Direct activation of telomerase by GH via phosphatidylinositol 3'-kinase

L Gómez-García, F M Sánchez, M T Vallejo-Cremades,
I A Gómez de Segura and E De Miguel del Campo

Research Unit, La Paz University Hospital, Madrid, Spain

(Requests for offprints should be addressed to E De Miguel del Campo, Research Unit Cirugía Experimental, Hospital Universitario La Paz, Castellana 261, 28046 Madrid, Spain; Email: emiguel.hulp@salud.madrid.org)

Abstract

Telomerase is a ribonucleoprotein DNA polymerase that has been associated with cell proliferation, cell survival and apoptosis inhibition. Telomerase is regulated by specific growth factors, cytokines and hormones. The present study examines the effect of GH on telomerase activity and identifies the signal transduction pathway involved in this process in Chinese hamster ovary (CHO)4 cells, which express rat GH receptor cDNA. Telomeric repeat amplification protocol assays demonstrated that treating CHO4 cells with increasingly high doses of GH up-regulated telomerase activity with the maximum activation at 24 h. Similarly, GH activated telomerase in another cell system,

primary cultures of rat hepatocytes. The telomerase activation in CHO4 cells was produced with an increase in hamster telomerase catalytic subunit (hamTERT) mRNA expression. The telomerase activity induced by GH was specifically blocked by the phosphatidylinositol 3'-kinase (PI3-K) inhibitor, LY294002, but not by the MAP kinase inhibitor, PD98059. These findings suggest that GH could activate telomerase through the direct activation of TERT transcription, as well as through the PI3-K signalling pathway.

Journal of Endocrinology (2005) **185**, 421–428

Introduction

Telomerase is a specialised RNA-dependent ribonucleoprotein polymerase that elongates the telomeres of chromosomes to compensate for the losses that occur in each round of DNA replication (Greider 1996). It has been suggested that telomerase may have other roles that are independent of telomere synthesis including cell survival, proliferation and apoptosis (Bodnar *et al.* 1998, Zhu *et al.* 2000, Minamino & Kourembanas 2001, Oh *et al.* 2001, Akiyama *et al.* 2002, Haendeler *et al.* 2003, Smith *et al.* 2003).

The telomerase enzyme consists of two essential components, the reverse transcriptase subunit, which contains telomerase catalytic activity (TERT), and the associated RNA component, which serves as the template to synthesise the telomeric sequence (Nugent & Lundblad 1998). There is evidence to indicate that the TERT, but not the RNA component, is highly correlated with the presence of telomerase activity (Koyanagi *et al.* 2000, Misiti *et al.* 2000). In fact, the telomerase activity in telomerase-negative cells can be restored by ectopic human TERT expression (Bodnar *et al.* 1998), which suggests that in certain cases, TERT is the only limiting factor for telomerase activation.

Telomerase activity is closely regulated by changes in gene transcription (Kyo *et al.* 2000, Mauro & Foster 2002), protein–protein interactions (Haendeler *et al.* 2003) and phosphorylation by protein kinase C, ERK1/2 and phosphatidylinositol 3'-kinase (PI3-K)/Akt (Li *et al.* 1998, Kang *et al.* 1999, Maida *et al.* 2002).

Human somatic cells normally lack telomerase activity (Ramakrishnan *et al.* 1998); however, approximately 90% of tumour-derived immortal cells (Kim *et al.* 1994), the germ-line cells, certain stem cells and cells from renewable tissues, such as intestinal epithelium and liver (Wright *et al.* 1996, Yasumoto *et al.* 1996, Inui *et al.* 2002), do contain telomerase activity. In these cells, telomerase activity is usually growth-regulated. In fact, growth signals induced, for example, by fibroblast growth factor (FGF), epidermal growth factor (EGF) or insulin growth factor (IGF)-I, are directly or indirectly involved in telomerase regulation (Haik *et al.* 2000, Akiyama *et al.* 2002, Wetterau *et al.* 2003).

Growth hormone (GH) is a peptide molecule that has diverse and pleiotropic actions on cellular metabolism, growth and differentiation (Carter *et al.* 1996). Other functions of GH are involved in apoptosis inhibition and cell survival (Gomez de Segura *et al.* 1998, Costoya *et al.* 1999, Vazquez *et al.* 1999, Frago *et al.* 2002, Liu *et al.* 2002, Madrid *et al.* 2002, Maida *et al.* 2002). The signal

transduction pathways triggered by GH in these processes have been analysed. Binding between GH and GH receptor (GHR) induces tyrosyl phosphorylation and activation of Janus kinase-2, a cytoplasmic kinase that is associated with GHR and mediates the cellular effects of GH (Carter *et al.* 1996). Subsequent signalling events include tyrosyl phosphorylation of the GHR and phosphorylation and activation of a number of cytoplasmic molecules, including signal transducer and activator of transcription-1 (STAT1), STAT3 and STAT5; mitogen activated protein kinases (MAPKs) (ERK-1 and ERK-2); insulin receptor substrate-1 (IRS1), IRS2 and IRS3; and PI3-K (Moller *et al.* 1992, Carter *et al.* 1996, Costoya *et al.* 1999).

In the present work we are interested in determining whether telomerase is regulated by GH. The findings suggest that GH can induce telomerase activation in Chinese hamster ovary (CHO)4 cells by up-regulating hamster (ham) TERT mRNA expression and by the signal transduction pathway that involves PI3-K.

Materials and Methods

Cell cultures and treatments

CHO4 cells, expressing the rat GHR cDNA, kindly provided by Dr C Moller (Center for Biotechnology, Karolinska Institute, Novum, Huddinge, Sweden; Moller *et al.* 1992), were cultured in Ham's F-12 medium containing 10% fetal calf serum and 1 mM L-glutamine, 50 units/ml penicillin and 50 µg/ml streptomycin (Gibco). To assay GH induction, 3×10^5 cells were plated and starved for 15–18 h in serum-free Ham's F12 medium, washed twice with PBS and then incubated with several doses of human recombinant GH (hGH) (Pharmacia SA, Spain) for the indicated time periods. To perform bleomycin induction, cells were treated as above, and instead of GH were stimulated with 10 mIU of bleomycin for 24 h in fresh serum-free Ham's F12 medium. For inhibitor studies, cells were treated with either LY294002 (PI3-K inhibitor) (Calbiochem-Novabiochem Corp., San Diego, CA, USA) or PD98059 (MAPK kinase (MEK)-1 inhibitor) (Calbiochem-Novabiochem) in the presence or absence of GH for the indicated period of time.

The study was approved by the Institutional Animal Care and Use Committee and met European criteria for protection of animals used for experimental and other scientific purposes (86/609/EU). Animals were purchased from an authorised breeder (Elevage Janvier, Le Genest-St-Isle, France) and housed in standard conditions of light and humidity and with a light/dark cycle of 12 h. Hepatocytes were isolated by perfusion of the livers of Wistar rats with collagenase (Sigma) (Feliu *et al.* 1983) and suspended in medium 199 supplemented with 10% fetal bovine serum (FBS), 0.1% BSA, 1 µM triiodothyronine, 1 µM dexamethasone and glucose up to 10 mM. Hepatocytes

were plated to attain a cell density of 1.2×10^5 cells/cm² and cultured in a cell incubator at 37 °C in an atmosphere of 5% CO₂. Four hours after plating, the medium was replaced by fresh supplemented medium 199. After 19 h of culture, hepatocyte monolayers were washed three times with PBS and incubated for an additional 2 h in medium 199 supplemented with 20 mM Hepes (pH 7.4), 0.1% BSA and 10 mM glucose; after this time 50 nM GH was added for another 24 h.

Telomerase activity assays

Telomerase activity was measured using the quantitative TeloTAGGG Telomerase PCR ELISA^{PLUS} kit (Roche Molecular Biochemicals, Indianapolis, IN, USA) and the TRAPeze Telomerase Detection kit (Intergen, New York, NY, USA). For each experiment, 3×10^5 CHO4 cells were plated in a 60 mm well in medium containing 10% FBS for 24 h before washing twice with phosphate buffer and changing to serum-free Ham's F12 medium for 16 h; after this, cells were stimulated with the indicated doses of hGH for the indicated time periods in fresh serum-free medium. After treatment, 2×10^5 were resuspended in 200 µl $1 \times$ lysis reagent and the extracts were obtained according to manufacturer's protocol for the TeloTAGGG Telomerase PCR ELISA^{PLUS} kit analysis. For each assay 1.5 µl (1500 cells) were used. For the TRAPeze Telomerase Detection kit, the cell extract was prepared with the CHAPS lysis buffer $\times 1$ according to manufacturer's protocol and an aliquot of cell extract containing 200 ng protein was used in each assay. The protein concentration was measured by the bicinchoninic acid protein assay (Pierce, Rockford, IL, USA). Once they had been prepared, the extracts were immediately frozen in liquid nitrogen and stored at -80 °C until use.

The Telomerase PCR ELISA^{PLUS} kit and TRAPeze Telomerase Detection kit are an extension of the original telomeric repeat amplification protocol (TRAP) (Kim *et al.* 1994). The ELISA kit allows the highly specific amplification of telomerase-mediated elongation products combined with non-radioactive detection following an ELISA protocol. The levels of telomerase activity were within the linear range of the TRAP assay. In the ELISA PCR, telomerase-mediated elongation was performed for 30 min at 25 °C. Afterwards the telomerase was heat inactivated for 5 min at 94 °C. PCR cycling conditions were 25 cycles (94 °C for 30 s, 50 °C for 30 s and at 72 °C for 90 s) followed by a cycle at 72 °C for 10 min. For TRAPeze, briefly, 200 ng protein were added to 50 µl reaction mixtures containing telomeric substrate oligonucleotide primer, a primer and template for amplification of internal standard, reverse oligonucleotide primer, Taq DNA polymerase (5 units/µl) (Roche), dNTP Mix and TRAP reaction buffer. After 30 min incubation at 30 °C for telomerase extension of the TS primer, the PCR cycling conditions were 33 cycles at 94 °C for 30 s, 59 °C

for 30 s and 72 °C for 90 s followed by a cycle at 72 °C for 10 min. A loading dye containing bromophenol blue and xylene cyanol (0.25% each in 50% glycerol/50 mM EDTA) was then added to each reaction tube and the sample (25 µl) was loaded on a 12.5% non-denaturing PAGE in 0.5 × TBE buffer. After electrophoresis, the gel with ethidium bromide (1:10 000 in deionised water) was stained for 30 min and destained for 20–30 min in deionised water at room temperature. Bands were visualised by ethidium bromide staining and quantified by densitometric scanning (1D Manager; TDI, Madrid, Spain). The telomerase activity was evaluated by the TRAP products/internal control ratio (data not shown).

In each set of experiments for each kit, aliquots of all samples were treated by RNase (37 °C for 20 min) in order to ascertain the specificity of the reaction. The two methods gave very similar results; accordingly, only the ELISA assay was performed in some experiments.

Real-time quantitative PCR for hamTERT mRNA detection

Total RNA was isolated from CHO4 cells using the aqueous phase obtained by the Tri Reagent method (Molecular Research Center, Cincinnati, OH, USA), following the manufacturer's instructions. Reverse transcription was performed with 1 µg total RNA, Oligo-p(dT) as primers, and AMV reverse transcriptase (Roche) following the manufacturer's instructions. Each PCR contained, in 20 µl, 1 × Fast Start DNA Master SYBR Green 1 mix (Roche), 4 mM MgCl₂, 0.5 µM (each) of the sense and antisense primers, and 1 µl of the corresponding cDNA. For each mRNA, hamTERT expression was corrected by the 18S content in each sample. PCR was performed in a LightCycler instrument (Roche) with 10 min preincubation at 95 °C followed by 45 cycles of 5 s at 95 °C (denaturation), 10 s at 70 °C or 60 °C (annealing) for hamTERT or 18S respectively, and 10 s at 72 °C (amplification). Standard curves were run with each set of samples and for each gene. Correlation coefficients (r^2) for standard curves were typically ≥ 0.98 . To confirm the specificity of the reaction product during each run, the melting profile of each sample was analysed using the LightCycler software. The melting profile was determined by keeping the reaction at 80 °C for hamTERT or at 87 °C for 18S, for 10 s, and then heating slowly to 95 °C at a linear rate of 0.1 °C/s to measure fluorescence.

The PCRs employed a set of primers that were specific for the hamTERT gene (Guo *et al.* 2001): hamTERT sense 5'-GACTCTGTTTCAGCGTGCTCAA3', and antisense 5'-AGAACTGGCCCATGTGTGGCT3'. The specific primers for 18S were from the Classic 18S Internal Standards kit (Ambion, Inc., Austin, TX, USA). In all cases, each PCR was repeated at least three times. Some of the samples were run in a gel to confirm that the specific band had the expected size (bp), as well as to rule out

possible contaminants that would not be distinguishable by their melting points (not shown).

Statistical analysis

All data were expressed as means $\pm$ S.D. unless otherwise indicated. Differences between means of various treatment and control groups were assessed for statistical significance by ANOVA followed by the post-hoc Fisher test for group comparison. A P value < 0.05 was considered to indicate statistical significance.

Results

Effect of GH on telomerase activity in CHO4 cells

We examined the effect of GH on telomerase activity in CHO4 cells. Cells were stimulated with different doses of GH (100, 200, 300, 400 nM) for 24 h before performing the TRAP-based ELISA assay. The maximum of telomerase activation was observed at 300 nM and was statistically different ($P < 0.05$) from the responses with 100, 200 and 400 nM (Fig. 1A). The highest dose did not stimulate telomerase activity. It is possible that this dose was toxic, as described previously (Madrid *et al.* 2002). The same result was confirmed with the TRAPeze Telomerase Detection kit (Fig. 1B). The levels of PCR amplification for the internal control were similar between treatments. The RNase treatment demonstrated the specificity of the telomerase reaction. Telomerase activation was also induced by a radiomimetic drug, bleomycin (data not shown), so telomerase activity can be regulated by different stimuli in CHO4 cells.

When CHO4 cells were stimulated with GH at 300 nM, telomerase activity was up-regulated within 6 h and persisted until at least 48 h later. Maximal activation was observed at 24 h after treatment (Fig. 2).

Effect of GH on telomerase in a primary culture of rat hepatocytes

We also sought to determine whether another cell type responded to GH with similar telomerase activation. We therefore treated a primary culture of rat hepatocytes with GH at 50 nM (Beauloye *et al.* 1999) for 24 h and then performed a TRAP assay with the TRAPeze Telomerase Detection kit (Fig. 3). This confirmed that GH was able to activate telomerase in a primary culture of rat hepatocytes and demonstrated that GH is able to induce telomerase activation in a different cell type.

Effect of GH on hamTERT mRNA expression

To investigate whether GH activation of telomerase could be explained by increases in hamTERT mRNA, we used

Figure 1 GH up-regulates telomerase in CHO4 cells. (A) CHO4 cells were starved in serum-free medium for 15–18 h, then incubated with GH (100–400 nM) for 24 h. After this, cells were harvested and TRAP-ELISA assays were performed. RNase treatment of all the samples served as a negative control. Results are means $\pm$ S.D. of five experiments performed in duplicate. * $P < 0.05$ vs non-stimulated control (100%); # $P < 0.05$ vs 100, 200 and 400 nM. There is a linear correlation between the 100, 200 and 300 nM doses, $r^2 = 0.988$. (B) Telomerase activity was visualised using the TRAPeze Telomerase Detection kit after exposing the CHO4 cells to the indicated doses of GH for 24 h. IC: internal control.

real-time quantitative PCR. Cells were treated with different doses of GH for 24 h. We did not observe a stimulatory effect of GH on the level of hamTERT mRNA at 24 h (data not shown). Some reports have described a rapid and transient induction of TERT mRNA (Liu *et al.* 2002). We therefore treated the cells with GH (300 nM) for 1–6 h and also observed up-regulation of hamTert mRNA expression (Table 1). The maximum induction was produced at 2 h after

Figure 2 Kinetics of the effect of GH on telomerase activity in CHO4 cells. CHO4 cells were treated with or without GH (300 nM) as described in Materials and Methods, for the indicated times. Cells were lysed and telomerase activity was assayed by the ELISA method. RNase-treatment of all the samples served as a negative control. Results shown are means $\pm$ S.D. of five experiments performed in duplicate. * $P < 0.05$ vs non-stimulated control (100%); # $P < 0.05$ vs 6, 48 and 72 h.

GH treatment. These results suggest that in CHO4 cells, GH regulation of telomerase activity is primarily transcriptional.

Mechanisms of GH-induced telomerase activity in CHO4 cells

We next characterised mechanisms of telomerase activity triggered by GH. PI3-K and MAPK are pathways that are activated by GH. Since telomerase activation can depend on both PI3-K and MAPK activation, we investigated whether these signalling pathways modified the telomerase activation induced by GH. For this purpose CHO4 cells were incubated with GH in the absence or presence of LY294002, a specific PI3-K inhibitor, or of PD98059, a specific inhibitor of MEK1, and then a TRAP-based ELISA assay was performed. The effect of GH was significantly inhibited by the PI3-K inhibitor, but not by the MAPK inhibitor. The PI3-K inhibitor did not affect constitutive telomerase activity (Fig. 4). To confirm that these concentrations of LY294002 and of PD98059 were efficacious in inhibiting both Akt/protein kinase B and ERK phosphorylation respectively, cells were treated with or without 10 μ M LY294002 or 30 μ M PD98059 and then treated for 10 min with or without 300 nM GH. Western blot analyses performed to determine the level of phospho-Akt or phospho-ERK demonstrated that the inhibitors used were effective (Fig. 4B).

Discussion

To the best of our knowledge, this is the first study showing that telomerase is a target of GH. We determined

Figure 3 GH up-regulates telomerase activity in hepatocytes. Cells were treated with 50 nM GH for 24 h and then telomerase activity was analysed using the TRAPeze Telomerase Detection kit, as described in Materials and Methods. This result is representative of the result of three independent experiments in duplicate, each using protein extracts prepared from hepatocytes obtained from a different rat. The levels of PCR amplification for the internal control (IC) were comparable between treatments.

that supraphysiological doses of GH induced telomerase activation in a CHO4 cell line that expresses rat GHR cDNA (Moller *et al.* 1992). We shown that the dose of 300 nM at 24 h induces a statistically different increase on telomerase activation. The doses used in this work were chosen based on the dose that induced cell survival in a prior study (Madrid *et al.* 2002). We have also noted that at the both lowest (100 nM) and highest dose (400 nM), the level of telomerase activation was below than the rest

of doses used. The highest doses (400 nM) used could be toxic for these cells, as has been described previously (Madrid *et al.* 2002).

In a primary culture of rat hepatocytes, which express GHR physiologically, we found that GH also triggered telomerase activity. Previously it has been shown that other growth factors such as EGF and hepatocyte growth factor also induce telomerase activation in hepatocytes (Inui *et al.* 2002). These events would confirm the regulation of telomerase by different growth factors, among which would be GH. On the other hand, GH does not activate telomerase in a prostate cancer cell line (Wetterau *et al.* 2003), perhaps indicating that the response would depend on cell type.

Expression of the catalytic subunit of telomerase is tightly linked to the regulation of its enzymatic activity and it is, in certain cases, a rate-limiting determinant of telomerase activity (Kyo *et al.* 1997, Minamino & Kourembanas 2001). TERT regulation involves transcriptional and post-transcriptional mechanisms (Mauro & Foster 2002). We have shown that the activation of telomerase by GH was induced by an increase in hamTERT mRNA expression. However, although the GH-induced telomerase activity was maximal at 24 h, it did not correlate with the TERT mRNA levels at this time. This discrepancy may be because GH elevates hamTERT mRNA levels rapidly and transiently, with a maximum effect between 2 and 4 h, that begins to decrease at 6 h and reaches the baseline level at 24 h (data not shown). Similar results have been described in response to oestrogen (Kyo *et al.* 1999), EGF (Maida *et al.* 2002), basic FGF in fibroblasts (Liu *et al.* 2002) or IGF-I (Wetterau *et al.* 2003), where telomerase activation has been induced through the up-regulation of TERT mRNA expression.

As mentioned above, telomerase activity may be regulated through PI3-K/Akt or ERK1/2 (Kang *et al.* 1999, Maida *et al.* 2002); in fact, PI3-K/Akt enhances human telomerase activity by phosphorylating the telomerase catalytic subunit (Kang *et al.* 1999, Breitschopf *et al.* 2001, Haendeler *et al.* 2003). Akt is one of the downstream targets of PI3-K, a signalling molecule associated with growth factors (Akiyama *et al.* 2002). In other

Table 1 Effect of GH on hamTERT mRNA expression. Total RNA was isolated from CHO4 cells that were serum starved for 15–18 h, and then stimulated with GH (300 nM) for the time periods indicated. hamTERT mRNA was measured using quantitative-PCR and normalised to 18S using the standard curve method of relative quantification. Results shown are means $\pm$ S.D. of at least six experiments performed in duplicate

	Time (h)			
	1	2	4	6
Baseline	0.58 $\pm$ 0.07	0.50 $\pm$ 0.09	0.58 $\pm$ 0.08	0.62 $\pm$ 0.09
GH (300 nM)	0.69 $\pm$ 0.1	1.28 $\pm$ 0.01*	1.08 $\pm$ 0.05*	0.78 $\pm$ 0.10

* $P < 0.05$ vs the respective baseline value.

Figure 4 Involvement of PI3-K signalling in GH-induced telomerase activation. (A) CHO4 cells that were starved in serum-free medium for 15–18 h, then incubated with LY294002 (10 μ M) or with PD98059 (30 μ M) in the presence or absence of GH (300 nM) for 24 h. Cells were then harvested and TRAP-ELISA assays were performed. Results are means $\pm$ s.d. of three experiments performed in duplicate. * P <0.05 vs control; # P <0.05 vs GH. (B) Effect of LY294002 (LY) on Akt phosphorylation and of PD98059 (PD) on ERK phosphorylation. CHO4 were treated as described in (A) for 10 min. Western blot analyses were performed as described in Materials and Methods using an anti-S473 phospho-Akt antibody (left panel) or anti-phospho-p44/p42 antibody (right panel). Membranes were stripped and reprobed with anti-Akt antibody (left panel) or anti-total-p44/p42 antibody (right panel).

GHR-transfected CHO cells, GH promoted growth by increasing cell survival and that effect depended on PI3-K/Akt activation (Costoya *et al.* 1999). Several pathways activated by GH are triggered through PI3-K and MAPK (Carter *et al.* 1996), so we examined the implication of both signalling pathways in regulating telomerase activity in CHO4 cells. We saw that the PI3-K inhibitor, LY294002, but not the MEK1 signalling pathway inhibitor, PD98059, decreased GH-induced telomerase activation. We also observed that LY294002 only down-regulated GH-induced telomerase activity in CHO4 cells, but not their constitutive activity. Our results indicate that the PI3-K/Akt pathway may mediate GH-induced telomerase up-regulation. Similar results have been described in a human multiple myeloma cell line, where telomerase activation would depend on PI3-K in response to other factors such as IGF-I or interleukin-6 (Akiyama *et al.* 2002).

The actions of GH on telomerase may be direct or mediated by IGF-I. The observed effect on CHO4 cells suggest a direct action since this system has been shown to not produce IGF-I in response to GH (Moller *et al.* 1992). Furthermore, this cell system was also employed to demonstrate the actin filament reorganisation in an IGF-I-independent response to GH (Goh *et al.* 1997). Therefore, although IGF-I can stimulate telomerase activity in other cell system like human multiple myeloma and DU-145 prostate cancer cells (Akiyama *et al.* 2002), we think that in our system GH activates telomerase independently of IGF-I.

GH has neuroprotective and radioprotective qualities and possesses the ability to inhibit apoptosis and induce cell survival (Gomez de Segura *et al.* 1998, Vazquez *et al.* 1999, Frago *et al.* 2002). In fact, in GHR-transfected CHO cells, GH may promote growth by increasing cell

survival and that effect may depend on PI3-K/Akt activation (Costoya *et al.* 1999). Using the same *in vitro* cell system used in this study, we have reported that GH promotes cell survival when the cells have been exposed to GH prior to irradiation (Madrid *et al.* 2002). Likewise, telomerase activity is also closely associated with cell proliferation (Minamino & Kourembanas 2001, Smith *et al.* 2003) in different types of cells and contributes to the life span and integrity of these cells by protecting against apoptosis as well as by inducing cell survival or neuroprotection (Bodnar *et al.* 1998, Zhu *et al.* 2000, Haendeler *et al.* 2003). In the light of these reports, we observed that not only are there are functional coincidences between GH and telomerase, but they also may share signal transduction pathways involving Akt (Costoya *et al.* 1999, Kang *et al.* 1999).

Here we have demonstrated that a PI3-K inhibitor, LY294002, can block Akt phosphorylation, a key step in activating the PI3-K signal pathway in response to GH. The identification of real Akt kinase substrates, such as telomerase, provided clues to understanding how GH could contribute to cell survival or proliferation via the PI3-K pathway. Current knowledge about the regulation of telomerase activity has shown that the modulation of this enzyme is critical for cell growth and survival.

In summary, we have shown that, in CHO4 cells, supraphysiological doses of GH activate telomerase. This activation involves mechanisms that are both transcriptional, by up-regulating hamTERT transcription, and post-transcriptional, through PI3-K signal pathways. Future studies are needed to identify the specific GH-induced signal transduction pathways that ultimately affect telomerase activity and the possible implication of this activation in cell proliferation or survival.

Acknowledgements

We thank Dr L Alvarez (Research Unit, La Paz University Hospital, Madrid, Spain) for critical reading of the manuscript. Hepatocytes were kindly provided by Dr E Feliu (Instituto de Investigaciones Biomédicas Alberto Sols, CSIC-UAM, Madrid, Spain).

Funding

This work has been supported by the Fondo de Investigaciones Sanitarias, project FIS PI020455 and by the Comunidad de Madrid, project CAM 08-6/0033/01-1. There is no conflict of interest in this article.

References

- Akiyama M, Hideshima T, Hayashi T, Tai YT, Mitsiades CS, Mitsiades N, Chauhan D, Richardson P, Munshi NC & Anderson KC 2002 Cytokines modulate telomerase activity in a human multiple myeloma cell line. *Cancer Research* **62** 3876–3882.
- Beauloye V, Ketelslegers JM, Moreau B & Thissen JP 1999 Dexamethasone inhibits both growth hormone (GH)-induction of insulin-like growth factor-I (IGF-I) mRNA and GH receptor (GHR) mRNA levels in rat primary cultured hepatocytes. *Growth Hormone and IGF Research* **9** 205–211.
- Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S & Wright WE 1998 Extension of life-span by introduction of telomerase into normal human cells. *Science* **279** 349–352.
- Breitschopf K, Zeiher AM & Dimmeler S 2001 Pro-atherogenic factors induce telomerase inactivation in endothelial cells through an Akt-dependent mechanism. *FEBS Letters* **493** 21–25.
- Carter S, Schwartz J & Smith LS 1996 Molecular mechanism of growth hormone action. *Annual Review of Physiology* **58** 187–207.
- Costoya JA, Finidori J, Moutoussamy S, Searis R, Devesa J & Arce VM 1999 Activation of growth hormone receptor delivers an antiapoptotic signal: evidence for a role of Akt in this pathway. *Endocrinology* **140** 5937–5943.
- Feliu JE, Mojena M, Silvestre RA, Monge L & Marco J 1983 Stimulatory effect of vasoactive intestinal peptide on glycogenolysis and gluconeogenesis in isolated rat hepatocytes: antagonism by insulin. *Endocrinology* **112** 2120–2127.
- Frago LM, Paneda C, Dickson SL, Hewson AK, Argente J & Chowen JA 2002 Growth hormone (GH) and GH-releasing peptide-6 increase brain insulin-like growth factor-I expression and activate intracellular signalling pathways involved in neuroprotection. *Endocrinology* **143** 4113–4122.
- Goh EL, Pircher TJ, Wood TJ, Norstedt G, Graichen R & Lobie PE 1997 Growth hormone-induced reorganisation of the actin cytoskeleton is not required for STAT5 (signal transducer and activator of transcription-5)-mediated transcription. *Endocrinology* **138** 3207–3215.
- Gomez de Segura IA, Prieto I, Grande AG, Garcia P, Guerra A, Mendez J & De Miguel E 1998 Growth hormone reduces mortality and bacterial translocation in irradiated rats. *Acta Oncologica* **37** 179–185.
- Greider CW 1996 Telomere length regulation. *Annual Review of Biochemistry* **65** 337–365.
- Guo W, Okamoto M, Lee YM, Baluda MA & Park NH 2001 Enhanced activity of cloned hamster TERT gene promoter in transformed cells. *Biochimica et Biophysica Acta* **1517** 398–409.
- Haendeler J, Hoffmann J, Rahman S, Zeiher AM & Dimmeler S 2003 Regulation of telomerase activity and anti-apoptotic function by protein–protein interaction and phosphorylation. *FEBS Letters* **536** 180–186.
- Haik S, Gauthier LR, Granotier C, Peyrin JM, Lages CS, Dormont D & Boussin FD 2000 Fibroblast growth factor 2 up regulates telomerase activity in neural precursor cells. *Oncogene* **19** 2957–2966.
- Inui T, Shinomiya N, Fukasawa M, Kobayashi M, Kuranaga N, Ohkura S & Seki S 2002 Growth-related signaling regulates activation of telomerase in regenerating hepatocytes. *Experimental Cell Research* **273** 147–156.
- Kang SS, Kwon T, Kwon DY & Do SI 1999 Akt protein kinase enhances human telomerase activity through phosphorylation of telomerase reverse transcriptase subunit. *Journal of Biological Chemistry* **274** 13085–13090.
- Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL & Shay JW 1994 Specific association of human telomerase activity with immortal cells and cancer. *Science* **266** 2011–2015.
- Koyanagi Y, Kobayashi D, Yajima T, Asanuma K, Kimura T, Sato T, Kida T, Yagihashi A, Kameshima H & Watanabe N 2000 Telomerase activity is down regulated via decreases in hTERT mRNA but not TEP1 mRNA or hTERC during the differentiation of leukemic cells. *Anticancer Research* **20** 773–778.

- Kyo S, Takakura M, Kohama T & Inoue M 1997 Telomerase activity in human endometrium. *Cancer Research* **57** 610–614.
- Kyo S, Takakura M, Kanaya T, Zhuo W, Fujimoto K, Nishio Y, Orimo A & Inoue M 1999 Estrogen activates telomerase. *Cancer Research* **59** 5917–5921.
- Kyo S, Takakura M, Taira T, Kanaya T, Itoh H, Yutsudo M, Ariga H & Inoue M 2000 Sp1 cooperates with c-Myc to activate transcription of the human telomerase reverse transcriptase gene (hTERT). *Nucleic Acids Research* **28** 669–677.
- Li H, Zhao L, Yang Z, Funder JW & Liu JP 1998 Telomerase is controlled by protein kinase Calpha in human breast cancer cells. *Journal of Biological Chemistry* **273** 33436–33442.
- Liu T, Nozaki Y & Phan SH 2002 Regulation of telomerase activity in rat lung fibroblasts. *American Journal of Respiratory Cell and Molecular Biology* **26** 534–540.
- Madrid O, Varea S, Sanchez-Perez I, Gómez-García I, De Miguel E, Gómez de Segura IA & Perona R 2002 Growth hormone protects against radiotherapy-induced cell death. *European Journal of Endocrinology* **147** 535–541.
- Maida Y, Kyo S, Kanaya T, Wang Z, Yatabe N, Tanaka M, Nakamura M, Ohmichi M, Gotoh N, Murakami S & Inoue M 2002 Direct activation of telomerase by EGF through Ets-mediated transactivation of TERT via MAP kinase signalling pathway. *Oncogene* **21** 4071–4079.
- Mauro LJ & Foster DN 2002 Regulators of telomerase activity. *American Journal of Respiratory Cell and Molecular Biology* **26** 521–524.
- Minamino T & Kourembanas S 2001 Mechanisms of telomerase induction during vascular smooth muscle cell proliferation. *Circulation Research* **89** 237–243.
- Misiti S, Nanni S, Fontemaggi G, Cong YS, Wen J, Hirte HW, Piaggio G, Sacchi A, Pontecorvi A, Bacchetti S *et al.* 2000 Induction of hTERT expression and telomerase activity by estrogens in human ovary epithelium cells. *Molecular and Cellular Biology* **20** 3764–3771.
- Moller C, Hansson A, Enberg B, Lobie PE & Norstedt G 1992 Growth hormone (GH) induction of tyrosine phosphorylation and activation of mitogen-activated protein kinases in cells transfected with rat GH receptor cDNA. *Journal of Biological Chemistry* **267** 23403–23408.
- Nugent CI & Lundblad V 1998 The telomerase reverse transcriptase: components and regulation. *Genes and Development* **12** 1073–1085.
- Oh H, Taffet GE, Youker KA, Entman ML, Overbeek PA, Michael LH & Schneider MD 2001 Telomerase reverse transcriptase promotes cardiac muscle cell proliferation, hypertrophy, and survival. *PNAS* **98** 10308–10313.
- Ramakrishnan S, Eppenberger U, Mueller H, Shinkai Y & Narayanan R 1998 Expression profile of the putative catalytic subunit of the telomerase gene. *Cancer Research* **58** 622–625.
- Smith LL, Collier HA & Roberts JM 2003 Telomerase modulates expression of growth-controlling genes and enhances cell proliferation. *Nature Cell Biology* **5** 474–479.
- Vazquez I, Gomez de Segura IA, Grande AG, Escribano A, Gonzalez Gancedo P, Gomez A & Diez R 1999 Protective effect of enriched diet plus growth hormone administration on radiation-induced intestinal injury and on its evolutionary pattern in the rat. *Digestive Diseases and Sciences* **44** 2350–2358.
- Wetterau LA, Francis MJ, Ma L & Cohen P 2003 Insulin-like growth factor I stimulates telomerase activity in prostate cancer cells. *Journal of Clinical Endocrinology and Metabolism* **88** 3354–3359.
- Wright WE, Piatyszek MA, Rainey WE, Byrd W & Shay JW 1996 Telomerase activity in human germline and embryonic tissues and cells. *Developmental Genetics* **18** 173–179.
- Yasumoto S, Kunitura C, Kikuchi K, Tahara H, Ohji H, Yamamoto H, Ide T & Utakoji T 1996 Telomerase activity in normal human epithelial cells. *Oncogene* **13** 433–439.
- Zhu H, Fu W & Mattson MP 2000 The catalytic subunit of telomerase protects neurons against amyloid beta-peptide-induced apoptosis. *Journal of Neurochemistry* **75** 117–124.

Received 8 February 2005

Accepted 24 February 2005

Made available online as an

Accepted Preprint 1 March 2005