

Extra-nuclear telomerase reverse transcriptase (TERT) regulates glucose transport in skeletal muscle cells

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Keywords: TERT, Glucose transporter, Insulin, Ageing, Diabetes, Muscle

Abstract

Telomerase reverse transcriptase (TERT) is a key component of the telomerase complex. By lengthening telomeres in DNA strands, TERT increases senescent cell lifespan. Mice that lack TERT age much faster and exhibit age-related conditions such as osteoporosis, diabetes and neurodegeneration. Accelerated telomere shortening in both human and animal models has been documented in conditions associated with insulin resistance, including T2DM. We investigated the role of TERT, in regulating cellular glucose utilisation by using the myoblastoma cell line C2C12, as well as primary mouse and human skeletal muscle cells. Inhibition of TERT expression or activity by using siRNA (100 nM) or specific inhibitors (100 nM) reduced basal 2-deoxyglucose uptake by ~50%, in all cell types, without altering insulin responsiveness. In contrast, TERT over-expression increased glucose uptake by 3.25-fold. In C2C12 cells TERT protein was mostly localised intracellularly and stimulation of cells with insulin induced translocation to the plasma membrane. Furthermore, co-immunoprecipitation experiments in C2C12 cells showed that TERT was constitutively associated with glucose transporters (GLUTs) 1, 4 and 12 via an insulin insensitive interaction that also did not require intact PI3-K and mTOR pathways. Collectively, these findings identified a novel extra-nuclear function of TERT that regulates an insulin-insensitive pathway involved in glucose uptake in human and mouse skeletal muscle cells.

1. Introduction

Ageing is an established risk factor for the development of the metabolic syndrome and type 2 diabetes (T2DM). At the cellular level, senescence and ageing are associated with a decrease in telomere length and accelerated telomere shortening has been documented in conditions associated with insulin resistance, including T2DM [1–3]. Maintenance of telomere length is dependent on the activity of the enzyme telomerase and in particular its catalytic protein TERT (telomerase reverse transcriptase), which can help reverse this process and extend the lifespan of cells [4,5]. Models of metabolic disorders have provided further evidence for a role of TERT in glucose metabolism; for example, mononuclear cells of diabetic patients display accelerated telomere shortening in comparison to age-matched healthy individuals, an effect correlated with poor glycaemic control [6,7]. Furthermore telomere shortening in the β -cells of mice has also been found to impair insulin secretion thus leading to glucose intolerance even though there was an intact number of β -cell numbers, and so demonstrating a role of telomeres in the pathogenesis of diabetes [8].

In cells TERT shuttles between the nucleus and the cytoplasm [9–11]. Nuclear TERT regulates telomere length by catalysing addition of repetitive tandem (TTAGGG) DNA sequences to telomeres, thereby monitoring cell turnover and maintaining telomere length [12–14]. Recently TERT has been identified in mitochondria [10], where it appears to protect mitochondrial DNA and function from damage induced by oxidative stress, thus preventing apoptosis and promoting cell survival [11, 15–17].

Since mitochondrial function is closely associated with cellular fuel selection, we hypothesised that extra-nuclear functions of TERT may target the signalling pathways controlling glucose utilisation [18,19]. Previous *in vivo* studies showed that telomerase deficiency impairs glucose metabolism and insulin secretion [20]. The availability of glucose itself has also been shown to have a direct effect on the epigenetic modulation of TERT, resulting in the modulation of cell lifespan [21]. In addition, TERT possesses a consensus sequence on Ser824 for phosphorylation by Akt, which leads to increased activity [22–24], whereas inhibition of PI3K activity reduces both TERT phosphorylation by Akt and nuclear translocation, resulting in decreased nuclear telomerase activity [25,26]. This raises the possibility that insulin may acutely affect the cytosolic functions of TERT.

Therefore, in the present study we investigated basal and insulin stimulated glucose uptake activity in cells with altered TERT expression or activity using mouse muscle-derived C2C12 as well as human and primary mouse skeletal muscle cells. In addition, we have studied the intracellular distribution of TERT protein in response to insulin in C2C12 cells. We find that basal but not insulin-stimulated glucose uptake requires adequate TERT expression and identified a novel interaction between TERT and the glucose transporters GLUT1, 4 and 12.

2. Materials and methods

2.1. Ethics statement

The experimental protocols for the animals were in accordance with the United Kingdom Animal (Scientific Procedures) Act of 1986 (ASPA). A Home Office project licence was not required for this study because no regulated procedures were carried out. Mice were humanely sacrificed by CO₂ asphyxiation, which is an appropriate method under Schedule 1 of the Act.

2.2. Cell culture

2.2.1. C2C12

The C2C12 mouse myoblast cells (American Type Culture Collection, ATCC) were maintained in DMEM (Invitrogen, Paisley, UK) supplemented with 5 mM glucose and 10% FCS (BioSera, Sussex, UK) at 37 °C in 5% CO₂.

2.2.2. Human skeletal muscle cells

The human skeletal muscle cells (PromoCell, UK Human Cell Culture Systems) were cultured in skeletal muscle growth medium provided by the supplier supplemented with 5% FCS, fetuin (50 µg/ml), basic fibroblast factor (1 ng/ml), epidermal growth factor (10 ng/ml), insulin (10 µg/ml) and dexamethasone (0.4 µg/ml) at 37 °C in 5% CO₂.

2.2.3. Primary mouse skeletal muscle cells

The primary mouse skeletal muscle cells were isolated from 3–7 week old male BLaC6 mice. Mice were sacrificed, and the gastrocnemius and soleus muscles were pooled, cut along the fibres and digested for 25 min at 37 °C in oxygenated collagenase (1.5 mg/ml, Worthington Biochemicals, Lorne Laboratories, Reading, UK) solution. Cells were isolated as previously described [27], and kept in DMEM supplemented with 5 mM glucose, 5% FCS and 1% penicillin/streptomycin solution (Sigma Aldrich, Dorset, UK) at 37 °C in 5% CO₂.

2.3. Pharmacological inhibitors

TERT activity was inhibited using several pharmacologically active agents, namely triethylene tetraamine (TETA, Fisher Scientific, Loughborough, UK), geldanamycin (GA, Alexis Biochemicals, Enzo Life Sciences, Exeter, UK) and 17-(allylamino)-17-demethoxygeldanamycin (17-AAG, Sigma Aldrich). On the day of treatment, the required agents were added to the cell culture medium at various concentration(s) (1–100 nM; unless otherwise stated). Cells were incubated with the inhibitors for 1–24 h prior to measurement of the biological effect(s).

2.4. Transfection of cells with TERT and siRNA

The TERT-pBABE-puro construct was kindly provided by Dr Woody E. Wright (University of Texas). Pre-designed siRNA for human and mouse TERT was obtained from Dharmacon (Thermo Scientific, Loughborough, UK). Cells were subcultured into 12-well culture plates for 24 h prior to transfection with increasing concentrations of either TERT plasmid or 50–500 nM siRNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Empty pBABE-puro construct and scrambled siRNA (Ambion Applied Biosystems, Warrington, UK) were used as controls. During the optimisation phase of the methodology, significant reduction of cell viability was observed when the concentration of siRNA heteroduplexes exceeded 200 nM (data not shown) and for that reason a concentration of 100 nM siRNA was employed in all subsequent experiments.

2.5. 2-Deoxyglucose uptake assay

2-Deoxyglucose uptake measurements were carried out as described previously, for 10 min in glucose-free DMEM supplemented with 0.1% BSA containing 20 µM 2-[³H]-deoxy-D-glucose (0.53 µCi/ml, AmershamGE Health, Buckinghamshire, UK) in the

absence or presence of insulin and inhibitors, unless otherwise stated [28]. Non-carrier mediated uptake was determined in parallel in the presence of 10 μ M cytochalasin B (Alexis Biochemicals) and the values subtracted from the total uptake per assay.

2.6. Immunofluorescence staining and confocal microscopy

Cells were grown on cover-slips and treated as required. They were subsequently fixed using 4% paraformaldehyde, permeabilised with 0.1% Triton X-100 and blocked for 1 h in 5% goat serum and BSA. Cells were stained with anti-TERT antibody (1:200, Novus Biologicals, Cambridge, UK), anti-GLUT1 (1:100, Abcam, Cambridge, UK), anti-GLUT4 (1:100, Abcam) and anti-GLUT12 (1:100 [29]) and detected using (1:400) AlexaFluor® 488 goat anti-rabbit fluorescent secondary antibody (Invitrogen). Samples were mounted in media containing DAPI (Vector Labs, Peterborough, UK). Negative controls were run with secondary antibody alone to exclude any non-specific binding/ autofluorescence. Images were acquired with an AxioVert 200M laser scanning confocal microscope under either a 20 \times or 40–63 \times 1.4 NA oil immersion objective. Images were captured using a Carl Zeiss laser scanning microscope and LSM 510 imaging software. Fluorescence intensity profiles were generated along multiple line axes, analysed, and quantified using ImageJ software developed at the National Institutes of Health (<http://rsb.info.nih.gov/ij/>). Quantification was carried out on thirty cells from randomly chosen fields (n= 3) for each condition. Both paired region of analysis and region of interest were used to monitor changes in protein intracellular distribution.

2.7. Western blotting

Cell protein extracts were resolved by SDS-PAGE (10%). The proteins were transferred onto nitrocellulose membrane and immunoblotted with the appropriate antibody and detected using chemiluminescence substrate (SuperSignalWest Pico, Thermo Scientific, UK).

2.8. Immunoprecipitation

Cells were lysed in ice-cold lysis buffer (20 mM HEPES, 500 mM NaCl, 25 mM DTT, 20% glycerol and 0.1% NP-40, and supplemented with 1:100 protease and phosphatase inhibitor cocktails). The extracts were incubated with each antibody and 15 μ l of protein G-beads (Millipore, Watford, UK), and the immunoprecipitates subjected to SDS-PAGE. To ensure use of equal amounts of protein per sample, blots were performed before immunoprecipitation experiments using β -actin (data not shown).

2.9. Statistical analysis

Values are represented as the means \pm SEM, for the number of independent experiments indicated in the legends of the figures. Data were tested for homogeneity and comparison between group means was performed by two-tailed Student's t-test with Prism software (v. 5.03, GraphPad Software). Significant difference among different treatment groups was denoted by p < 0.05.

3. Results

3.1. Expression characteristics of TERT in C2C12

In preliminary experiments we investigated the expression and subcellular localisation characteristics of TERT in C2C12 myoblasts. Using RT-PCR we detected significant levels of TERT mRNA; furthermore these cells express a protein with a molecular

mass of 120 kDa recognized by a specific TERT antibody (data not shown). We also used indirect immunofluorescent signal detection by confocal microscopy to investigate TERT expression characteristics. As previously described [30], in cells such as R-cells (L5178Y murine lymphoma cell line) that possess very long telomeres (~48 kb), TERT is highly expressed and exclusively localised in the nucleus. By contrast, in C2C12 cells, we detected a different expression pattern; under unstimulated conditions, basal TERT immunostaining was distributed diffusely throughout the cytoplasm with only weak signal in the nucleus, raising the possibility of extra-nuclear actions of TERT in these cells (Fig. 1a).

C2C12 cells respond to insulin actions via an extensive signalling network that involves activation of the PI3-K and mTOR signalling cascades [31–34]. Exposure of C2C12 cells to insulin (100nM for 1 h) induced TERT intracellular movement evident from increased TERT signal redistribution near or at the plasma membrane by 35% (Fig. 1b). Similar experiments were repeated in C2C12 cells engineered to overexpress exogenous TERT (C2C12-TERT). These cells expressed 5–7 fold higher levels of TERT compared to native C2C12 cells (data not shown) and immunofluorescent signal detection showed that plasmid transfection resulted in a significant increase in TERT signal without any changes in subcellular localisation characteristics with the majority of the intracellular signal detected in the cytoplasm but not the nucleus (Fig. 1c). Exposing these cells to insulin led in a 2-fold increase in TERT signal translocated near or at the plasma membrane compared to control untreated cells. Insulin actions required intact PI3-K and mTOR activity since pre-treatment of C2C12-TERT cells with either wortmannin or rapamycin abolished insulin effect on TERT translocation (Fig. 1d).

3.2. TERT co-localises with GLUTs in C2C12 cells

The insulin-regulated localisation of TERT in C2C12 prompted us to study potential interactions between TERT and GLUTs, which represent major targets of insulin in the control of glucose uptake by skeletal muscle cells. Preliminary RT-PCR experiments using sequence-specific primers for GLUT1, GLUT4 and GLUT12 amplified DNA fragments for each GLUT in both undifferentiated and differentiated C2C12 myoblasts (Fig. 2a). Moreover, immunostaining experiments using specific antibodies detected strong immunofluorescent signal for each of the three GLUTs in C2C12 cells (Fig. 2b). Similar to other cell types [35–37], the housekeeping transporter GLUT1 signal was detected in both cytoplasm and plasma membrane, whereas GLUT4 and GLUT12 staining was primarily in the cytoplasm. Insulin treatment of C2C12 cells led to significant redistribution of GLUT4 and GLUT12 signal; however although GLUT4 signal showed the expected translocation to the cell surface, GLUT12 signal was localised to peri-nuclear structures that were also stained by an antibody against a Golgi marker (Fig. 2b).

The possibility of direct interaction between TERT and GLUTs was further investigated in C2C12 cells. Co-immunoprecipitation studies with an anti-TERT antibody in whole cell extracts identified a constitutive association between TERT and all three glucose transporters (GLUT1, GLUT4 and GLUT12). Under insulin stimulated conditions, TERT association with GLUT12 but not GLUTs 1 and 4 was enhanced by insulin stimulation. However, basal or insulin-induced interaction appeared to be independent of PI3-K and mTOR activity since pretreatment of cells with either wortmannin or rapamycin had no effect on TERT–GLUT associations (Fig. 3). In addition, TETA a telomerase inhibitor did not affect TERT association with GLUT1, GLUT4 and GLUT12. Interestingly, exposure of C2C12 cells to geldanamycin (which inhibits TERT

through inhibition of the hsp90–TERT complex formation) appeared to reduce the amount of GLUT12 co-immunoprecipitated by the TERT antibody, without altering immunoprecipitated GLUT1 and GLUT4 levels.

3.3. TERT and 2-deoxyglucose (2-DG) uptake in C2C12

The studies described above raised the possibility that TERT is involved in the regulation of glucose uptake in skeletal muscle cells. This was investigated in C2C12 myoblasts by a 2-DG uptake assay; unstimulated cells exhibited significant 2-DG uptake (71 ± 5 pmol/ 10 min/mg protein). In agreement with previous studies [36] treatment with insulin (100nM) for 10minsignificantly increased 2-DGuptake by 44% compared to non-treated cells. To test the effect of TERT on basal glucose uptake, we employed knock-down of TERT expression by short interfering RNA sequences complementary to the TERT transcript.. Preliminary experiments (Fig. 4a) demonstrated that at a concentration of 100 nM, specific siRNA effectively reduced TERT mRNA expression by 40–50% and protein by 80–90% in C2C12 cells. In contrast, transfection of cells with random sequence siRNA had no effect on TERT expression.

Knock-down of TERT expression in the C2C12 cells using siRNA resulted in a significant reduction in basal 2-DG uptake by approximately 20% (Fig. 4b). The difference in the efficiency of the siRNA to down-regulate mRNA and protein expression may reflect the manner in which siRNA targets mRNA and interferes with its degradation and therefore the impact on the linked but independent gene transcription and subsequent protein translation could be quite different. Cell viability assays confirmed that this inhibitory effect did not result from increased cell death (Fig. 4c). Using a complementary approach we also investigated the effects of TERT inhibitors; all inhibitors tested (geldanamycin, 17-AAG and TETA) significantly decreased basal 2-DG uptake in C2C12 cells by varying degrees of potency of 32–71%. Interestingly, we also observed inhibitory effects between 27 and 55% of these compounds on TERT mRNA expression raising the possibility that their action might involve regulation of TERT transcription (Fig. 4d). The inhibitory effects of siRNA or chemical TERT inhibitors were not due to non-specific toxic effects of these inhibitors as both cell viability (Fig. 4c) and total cell protein remained unchanged.

Follow-up experiments investigated also the effect of TERT overexpression on basal 2-DG uptake in C2C12-TERT cells. In these cells, increased intracellular TERT expression led to a 53% increase in basal 2-DG uptake (Fig. 5).

Since our previous experiments identified insulin-dependent regulation of TERT intracellular localisation, we next investigated whether TERT was involved in insulin induced glucose transport. Although maximal stimulation was attenuated in TERT inhibitor-treated cells, insulin effect on 2-DG uptake was comparable between TERT inhibitor treated and control cells (Fig. 6a) suggesting that TERT inhibition does not affect the ability of insulin to stimulate glucose transport. Moreover, insulin effects were not affected by TERT knocked-down or overexpression (data not shown) confirming independence from TERT expression levels.

To investigate whether TERT effects were cell type specific, we carried out similar experiments in primary mouse skeletal muscle cells and in human muscle cells. In both types of cells overexpression of TERT increased basal 2-DG uptake by 3–3.5 fold but did not affect the magnitude of insulin responses, suggesting a similar mode of TERT actions in these cells (Fig. 6b). Moreover, silencing TERT mRNA expression by siRNA in both human and primary mouse skeletal muscle cells reduced the basal

rate of glucose uptake by 37% and 43% respectively (Fig. 6c), but did not alter insulin effects (data not shown).

4. Discussion

The present work identified a novel extra-nuclear function of TERT that contributes in the regulation of basal glucose transport in C2C12 myoblasts. Similar findings in human and primary mouse skeletal muscle cells provide further support for the physiological relevance of TERT actions. Muscle cells take up ~65% of the glucose produced by the liver in the basal, low-insulin concentration, and are responsible for 80% of the insulin-stimulated glucose disposal. To exert this action skeletal muscle TERT is primarily localised outside the nucleus, in the cytoplasm and its subcellular localisation and trafficking to the cell surface appear to be regulated by the insulin signalling machinery, in particular the mTOR and PI3-K cascades. At present the functional consequences of TERT–insulin interactions are unknown but our studies suggest that it is unlikely to involve regulation of insulin-induced glucose uptake, a mechanism which is not affected by changes in TERT expression.

One potential mechanism that would allow TERT to regulate basal glucose uptake might involve protein–protein interactions with the various glucose transporters, which contributes to basal as well as extracellular signal-stimulated glucose uptake, such as the GLUT1, GLUT4 and GLUT12, as indicated by their co-immunoprecipitation with TERT in unstimulated C2C12 cells. TERT–GLUT interaction appears to be independent of insulin signalling mediators such as mTOR and PI3-K activity. To date, several GLUT-binding proteins have been identified [36], including aldolase and α -actinin 4 which may work as scaffolds linking GLUT to actin cytoskeleton or other specific intracellular anchors such as: Ubc 9, the sentrin-conjugated enzyme, predicted to regulate GLUT4 and GLUT1 protein turnover; Daxx, the adapter protein associated with Fas; and the type II TGF- β receptors, which also binds GLUT4 (but not GLUT1) and may lead to GLUT4 SUMOlation. Interactions of these proteins with GLUT C-terminus are also found to occur independent of insulin. Other GLUT partners such as the aminopeptidase IRAP might regulate endosomal retention and regulate exocytosis of GLUTs in the unstimulated state, either through static or dynamic retention within endosomes, therefore TERT might play similar regulatory roles in C2C12 cells.

The physiological significance of these findings may reside in the changes that occur in TERT expression during ageing and cell senescence. TERT activity decreases with age, resulting in telomere shortening. It is well-established that impaired telomerase activity (and accompanying reduction in telomere length) is strongly associated with the process of ageing and senescence. In this context, it may be relevant that in various models accelerated telomere shortening is also associated with insulin resistance and type-2 diabetes [6–8]. Similarly, ageing is linked to increased insulin resistance of which one of the main metabolic characteristics of muscle is its loss of its ability to up-regulate glucose uptake in response to insulin. Therefore, loss of TERT activity (e.g. in diabetes, ageing) and insulin sensitivity of glucose uptake might occur in parallel. Our observations may point towards a potential link as TERT interacts with the molecular machinery controlling glucose uptake. Our data may explain why conditions of diminished TERT expression (associated with accelerated telomere shortening) may also adversely affect glucose transport.

In conclusion, the present data identified a novel extra-nuclear function of TERT mediated via association of TERT with GLUT proteins to regulate glucose transport. Further studies will now focus on the precise interactions of TERT with the insulin signalling cascade. These findings might provide clues and possible mechanistic information for the diminished insulin-responsiveness, with ageing, of glucose uptake by muscle cells.

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Figure Legends:

Fig. 1. Confocal micrographs of C2C12 cells immunostained for endogenous TERT protein and in the presence of insulin, wortmannin and rapamycin. a) C2C12 and R cells were immunostained for endogenous TERT using an Alexa-Fluor® 488 (green) secondary antibody. b) C2C12 cells treated with 100 nM insulin for 1 h and immunostained for endogenous TERT protein. c) C2C12 cells were transfected with 2 µg TERT plasmid and subsequently treated with 100 nM insulin for 10 min alone or with 100 nM wortmannin or rapamycin for 1 h and then insulin for 10 min and after 24 h immunostained for TERT protein. Cell nuclei were respectively stained with DAPI (blue) and images captured using confocal microscopy (C2C12 cells at 63× objective). Micrographs are representative of three separate experiments. d) 30 random cells per treatment (n= 3) were processed using ImageJ, where the total fluorescent intensity and change in protein distribution were determined by measuring paired regions of interest. Images were analysed for changes in protein localisation between the nucleus and cytoplasm by defining a cross-sectional area and plotting profiles of fluorescent intensity across that area. Data is representative for fluorescent intensities averaged from 30 random cells (n= 3). Values presented are mean (±SEM) of 3 independent experiments. Asterisks indicate statistical significance (t-test) of difference from control samples: **p < 0.01 and ***p < 0.001.

Fig. 2. Glucose transporter 1, 4 and 12 mRNA expression in C2C12 and confocal micrographs of C2C12 cells showing immunostaining of GLUT1, 4 and 12 in the presence and absence of insulin. a) Graphs show the mRNA expression of GLUTs 1, 4 and 12 in undifferentiated (U) and differentiated (D) C2C12 cells. Reverse transcription PCR (RT-PCR) was performed on RNA extracted from treated cells. GAPDH mRNA was used as an internal control and an image of the gel was captured and used to quantify GLUT mRNA expression using ImageJ software. C2C12 cells immunostained for endogenous b) GLUT1, GLUT4 and GLUT12 proteins. Cells were treated with 100 nM insulin for 1 h and immunostained. Cells were imaged using an Alexa-Fluor® 488 (green) secondary antibody. Cell nuclei were respectively stained with DAPI (blue) and images captured using confocal microscopy. Micrographs are representative of three separate experiments. Images were analysed for changes in protein localisation between the nucleus and cytoplasm by defining a cross-sectional area and plotting profiles of fluorescent intensity across that area.

Fig. 3. Association of TERT protein with GLUT1, GLUT4 and GLUT12 proteins in C2C12 cells and the effect of inhibitors on this interaction. C2C12 cell lysates were immunoprecipitated with TERT antibody and probed for GLUT1, GLUT4 and GLUT12 proteins in control cells and in cells incubated with 100 nM insulin. C2C12 cell lysates were prepared from control cells and cells treated with 100 nM rapamycin (for 24 h), wortmannin (1 h), geldanamycin or TETA (24 h). 20 µg of each cell lysate was immunoprecipitated with TERT antibody and probed for GLUT1, GLUT4 and GLUT12

proteins. Protein G beads were used as a control. β -Actin probing before IP was used as a loading control. Results are representative of three independent experiments (IP: immunoprecipitation and WB: western blotted).

Fig. 4. Effect of TERT siRNA and inhibitors on [3H] 2-deoxyglucose uptake and TERT mRNA and protein expression in C2C12 cells. The effect of TERT siRNA on a) TERT mRNA and protein expression was assessed. C2C12 cells were transfected with 100 nM TERT siRNA and random siRNA (as control) and analysed 24 h after transfection. Reverse transcription PCR (RT-PCR) was performed on RNA extracted from treated cells. GAPDH mRNA was used as an internal control and an image of the gel was captured and used to quantify TERT mRNA expression using ImageJ software. Western blotting was performed to analyse TERT protein expression and β -actin was used as a loading control. b) The effect of TERT knock-down on 2-deoxyglucose uptake was assessed; cells were transfected with 100 nM TERT siRNA. Random siRNA was used as a negative control and cells were analysed 24 h after transfection. Cells were incubated in serum free medium for 4 h and 2-deoxyglucose uptake was measured over 10 min and is represented as pmol/10 min/mg protein. c) The effect of 100 nM TERT siRNA, random siRNA and transfection reagent (Lipofectamine) on cell viability was assessed using MTT assay. d) Reverse transcription PCR (RT-PCR) was performed on RNA extracted from C2C12 cells treated with 100 nM TETA, geldanamycin or 17-AAG for 24 h. GAPDH mRNA was used as an internal control and an image of the gel was captured and used to quantify TERT mRNA expression using ImageJ software. Values are means (\pm SEM) for 5 separate experiments, performed in duplicate. Asterisks indicate statistical significance (t-test) of difference from control: * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$.

Fig. 5. Effect of TERT over-expression on [3H] 2-deoxyglucose uptake in C2C12 cells. The effect of TERT over-expression on 2-deoxyglucose uptake was assessed; C2C12 cells were transfected with pBABE-puro-TERT plasmid (6 μ g). Lipofectamine and empty pBABE-puro plasmid (control vector) were used as negative controls. After 24 h the cells were incubated in serum free medium for 4 h and 2-deoxyglucose uptake was measured over 10 min. 2-Deoxyglucose uptake is represented as pmol/10 min/mg protein. Values presented are means (\pm SEM) for 5 separate experiments, performed in duplicate. Asterisk indicates statistical significance (t-test) of difference from control: * $p \leq 0.05$

Fig. 6. Effect of TERT inhibition in C2C12 cells and over-expression and knock-down on [3H] 2-deoxyglucose uptake in human and primary mouse skeletal muscle cells. The effect of TERT inhibition on 2-deoxyglucose uptake was assessed in: a) C2C12 cells treated with 100 nM geldanamycin (GA), TETA or 17-AAG for 24 h and then 100 nM insulin for 10 min. b) Human and primary mouse skeletal muscle cells were transfected with 4 μ g and 6 μ g pBABE-puro-TERT plasmid, respectively. Lipofectamine and empty pBABE-puro plasmid (control vector) were used as negative

controls. c) Next the effect of TERT knock-down on 2-deoxyglucose was assessed. Primary mouse and human skeletal muscle cells were transfected with 100 nM TERT siRNA and random siRNA was used as a negative control. After 24 h the cells were incubated in serum free medium for 4 h and 2-deoxyglucose uptake was measured over 10min. 2-Deoxyglucose uptake is represented as pmol/10 min/mg protein. Values are means (\pm SEM) for 4 independent experiments, performed in duplicate. Asterisks indicate statistical significance (t-test) of difference from control: * $p \leq 0.05$ and ** $p \leq 0.01$.

Figure 1

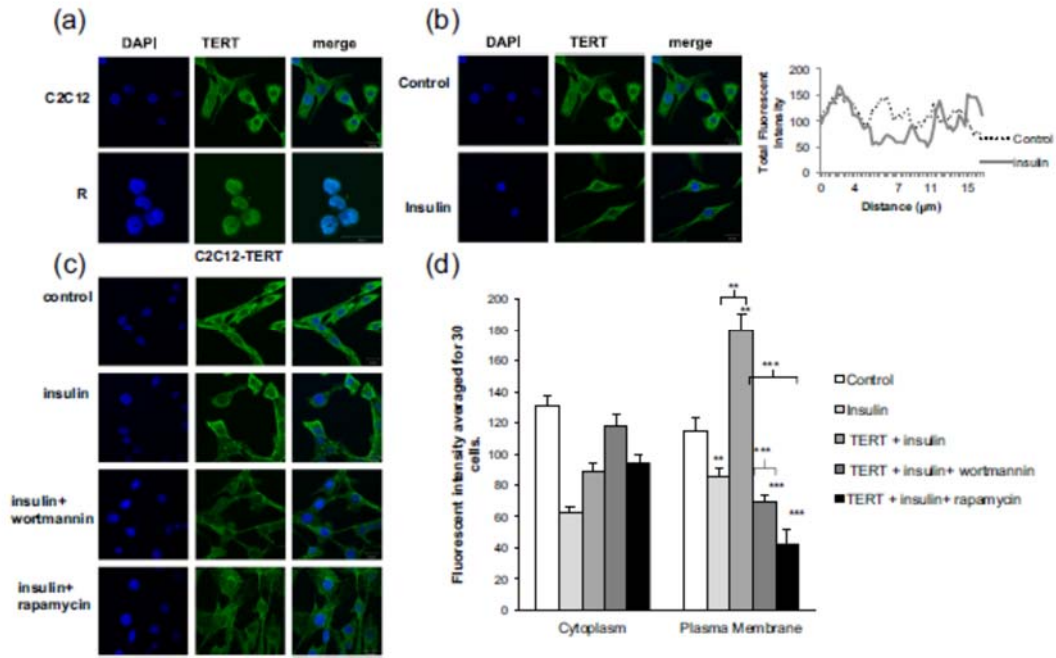


Figure 2

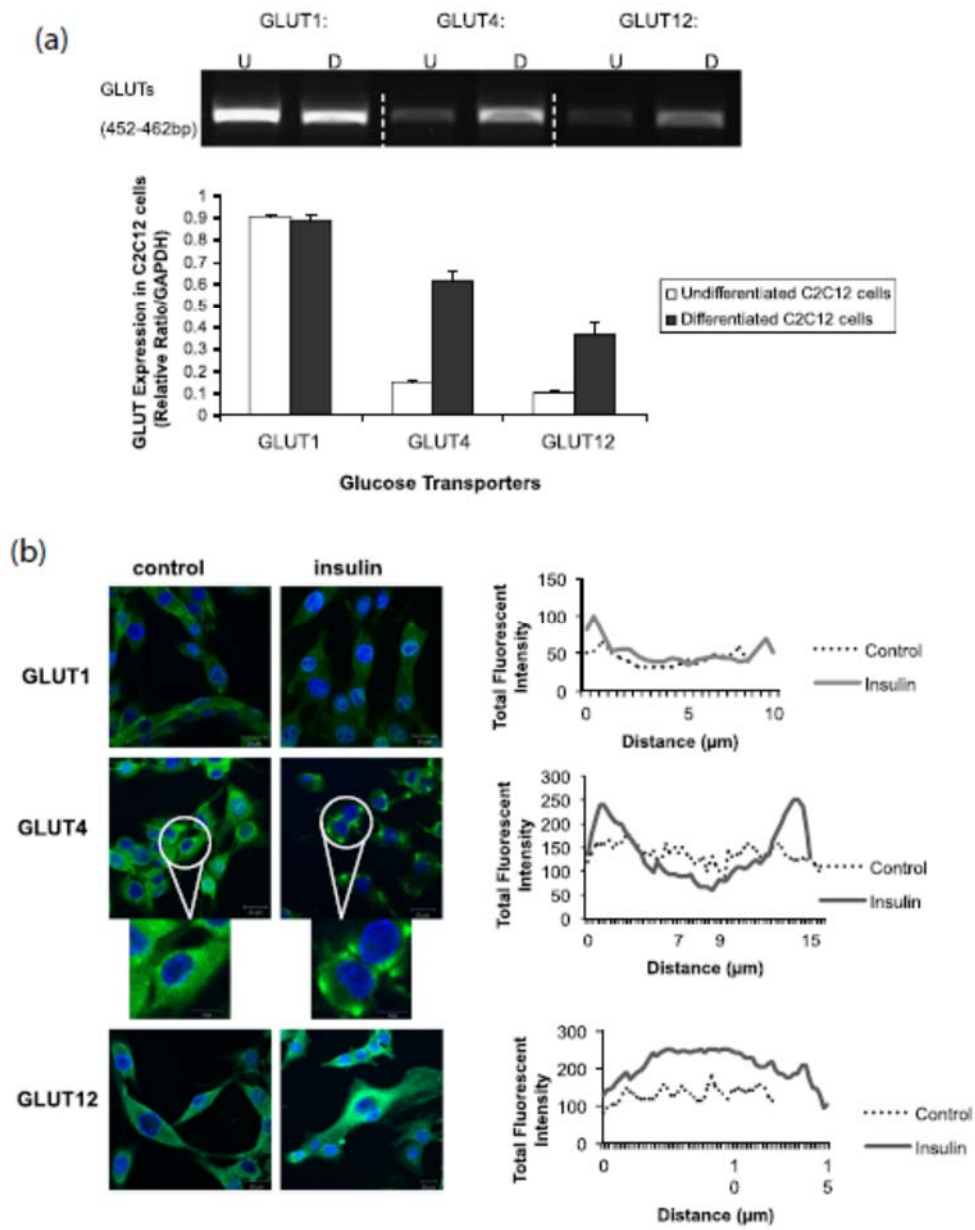


Figure 3

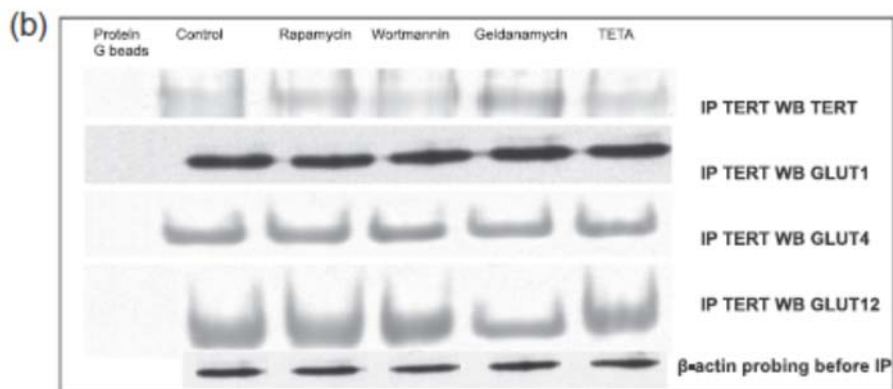
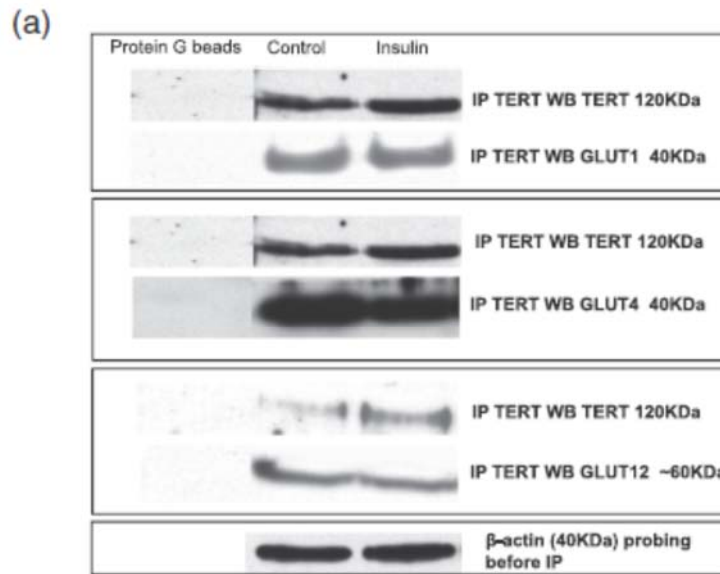


Figure 4

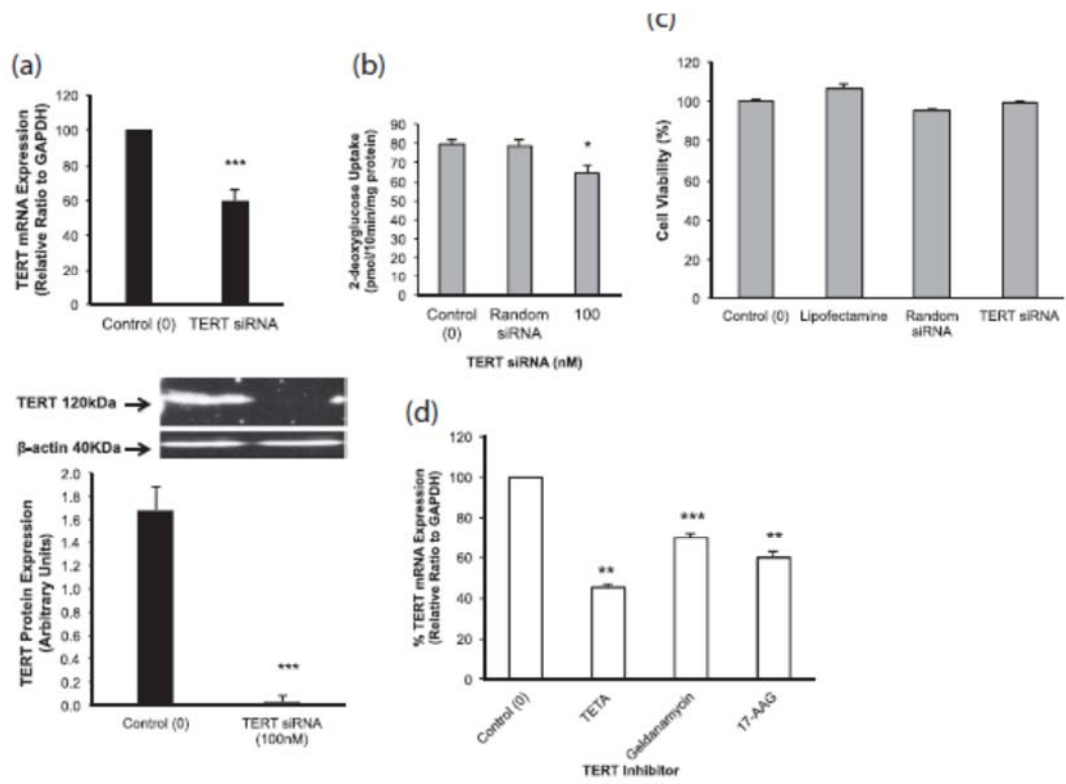


Figure 5

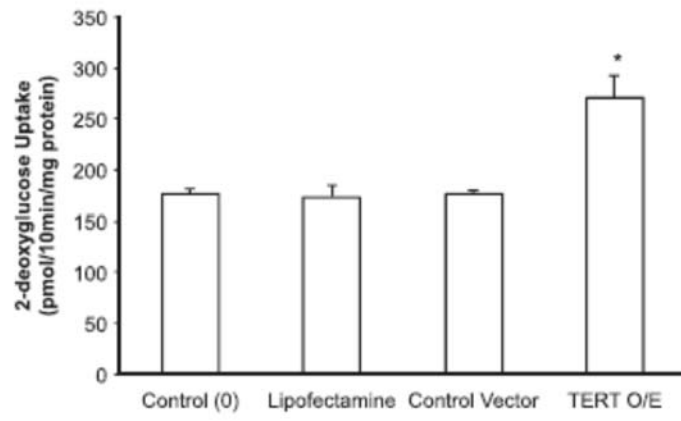


Figure 6

