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Low telomerase activity: Possible role in the progression of human medullary thyroid carcinoma

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ABSTRACT

Maintenance of telomere length has been reported to be an absolute requirement for unlimited growth of human tumour cells and in about 85% of cases, this is achieved by reactivation of telomerase, the enzyme that elongates telomeres. Only in rare cases, like in human medullary thyroid carcinomas (MTC), telomerase activity (TA) is low or undetectable; however, this does not limit tumours to become clinically significant. Here, we report that very low TA (below 5% of HEK293) observed in MTC cell strains derived from different patients, although not sufficient for immortalising the cells, is necessary for prolonging their replicative life span. Telomere erosion led to induction of a crisis period after long-term *in vitro* cultivation, which was reached earlier when treating the cells with MST-312, a telomerase inhibitor at non-toxic concentrations. Crisis was bypassed either by ectopic hTERT introduction or by infrequent spontaneous immortalisation, the latter of which was always associated with telomerase reactivation and changes of the cellular phenotype. While confirming the high importance of telomerase for tumour development, these data draw attention to the relevance of low TA: although insufficient for telomere stabilisation, it allows MTC cells to reach more population doublings, increasing both cell numbers as well as the risk of accumulating mutations and thus might support the development of clinically significant MTC.

1. Introduction

Normal human cells can only be propagated for a limited number of population doublings (PDs) *in vitro* before entering the phase of replicative senescence[1] This senescent state is supposed to be the result of progressive telomere shortening with each cell division,[2] because critically short telomeres are recognised as DNA damage and subsequently induce this irreversible growth arrest.[3] In contrast, most human tumour cells have gained a mechanism to prevent telomere erosion, which is usually achieved by reactivation of telomerase, a ribonucleoprotein enzyme complex that elongates telomeres.[4] Since approximately 85% of tumours are characterised by elevated telomerase activity (TA), TA can be regarded

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as the most universal characteristic marker for human cancers.[5] However, besides telomerase reactivation, an alternative telomere lengthening mechanism (ALT) has been described as well,[6] which is discussed to be based on homologous recombination.[7] Recent data also demonstrate that telomere maintenance in human cells may be more diverse than previously thought.[8–12] As described recently, human liposarcomas, although showing an incidence for ALT and to the same extent for telomerase reactivation, can also develop without, or with a yet unknown, telomere maintenance mechanism.[13] Similar results have been shown for a non-small cell lung cancer cell line[8] as well as thyroid neoplasia.[14] This raises the question as to whether a constitutive telomere length maintenance mechanism is a prerequisite for all tumour cells to form clinically significant tumours.

In order to address this question, we have used previously established, well characterised human medullary thyroid carcinoma (MTC) cell strains derived from eight different patients.[15–17] MTC is a rare tumour arising from parafollicular C cells of the thyroid gland which in many cases is characterised by low to undetectable TA *in vivo*.[18–26] Accordingly, seven out of eight cell strains showed low TA at early population doubling level (PDL) after establishment of primary cell cultures.

Here we present evidence that even this low TA is sufficient to increase the replicative life span of human MTC cells and therefore its inhibition might contribute to reducing the size of tumours *in vivo*.

2. Materials and methods

2.1. Cells and culture conditions

An overview of the MTC cell strains used within this study is given in Table 1. All cell strains but BOJO (grown in Ham's F12 alone) were grown in Ham's F12 / M199 (1:1) supplemented with 4 mM L-Glutamine and 10% w/v foetal bovine serum (FBS, Hyclone). After establishment of primary cultures, aliquots were frozen as soon as possible. Each cell strain was thawed and cultivated until crisis at least in three independent experiments. All MTC cell strains used within this study grew as suspensions of single cells and multicellular aggregates.[27] Cell numbers and viability were determined using a haemocytometer and trypan blue. PDs were calculated with the following formula: PD = ln[(final number of cells) / (initial number of cells)] / ln.[28] PDL resulted from cumulative PDs.

2.2. Introduction of hTERT into MTC cells

The vector pCIneo (Clontech Laboratories Inc.) was used as backbone for construction of the plasmid pCI/hTERTβ carrying the cDNA of hTERT, cut out from the vector pGRN145 (kindly provided by Geron Corporation), under the control of the constitutive β-actin promoter. Gene delivery was performed using the Nucleofector™-technology (solution V, program G16 for SHER-I and T01 for all other cell strains; Amaxa Biosystems). Stable transfectants were selected using the following concentrations of G418: 400 μg/ml for SHER-I, MTC-SK and GRS V; 800 μg/ml for RARE. When stable transfectants reached the cell numbers that had been used for nucleofection, the PDL was reset to zero.

2.3. Determination of telomerase activity and telomere lengths

TA was determined using a modified real-time TRAP assay[29] and TA was expressed relative to HEK293. To exclude possible false-negative results from Taq polymerase inhibitors, lysates equivalent of 4000 telomerase-negative cells were mixed with lysates equivalent to 1000 telomerase-positive GSJO cells and subjected to analysis. Telomere lengths were determined using flow FISH.[29] Quantum™24 FITC-labelled standard-beads (Bangs Laboratories, Inc.) were used to set up a standard curve for conversion of measured fluorescence values (FL1) to molecules of equivalent soluble fluorochrome (MESF).

2.4. Qualitative and quantitative reverse transcription PCR

Total RNA was extracted using TRIZOL® reagent (Invitrogen). After DNA digestion with 2 U DNAse I (Amersham), reverse transcription was performed with 2.5 μg total RNA and 200 U Superscript III (Invitrogen) in a reaction volume of 20 μl. RNA was hydrolysed by addition of 2 μl 2.5 M NaOH (10 min, 37 °C). After neutralisation with 10 μl 2 M HEPES free acid, cDNA was purified with Qiaquick columns (Qiagen) and eluted in 100 μl. Quantitative real-time PCR was performed with 1 μl of cDNA per 20 μl reaction volume using Platinum®

Table 1 – Summary of the cell strains used within this study

Cell strain	Tumour stage at time of operation / tissue	Immunocytochemical characterisation				
		CT	CGRP	GRP	NSE	CgA
SHER-I	pT4N1MX / primary tumour	+	n.d.	+	+	–
GSJO	pT2N1MX / primary tumour	+	n.d.	+	+	+
RARE	pT4N1MX / lymph node	+	+	+	+	+
BOJO	pT4N2M1 / lymph node	+	+	+	+	+
GRS V	pT4N1M1 / lymph node	+	+	+	+	+
GRS IV	pT4N1M1 / lymph node	+	+	+	+	+
SINJ	pT2N1M0 / lymph node	+	+	+	+	+
MTC-SK	pT4N1MX / lymph node	+	+	+	+	+

CT: calcitonin; CGRP: calcitonin gene related peptide; GRP: gastrin related peptide; NSE: neurone-specific enolase; CgA: Chromogranin A; n.d.: not done.

SYBR Green qPCR SuperMix-UDG (Invitrogen) and the Rotorgene 2000 thermal cycler (Corbett Research). PCR conditions were 2 min at 50 °C, 2 min at 95 °C and 40 cycles of 10 s 94 °C, 15 s 55 °C, 15 s 72 °C and 5 s 80 °C (data acquisition). The following primer pairs were used in a final concentration of 0.5 μM: Myc-sense (CGACGCGGGGAGGCTATTCTGC) and Myc-antisense (CTCGCTCTGCTGCTGCTGCTGGTA) for c-Myc (Accession NM002467), Mad-sense (GACGGGCTCATCTTCGCTTGTG) and Mad-antisense (AGGTGTCGCTGCTCTCGCTGAA) for Mad1 (Accession NM002357), GAPD-sense (TGCACCACCAACTGCTTAGC) and GAPD-antisense (GGCATGGACTGTGGTCATGAG) for GAPD (Accession NM002046), ACTB-sense (CTGGAACGGTGAAGGTGACA) and ACTB-antisense (AAGGGACTTCCTGTAACAATGCA) for ACTB (Accession NM001101). Standard curves for each amplicon were generated with a serial dilution of dsDNA PCR-product in duplicate. Each sample was analysed in triplicate. Relative quantification was done by calculating the geometric average ratios of the target gene copies to the reference housekeeping gene copies of GAPD and ACTB and these values were normalised to the expression levels of SHER-I / PDL 22, which was included in each run.

Qualitative PCR was done for 35 cycles of 30 s 94 °C, 30 s 55 °C and 45 s 72 °C, using following primer pairs: hTERT-sense (GGGTGGCACGGCTTTTGTTCAGAT), hTERT-antisense (TTC AGCCGCAAGACCCCAAAGAGT), NEO-for (ATGATTGAACAAG ATGGATTGCAC), NEO-back (TCAGAAGAACTCGTCAAGAAG GC). Amplification products were analysed on a 2% w/v agarose gel.

2.5. Determination of cell proliferation

Cells were incubated with 10 μM BrdU for 17 h, washed with 1% w/v BSA in PBS and fixed with 70% v/v ethanol (–20 °C, 30 min, 4 °C). After treatment with 2 M HCl / 1% v/v Triton for 30 min, neutralisation was done with 0.1 M Na-Borat pH 8.5. Primary (mouse anti-BrdU, Becton Dickinson) and secondary (goat anti-mouse IgG-FITC, Sigma) antibodies were applied in TBS (0.5% v/v Tween20 / 1% w/v BSA in PBS). Cells were then washed with PBS, centrifuged and resuspended in 2.5 μg/ml propidium iodide (PI, Roche) in PBS. 10000 events were analysed using a FACS-Calibur and the CellQuest Pro software (Becton Dickinson).

2.6. Apoptosis assay (Annexin-V-FITC/PI staining)

For determination of apoptosis and necrosis, cells were stained with Annexin-V-FITC and PI (Roche). Analysis was performed on a FACS-Calibur measuring 10000 events per experiment.

2.7. Western blot analysis

Cells were lysed in 50 mM Tris, 0.5 M NaCl, 1% w/v Sodium Deoxicholate, 0.1% w/v SDS, 2 mM EDTA containing Complete Protease Inhibitor Cocktail Tablets (Roche). Equal amounts of proteins were separated on a NuPAGE 12% Bis-Tris polyacrylamide gel (Invitrogen) and transferred to a PVDF membrane (Millipore). Following primary antibodies were used: mouse anti-human p16 (Santa Cruz Biotechnology, 1:1000), mouse anti-human p21 (USB, 1:2000), rabbit anti-human p27 (USB, 1:1000) and mouse anti-human β-actin (Sigma, 1:10000). After incubation with the appropriate secondary antibody (goat anti-mouse IgG or goat anti-rabbit IgG, both peroxidase conjugates, Sigma, 1:5000), detection was performed with ECL-Plus (AmershamBiosciences) and a Lumi-Imager (Roche).

3. Results

3.1. Human MTC cells show low TA which is associated with progressive telomere erosion

In order to evaluate the role of telomerase in the development and pathogenesis of human MTC, we started to investigate TA in already established MTC cell strains. Whereas only GSJO displayed high TA (46% of HEK293), all other cell strains displayed TA in a range of 0.2 – 2.8% of HEK293 at early PDL as analysed by quantitative real-time TRAP assay (Fig. 1A). Since the establishment of the cell strain GSJO from primary culture was a long process that took over one year, compared to 1 – 4.5 months for all other cell strains, spontaneous reactivation of telomerase or selection of a cell clone with high TA might have been responsible for the exceptional high TA of GSJO. Interestingly, human normal umbilical vein endothelial cells (HUVECs / PDL 12) showed no TA under the described assay conditions. The absence of substantial TA in the analysed MTC cell strains was not due to an intrinsic inhibitor of telomerase or Taq polymerase, as was confirmed by mixing GSJO with all other MTC cell extracts (Fig. 1B).

Accordingly, GSJO cells showed low, stabilised telomere lengths, whereas telomeres shortened progressively in all other cell strains. GRS V and SINJ are shown as representative examples (Fig. 1C). Interestingly, the telomere lengths of around 40–50 kMESF that we have observed in early passage MTC cells lie in the range of senescent HUVECs[30], indicating that telomeres in MTC cells shorten beyond that of normal human cells. In accordance, human cancer cells often have been described to maintain shorter telomeres than do cells in surrounding normal tissues.[31]

3.2. MTC cells enter a crisis phase

MTC cells displaying telomere erosion during *in vitro* propagation showed a growth plateau after a defined number of PDs (Fig. 2A, GRS V, SINJ and SHER-I are shown as representative examples). However, the number of PDs reached differed significantly between the MTC cell strains. Whereas GRS V cells could be propagated up to PDL 150, SINJ entered growth arrest around PDL 100 und SHER-I around PDL 70, which might be attributed to differences in the success of the establishment of primary cultures or a different genetic background. Nevertheless, in all cases the decline in growth potential was characterised by increased acidification of the cell culture medium and all hallmarks of the typical crisis period.[32] Pre-crisis GRS V cells (PDL 77) showed a low rate of spontaneous apoptosis of about 5% and a high BrdU labelling index (69.3% of the cells have incorporated BrdU after 17 h). In contrast, during crisis only 26.9% of the cells were actively dividing, which was counter-balanced by high percentages of early apoptotic (29.4%) and late apoptotic/necrotic (33.1%) cell

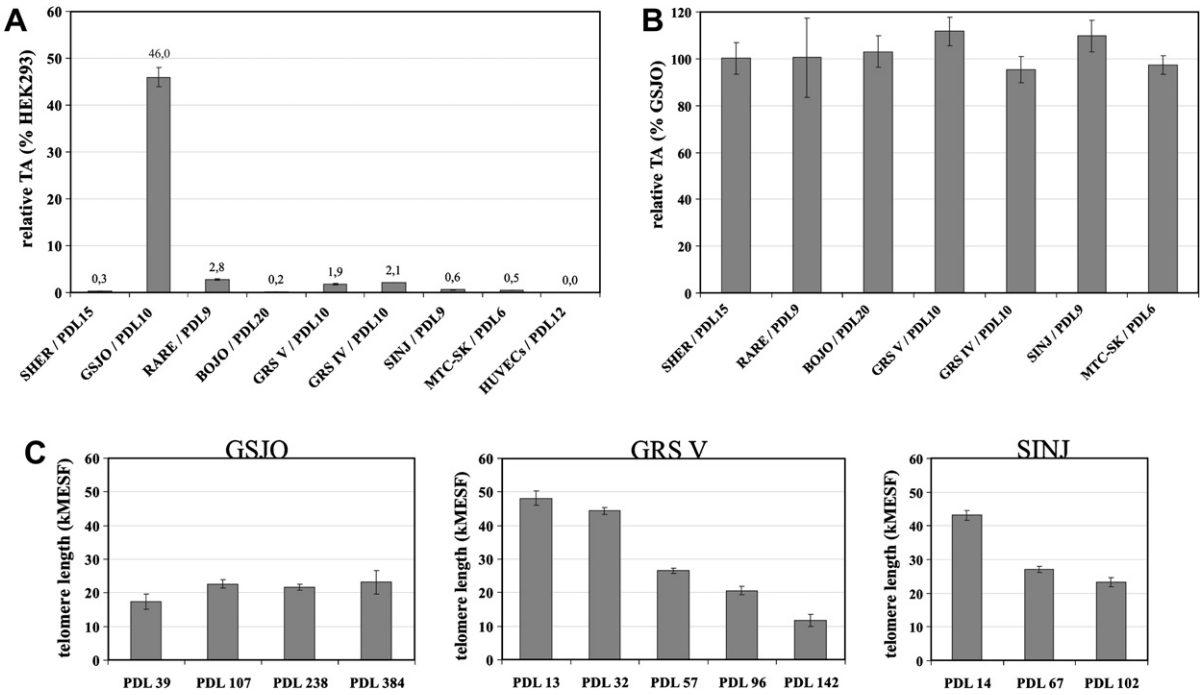

Fig. 1 – MTC cells are characterised by low telomerase activity at early passage and progressive telomere erosion upon *in vitro* cultivation. (A) Telomerase activity was analysed using real-time TRAP assay and is expressed relative to HEK293. Error bars indicate standard deviations of three measurements. (B) Cell lysates of GSJO were mixed with lysates of all other MTC cell strains and with lysis buffer as a control and subjected to real-time TRAP assay. Error bars indicate standard deviations of three measurements. (C) Telomere lengths were analysed using flow FISH. Relative telomere lengths are expressed as kMESF. Error bars indicate standard deviations of three measurements. *MESF*, molecules of equivalent soluble fluorochrome.

fractions (Fig. 2B). Compelling evidence implicates that gradual shortening of telomeres induces the expression of certain cyclin-dependent kinase (CDK) inhibitors.[33] Specifically, p16, p21 and p27 play an essential role in triggering growth arrest as has been shown in SV40 early region immortalised human fibroblasts during M2-phase.[34] Accordingly, p21 expression increased dramatically at crisis (PDL 142) (Fig. 2C), compared to both, pre-crisis (PDL 40) as well as post-crisis cells (PDL 360), whereas p27 seemed to be only slightly elevated. In contrast, p16 levels remained unaltered when GRS V reached the growth plateau, but its expression was undetectable in spontaneously immortalised, post-crisis cells.

3.3. *Spontaneous immortalisation of MTC cells is associated with induction of TA and changes of the cellular phenotype*

As described for cells in crisis, spontaneously immortalised cell clones arose in rare cases, concomitant with reactivation of telomerase[35] (Fig. 3A, GRS V and SINJ are shown as representative examples). Interestingly, the amount of TA essential for immortalisation varies significantly between cell strains. Whereas TA in GRS V increased from 1.9% of HEK293 up to 10%, TA in SINJ increased from 0.6% to around 150%. Only GSJO never exhibited a growth arrest up to 400 PDs and TA (around 50% of HEK293) remained constant over the whole cultivation period. Furthermore, the frequency of spontaneous immortalisation was dependent on the cell strain analysed. SINJ and BOJO spontaneously immortalised only in one out of three and six independent experiments, respectively, with an initial cell number of about 5×10^6 at the onset of crisis. On the contrary, RARE consistently induced TA in three independent experiments. Thus, immortalisation frequency of most MTC cell strains was in the range of that found for SV40 large T transfected cells in crisis[36] or cells harbouring mutations in the p53 gene.[37]

Spontaneous immortalisation was associated with changes of the cellular phenotype as can be seen in Fig. 3B for SINJ and BOJO. Whereas SINJ changed to a more uniform, rounded morphology, BOJO stopped growing in the typical large aggregates but rather grew in smaller, loose aggregates indicating less cell junctions. Additionally, when analysing the expression levels of c-myc, whose activation plays an essential role in tumour progression and maintenance[38], we found elevated c-myc transcription levels in post-crisis when compared to pre-crisis cells (Fig. 3C, RARE, BOJO, SINJ and MTC-SK are shown as representative examples). In contrast, only minor upregulation of c-myc could be detected in GSJO.

The c-myc antagonist mad1 was kept at constant levels after immortalisation in three of the analysed cell strains. Nevertheless, RARE and MTC-SK showed a significant downregulation of mad1, which could have an additive effect on c-myc upregulation.

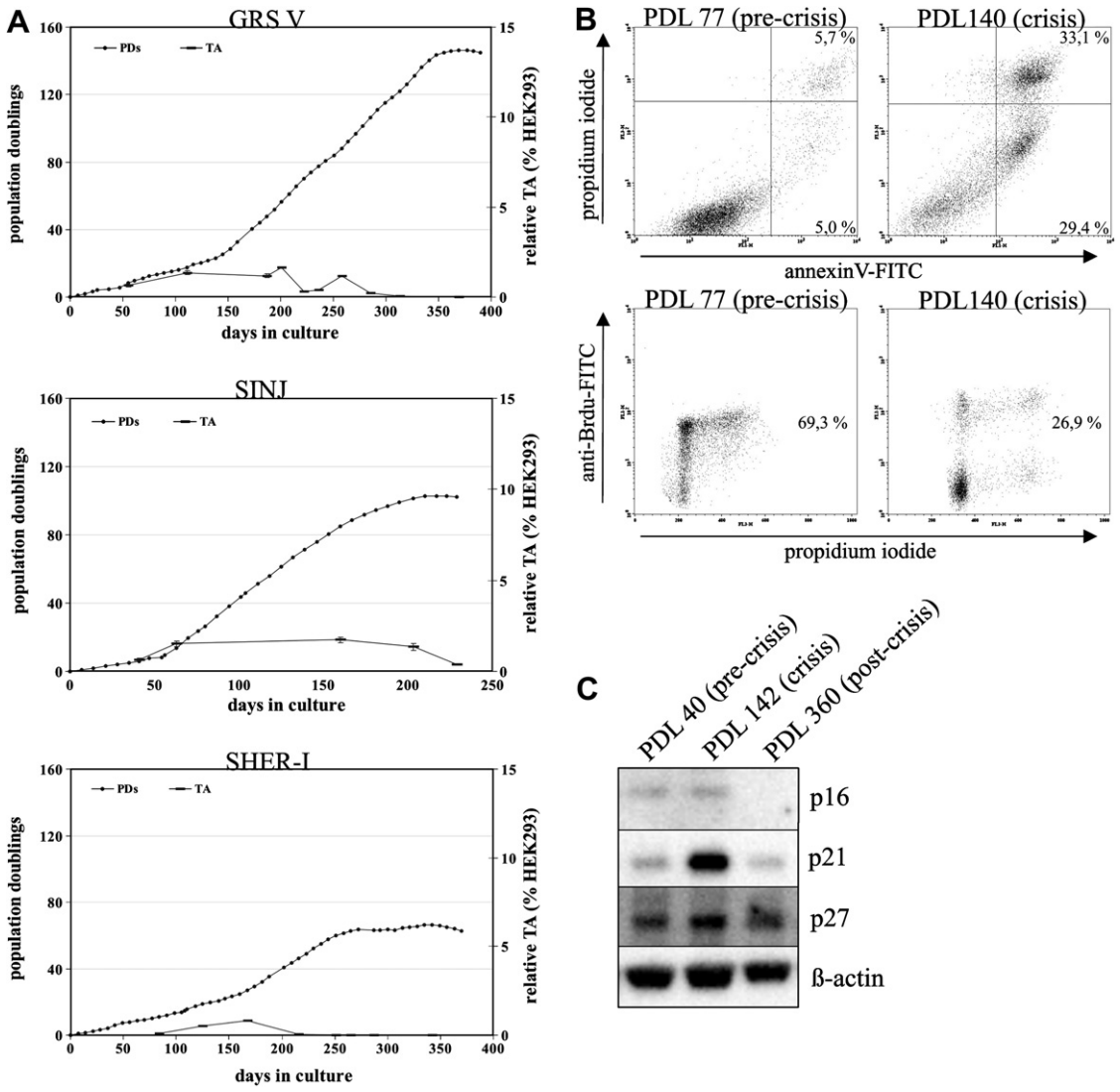

Fig. 2 – MTC cells show a limited replicative life span *in vitro*. (A) Growth curves and telomerase activity of GRS V, SINJ and SHER-I were monitored continuously. (B) The percentages of apoptotic/necrotic cells (upper panels) and of cells that have incorporated BrdU (lower panels) were analysed at different PDL. Therefore, pre-crisis (PDL 77) or crisis (PDL 140) GRS V cells were either stained with Annexin-V-FITC and propidium iodide or fixed and stained with anti-BrdU antibody and a secondary FITC-labelled antibody, followed by flow cytometric analysis. (C) Pre-crisis, crisis and post-crisis cells were analysed for expression of p16, p21 and p27 using western blotting. ß-actin was used as a loading control.

3.4. *Ectopic expression of hTERT induces immortalisation*

To test whether an enhancement of TA could counteract telomere erosion, hTERT or the empty vector were overexpressed in RARE, GRS V, MTC-SK and SHER-I (Fig. 4, SHER-I is shown as representative example). After selection of positive transfectants using G418, transcription of the selection marker neo^R was confirmed by reverse transcription-PCR for SHER-I/hTERT and SHER-I/pCIneo. In contrast, the hTERT transcript was only detected in SHER-I/hTERT (Fig. 4A). Consequently, TA was only induced in SHER-I/hTERT (29.5% of HEK293) (Fig. 4B), which was associated with continuous growth up to PDL 100 so far (Fig. 4C). Similar results were obtained for all other MTC cell strains transfected. In contrast to the cell strains that spontaneously induced TA, ectopic expression of hTERT did not induce, but rather reduced c-myc expression level, similar to mad1 expression (Fig. 4D). These data might suggest that activation of c-myc has been responsible for the spontaneous induction of TA in our MTC cell strains. Additionally, analysis of the cellular karyotype of hTERT transfected cells and the corresponding pre-crisis and post-crisis cells revealed a higher karyotypic stability after expression of hTERT: whereas pre-crisis SHER-I at PDL 19 presented a normal karyotype [46, XY] with a fraction (35%) of tetraploid cells, spontaneously immortalised SHER-I (PDL 145) were aneuploid harbouring chromosomal rearrangements [44,X, -Y,der(6)t(6;Y)(p23;q11),-8,der(12)t(8;12)(q12;p11)] with 70% of the cells having undergone endoreduplication of the aneuploid tumour cell genome. Analysis of SHER-I/hTERT, at similar PDL as the spontaneously immortalised counterpart,

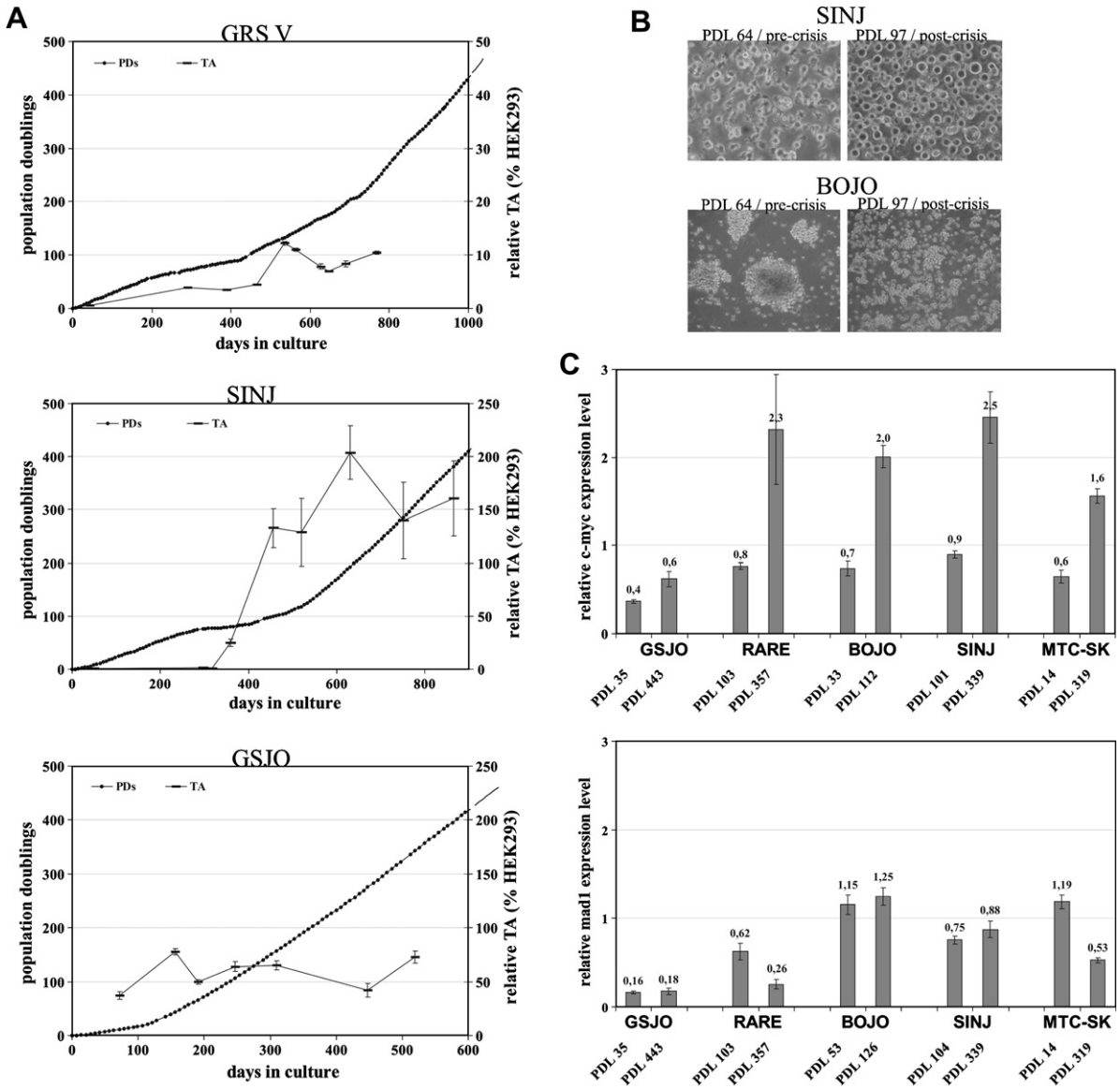

Fig. 3 – By spontaneous reactivation of telomerase, MTC cells escape the observed growth arrest and turn into continuously growing cell lines with changed phenotype. (A) Growth curves and telomerase activity of spontaneously immortalised GRS V, SINJ and GSJO were monitored continuously. (B) The morphology of SINJ and BOJO cells pre-crisis and post-crisis was monitored using phase contrast microscopy. (C) Expression levels of c-myc (upper panel) and mad1 (lower panel) in GSJO, RARE, BOJO, SINJ, MTC-SK were determined using quantitative real-time PCR. Cells were analysed before (left bars) and after (right bars) reactivation of telomerase. Values were normalised to two housekeeping genes GAPD and ACTB as well as to the expression levels of SHER-I (PDL 22) as an internal standard (expression of SHER-I was arbitrarily set to 1). The cells were analysed at the indicated PDs. Error bars indicate standard deviations of three measurements.

revealed a perfectly normal diploid karyotype, which was reproduced in another independent experiment.

3.5. *Treatment of MTC cells with MST-312 reduces the number of PDs reached*

In order to test whether the observed low TA influences the progression of human MTC, we investigated the cellular behaviour of pre-crisis MTC cells after prolonged growth in the presence of the telomerase inhibitor MST-312. It has been shown that cultivation of various human tumour cell lines in the presence of non-toxic concentrations of MST-312 leads to telomere shortening and the induction of cellular growth arrest.[39,40] A short term experiment over a period of 7 days revealed that the highest MST-312 concentration that had no toxic effect on MTC cells was 150 nM (Fig. 5A), which was used in all subsequent experiments. Additionally, no difference in growth characteristics between spontaneously immortalised MTC-SK, GRS V and GSJO treated with MST-312 and the corresponding control cells could be detected over at least 60 PDs (Fig. 5B), further demonstrating no acute cytotoxicity of the telomerase

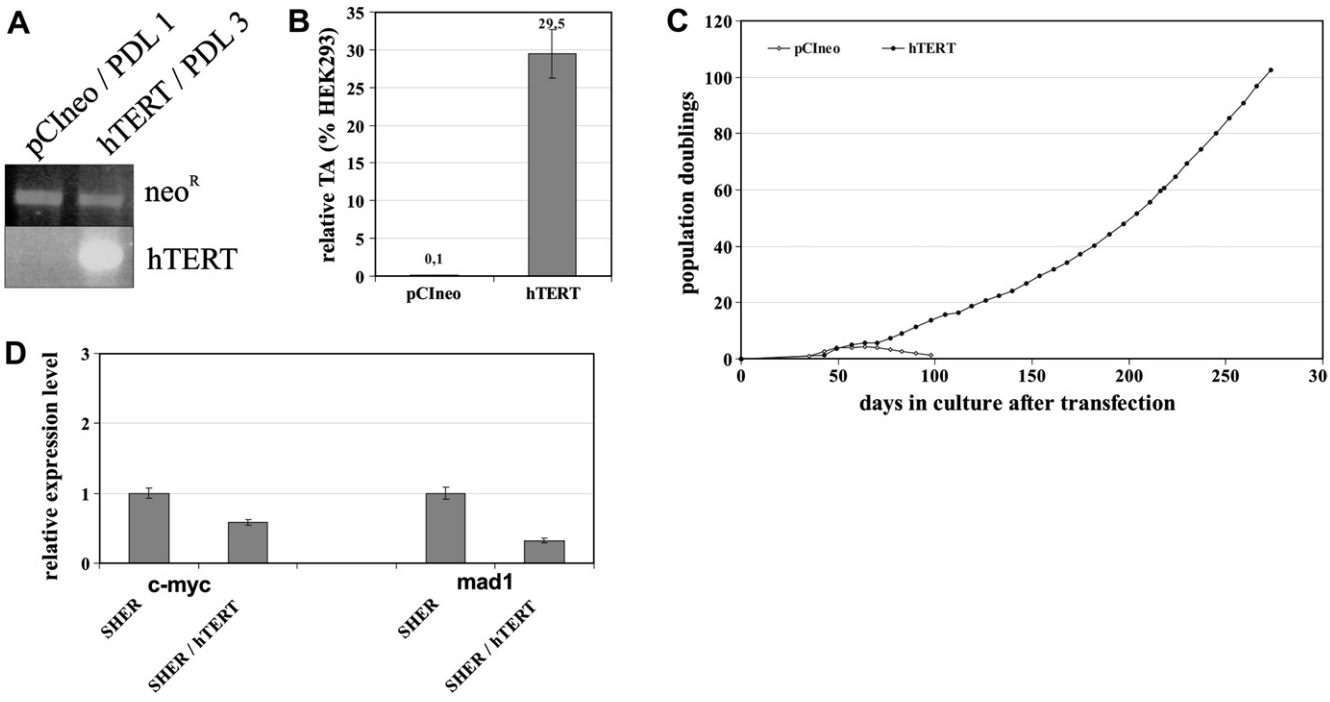

Fig. 4 – Ectopic expression of hTERT induces cell immortalisation. (A) The expression of neo^R and hTERT was determined using reverse transcription PCR after transfection with hTERT and a vector control. (B) Telomerase activity was analysed using real-time TRAP assay. (C) Growth curve of hTERT transfected cells and the corresponding vector control line are shown. (D) c-myc (left panel) and mad1 (right panel) expression levels in SHER-I before and after hTERT introduction was determined using quantitative real-time PCR. Values were normalised to two housekeeping genes GAPD and ACTB as well as to the expression levels of SHER-I (PDL 22) as an internal standard (expression of SHER-I was arbitrarily set to 1).

inhibitor. However, when cultivating mortal MTC-SK cells with MST-312, a significant reduction of the growth rate was observed as cells approach crisis period (Fig. 5C). In order to test whether this growth retardation can be attributed to an accelerated telomere shortening rate, we analysed telomere lengths of treated and untreated cells at the indicated time points. As shown in Fig. 5D, cells propagated in MST-312 containing medium were characterised by significantly ($p < 0.01$) shorter telomeres after 41 days, when compared to untreated cells. However, similar telomere lengths were observed at the end of the cellular life spans.

4. Discussion

Telomerase has been shown to play an essential role in the development and progression of human cancer. In the case of human MTC, data on the role of telomerase are rare and somehow controversial. Qualitative studies on tumour samples detected TA in only six out of eleven MTCs.[18–25] Additionally, Bockhorn and colleagues quantified TA in six MTCs ranking from 5% – 20% of HEK293, with five of them at the lower boundary.[26] Since *in vitro* propagated tumour cell lines have the potential to contribute essentially to our understanding of the role of neoplastic transformation, we here made use of eight previously established, well characterised MTC cell strains in order to get a deeper understanding of MTC development and the involvement of telomerase.

The results obtained showed that all but one MTC cell strains analysed so far were characterised by low TA. Thus, there seems to exist a general trend that within the thyroid the number of carcinomas with low to undetectable TA is extraordinarily high, supporting the idea that high TA is not important for the tumourigenesis of MTC. This is in contrast to the data obtained in other carcinomas, where high TA has been correlated with malignant transformation. Determination of TA and telomere lengths in normal and tumour thyroid tissues as well as primary cell cultures other than MTC have given similar results,[14] further indicating that telomere maintenance is not a prerequisite for the progression of thyroid cancer. It has been shown previously that transformation of human cells is possible in the absence of a telomere maintenance mechanism and that these cells reached crisis.[41] Thus, the original tumour source of our MTC cell strains could have been characterised by low TA as well and recently, similar results have been described for human squamous cell carcinomas[42]: cell lines from tumours that showed characteristics of crisis *in vivo* lacked significant TA and senesced *in vitro*, which could be prevented by ectopic expression of hTERT. Thus, these cells must have gained mechanisms to prevent the induction of senescence or apoptosis due to critically short telomeres. Indeed, abrogation of tumour suppressor protein function such as p53 and pRb[43,44] as well as

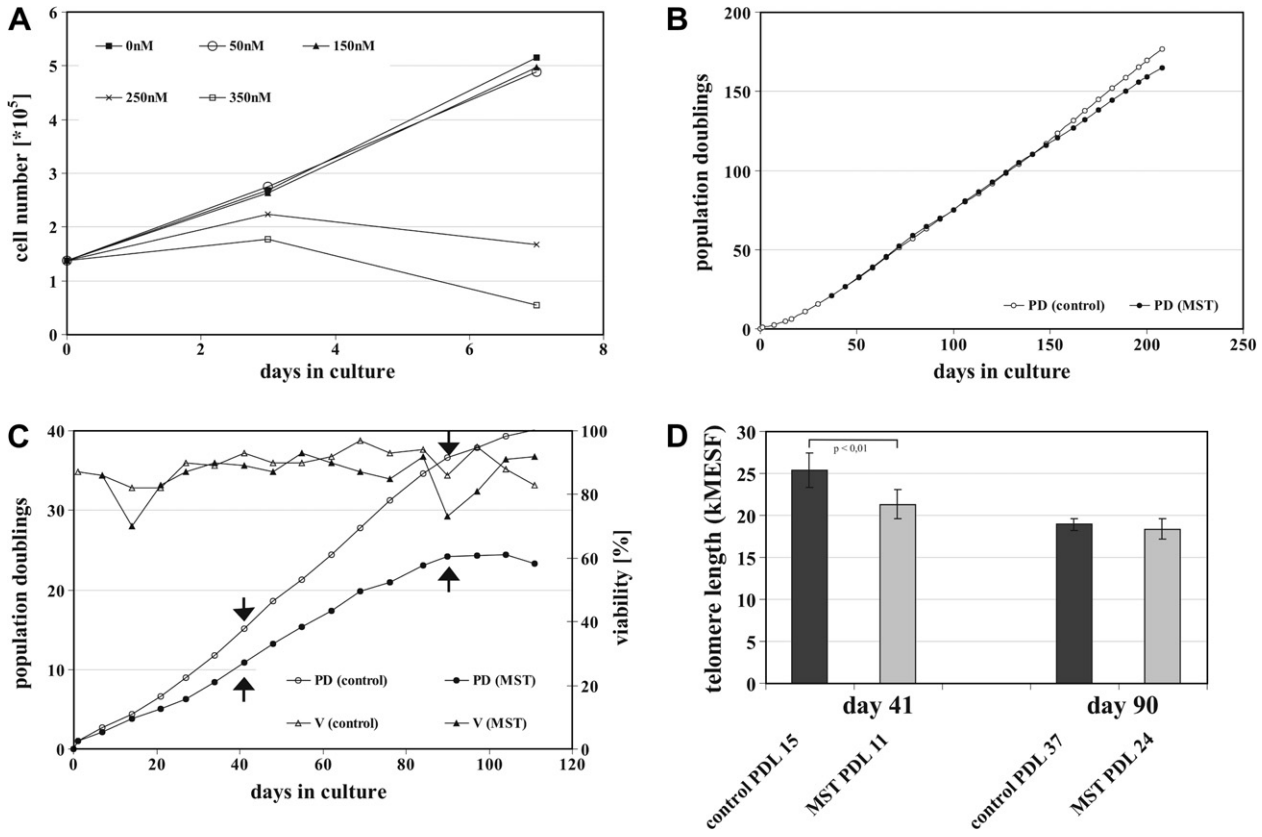

Fig. 5 – Propagation of MTC cells in the presence of MST-312 telomerase inhibitor reduces the numbers of PDs reached. (A) Pre-crisis MTC-SK (PDL 38) was cultivated for 7 days with different concentrations of MST-312 and cell numbers were determined. (B) Growth curves of spontaneously immortalised and (C) mortal MTC-SK cells treated with 150 nM MST-312 and the untreated control cells are shown. Viability (V) was determined by trypan blue-staining. (D) Telomere lengths of MST-312 treated and untreated mortal MTC-SK cells after day 41 and 90 were determined using flow FISH.

frequent expression of the anti-apoptotic protein bcl-2[45] have been described in the case of MTC. These data could explain why the apoptotic rate is particularly low in MTC,[46,47] which might also contribute to the fact that these carcinomas reach a clinically significant size without the necessity to activate a telomere-stabilising mechanism.

Interestingly, the MTC cell strains analysed here displayed significantly higher levels of TA when compared to normal human diploid cells. Moreover, cultivation of the cells in the presence of the telomerase inhibitor MST-312 significantly influences the number of PDs reached. This might indicate that even a low TA shifts the balance of telomere erosion/elongation slightly to the elongation side resulting in more pre-crisis cells, without, however, being sufficient to fully immortalise the cells. On the other hand, we cannot exclude that the low TA could also exert a protective role against cell death as described previously[48] or stabilise the karyotype.[47] These data are also in accordance with our observation that ectopic expression of hTERT protects the original cellular phenotype. Furthermore, in contrast to cells that had spontaneously bypassed crisis, no reactivation of the proto-oncogene c-myc was observed. Thus, we hypothesise that spontaneous reactivation of TA might be due to induction of c-myc as described before.[49]

In summary, these data suggest that interfering with telomerase in tumour cells with low TA might help reduce the number of cell divisions that single tumour cells can undergo and thus limit the size of tumours, which in turn could lower the risk for further spontaneous mutations. Therefore, reducing TA might be a valuable supportive treatment strategy in anti-tumour therapies.

Conflict of interest statement

None declared.

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