The hTERT-protein and Ki-67 labelling index in recurrent and non-recurrent meningiomas

L. Maes*, E. Lippens*, J. P. O. Kalala† and L. de Ridder*

*Department of Anatomy, Embryology, Histology and Medical Physics, Section Histology, Ghent, Belgium and

†Department of Neurosurgery, University Hospital, Ghent, Belgium

Received 2 August 2004; revision accepted 11 October 2004

Abstract. Meningiomas are considered as benign neoplasms affecting the coverings of the central nervous system and compromise approximately 20% of all intracranial tumours. However, a number of these tumours recur even after total resection. The aim of this study is to evaluate the prognostic significance for recurrence of the human telomerase catalytic subunit (hTERT) in the cells of meningiomas. The expression of hTERT-protein can be evaluated by immunohistochemical staining using a monoclonal antibody against hTERT (clone 44F42, NCL-L-hTERT). The interdependence between tumour recurrence and cell proliferation in this study is analysed by Ki-67 immunoreactivity (clone MIB-1). Archival material from 29 non-recurrent and 32 recurrent tumours has been evaluated, including specimens from World Health Organization (WHO) stages I ($n = 73$), II ($n = 2$) and III ($n = 12$). Although the tumours were categorized as benign meningiomas following the WHO classification, recurrence in 22 of 50 cases did not correlate with the tumour stage. For hTERT staining, the following results were found for nucleolar and total nuclear staining, respectively: non-recurrent meningiomas, 2.9% (± 7.7) and 3.0% (± 8.0); recurrent meningiomas at first resection, 16.8% (± 19.7) and 31.6% (± 30.2). Concerning the Ki-67 labelling index (LI): for the group of non-recurrent meningiomas, results were 2.1% (± 1.7) and for the recurrent group at first resection, 1.7% (± 2.0). A significant difference was seen for the hTERT staining ($P < 0.001$) between the non-recurrent and recurrent meningiomas, whereas no statistical significance was found for Ki-67. In conclusion hTERT-positive meningiomas had a high incidence for recurrence. Ki-67 was a good marker of cell proliferation status of the tumours, but did not correlate with recurrence; thus, hTERT alone seemed to be a potential predictor for recurrence.

INTRODUCTION

Meningiomas are the most common benign neoplasms affecting the coverings of the nervous system and are estimated to constitute up to 20% of primary intracranial tumours. Most meningiomas do not relapse after total resection, with the exception of a limited number (6–19%).

Correspondence: Prof. Dr L. de Ridder, Department Histology, Ghent University, Louis Pasteurlaan 2, B-9000 Gent, Belgium. Tel.: + 32(9)2649240; Fax: + 32(9)2649498; E-mail: leo.deridder@ugent.be

Meningiomas are classified following the World Health Organization (WHO) system into three grades, benign meningioma, atypical meningioma and anaplastic meningioma (Kleihues & Cavenee 2000). Although various histopathological features have been associated with aggressive behaviour or recurrence, there is little agreement on which features are most important to prognosis (Jaaskelainen *et al.* 1986; Mahmood *et al.* 1993; Carroll *et al.* 1999). Several studies have been undertaken concerning the proliferation potential of meningioma cells using antibodies against Ki-67. In correlation with recurrence, none of the current articles has been able to predict recurrence at an individual-patient level (Abramovich & Prayson 1999; Ho *et al.* 2002). On the other hand, telomerase activity on telomeres has been linked to malignancy, recurrence and cellular indefinite life span. Telomeres are repetitive DNA sequences at the ends of chromosomal DNA (Blackburn 1991; Nugent & Lundblad 1998). Telomerase is a ribonucleoprotein polymerase enzyme, composed of an integral RNA component (hTR) and several other proteins, including a reverse transcriptase subunit (hTERT). Telomerase activity is thought to be essential for maintaining chromosome ends by adding repetitive TTAGGG sequences to the telomeres (Weinrich *et al.* 1997; Masutomi *et al.* 2000); it is linked to cell immortality. Several studies have found that telomerase activity and hTERT mRNA are up-regulated in atypical, malignant, or recurrent meningiomas, but remain low or absent in benign meningiomas (Simon *et al.* 2000; Cabuy & de Ridder 2001). Because of the current dilemma for neurosurgeons and neuropathologists when predicting which patients have a higher incidence of relapse after total resection of a meningioma, relating to the present WHO classification, we have analysed a selected group of samples from our archival, paraffin-embedded material, taken from patients with recurrent meningeal tumour. First, we checked the biopsies for their WHO classification. Second, the expression of the reverse transcriptase subunit (hTERT-protein) was evaluated from the telomerase complex, and finally, an immunohistochemical analysis was performed to determine the cell proliferation status. These data were compared to data from a group of non-recurrent meningiomas and statistically analysed for a correlation with recurrence.

MATERIALS AND METHODS

Tumour samples, clinical data and histopathological classification

A series of biopsies from 446 patients operated on for relapsed meningioma was selected from our archival material. Surgery had been performed between 1985 and 1999 at the University Hospital, St Lucas Hospital and Maria-Middelares, Ghent, Belgium. The selected material referred to intracranial meningiomas that had been totally resected at the initial operation and that were supported by complete histopathological records. Pre-operative radiation therapy had not been administered in any of the analysed cases. Total resection was based on the surgeon's assessment at the time of surgery and on post-operative scanning. Pertinent clinical data were obtained from the patients' medical records. Fifty-eight biopsies, including initial resections and recurrences, from 32 recurrent patients and 29 biopsies from non-recurrent patients were collected and classified according to the WHO criteria (Kleihues & Cavenee 2000). All available histological slides were reinterpreted and evaluated for tumour grade, including WHO stage I ($n = 73$), II ($n = 2$) and III ($n = 12$). Cases were immunocytochemically stained for vimentin (an intermediate filament), glial fibrillary acidic protein (GFAP) for the cytoplasmic filament of astrocytomas, S-100 protein for glial and neural tumours and NSE for neurone-specific enolase, to confirm the diagnosis. The median patient age was 56.5 (range 25–86 years) and there were 42 women and 19 men.

Recurrent meningiomas

The 58 archival biopsies from the 32 relapsing patients included 27 paraffin-embedded biopsies from the initial resection, 27 from the first recurrence, three from a second relapse and one from a third relapse. The presence of recurrence was based on data obtained from clinical and histological records. The tumours comprised 22 benign and five malignant meningiomas at initial resection. The group of five recurrent malignant meningiomas was considered as the control group for recurrence. In five other cases there was histopathological malignant development of benign meningiomas towards a higher WHO grade after the initial resection in the first recurrence.

Non-recurrent meningiomas

In addition, a group of 29 non-recurrent meningiomas was chosen randomly as control. These comprised 28 benign meningiomas and one non-recurrent atypical meningioma.

Immunohistochemical analysis

hTERT analysis

Paraffin-embedded tissue sections (4- μ m) from intracranial tumours were prepared for examination by standard immunohistochemical procedures. Slides were heated in sodium citrate buffer (10 mM, pH 6.0) for 30 min in a microwave oven at the highest power (700 W, Amana, Newton, IA, USA) for high-temperature antigen unmasking. Endogenous peroxidase activity was blocked by treatment with 3% H₂O₂ in distilled water for 10 min at room temperature. The standard streptavidin–biotin–peroxidase complex method was performed, employing antibodies against hTERT (1 : 50 dilution; clone 44F12; Novocastra, Newcastle, UK). Expression of hTERT was defined as the ratio of cells with immunoreactivity per 1000 tumour cells. Subsequently, sections were incubated with biotinylated rabbit anti-mouse immunoglobulin G, 1 : 200 for 30 min, followed by a third incubation with streptavidin–biotin–peroxidase complex, 1 : 200 for 30 min. Visualization of the complex was realized with diaminobenzidine (DAB) and sections were counterstained with haematoxylin. A glioblastoma multiforme, positive for hTERT, was used as positive control and normal brain tissue and antigen-free buffer of the positive control were used as the negative control. The hTERT percentages were calculated as the mean percentage values by counting the nuclei of tumour cells in three positive areas of a single tumour for 1000 cells.

Ki-67 analysis

To evaluate the proliferative activity of tumour cells, Ki-67 immunohistochemistry was utilized. The same staining technique was performed as described for hTERT immunoreactivity with minor modifications. Slides were heated in sodium citrate buffer (10 mM, pH 6.0) for 15 min in a microwave oven at the highest power (700 W, Amana) for high-temperature antigen unmasking. The antibody against Ki-67 (clone MIB-1, 1 : 50 dilution; DAKO, Glostrup, Denmark) was employed. A glioblastoma multiforme was again used as a positive control and primary antigen-free buffer of the positive control served as the negative control. The Ki-67 labelling index (LI) was calculated as mean percentage values by counting the nuclei of tumour cells in three positive areas of a single tumour, for 1000 cells.

Statistical analysis

Statistical analysis was performed with SPSS FOR WINDOWS version 10.0 (SPSS Inc., Chicago, IL, USA). The categorized variables entered into the analysis were patients' histological subtype, tumour grade, recurrence, Ki-67 LI and hTERT LI. Tumour recurrence was the major

response variable. The initial resection in the group of recurrent benign meningiomas, and the biopsies from non-recurrent benign meningiomas were both evaluated statistically. Values were expressed as mean $\pm$ SD and as percentages when appropriate. The Spearman correlation test was used to measure the strength of association between the variation in WHO grade, Ki-67 LI and hTERT LI and the recurrence at the time of initial resection for relapsing meningiomas and the biopsies from the non-recurrent group. A P -value < 0.05 was considered statistically significant. All reported P -values are two-tailed.

RESULTS

Although classified as benign, 22 of the 50 WHO grade I meningiomas recurred after initial resection. Sixteen of these recurred within 5 years, together with five atypical or malignant meningiomas (Fig. 1).

All except one of the 27 recurrent benign meningiomas at initial resection were positive for the 44F12 antibody against hTERT. In contrast, six out of 28 cases within the group of non-recurrent benign meningiomas (21%) were positive for hTERT. From these six cases, two expressed the molecule less than 5%, two expressed it between 10 and 20% and two of them expressed it $> 20\%$. In their second and later resections, all recurrent meningiomas were positive for hTERT at higher levels. Positive staining of the tumour cells was seen in the nucleolar region (or it had some speckles around it) and in the nucleus, with or without staining of the nucleolus. Tumour cell staining of nucleoli and total nucleoplasm was analysed. Overall results pertaining to scoring, staining pattern and recurrence for all meningiomas are summarized in Table 1 and Figs 2, 3 and 4. Concerning the benign meningiomas, nucleolar hTERT LI was 3.0% (SD = 7.8) for non-recurrent and 16.3% (SD = 21.1) for the recurrent meningiomas. For total staining of the nucleoplasm, nucleoli included, hTERT LI was 3.1% (SD = 8.1) for non-recurrent benign meningiomas and 31.0% (SD = 31.0) for recurrent benign meningiomas. Heterogeneity and intensity of hTERT staining differed depending on the tumour, and positive cells were clustered together in islands surrounded by non-staining cells (Fig. 5). A significant correlation was found between hTERT protein staining and recurrence in the benign meningiomas. For nucleolar

Figure 1. Cumulative proportions of patients showing tumour relapse against the years since the first operation (total $n = 27$).

Table 1. Comparison of Ki-67 and hTERT expression in non-recurrent versus recurrent meningiomas

	Non-recurrent (n = 29)	Recurrent			
		Initial resection (n = 27)	Recurrence 1 (n = 27)	Recurrence 2 (n = 3)	Recurrence 3 (n = 1)
% Ki-67 labelling ($\pm$ SD)	2.1 ($\pm$ 1.7)	1.7 ($\pm$ 2.0)	2.2 ($\pm$ 3.8)	4.8 ($\pm$ 1.7)	3.5
% hTERT staining ($\pm$ SD) in nucleolus	2.9 ($\pm$ 7.7)	16.8 ($\pm$ 19.7)	20.1 ($\pm$ 16.8)	50.3 ($\pm$ 13.4)	32.4
% hTERT staining ($\pm$ SD) in nucleus	1.6 ($\pm$ 5.6)	20.9 ($\pm$ 33.9)	21.7 ($\pm$ 30.5)	8.2 ($\pm$ 13.6)	41.4
Total % hTERT staining ($\pm$ SD)	3.0 ($\pm$ 8.0)	31.6 ($\pm$ 30.2)	40.4 ($\pm$ 26.2)	57.8 ($\pm$ 3.1)	53.2

SD = standard deviation.

Figure 2. Relation between Ki-67, hTERT-protein and recurrence. **Figure 3.** Correlation pattern for Ki-67 and total hTERT staining in non-recurrent meningiomas.

Figure 4. Correlation pattern for Ki-67 and total hTERT staining in recurrent meningiomas (first resection).

Figure 5. Immunohistochemical staining for hTERT, deeply stained nucleoli and showing nucleolar staining with speckles in some cells (arrow), of a malignant meningioma.

staining, the correlation coefficient was 0.67 ($P < 0.001$) and for staining of the total nucleoplasm, nucleoli included, it was 0.74 ($P < 0.001$).

Results of comparison of immunohistochemical expression of Ki-67 between the recurrent and non-recurrent meningiomas are also presented in Table 1 and Figs 2, 3 and 4. A representative image of Ki-67 immunostaining localized to whole nuclei is shown in Fig. 6. Ki-67 immunostaining was relatively homogeneous and all samples had indicated expression. The mean Ki-67 LI was 2.5% (SD = 3.1) for atypical and malignant meningiomas, 1.9% (SD = 1.4) for non-recurrent benign meningiomas and 1.7% (SD = 2.0) for recurrent benign meningiomas. The statistical investigation was performed for Ki-67 LI between the group of recurrent benign meningiomas at initial resection and the benign non-recurrent tumours. No correlation was found between Ki-67 LI and recurrence for our benign meningiomas (correlation coefficient = -0.21 ; $P = 0.14$).

Figure 6. Immunohistochemical staining for Ki-67 showing nuclear staining of the neoplastic cells from a malignant meningioma.

DISCUSSION

Meningiomas are considered to be benign neoplasms. A particular subset has a tendency to recur despite macroscopic radical surgery. Some hypotheses have appeared in the literature concerning the possible mechanisms for recurrence of these meningiomas (Jaaskelainen 1986). It is conceivable that neurosurgeons may miss a micro-tumour or isolated tumour cells, resulting in the regional regrowth of a meningioma. Although various histopathological features have been associated with more aggressive behaviour and recurrence of meningiomas, there is limited agreement about which features are the most important. These features include loss of architecture, hypercellularity, nuclear pleomorphism, prominent nucleoli, high nuclear : cytoplasmic ratios, increased mitotic activity, hypervascularity, necrosis and invasion of the brain (Jaaskelainen *et al.* 1986; de la Monte *et al.* 1986; Kleihues & Cavenee 2000). The lack of consensus regarding the characteristics associated with aggressive tumour behaviour and recurrence for meningiomas makes it difficult for histopathologists and neurosurgeons to predict recurrence. In meningiomas, the Ki-67 LI, telomerase activity and hTERT mRNA have been correlated with clinical tumour recurrence without correlation with the clinical evolution (Simon *et al.* 2000; Cabuy & de Ridder 2001). Here, we therefore studied the immunohistochemical hTERT-protein expression in comparison with the Ki-67 LI linked to the WHO classification within our selected group of recurrent meningiomas.

Our study indicates the problem for neuropathologists when they predict the recurrence of meningiomas and when they classify a meningioma as atypical. In the WHO classification, the atypical form is linked to the next common tumour-associated features: frequent mitosis, increased cellularity, small cells with high nuclear : cytoplasmic ratios and/or prominent nucleoli, uninterrupted patternless or sheet-like growth and foci of 'spontaneous' or geographical necrosis (Kleihues & Cavenee 2000). Meningiomas categorized as 'atypical' or 'malignant' have the tendency to recur after total resection. The problem is the behaviour of meningiomas diagnosed as benign. They are considered as quiescent tumours but nevertheless up to one-fifth of them recur after total resection (Jaaskelainen 1986; Mahmood *et al.* 1993). Although they had been classified as benign histopathologically, 22 of our meningiomas recurred after total initial resection.

The use of cell proliferation markers in the evaluation of meningiomas to identify those tumours that have an increased cell proliferation rate and a putative higher risk for recurrence has been well investigated (Abramovich & Prayson 1999). Some researchers have found a higher Ki-67 LI at times of initial resection in tumours that relapsed later, while others obtained no statistically significant difference in Ki-67 LI between non-recurrent and recurrent tumours (Abramovich & Prayson 1999; Scholzen & Gerdes 2000; Ho *et al.* 2002). Our results have shown no statistical difference for Ki-67 LI between the non-recurrent and recurrent benign meningiomas (correlation coefficient = -0.21). The Ki-67 expression concurs with classification by WHO categorization and tends to correlate with the biological behaviour of these different groups (i.e. benign, atypical and malignant), this however, does not indicate whether a tumour has a tendency to recur. Therefore, Ki-67 is a good marker of the proliferation status of the cells of a biopsy, and for studies between different histopathological groups of meningiomas devised by WHO. At the individual level though, it has been found to not be an indicator of possible recurrence and, as such, its expression has no predictive value.

We investigated hTERT-protein expression in our series of meningiomas. Several other groups have found a correlation between hTERT mRNA and recurrence (Simon *et al.* 2000; Cabuy & de Ridder 2001). Simon *et al.* found that all nine biopsies from recurrent patients analysed by reverse transcriptase–polymerase chain reaction (RT-PCR) expressed hTERT mRNA. However, expression of hTERT-protein has not previously been investigated systematically in series of recurrent meningiomas. Twenty-six of our 27 recurrent meningiomas were positive for hTERT-protein expression at the time of initial resection and at the moment of recurrence all were positive at even higher levels. In contrast, looking at the group of non-recurrent meningiomas, only six of them were positive (20%) at the initial biopsy. Further follow-up would be required to determine any potential recurrence. Statistically, there is a significant correlation of 0.74 between initial resections of benign meningiomas and recurrence concerning their hTERT expression. Thus, hTERT positivity demonstrated with monoclonal antibody 44F12 provides valuable information concerning the biological behaviour of the tumour, as it seems to predict recurrence and clinically malignant behaviour, even in histopathologically diagnosed benign meningiomas. hTERT may be considered as a hallmark for the biological transformation of benign meningiomas towards malignancy and recurrence. Moreover, a progression towards a higher grade of positivity for hTERT has been demonstrated in the later recurrences and with a lower variation in the results.

The advantage of immunohistochemical staining for the hTERT-protein with 44F12 antibody, in comparison to the analysis for hTERT mRNA with RT-PCR, is that it demonstrates cellular distribution of the molecule *in situ*. The procedure and handling of biopsies for RT-PCR render it difficult to compare with immunostaining, because of RNase activity, temperature inactivation, contamination and time–labour relationships. Studies using *in situ* hybridization for hTERT mRNA have also been successful but these techniques are more time consuming and detect the mRNA instead of the protein itself (Saldanha *et al.* 2003).

In our case specimens, the distribution of hTERT was mainly nucleolar, although in some samples there was generalized total staining of the nucleoplasm, with or without special darker staining of the nucleolus. Concerning cellular localization of the hTERT-protein, there are varying hypotheses in the literature, amongst which the most important seems to us to be staining of the nucleolus (Etheridge *et al.* 2002; Smith *et al.* 2003). Wong *et al.* showed that the active telomerase complex is formed within the nucleolus, where it is sequestered and protected and prevents chromosomes from nonsense transcriptions. Next, that group concluded that telomerase is active on chromosomes during the S and early G₂ phases of mitosis, the time at which human telomeres are thought to be replicated (Wong *et al.* 2002). Besides nucleolar staining,

staining which we have termed 'speckling' is seen around the nucleoli. A possible explanation is that these speckles are the active telomerase complex at the chromosomal ends. To summarize, after transcription, hTERT mRNA moves into the cytoplasm where it is translated into the protein. Next, there is phosphorylation of the protein, enabling it to re-enter the nucleus where it locates to the nucleolar region. Finally, it becomes active on the chromosomal ends as they are being released from the nucleolus.

In conclusion, present clinical indicators are insufficient to predict recurrence after resection of benign or atypical meningiomas. Ki-67 expression provides information on the proliferative status of the cells of a tumour. At group level a straight correlation with the histopathological WHO grade of malignancy is seen. Meningiomas that are hTERT-protein positive have a high incidence for recurrence. Thus, hTERT seems to be a predictor of potential recurrence.

ACKNOWLEDGEMENTS

We would like to thank the neurosurgeons from the University Hospital, St Lucas Hospital and Maria-Middelares in Ghent, who kindly provided us with tumour samples, and Klaus Bacher from Medical Physics, Ghent University, for statistical analysis. We are also grateful to N. François, B. Hillaert, R. De Vos and L. Pieters for technical assistance.

REFERENCES

- ABRAMOVICH CM, PRAYSON RA (1999) Histopathologic features and MIB-1 labeling indices in recurrent and nonrecurrent meningiomas. *Arch. Pathol. Lab. Med.* **123**, 793.
- BLACKBURN EH (1991) Structure and function of telomeres. *Nature* **350**, 569.
- CABUY E, DE RIDDER L (2001) Telomerase activity and expression of telomerase reverse transcriptase correlated with cell proliferation in meningiomas and malignant brain tumors *in vivo*. *Virchows Arch.* **439**, 176.
- CARROLL T, MALTBY E, BROCK I, ROYDS J, TIMPERLEY W, JELLINEK D (1999) Meningiomas, dicentric chromosomes, gliomas, and telomerase activity. *J. Pathol.* **188**, 395.
- ETHERIDGE KT, BANIK SS, ARMBRUSTER BN, ZHU Y, TERNS RM, TERNS MP, COUNTER CM (2002) The nucleolar localization domain of the catalytic subunit of human telomerase. *J. Biol. Chem.* **277**, 24764.
- HO DM, HSU CY, TING LT, CHIANG H (2002) Histopathology and MIB-1 labeling index predicted recurrence of meningiomas. *Cancer* **94**, 1538.
- JAASKELAINEN J (1986) Seemingly complete removal of histologically benign intracranial meningioma: late recurrence rate and factors predicting recurrence in 657 patients. *Surg. Neurol.* **26**, 461.
- JAASKELAINEN J, HALTIA M, SERVO M (1986) Atypical and anaplastic meningiomas: radiology, surgery, radiotherapy, and outcome. *Surg. Neurol.* **25**, 233.
- KLEIHUES P, CAVENEE WK (2000) *WHO Classification of Tumors. Pathology and Genetics. Tumours of the Nervous System*. Lyon: IARC Press.
- MAHMOOD A, CACCAMO DV, TOMECEK FJ, MALIK GM (1993) Atypical and malignant meningiomas: a clinicopathological (review). *Neurosurgery* **33**, 955.
- MASUTOMI K, KANEKO S, HAYASHI N, YAMASHITA T, SHIROTA Y, KOBAYASHI K, MURAKAMI S (2000) Telomerase activity reconstituted *in vitro* with purified human reverse transcriptase and human telomerase RNA component. *J. Biol. Chem.* **275**, 22568.
- DE LA MONTE SM, FLICKINGER J, LINGGOOD RM (1986) Histopathologic features predicting recurrence of meningiomas following subtotal resection. *Am. J. Surg. Pathol.* **10**, 836.
- NUGENT CI, LUNDBLAD V (1998) The telomerase reverse transcriptase: components and regulation. *Genes Dev.* **12**, 1073.
- SALDANHA SN, ANDREWS LG, TOLLEFSBOL TO (2003) Analysis of telomerase activity and detection of its catalytic subunit, hTERT (Review). *Anal. Biochem.* **315**, 1.

- SCHOLZEN T, GERDES J (2000) The Ki-67 protein: from the known and the unknown. *J. Cell Physiol.* **182**, 311.
- SIMON M, PARK TW, LEUENROTH S, HANS VH, LONING T, SCHRAMM J (2000) Telomerase activity and expression of the telomerase catalytic subunit, hTERT, in meningioma progression. *J. Neurosurg.* **92**, 832.
- SMITH DL, SORIA JC, MORAT L, YANG Q, SABATIER L, LIU DD, NEMR RA, RASHID A, VAUTHEY JN (2003) Human telomerase reverse transcriptase (hTERT) and Ki-67 are better predictors of survival than established clinical indicators in patients undergoing curative hepatic resection for colorectal metastases. *Ann. Surg. Oncol.* **11**, 45.
- WEINRICH SL, PRUZAN R, MA L, OUELLETTE M, TESMER VM, HOLT SE, BODNAR AG, LICHTSTEINER KIM NW, TRAGER JB, TAYLOR RD, CARLOS R, ANDREWS WH, WRIGHT WE, SHAY JW, HARLEY CB, MORIN GB (1997) Reconstitution of human telomerase with the template RNA component hTR and the catalytic protein subunit hTERT. *Nat. Genet.* **17**, 498.
- WONG JM, KUSDRA L, COLLINS K (2002) Subnuclear shuttling of human telomerase induced by transformation and DNA damage. *Nat. Cell. Biol.* **4**, 731.