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Telomerase activity in relation to pro- and anti-apoptotic protein expression in high grade non-Hodgkin's lymphomas

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Background and Objectives. Telomerase activity (TA) is determined by the catalytic unit telomerase reverse transcriptase (hTERT). *In vitro* studies show that hTERT is downregulated by wild type *p53* and TA is upregulated by BCL-2 expression. The aim of this study was to investigate the relationship of TA and mRNA expression of hTERT, telomerase RNA (hTER) and Tankyrase in 31 samples from patients with high-grade non-Hodgkin's lymphoma (HG-NHL). The results were then related to apoptosis and proliferation and the expression of p53 and BCL-2 family member proteins.

Design and Methods. The telomeric repeat amplification protocol (TRAP) assay and reverse transcription-polymerase chain reaction (RT-PCR) were used to quantify TA, and hTERT, hTER and Tankyrase mRNA expression. Proliferation (Ki67), p53, BCL-2, MCL-1, BAX and BAK protein expression were evaluated by immunohistochemistry. Apoptosis was evaluated by TUNEL staining.

Results. TA was detected in 93% of HG-NHL and tended to be higher in p53+ lymphomas. A positive correlation existed between mRNA expression of hTERT, hTER and Tankyrase. hTERT mRNA expression tended to be higher with increasing levels of apoptosis and proliferation, in HG-NHL samples lacking BAX expression and in samples from patients with survival shorter than 3.5 years. hTER mRNA expression was significantly higher in BAX and BAK negative samples.

Interpretation and Conclusions. Telomerase is activated or upregulated in the majority of HG-NHL. Enhanced TA combined with deregulation of the factors responsible for cell survival and proliferation may contribute to the development and progression of lymphomas. Observation that high hTERT mRNA expression may be related to shorter survival should prompt further investigation of the clinical significance of TA and its components in HG-NHL.

Key words: lymphoma, telomerase, proliferation, apoptosis, BCL-2, p53

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Telomerase is a multi-component ribonucleoprotein polymerase that synthesizes telomeric repeat sequences (TTAGGG)n essential for the stability and integrity of chromosomes. Telomerase activity (TA) prevents the loss of terminal DNA which occurs during linear DNA replication (i.e. the end replication problem), allowing continued proliferation and hence, contributing to cellular immortalization.[1,2] Telomerase is believed to play an important role in tumorigenesis as it provides a mechanism for telomere maintenance, which is a requirement for continuous cell proliferation.[2-5] TA is generally absent in normal somatic tissues, but is detectable in >80% of all malignant human tumors[6-8] including non-Hodgkin´s lymphomas (NHL).[9,10]

Human telomerase consists of telomerase RNA template (hTER) which provides an intrinsic template for addition of telomeric repeat sequences[11] and telomerase reverse transcriptase (hTERT) which is a key component for the control of TA.[12-14] Induction of hTERT expression is essential for telomerase activation during cellular immortalization and tumor progression.[12] Ectopic expression of hTERT is capable of reconstituting TA in normal human fibroblasts.[14] *In vitro* studies have shown that the tumor suppressor gene *p53* downregulates the transcription of hTERT in human cancer cells.[15-17] Interestingly, the *p53* gene is frequently inactivated during oncogenesis. In NHL, *p53* mutations are frequently related to tumor progression[18] and are associated with poor prognosis.[19,20] However, the few hitherto published studies on telomerase expression in NHL did not compare TA in p53 positive and p53 negative tumors.[10,21,22]

Tankyrase (Tank) is a poly (ADP-Ribose) polymerase, a DNA repair enzyme, found at human telomeres. It plays a role in genomic DNA stability.[23] Tank has been shown to be a positive regulator of telomere elongation *in vivo*.[24] It binds to the telomeric protein TRF-1 (telomeric repeat binding factor 1) and inhibits TRF-1 function by a process of poly-ADP/ribosylation. TRF-1 is a negative regulator of telomere extension. It does not control the expression of telomerase itself but is believed

to inhibit the action of telomerase at the telomere termini.[24] Thus, Tank may indirectly facilitate TA. Studies relating TA to Tank expression in malignant tumors are very scarce.[25]

The *bcl-2* gene was first discovered in follicular lymphomas that bear a t(14;18)(q32; q21) translocation, which results in the over-expression of a BCL-2 protein in germinal center cells.[26] Over-expression of BCL-2 contributes to oncogenesis by blocking apoptosis thereby promoting cell survival.[27] The BCL-2 protein family consists of pro-apoptotic (e.g. BAX, BAK, BCL-xS) and anti-apoptotic (BCL-xL, MCL-1) proteins which share homology with BCL-2 and regulate programmed cell death (PCD) by protein-protein interactions.[28] Interestingly, over-expression of BCL-2 has been shown *in vitro* to upregulate TA.[29] *In vitro* studies have shown that telomerase may also play a role in suppressing the apoptotic signaling cascade.[30] Therefore, it was of interest to assess whether there is any relationship between BCL-2 protein family expression and TA in high-grade (HG)-NHL.

Information on the prognostic significance of telomerase activity in HG-NHL is very scarce. It has been reported that TA is higher in aggressive NHL than in low-grade (LG) lymphomas.[10,22] However, high telomerase activity has been reported to have an adverse prognostic significance in several malignancies such as neuroblastoma,[31] gastric cancer,[32] breast cancer,[33] meningiomas,[34] B-chronic lymphocytic leukemia (B-CLL)[35] and acute myeloid leukemia (AML).[36,37]

In the present study we investigated whether a relationship exists between activity of telomerase, its components and Tank with the expression of p53 and BCL-2 family proteins, proliferation and apoptosis in 31 samples from patients with HG-NHL. Preliminary data on patient outcome in relation to activity of telomerase and its components are also presented.

Design and Methods

Biopsies

Formalin-or B5-fixed, paraffin-embedded and fresh-frozen biopsies from 31 patients with HG-NHL collected during the period June 1994 to February 1996 at the Hematopathology Laboratory, Department of Pathology at Karolinska Hospital in Stockholm were investigated. Twenty-seven biopsies were obtained at diagnosis and 4 biopsies at recurrence of the disease. The biopsies comprise the consecutive cases diagnosed during this period with available frozen material. This study was approved by the local ethics committee.

The biopsy samples were reviewed by one of us (APM) and re-classified according to the REAL classification.[38] Twenty-one cases were B-cell derived: 8 diffuse large cell (DLCB), 8 follicular center cell derived type III (FCC III) and 5 other B-cell derived [2 high-grade mantle cell (MAC), 2 T-cell rich B-NHL (TCR-B) and 1 lymphoblastic (LB-B)]. Seven cases were T-cell derived: 1 lymphoblastic (LB-T) and 6 peripheral medium and large mixed cell (P-T). Three cases were large cell anaplastic (LCA) NHL. Samples from 7 reactive hyperplastic tonsils and peripheral blood mononuclear cells (PBMC) from 8 healthy donors were also investigated.

Clinical data

The mean age of the patients was 65 years (range: 22-86, median 71). There were 19 males and 12 females (M: F= 1.6). Data referring to stage of disease were available for 25 patients: 9 (36%) of the patients presented with stage I disease, 3 (12%) with stage II disease, 4 (16%) with stage III disease and 9 (36%) with stage IV disease.[39] Twenty-eight patients received chemotherapy, however, information concerning the specific therapy protocols applied was available for only 21 patients: of these, 11 patients received CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone,[40] 6 patients received CHOP in combination with other cytostatic drugs and 4 patients received other treatment protocols (CNOP, DexaBEAM). Three patients with stage I disease were treated with radiotherapy.

Response to primary treatment was estimated as a complete remission (CR) or no complete remission (i.e. partial remission or no response)(NR). CR was defined as the resolution of clinical and radiologic signs of disease for at least 4 weeks. In the group of patients who obtained CR, disease-free survival (DFS) was estimated as the time to relapse or last follow-up. The follow-up time for surviving patients was at least 4 years. The overall survival was calculated from the date of diagnosis until death or the last follow-up evaluation.

Preparation of frozen tissue

In order to ensure that the frozen material used to assess TA, and mRNA expression of hTER, hTERT and Tank corresponded to the paraffin-embedded material used for TUNEL staining and immunohistochemistry, 5 μm sections were cut from frozen samples and stained with Wright´s stain. A suitable area was then located by comparing the histologic features of the frozen material to those of a hematoxylin and eosin stained section from the paraffin block. This area was then cut away from the frozen biopsy and further sectioned using a cryostat or sterile scalpel.

Telomerase activity assay

A commercial telomerase PCR ELISA kit (Boehringer Mannheim, Mannheim, Germany) based on a telomeric repeat amplification protocol (TRAP assay) was used to determine TA in the fresh-frozen biopsies from HG-NHL lymph nodes.[6] Since the first analysis gave negative results in most samples, suggesting the presence of abundant RNase, 20υ (units) of RNase inhibitors were added in 100 μL of buffer. Then, 0.5 μg of protein extract was used according to the manufacturer´s protocol. Protein extracts from a REH cell line and lysis buffer were always tested in parallel as positive and negative controls, respectively. The value of TA in duplicate studied samples was expressed as the percentage of enzymatic activity exhibited in 0.5 μg of REH cellular protein extract, considered as 100%.

RT-PCR detection of hTERT, hTER and Tank mRNA

Relative mRNA expressions of hTERT, hTER and Tank were determined in the fresh frozen biopsy material by RT-PCR. RNA extraction, cDNA synthesis and PCR were performed as described elsewhere.[37] PCR products were resolved in 2% ethidium bromide stained agarose gels, visualized in UV light and photographed. Volumetric integration of signal intensities was performed using NIH Image software (version 1.58). The relative levels of hTERT, hTER and Tank mRNA expression were given by the ratio of their individual signal density to that of β_2-microglobulin (β_2M) (i.e. normalized to β_2M expression).

Detection of apoptotic cells by TUNEL staining

TdT-mediated dUTP nick labeling (TUNEL) (Apoptag Kit, Oncor, Gaithersburg, MD) was performed on paraffin sections as previously described.[41] Sections from reactive, hyperplastic tonsils containing apoptotic cells in the germinal centers were used as positive controls in every staining batch. In negative control slides, TdT enzyme was substituted by double distilled (dd)$H_2$0. For each TUNEL stained section, 40 microscopic fields were assessed using the ×63 objective and a 42 Weibel grid. Results were expressed as percentages of TUNEL positive cells.

Immunohistochemistry

The expression of p53, BCL-2, BAX, BAK, MCL-1, and Ki-67 was evaluated by immunohistochemistry. Briefly, 5 μm sections were deparaffinized, rehydrated and cooked in citric buffer, pH 6.0 at 700W in a microwave oven for the predetermined time period. After cooling and washing in Tris buffer (pH 7.6), endogenous peroxidase activity was quenched by rinsing in 3% hydrogen peroxide for 30 minutes. Sections were incubated with the working dilution of the primary antibody: BCL-2 (DAKO, Clone 124) 1:40, BAX (Pharmingen, 13666E, Rabbit Polyclonal) 1:800, BAK (Oncogene Research Products, TC102) 1:60, MCL-1 (Pharmingen, 13656, Rabbit Polyclonal) 1:600, p53 (Santa Cruz Biotechnology, DO-1) 1:100, Ki-67 (Immunotec, MIB-1) 1:100. Incubation with secondary antibodies, rabbit anti-mouse (RAM, DAKO, Glostrup, Denmark, code E0413, 1:200) or swine anti-rabbit [(SWAR, DAKO, Glostrup, Denmark, code E0431, 1:450 for BAX and 1:800 for MCL-1 staining)], was carried out for 30 minutes at room temperature. Avidin-biotin-immunoperoxidase complex (ABC/HRP, DAKO, Glostrup, Denmark) was then applied for 30 min, at room temperature, followed by diaminobenzidine chromogen (DAB, Sigma, St. Louis, MO, USA). Bovine serum albumin (5% BSA) and 5% swine serum were used to block non-specific reactions with the secondary antibodies RAM or SWAR, respectively. Sections were rinsed in Tris buffer (2×5 minutes) after all incubations with the exception of the blocking agent step, when only excess reagent was removed. Sections of tonsils with reactive follicular hyperplasia and high proliferation in germinal centers were stained as a control together with each staining batch.

Evaluation of immunostainings

The MIB-1 antibody yielded distinct and homogeneous nuclear staining of the Ki-67 antigen without any background. Ki-67 expression was determined quantitatively by calculating the number of positive Ki-67 neoplastic cells in 22 fields.[42] The immunohistochemical stains for p53, BCL-2, BAK, BAX and MCL-1 were evaluated semi-quantitatively as follows: (-) negative, (+/–) a fraction of cells (<30%) positive, (+) most cells (>80%) weakly positive, (++) most cells strongly positive and (+++) most cells with exceptionally strong staining/over-expression. For evaluation of BCL-2 expression a strong positive cytoplasmic staining seen in the infiltrating, reactive T-cells was used as an internal control and considered as (++). NHL cells with weaker expression were considered as (+), and cells with expression stronger than that of T- cells as over-expression (+++). BAK staining had a distinct dot-like pattern. The expression found in the mantle zones of reactive germinal centers was considered as (+). BAX and MCL-1 giving diffuse cytoplasmic staining and positivity similar to that of the reactive germinal centers was considered as (+). As controls, sections from other NHL samples with known strong BAK, BAX and MCL-1 expression (++) were also stained with every batch. p53 showed a distinct nuclear pattern of expression. Single positive cells always found in sections were considered as a positive control.

Statistical analysis

Relationships between continuous variables were analyzed by Pearson's correlation coefficient. The Mann-Whitney U test was used for continuous data and chi-squared or Fisher's exact tests were used for categorical data. A Cox proportional hazard model was used in survival analysis. A two-sided *p*-value of 0.05 was used as the criterion for statistical significance. All the above tests were applied in a SPSS 8 statistical package.

Results

Telomerase activity and its components in normal and reactive lymphatic cells

The mean TA in normal PBMC was only 5% (range 3-10%, SD 3%). The expression of hTERT was also low - mean 0.01 (range 0-0.03, SD 0.015). The expression of hTER was higher with a mean of 0.46 (range 0.30-0.60, SD 0.19). The expression of Tank mRNA was not evaluated in PBMC. In reactive tonsil samples the mean TA was high i.e. 54% (range 4-100%, SD 35%). The mean expression of hTERT, hTER and Tank in the tonsil tissue was 0.32 (range 0.10-0.60, SD 0.16), 0.85 (range 0.73-1.02, SD 0.10) and 0.93 (range 0.77-1.03, SD 0.10), respectively.

Telomerase activity and expression of hTERT, hTER and Tank mRNA in HG-NHL

TA could not be evaluated in three HG-NHL samples, due to low protein concentration. Of the remaining 28 HG-NHL samples, 26 (93%) had demonstrable telomerase activity (mean 36%, median 33%, range 0.005%-

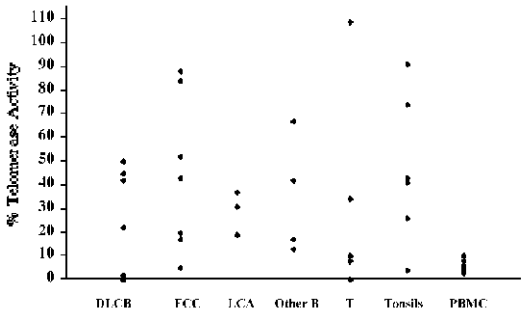


Figure 1. The percentages of relative telomerase activity in various subgroups of HG-NHL as compared to results obtained in tonsils and PBMC. Telomerase activity is expressed as a percentage of the activity of the REH control cell line which is considered as having 100% telomerase activity.

109%) (Figure 1).

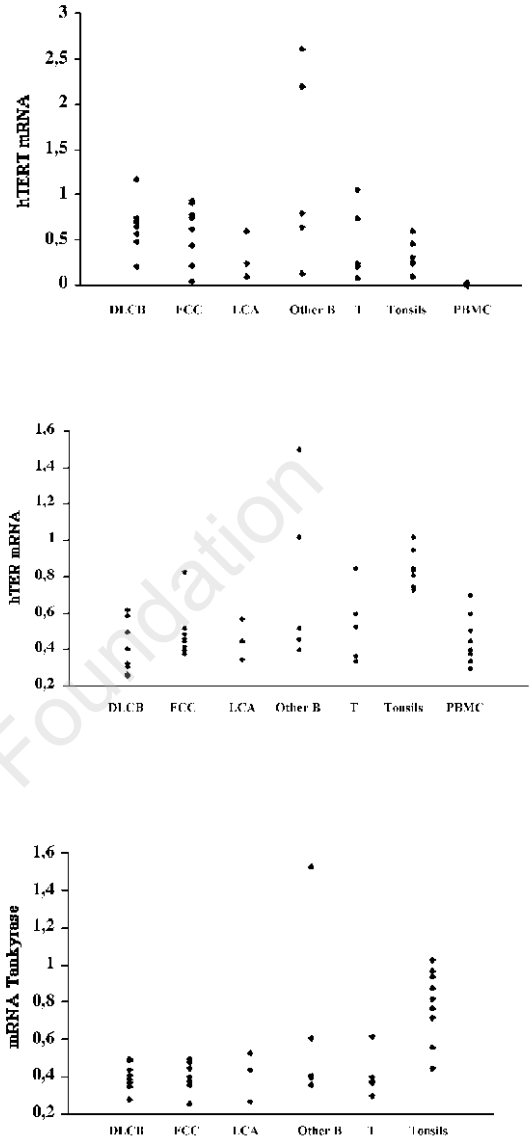
HTERT, hTER and Tank mRNA expression was found in all 30 evaluated HG-NHL samples (one sample had to be excluded due to RNA degradation) (Figures 2a, 2b, 2c, Figure 3). The mean expression of hTERT mRNA was 0.67 (range 0.07-2.62, median 0.64), hTER mRNA 0.52 (range 0.26-1.49, median 0.45) and the mean relative Tank mRNA expression was 0.45 (range 0.26-1.53, median 0.40). A positive correlation was found between hTERT and hTER mRNA (r= 0.588, *p*= 0.01), hTERT and Tank mRNA (r= 0.615, *p*= 0.01) and, hTER and Tank mRNA (r= 0.712, *p*= 0.01) expression. However, there was no significant correlation between TA and expression of hTERT, hTER and Tank mRNA. There were no significant differences in TA and expression of hTERT, hTER and Tank mRNA between B- and T-HG-NHL. HG-NHL samples taken at relapse of lymphoma did not differ from the remainder as far as concerns TA and expression of hTERT, hTER and Tank mRNA.

Apoptotic and proliferative fractions

Optimal quality TUNEL staining of apoptotic cells was obtained in 29 HG-NHL samples. The mean size of the apoptotic fraction was 2.6% (range 0.3-7.2%, median 1.7%). Ki-67 expression was evaluated in all 31 studied samples. The mean size of the proliferative fraction was 56% (range 29-91%, median 56%). There was no correlation between TA and the levels of proliferation or apoptosis. However, there was a tendency for a positive correlation between hTERT mRNA expression and the size of the apoptotic and proliferative fractions (r= 0.353, *p*= 0.06 and r= 0.314, *p*= 0.09, respectively). In contrast, most samples with high Tank mRNA expression (i.e. > median 0.40) had apoptotic and proliferative fraction levels under the mean values for the whole group (*p*= 0.033 and *p*= 0.038, respectively).

Expression of apoptosis-related proteins

The distribution of p53, BCL-2, BAK and MCL-1 protein expression in studied HG-NHL samples is given in



Figures 2a, b, c. The relative mRNA expression of hTERT, hTER and Tankyrase in various subgroups of HG-NHL as compared to results obtained in tonsils and PBMC. mRNA level is expressed as a signal density relative to the β2-microglobulin (β_2M) control.

Table 1. TA tended to be higher in p53 positive than in p53 negative samples (47% vs. 27%, respectively, *p*= 0.089). hTER levels were significantly higher in lymphomas that lacked expression of the pro-apoptotic proteins BAK and BAX than in samples positive for BAK (*p*<0.05) and BAX (*p*= 0.006), respectively. BAX-negative lymphomas tended to have a higher mean mRNA hTERT expression than BAX-positive ones (*p*= 0.07) (Table 2).

Figure 3. hTERT, hTER and Tankyrase mRNA expression in 8 representative HG-NHL biopsy samples as determined using RT-PCR. β_2M mRNA expression was also included as a loading control.

There was no correlation between BCL-2 and MCL-1 expression and TA or studied mRNA expression.

Clinical data

hTERT mRNA expression was higher in samples from older patients (r=0.361, p =0.05). No significant association was found between TA or mRNA expression of hTERT, hTER and Tank and disease stage, response to treatment (CR vs. NR) or disease-free survival. However, patients alive at the last follow-up (n=11, mean follow-up 53 months, range 42-70 months) had a lower mean mRNA hTERT expression (mean 0.47) than patients who died of NHL (n=17, mean survival 15.6 months, range 1-38 months, mean hTERT mRNA expression 0.80, not significant).

Discussion

It has been previously established that telomerase activity may be upregulated in NHL and that it is significantly higher in HG than in LG lymphomas.[9,22,43-45] Our finding that 93% of HG-NHL express TA is similar to previous reports by Norrback *et al.*[45] and Ely *et al.*[22] who found TA in virtually all studied NHL cases. Harada *et al.* reported a somewhat lower percentage of NHL expressing TA (83%).[10] However, all cited authors used different methodologies to determine TA, thus, a direct comparison of the results of various studies is not possible. Our finding that the range of TA expressed by reactive tonsil samples is similiar to that of HG-NHL may be attributed to the fact that TA is not solely a marker for malignancy but may also be expressed by germinal center cells and activated T-lymphocytes present in tonsillar material.[44,45]

hTERT is the catalytic subunit of telomerase and determines TA.[12,13] hTERT mRNA expression and TA have been found to be correlated in some malignant tumors[46] and in AML.[37] In HG-NHL of the central nervous system, hTERT mRNA expression has also been shown to correlate to TA.[10,47] However, in a mouse leukemia cell line an increase of TA caused by X-irradiation was shown to be independent of hTERT, hTER or Tank.[48] Increased hTER expression has been previously associated with cell activation and proliferation but not always with an increase of telomerase activity.[11,49] The lack of correlation between hTERT mRNA expression and TA in this study may be due to the presence of endogenous RNase in the samples influencing the results obtained by the TRAP assay.[21] Furthermore, splice variants of hTERT mRNA lacking catalytic activity[50] and telomerase inhibitors yet to be identified may also be present.[51] Since we included RNase inhibitors in the TRAP assay protocol and our results were comparable to other studies,[10,22,45] this lack

Table 1. Expression of p53 and BCL-2 family member proteins in studied HG-NHL.

	p53 *(n= 31)*	*BCL-2* *(n= 31)*	*BAK* *(n= 31)*	*BAX* *(n=30)*	*MCL-1* *(n= 31)*
–	18 (58%)	14(45%)	10 (32%)	9 (30%)	5 (6%)
–/+	0	0	8 (26%)	4 (13%)	8 (26%)
+	10 (32%)	8 (26%)	5 (16%)	15 (50%)	18 (58%)
++	3 (10%)	8 (26%)	8 (26%)	1 (3.5%)	0
+++	0	1 (3%)	0	1 (3.5%)	0

Table 2. hTER and hTERT mRNA expression in relation to BAK and BAX protein expression.

	hTER *Mean, (SD)*	*[p value]*	*hTERT* *Mean, (SD)*	*[p value]*
BAK Negative	0.59, (0.29)	[p< 0.05]	0.54, (0.26)	NS
BAK Positive	0.43, (0.15)		0.46, (0.27)	
BAX Negative	0.71, (0.35)	[p= 0.006]	0.96, (0.86)	[p= 0.07]
BAX Positive	0.44, (0.13)		0.54, (0.34)	

of correlation is probably not due to endogenous RNase but to one of the above mentioned phenomena.

The tumor suppressor gene *p53* has been suggested to repress TA through downregulation of hTERT transcription. The interaction of p53 with other transcription factors may be involved in this regulation.[17,52] This suggests that the downregulation of *p53* may favor upregulation of telomerase activity in cancer cell development. Over-expression of p53 detected by immunohistochemistry is usually due to a mutation of the *p53* gene or abnormal stabilization of p53 protein, thus reflecting downregulation of the *p53* gene.[17] A positive correlation between telomerase activity and p53 expression has been reported in non-small cell lung cancer[53] and breast cancer.[33] We also showed a tendency for there to be higher TA in p53 positive than in p53 negative HG-NHL samples. This observation suggests that high TA in HG-NHL could be linked to p53 protein accumulation.

Tank is a DNA repair enzyme that may indirectly control TA by facilitating the access of telomerase to the telomeric ends. This action may be mediated by the removal of TRF1 from telomeres by a process of poly-ADP/ ribosylation.[23] We were able to show that mRNA expression of telomerase components (hTERT and hTER) is correlated to mRNA expression of Tank in HG-NHL. Similar correlations have also been shown in multiple myeloma.[25] Our finding that most samples with higher Tank levels had a lower mean of apoptotic and proliferative fractions is in agreement with the suggested DNA repair role of Tank. However, we could not find any published studies clarifying whether Tank is involved in the regulation of apoptosis.

A relationship between TA and tumor cell proliferation has been reported in many malignancies (reviewed in ref.#2). Ely *et al.* found a strong correlation between TA and proliferative fraction in material that included both LG and HG-B NHL.[22]) We found that hTERT mRNA expression, but not TA, was correlated to the size of the proliferative fraction. Similarly, Norrback *et al*. did not find any correlation between TA and S-phase fraction in a series of 26 NHL.[45] However, the same authors did find a correlation between S-phase and both TA and hTERT expression in a larger series of 63 NHL including both HG and LG NHL.[21]

Both a decrease in the rate of apoptosis and an increase in cell proliferation can promote tumorigenesis.[54] Cellular immortalization may be associated with an increased resistance to apoptosis along with an increase in TA.[1,55] Nevertheless, the relationship between TA and apoptosis remains unclear. Some *in vitro* studies have shown that TA can confer resistance to apoptosis in glioblastoma cell lines.[55] Other studies suggested that TA was not related to drug-induced apoptosis in leukemic cell lines.[56] We did not find any correlation between TA and the size of apoptotic fractions in our series of HG-NHL. However, there was a tendency for a positive correlation between hTERT mRNA expression and apoptosis. Although a number of *in vitro* experiments have shown that high TA can protect cells from apoptosis, the *in vivo* situation may be much more complicated e.g. the growth of tumors can be restricted by local blood supply. Moreover, tumors (including HG-NHL) with high proliferation rates often exhibit enhanced apoptosis.[57,58] Since high proliferation is in general associated with increased telomerase activity and hTERT expression, it is not surprising that higher hTERT expression may, in tumor samples, be correlated with a higher rate of apoptosis.

We did not find any correlation between TA and BCL-2, MCL-1 BAX or BAK expression in HG-NHL. This lack of correlation between TA and BCL-2 expression was also reported in a Jurkat T-cell system,[59] non-small cell lung cancer,[53] lung adenocarcinomas[60] and colorectal carcinomas.[61] However, we found that hTERT and hTER mRNA expression was higher in cases lacking expression of pro-apoptotic protein BAX and in the case of hTER also BAK, which may suggest an association with resistance to apoptosis.

The negative prognostic significance of high TA has been reported in several malignancies such as central nervous system tumors,[34] colorectal cancer[62] and gastric cancer.[32] Detectable TA has been related to shorter survival in B-CLL.[35,63] In previous studies, increased TA and hTERT mRNA in NHL were associated with aggressive features such as HG-NHL morphology and a high proliferative fraction.[21,22]

Our preliminary results suggest that high hTERT mRNA expression may be related to shorter survival in highly malignant NHL. Thus, further investigation of the clinical relevance of telomerase activity in HG-NHL in a larger patient cohort and in relation to other prognostic variables would be of interest.

Contributions and Acknowledgments

BMN, DX, AG and APMD designed the study. BMN, WW, ZC and MH performed the experimental procedures. JM was responsible for the statistical analysis. BMN, DX, AG and APMD evaluated and interpreted the data. BMN wrote the first version of this paper. APMD, AG and DX revised the paper and gave final approval for its submission.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Rosario Notaro, who acted as an Associate Editor. The final decision to accept this paper was taken jointly by Dr. Notaro and the Editors.

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Potential implications for clinical practice

Treatment of high-grade NHL is based on the International Prognostic Index which does not include any biological properties of the tumors. Our preliminary results suggest that high hTERT mRNA expression may be related to shorter survival in highly malignant NHL. The inclusion of telomerase studies in future clinical trials should, therefore, be considered to see whether they could improve predictive accuracy.

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