other exhibited less nuclear pleomorphism. Although there is controversy regarding the relationship between pleomorphism and malignant potential of pheochromocytomas, some pathologists have proposed that less nuclear pleomorphism is often observed in malignant pheochromocytomas [10]. In addition, it is noteworthy that both the telomerase-positive pheochromocytomas apparently arose from extra-adrenal tissues adjacent to the normal glands. Approximately 10% of pheochromocytomas are derived from orthosympathetic-related paraganglia, and extra-adrenal pheochromocytomas are reported to show malignant potential at a higher frequency than those derived from the adrenal gland [16]. Our observations may reflect the difference between adrenal and extra-adrenal pheochromocytomas in terms of pathogenesis or biologic properties.

Telomerase activity has been proposed to be important in the proliferation of malignant cells and is closely correlated with the malignant phenotype [9]. Recent reports, however, have demonstrated telomerase activity in more than 80% of colorectal adenomas [15] and in almost half the fibroadenomas in young women [6].

According to these observations, this enzyme might be reactivated in the earlier stage of tumorigenesis in certain specific tumors regardless of whether they are benign or malignant. Although we clarified the fact that a subset of adrenal cortical tumors and pheochromocytomas showed positive telomerase activity, additional studies on a larger number of tumors or metastatic lesions are required to elucidate the exact role of telomerase reactivation in tumorigenesis and in the progression of these tumors.

With the advances in diagnostic and treatment technology, adrenal tumors have become detectable at an early stage of development, and also easily removed by less invasive techniques such as laparoscopic surgery [11]. In this context, more reliable markers for determining the biologic potential of adrenal tumors are required to decide the strategy for treatment or follow-up. Telomerase activity might be a potential marker for estimating the biologic characteristics of adrenal cortical tumors and pheochromocytomas.

Acknowledgements This work was supported in part by Grants-in-Aid from the Ministry of Education, Science, and Culture of Japan.

References

1. Chadeneau C, Hay K, HirteI HW, Gallinger S, Bacchetti S (1995) Telomerase activity associated with acquisition of malignancy in human colorectal cancer. Cancer Res 55:2533
2. Courter CM, Avillon AA, Lefeuvre CE, Stewart NG, Greider CW, Harley CB, Bacchetti S (1992) Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. EMBO J 11:1921
3. Courter CM, Hirte HW, Bacchetti S, Harley CB (1994) Telomerase activity in human ovarian carcinoma. Proc Natl Acad Sci USA 91:2900
4. Harley CB, Futcher AB, Greider AW (1990) Telomeres shorten during aging of human fibroblasts. Nature 345:458
5. Hiyama E, Hiyama K, Yokoyama T, Matsuura Y, Piatyszek MA, Shay JW (1995) Correlating telomerase activity levels with human neuroblastoma outcomes. Nature Med 1:249
6. Hiyama E, Gollahon L, Kataoka T, Kuroi K, Yokoyama T, Gazdar AF, Hiyama K, Piatyszek MA, Shay JW (1996) Telomerase activity in human breast tumors. J Natl Cancer Inst 88:116
7. Hiyama K, Hiyama E, Ishioka S, Yamakido M, Inai K, Gazdar AF, Piatyszek MA, Shay JW (1995) Telomerase activity in small-cell and non-small-cell lung cancer. J Natl Cancer Inst 87:895
8. Hough AJ, Hollifield JW, Page DL, Hartmann WH (1979) Prognostic factors in adrenal cortical tumours: a mathematical analysis of clinical and morphologic data. Am J Clin Pathol 72:390
9. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PLC, Coviello GM, Wright WE, Weinrich SL, Shay JW (1994) Specific association of human telomerase activity with immortal cells and cancer. Science 266:2011
10. Madeiros LJ, Wolf BC, Balogh K, Federman M (1984) Adrenal pheochromocytoma: a clinicopathologic review of 60 cases. Hum Pathol 16:580
11. Matsuda T, Terachi T, Yoshida O (1993) Laparoscopic adrenalectomy: the surgical technique and initial results of 13 cases. Min Invas Ther 2:123
12. Morin GB (1989) The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. Cell 59:521
13. Page DL, Delellis RA, Hough AJ (1986) Tumours of the adrenal. In: Hartmann WH (ed) Atlas of tumour pathology, second series, fascicle 23. Armed Forces Institute of Pathology, Washington DC, p 81
14. Piatyszek MA, Kim NW, Weinrich S, Hiyama K, Hiyama E, Wright WE, Shay JW (1995) Detection of telomerase activity in human cells and tumors by a telomeric repeat amplification protocol (TRAP). Methods Cell Sci 17:1
15. Tahara H, Kuniyasu H, Yokozaki H, Yasui W, Shay JW, Ide T, Tahara E (1995) Telomerase activity in preneoplastic and neoplastic gastric and colorectal lesions. Clin Cancer Res 1:124
16. Van Heerden JA, Sheps SG, Hamberger B, Sheedy PF II, Poston JG, ReMine WH (1982) Pheochromocytoma: current status and changing trends. Surgery 91:367
17. Weiss LM (1984) Comparative histologic study of 43 metastasizing and nonmetastasizing adrenal cortical tumors. Am J Surg Pathol 8:163