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Research article

The effects of Rapamycin on Telomerase Activity and Regulation in Cancer Cells

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Abstract

Telomeres, DNA-protein structures located at the ends of eukaryotic chromosomes are shortened with each DNA synthesis in somatic cells, thus limiting their life span. Telomere shortening is prevented by the activity of telomerase, a reverse transcriptase which is expressed mainly in cancer cells, providing them with a limitless life span. Tor1 and Tor2 (targets of Rapamycin), two similar kinases that regulate cell growth are part of the TOR complex 1 (TORC1) which coordinates the response to nutrient starvation and is sensitive to Rapamycin. Signaling pathways involving mTOR and telomeres regulation where found to be overlapped in yeast, where TORC1 signal controls the Ku heterodimer, a telomere regulator. In the light of the importance of telomerase activity in malignancies and the anti-cancer activity of Rapamicin, we studied the potential implications of using Rapamycin as a therapeutic agent against cancer by analyzing the effect of the drug on telomerase in cancer cells. Our study was therefore aimed at the characterization of the effect of Rapamycin on telomerase activity (TA) and the elucidation of the possible regulatory mechanisms affected by this drug in vitro. The results show a cytostatic effect of Rapamycin on the proliferation of Jurkat and MCF-7 cell lines and an inhibitory effect on TA in these cells. The ability of Rapamycin to inhibit TA was mediated by two separate mechanisms: In Jurkat cells the inhibition was on the transcriptional level, controlled by the reduced binding of transcription factors to the hTERT promoter, while in MCF-7 cells the inhibition of TA was mediated on the post translational level, by its kinases, AKT and PKCα. A non-critical dose (1nM) of Rapamycin induced only a small decrease of about 25% in telomere length after six months of exposure to the drug. We hope that the knowledge gained from this work will lead to develop new combinations of Rapamycin and other anti-cancerous agents that target intra-cellular pathways, to gain further advances in the never-ending quest to find effective anti-cancer treatments.

Keywords: telomeres; telomerase; rapamycin, mTOR

Introduction

Mammalian target of Rapamycin (mTOR) is a serine-threonine protein kinase that belongs to the phosphoinositide 3-kinase (PI3K)-related kinase family [1]. Through kinase cascades, mTOR regulates cell size, progression of the cell cycle, cell survival, and is considered a master regulator of protein synthesis [2]. As such - it is deregulated in various cancer cells. In mammals, there are two mTOR protein complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTOR forms a physical and functional complex with several proteins including Hsp90, Akt, TERT, p70 S6 kinase (S6K), providing a compelling evidence for another level of regulation, that of telomerase activity [2,3].

The classic mTOR inhibitor is the macrolide antibiotic Rapamycin which indirectly inhibits its kinase activity [4]. In yeast, exposure to Rapamycin inhibits TORC1 signaling pathway, blocking the recruitment of telomerase to the telomeres and ultimately resulting in rapid telomere shortening [3].

The mTOR pathway is frequently constitutively active in cancer cells, leading to aberrant protein translation, cell growth and proliferation. The tumor suppressor phosphatase and tensin homologue (PTEN) is often deleted or truncated in endometrial, breast, prostate, and ovarian cancers leading to an overexpression of the PI3K–Akt–mTOR pathway [5].

The importance of telomerase in the biology and prognosis of many types of cancers is well established [6]. Telomerase is a unique reverse transcriptase expressed almost exclusively in

> 90% of cancer cells. It compensates for telomeric loss in each DNA replication thus conferring endless replicative potential to the cancer cell [7]. Due to its essentiality and specificity to the malignant cell it may serve as a valid anticancer drug target and indeed active compounds that target telomerase are already in advanced phases of clinical trials [8]. Numerous cytotoxic drugs target telomerase [9-11] including proteasome inhibitors [12,13]. In the light of the importance of both the mTOR and telomerase to the growth and perpetuation of the malignant clone abd the regulatory croos talk between mTOR and the enzyme, we asked whether the inhibition of mTOR in cancer cells will have an additional inhibitory effect on telomerase activity. Additionally, we aimed at deciphering the possible regulatory mechanisms contributing to this inhibitory effect (if exists).

Real-Time RT-PCR

The expression of the hTERT gene was detected by Real Time PCR. Total RNA was extracted using EZ-RNA Isolation Kit reagent (Biological Industries Beit Haemek, Israel). The extracted RNA was reverse transcribed according to the manufacturer's instructions of the High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, CA. USA). The real-time quantitative RT-PCR is based on the TaqMan methodology, ABI PRISM 7000 Sequence Detection System (Applied Biosystems, CA. USA), and StepOnePlus™ Real-Time PCR Systems (Applied Biosystems, CA. USA). The expression of the hTERT gene was calculated relatively to the expression of the control gene, HPRT-1.

Chromatin immunoprecipitation (ChIP) assay

The binding of NFκB, c-Myc, SP1, and WT1 to the hTERT promoter was assessed by the Chromatin Immunoprecipitation (ChIP) assay47 in the Jurkat cell line. Chromatin Immunoprecipitation was performed with the EZ ChIP kit according to the manufacturer's directions (Upstate, Temecula, CA) and as described previously [14]. Cells were harvested and fixed in 37% formaldehyde at 37°C for 10min. After being quenched by glycine and washed in PBS, the cells were lysed in 1ml SDS lysis buffer. The DNA was sheared by sonication to an average size of 0.2kb to 1kb. A 100µl extract was diluted, immunoprecipitated with the indicated antibodies (anti-SP1 and anti-c-Myc: 1:50, Millipore MA, USA; anti-NFκB: 1:50, Abcam MA, USA; anti-WT1: 3:100, Santa Cruz Biotech, TX, USA) for 16h at 4°C with agitation and was supplemented with 60µl of protein G agarose beads for 1h at 4°C. The immunocomplexes were washed with the serial washing buffers, eluted, and heated at 65°C for 4h to reverse the cross-links. After DNA purification (by QIAquick PCR Purification kit), the immunoprecipitated DNA was subjected to a real-time PCR reaction to amplify the binding sites of the various transcription factors. The levels of the DNA binding of SP1, c-Myc, NFκB, and WT1 were evaluated relatively to the total DNA input of each sample that was not immunoprecipitated with any of the antibodies. The antibodies against SP1 and c-Myc were purchased from Millipore MA, USA.

The relevant primers with the following sequences were used:
For the region of c-Myc and SP1 binding site:
Forward primer: AGTGGATTCGCGGGCACAGA;
Reverse primer: TTCCCACGTGCGCAGCAGGA
For the region of NFκB binding site:
Forward primer: GCCTCCTAGCTCTGCAGT
Reverse primer: ACCCGAGGACGCATTGCT
For the region of WT1 binding site:
Forward primer: TTTGCCCTAGTGGCAGAGAC
Reverse primer: GCCGGAGGAAATTG

Due to the high GC content in the hTERT promoter region, real-time PCR reactions included several additional temperature steps (in the polymerization stage) to ensure proper products formation. PCR conditions were as follows 940C – 3min, followed by 39 cycles of: 940C- 20 sec, 580C- 30 sec, 720C- 20 sec, 760C- 20 sec, 800C- 20 sec 840C- 20 sec and finally 720C- 5 min. The products were separated on a 1% agarose gels and analyzed by the VersaDoc software on the Gel Doc documentation system (BioRad, Israel).

Immunoprecipitation and western blot analysis

The phosphorylation levels of telomerase and its kinases, (phospho)-pAKT and pPKCα in MCF-7 cells and the cellular levels of SP1, c-Myc, NFκB and WT1 in Jurkat cells were evaluated by Western blotting. The cells were grown for 24, 48 and 72 hours in the presence of Rapamicin (IC50) and lysed by using a lysis buffer containing phosphatase and protease inhibitors. Identical protein amounts (100µg) were separated by 10% SDS-PAGE, transferred to nitrocellulose membrane and detected by the following specific antibodies: anti-pAKT Ser473/ pPKCα Ser657 and anti-total AKT/ PKCα. (anti- AKT: 1:1000, Cell signaling SC, USA; anti-PKCα: 1:500, Santa Cruz Biotechnology, CA, USA; anti-SP1 and anti-c-Myc: 1:500, Millipore MA, USA; anti-NFκB: 1:1000, Abcam MA, USA; anti-WT1: 1:500, Santa Cruz Biotech, TX, USA). Signals were visualized after exposing the membranes to 2nd fluorescent antibodies and quantified by the Odyssey analysis software (Licor).

To determine the levels of phosphorylated hTERT, an Immunoprecipitation assay was used. 500-1000µgs of protein were precipitated with 10µg/mg anti-phosphoserine antibody (StressMarq Biosciences Inc.) or with 20µg/mg anti-total hTERT antibody (Santa Cruz, CA, USA). The protein-antibody complexes were mixed with 20µg of protein G agarose beads for 16h while agitation at 4oC. The immunoprecipitated lysates were washed with lysis buffer, boiled for 3 min and subjected to standard Western blot analysis, as described above. The primary anti-hTERT antibody for the immunoblotting (1:500-1:1000) was purchased from Epitomics, CA, USA.

Southern blot assay

To perform a Southern Blot, DNA was extracted by using ArchivePure™ DNA purification kit (5 prime, MD, USA) and quantified with NanoDrop™ (Thermo). Genomic DNA was then digested with the restriction enzymes *RSAI, HINFI* which digest DNA to segments of less than 2kb without cutting the telomeres. After digestion the DNA was separated by electrophoresis on 0.8% Agarose gel and later soaked in 0.25M HCl depurination solution, denaturing solution containing 0.5M NaOH and 1.5M NaCl, and finally neutralization solution, containing 0.5M Tris HCl and 3M NaCl pH=7.5. DNA binding to the membrane was performed by the force of capillarity in 20xSSC. The DNA was cross-linked to the membrane by exposure to UV radia-

tion and then incubated with pre-hybridization solution for 1h at 420C, followed by soaking in digoxigenin (DIG) hybridization solution that contains a telomeric probe marked with DIG (CCCTAA)4 (Hy Laboratories, Rehovot, Israel). After hybridization, the membrane was exposed to anti-DIG antibody conjugated to an alkaline phosphatase, and the substrate CSPD. The chemiluminescent signal was captured on a film and the sizes of telomeric smears were compared with those of the DIG labelled marker.

Statistical analysis

A two-tailed One-sample Student t test with unequal variance and ANOVA one way were used to calculate the P values in SPSS for Windows version 11.5 software (SPSS, Inc., Chicago, IL). In all assays, P values of less than 0.05 and 0.001 were considered statistically significant and highly significant, respectively.

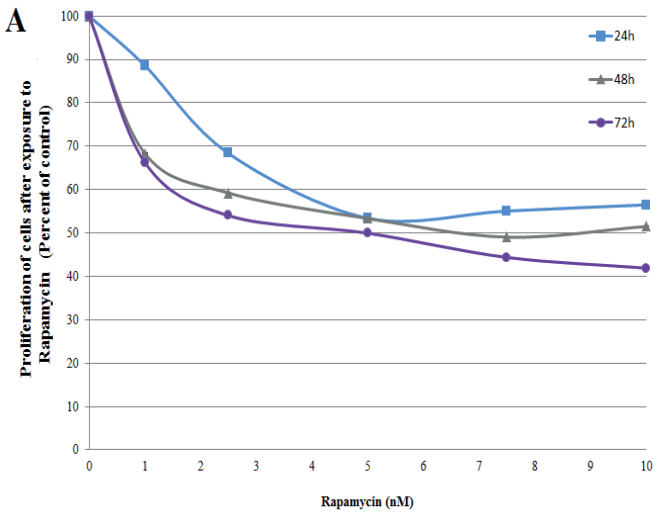
Results

The effect of Rapamycin on the proliferation of cancer cells

The proliferation of the Jurkat cell line was monitored after admission of Rapamycin for 24h, 48h, 72h, and 96h. The drug differentially inhibited cell proliferation in a dose and time dependent manners. From 24h onward, the maximal decrease of 50% in proliferation in these cells was found to be at 4.57nM (Figure 1A). The proliferation of the MCF-7 cell was also monitored after admission of Rapamycin for 24h, 48h, 72h, and 96h. However, the maximal decrease in cell number reached 48% at 9.17nM (Figure 1B) and was obtained after exposure to Rapamycin for over 24h. Higher doses of Rapamycin did not decrease cell proliferation beyond 50% in these cells.

Telomerase activity following treatment with Rapamycin

After inspecting the inhibitory effect of epoxomicin on cell proliferation we assessed its effect on telomerase activity (TA) by the quantitative TRAP assay. Both cell types exhibited a differential decrease in TA in response to Rapamycin. Following the treatment of Jurkat cells for 24h TA increased by 32%. After 48h TA increased by only 10%. Treatment of cells for 72h and 96h led to a reduction in TA by 20% and 32% respectively (Figure 2A). In MCF-7 cells an increase of 20% in TA was observed following exposure of the cells to Rapamycin for 24h. Only after



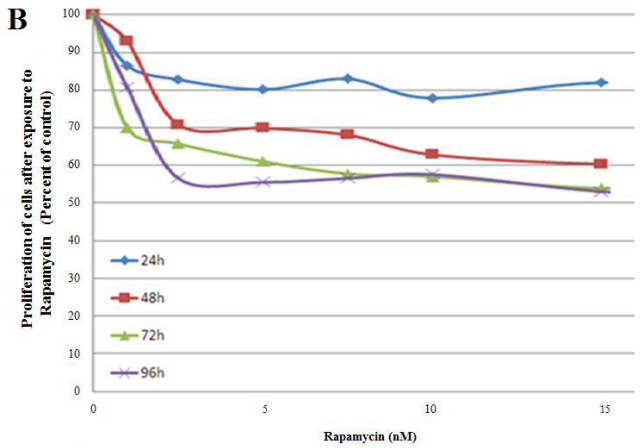
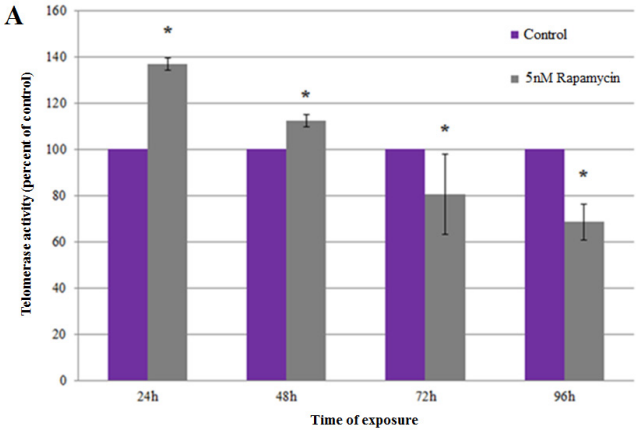


Figure 1. The proliferation of Jurkat and MCF7 cells after exposure to Rapamycin. Jurkat (A) and MCF7 (B) cells were grown in the presence of Rapamycin at 0 – 15 nM for 24h, 48h, 72h, and 96h, and their proliferation was evaluated by the WST-1 (Jurkat) or SRB (MCF7) methods. Each dose represents mean (%) ± S.E of three or more independent experiments conducted in quadruplicates. P-value for each data series – < 0.05.



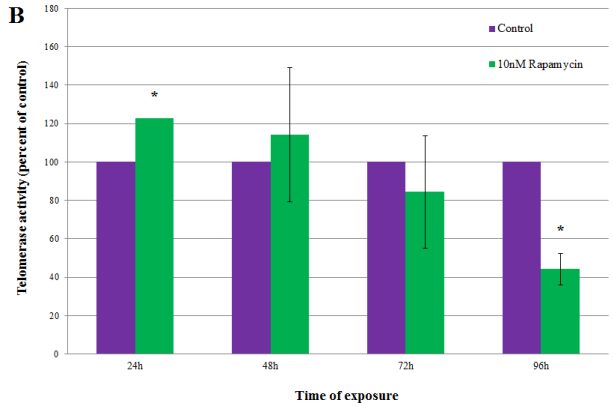


Figure 2. Telomerase activity following exposure to Rapamycin. Jurkat (A) and MCF7 (B) cells were grown in the presence of 5nM and 10nM Rapamycin, respectively, for 24h, 48h, 72h, and 96h. The kinetics of telomerase activity (TA) was assessed via a quantitative TRAP assay. Each dose represents mean (%) ± S.E of three or more independent experiments conducted in duplicates. *Indicates P-value < 0.05

as 96h of treatment a marked decrease (56%) in TA was obtained (Figure 2B). In order to further explore regulatory aspects of TA following Rapamycin treatment we analyzed the cells at early stages occurring prior to TA inhibition in both cell types.

The effect of Rapamycin on telomerase regulation

To decipher the mechanisms underlie the effect of Rapamycin on telomerase regulation, we assessed the putative effects of the drugs in both cell types. We measured the two levels of telomerase regulation that are considered the major regulatory modalities on telomerase activity: the transcriptional level and the post translational.

hTERT gene expression following Rapamycin treatment of Jurkat and MCF-7 cells

The expression of hTERT was evaluated by Q-RT-PCR. Rapamycin treatment for 24h reduced hTERT mRNA levels in Jurkat cells by 24% (Figure 3A). However, the levels of hTERT mRNA expression in MCF-7 did not decrease but possessed a small, not statistically significant, increase of 18% (Figure 3B).

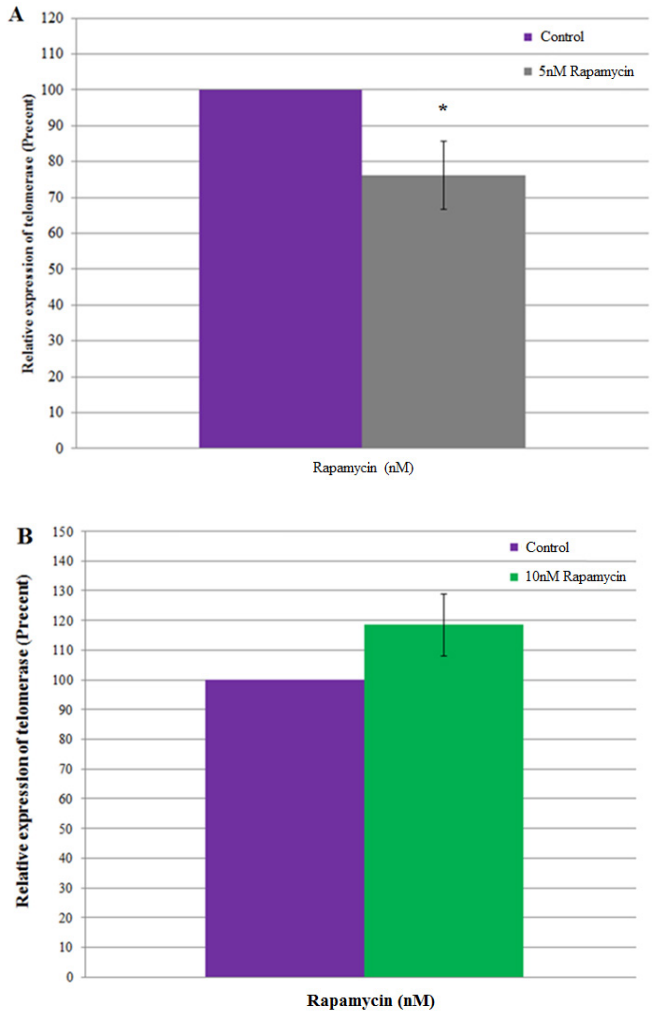


Figure 3. hTERT mRNA gene expression following 24h exposure to Rapamycin. Jurkat (A) and MCF7 (B) cells were grown with and without Rapamycin. Telomerase activity was assessed by the TRAP (Telomere Repeat Amplification Protocol) assay according to the manufacturer's instructions. Each column represents mean of TA (%) ± S.E of three or more independent experiments conducted in duplicates. *Implies statistical significance.

These differences in hTERT mRNA expression may suggest that the decrease in TA observed in the Jurkat cells after Rapamycin exposure was transcriptionally mediated. In the MCF-7 cells the regulation seemed to occur at a different level. Following this observation and to further understand why the transcription of the hTERT gene was reduced in response to the drug we explored the effect of Rapamycin on the binding of transcription factors to the hTERT promoter by the ChIP assay and compare them to the total cellular levels of these factors.

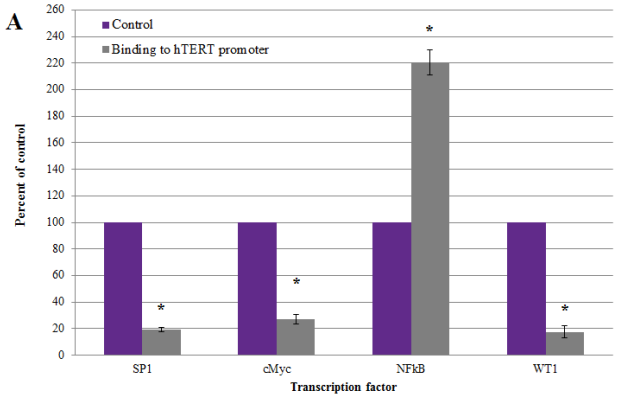
The levels of transcription factors binding to the hTERT promoter and their cellular levels following Rapamycin treatment

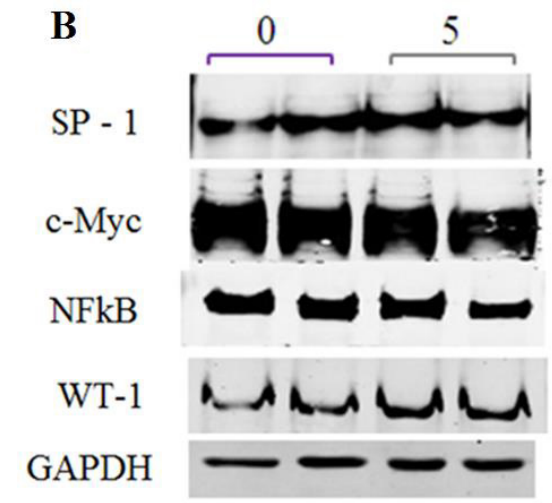
To understand which mechanism induced the decrease in hTERT expression in the Jurkat cells, we followed the levels of binding of the four major transcription factors WT1, NF κB, c-Myc and SP1 to the hTERT core promoter by the Chromatin Immunoprecipitation (ChIP) assay. Rapamycin reduced the binding of SP1, c-Myc, and WT1 (a negative regulator of TA) and increased the binding of NFκB to the promoter of hTERT in Jurkat cells (Figure 4A).

To understand whether the decreased binding of the various transcription factors to the hTERT promoter after Rapamycin treatment stemmed from a general reduction in their cellular levels, we measured their protein levels by Western blotting using specific antibodies. The results showed that Rapamycin induced a change in the expression of these factors of +82%, -28%, -28%, and +11%, for WT1, NFκB, c-Myc, and SP1 respectively (Figure 4C). The decrease in expression of c-Myc and SP1 was much smaller than that of the actual binding of the transcription factors to the hTERT promoter. We therefore attribute the effect of Rapamycin on telomerase activity to the decreased binding of these transcription factors to the hTERT promoter. The increase in NFκB binding to the hTERT promoter in light of a small decrease in its cellular levels emphasizes the importance of this specific binding and defines NFκB as a regulator of TA in response to Rapamycin.

The effect of Rapamycin on the phosphorylation of telomerase and its upstream kinases

Telomerase is phosphorylated and thus becomes activated by two kinases - AKT and PKCα. In order to explore whether the effects of Rapamycin on TA were mediated by affecting the post-translational regulation of the enzyme we assessed the levels of the phosphorylated form of telomerase and its kinases in response to Rapamicin treatment by immunoprecipitation





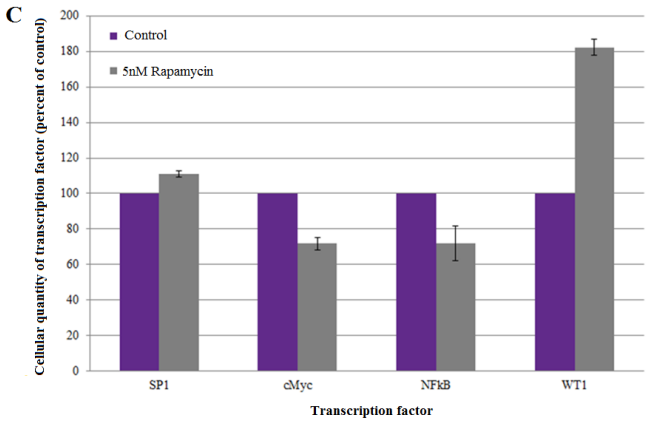
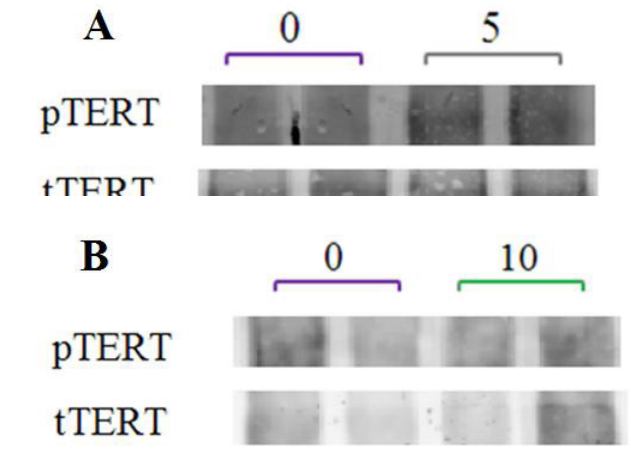


Figure 4. hTERT transcription factors in Jurkat cells after Rapamycin treatment. (A). Relative binding of the transcription factors SP1, c-Myc, WT1, and NFkB to the hTERT promoter; (B). Example of one representative Western blot, out of three, of transcription factor levels in Jurkat cells exposed to 5ng/ml Rapamycin for 24h; (C). Quantification of the cellular levels of SP1, c-Myc, WT1, and NFkB. Transcription factor binding was measured by ChIP assay; quantity of transcription factor in the cells was measured by Western blot. Each column represents mean of TA (%) ± S.E of three or more independent experiments conducted in duplicates. *Implies statistical significance. P-value < 0.05



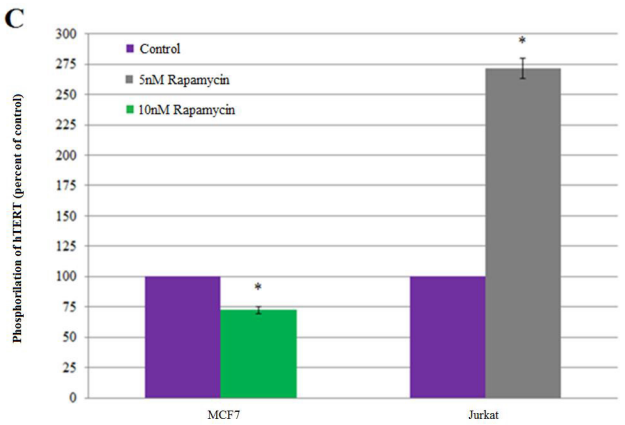
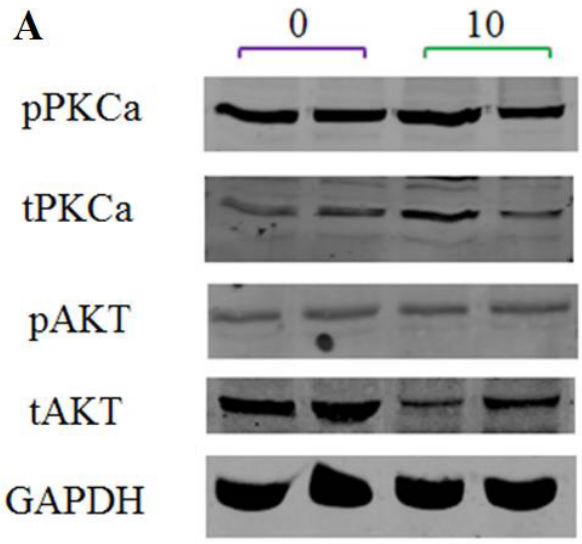


Figure 5. The levels of phosphorylated proteins following exposure to Rapamycin. (A). Examples of one representative Western blot experiment, of hTERT protein levels in Jurkat cells after exposure to 5nM Rapamycin; (B). Examples of one representative experiment of hTERT protein levels in MCF7 cells after exposure to 10ng/ml Rapamycin; (C). Quantitation of the phosphorylated levels of phospho-hTERT in response to Rapamycin for 24h. p - phosphorylated; t - total. Each column represents mean (%) ± S.E of three or more independent experiments conducted in duplicates. *Implies statistical significance of P-value < 0.05.

and Western immunoblot analysis. An increase of 171% in the phosphorylation of hTERT in Jurkat cells (Figure 5A, C) and a reduction of approximately 28% of the enzyme's phosphorylation in MCF-7 cells (Figure 5B, C) were observed following 24h exposure of the cells to the IC50 values of Rapamycin in each cell type. Exposure of cells to Rapamycin for 24h induced 18% reduction in the level of the phosphorylated form of AKT in MCF-7 cells, while the phosphorylation levels of PKCα increased by 40% (Figure 6).



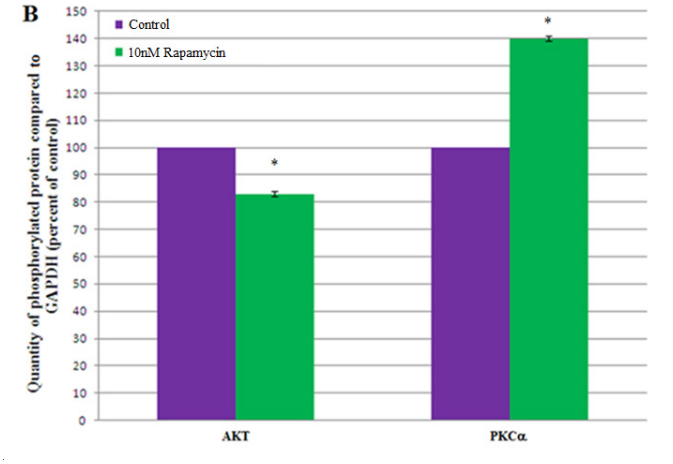


Figure 6. The levels of phosphorylated proteins following exposure to Rapamycin. (A). Examples of one representative Western blot experiment out of three of protein levels in MCF7 cells after exposure to 10nM Rapamycin; (B) Levels of Phosho-AKT and phosphor-PKC proteins levels after exposure of MCF7 cells to 10nM Rapamycin for 24h. Each column represents mean (%) ± S.E of three or more independent experiments conducted in duplicates. p - phosphorylated; t - total. P-value < 0.05

Telomere length after exposure to Rapamycin

Long-term effects of Rapamycin on telomere length were followed in both cell lines. Jurkat and MCF-7 cell lines were exposed to a non-lethal (1nM) dose of Rapamycin over several months and DNA was extracted after 1, 2, and 4 months of growth of MCF-7 cells, and 2, 4, and 6 months of growth of Jurkat cells. This non-lethal dosage decreased TA by 15% - 20% in both cell lines after 96h of Rapamycin exposure (not shown). Accordingly, telomeres were mildly shortened following the drug exposure, both in Jurkat and MCF-7 cells (Figure 7). Telomere length in Jurkat cells decreased by 25% over the course of six months (from 5.1kb to 3.8kb) and in MCF-7 cells the length of telomeres decreased by 28% during that time (from 5.4kb to 3.9kb).

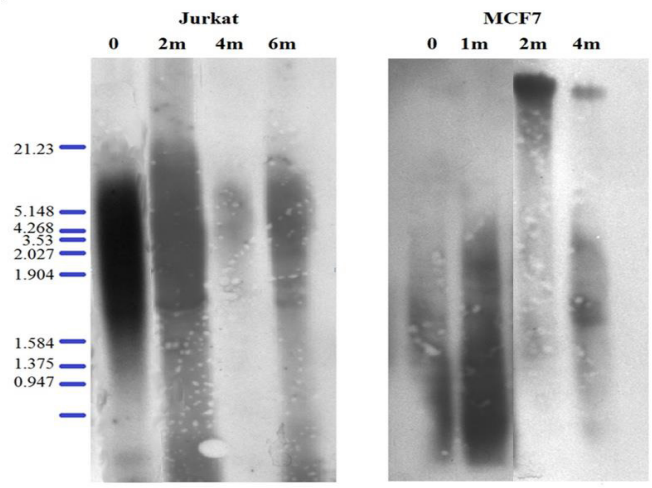


Figure 7. Telomere length after long- term exposure to a non-lethal dose of Rapamycin. Telomere lengths of Jurkat (left) and MCF7 (right) after exposure to 1 ng/ml Rapamycin over a period of 1, 2, 4, and 6 months.

Discussion

One of the major aspirations in cancer research is the development of agents capable of selectively targeting aberrantly regulated pathways which are essential to the malignant cell. Telomerase is such a target since it fulfils these two conditions: specificity and essentiality for cancer cells34. Therefore and in the light of its remarkable importance in the biology of malignancy, the effects of various drugs on its activity may be especially important.

Rapamycin targets the mTOR pathway which regulates the translation of ribosomal proteins. Rapamicin was approved as an immunosuppressant by both the FDA and the European Commission by the year 2000 and its anticancer activity was shown against several kinds of solid cancers3. However, not all of Rapamicin targets were identified so far [15].

The overlap between the mTOR pathway and telomerase regulation lies among other places with AKT which phosphorylates telomerase on one hand, and is a downstream target of mTOR on the other hand. Therefore, we focused in this research on the possible crosstalk between telomerase activity and the mTOR pathway, as both play a significant role in various cancer types and due to the added value of identifying telomerase as another possible target for Rapamycin.

Our results show that Jurkat and MCF-7 cells exhibited deviating sensitivities to the anti-proliferative effect triggered by Rapamycin. The effect on both cell types appeared to be cytostatic, while Jurkat cells possessed a higher sensitivity to the drug than MCF-7. Rapamycin differently affected TA in the two cell types. While Jurkat cells showed a 20%-32% decrease in TA after 72h- 96h drug's exposure, TA in MCF-7 cells was substantially decreased by 56% after 96h of exposure. The decrease in TA in these cell lines supports previous studies showing that the effect of Rapamycin is not limited to cancerous cells apoptosis but may affect other targets [2, 16, 17]. The drug interferes in various ways to inhibit TA in our setting: one pathway being the transcriptional level (Jurkat cells) by the decrease in hTERT mRNA expression and the other is the post-translational modifications level of its catalytic subunit by its kinases (MCF-7 cells).

Previous studies conducted by others and our laboratory have shown that TERT promoter activity is usually regulated by a variety of transcription factors. The most important which target its core promoter are WT1, NFκB, c-Myc, and SP1 [13, 18- 19]. In Jurkat cells, the down-regulation of TA is mediated by a reduced mRNA expression of hTERT. This reduction was controlled by a decrease in the binding of the c-Myc and SP1 transcription factors, and an increase in NFkB binding. The decrease in the expression of the hTERT gene after Rapamycin treatment stems from the summation of the binding of these transcription factors: whereas the binding of two out of three positive regulators: c-Myc and SP1 markedly decreased in response to the drug, the binding of the third factor NF□B increased, accompanied by a decrease in the negative regulator WT1. These contradictory changes resulted in a relative small decrease in the hTERT expression. Western blot analysis revealed a decrease in c-Myc and NFkB levels and an increase in WT1 level following Rapamycin exposure. However – ChIP analysis proved that while NFkB decreased in its cellular quantity, it bound more affectively to the hTERT promoter in response to the drug. This contradict-

ing effect on quantity vs. binding pattern was also seen in WT1 which showed a far lower binding ability. Lower binding ability was also seen in SP1 and c-Myc. In their study, Shapira et al. and Sitaram et al. described the same phenomenon, regarding the changes in SP1 and WT1 binding to the gene's promoter respectively [20, 21]. The common roles of c-Myc SP1 and WT1 have also been well documented over the years. c-Myc and WT1 are known to activate proto-oncogenes [22], while SP1 and c-Myc are positive activators of hTERT transcription [19, 23].

In contrast to Jurkat cells, the regulation on TA in response to Rapamycin in MCF-7 cells was mainly post translational. Rather, the decrease in TA after Rapamycin exposure was mediated post-translationally.

Rapamycin induced a slight decrease in phospho-AKT levels and a marked increase in phospho-PKC in MCF-7 cells, accompanied by a decrease in the phosphorylated form of hTERT. We therefore concluded that the decrease in telomerase phosphorylation may be mediated mainly by a decrease in the level of pPKCα. These findings support those of Daniel, Peek, and Tollefsbol as well as Sasaki et al. [24, 25], which suggested that AKT is an up-regulator of hTERT transcription and numerous articles published by Yamada et al. regarding the ability PKC to activate telomerase [26-28]. However, the fact that in our setting the phosphorylation levels of PKCα increased, may explain why the phosphorylation of hTERT, as a sum effect of these two kinases, decreased by ~25% and not more in response to Rapamycin exposure. The notion that, out of the two tested kinases, PKC is responsible for the change in telomerase activity is strengthened when taking into account the findings of Sarbassov et al., who showed that after 24h of exposure the levels of AKT are reduced below the point needed to maintain an active signaling pathway [29]. Bae-Jump et al. in type I and type II endometrial carcinomas showed that in these cancers Rapamycin sensitivity was independent of AKT status. Though their work also showed a decrease in hTERT levels that was not seen in this cell line [30].

In the Jurkat cell line the levels of phosphorylated hTERT were far higher after exposure to Rapamycin. This may provide an explanation as to why we found an increase in TA, but a decrease in expression of hTERT mRNA after exposure to Rapamycin. It seems that the increase in phosphorylation was able to overcome the increase of the greater binding of the inhibitory factor NFκB, and the dwindling in the binding of the hTERT activating factors and therefore the overall decrease in telomerase activity did not exceeded 30% in response to Rapamycin. More experiments are probably needed in order to explore the effects of the complex dynamics of the various transcription factors binding, cellular levels and the phosphorylation of TERT and its kinases in this setting.

In both cell types telomere length, after exposure to a non-lethal dose of Rapamycin, slightly decreased by 25%. This relatively small change may derive from the cells attempt to protect themselves from the onslaught imposed by Rapamycin treatment. This protection mechanism is more pronounced in the MCF-7 line than in Jurkat cells. In addition, the minimal dosage of Rapamycin inhibits telomerase activity only by 20%, which may not be sufficient to shorten telomeres even after longer exposure to the drug. In contrast to our findings, in a recent study published at 2011, Ungar et al. showed that upon exposure to Rapamycin yeast telomeres shorten noticeably [3].

In the light of the crucial role of telomerase in cancer cells, it was important to characterize the possible relation between telomerase activity and Rapamycin and distinguish the biochemical mechanism of its regulation. However, the changes in cellular phenotype after exposure to this macrolide antibiotic result from the effects of many signal transduction processes that are either inhibited or catalyzed by the treatment. In this respect, the effect of the drug on the kinetics of cell's proliferation differed from its effect on telomerase activity kinetics. This difference may imply that the two signals transduction pathways leading to telomerase activation and cell proliferation are mutually exclusive and that proliferation of these cells is probably not dependent on telomerase activity. A similar phenomenon has been shown by us [31, 32] as well as others [33, 34]. The relatively small change following prolonged exposure of the cells to a minimal dosage of Rapamycin (which minimally decrease telomerase activity) may point to other consequences of the inhibition of telomerase in response to the drug. Such consequences may be attributed to telomerase's extracurricular activities which affect numerous cellular pathways in non-telomeres dependent pathways. These include, among others, an RNA-dependent RNA polymerase (RdRP) activity in the mitochondria, involvement in apoptosis [35] and in the WNT signaling pathway [35-37. Therefore, the inhibition of telomerase activity may cause damage to other cellular features including those that will perturb its extracurricular activities. It is therefore suggested to study these effects in a future study.

We hope that the knowledge gained from this work will lead to the examination of new combinations of Rapamycin and other anti-cancerous agents, thus enhancing Rapamycin's effect, leading to further advances in the never-ending quest to find effective anti-cancer treatments.

References

1. Peponi E, Drakos E, Reyes G, et al. Activation of mammalian target of rapamycin signaling promotes cell cycle progression and protects cells from apoptosis in mantle cell lymphoma. Am J Pathol. 2006; 169, 2171-80.
2. Sundin T, Hentosh P. InTERTesting association between telomerase, mTOR and phytochemicals. Expert Rev Mol Med.2012; 14:e8.
3. Ungar L, Harari Y, Toren A, et al. Tor complex 1 controls telomere length by affecting the level of Ku. Curr Biol. 2011; 21: 2115-20.
4. Kim DH, Sarbassov DD, Ali SM, et al. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Cell. 2002; 110: 163-75.
5. Sundin T, Hentosh P. InTERTesting association between telomerase, mTOR and phytochemicals. Expert Rev Mol Med. 2012; 14: e8.
6. Olovnikov AM. Principle of marginotomy in template synthesis of polynucleotides. Dokl Akad Nauk SSSR. 1971; 201: 1496-1499.
7. Renaud S, de Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet. 1992; 339:1523-1526.
8. Verdun RE, Karlseder J. Replication and protection of telomeres. Nature. 2007; 447: 924-31.
9. Wright WE, Tesmer VM, Huffman KE, et al. Normal human chromosomes have long G-rich telomeric overhangs at one end. Genes Dev. 1997; 11: 2801-2809.
10. Griffith JD, Comeau L, Rosenfield S, et al. Mammalian telomeres end in a large duplex loop. Cell. 1999; 97: 503-14.

11. Dionne I, Wellinger RJ. Cell cycle-regulated generation of single-stranded G-rich DNA in the absence of telomerase. Proc Natl Acad Sci U SA. 1996; 93: 13902-7.
12. De Lange T, Jacks T. For better or worse? Telomerase inhibition and cancer. Cell. 1999; 98: 273-5.
13. Shalem-Cohavi N, Beery E, Nordenberg J, et al. The effects of Proteasome Inhibitors on Telomerase Activity and Regulation in Multiple Myeloma Cells. Int J Mol Sci. 2019; 20: 2509-21.
14. Weiss C, Uziel O, Wolach O, et al. Differential downregulation of telomerase activity by bortezomib in multiple myeloma cells-multiple regulatory pathways in vitro and ex vivo. Br J Cancer. 2012; 107: 1844-1852.
15. Koehl GE, Schlitt HJ, Geissler EK. Rapamycin and tumor growth: mechanisms behind its anticancer activity. Transplantation Rev. 2005; 19: 20-31.
16. Bjornsti MA, Houghton P J. The TOR pathway: a target for cancer therapy. Nat Rev Cancer. 2004; 4:335-48.
17. Fingar DC, Blenis J. Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. Oncogene. 2004; 23: 3151-71.
18. 18. Suske G. The Sp-family of transcription factors. Gene. 1999; 238: 291-300.
19. Kyo S, Takakura M, Taira T, et al. Sp1 cooperates with c-Myc to activate transcription of the human telomerase reverse transcriptase gene (hTERT). Nucleic Acids Res. 2000; 28: 669-77 .
20. Shapira S, Granot G, Mor-Tzuntz R, et al. Second-generation tyrosine kinase inhibitors reduce telomerase activity in K562 cells. Cancer Lett. 2012; 323: 223–31.
21. Sitaram RT, Degerman S, Ljungberg B, et al. Wilms' tumour 1 can suppress hTERT gene expression and telomerase activity in clear cell renal cell carcinoma via multiple pathways. Br J Cancer. 2010; 103: 1255-62.
22. Han Y, San-Marina S, Liu J, et al. Transcriptional activation of c-myc proto-oncogene by WT1 protein. Oncogene. 2004; 23: 6933-41.
23. Lue N, Autexier C. Telomerases. 318 (John Wiley & Sons, Inc., 2012).
24. Sasaki T, Kuniyasu H, Luo Y, et al. AKT activation and telomerase reverse transcriptase expression are concurrently associated with prognosis of gastric cancer. Pathobiology. 2014; 81: 36-41.
25. Daniel M, Peek GW, Tollefsbol TO. Regulation of the human catalytic subunit of telomerase (hTERT). Gene. 2012; 498: 135-46.
26. 26. Yamada O, Kawauchi K. The role of the JAK-STAT pathway and related signal cascades in telomerase activation during the development of hematologic malignancies. Jak-Stat. 2013; 2:e25256.
27. Yamada O, Ozaki K, Nakatake M, et al. Akt and PKC are involved not only in upregulation of telomerase activity but also in cell differentiation-related function via mTORC2 in leukemia cells. Histochem. Cell Biol. 2010; 134: 555-63.
28. Kawauchi K, Ihjima K, Yamada O. IL-2 increases human telomerase reverse transcriptase activity transcriptionally and posttranslationally through phosphatidylinositol 3'-kinase/Akt, heat shock protein 90, and mammalian target of rapamycin in transformed NK cells. J Immunol. 2005; 174: 5261-9.
29. Sarbassov DD, Ali SM, Sengupta S, et al. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell. 2006; 22:159-68.
30. Bae-Jump VL, Zhou C, Boggess JF, et al. Rapamycin inhibits cell proliferation in type I and type II endometrial carcinomas: a search for biomarkers of sensitivity to treatment. Gynecol Oncol. 2010;119: 579-85.
31. Weiss C, Uziel O, Wolach O, et al. Differential downregulation of telomerase activity by bortezomib in multiple myeloma cells-multiple regulatory pathways in vitro and ex vivo. Br J Cancer. 2012; 107: 1844-52.
32. Uziel O, Fenig E, Nordenberg J, et al. Imatinib mesylate (Gleevec) downregulates telomerase activity and inhibits proliferation in telomerase-expressing cell lines. Br J Cancer. 2005; 92: 1881-91.
33. Rizki A, Lundblad V. Defects in mismatch repair promote telomerase-independent proliferation. Nature. 2001; 411:713-6.
34. Bailey SM, Brenneman M, Goodwin EH. Frequent recombination in telomeric DNA may extend the proliferative life of telomerase-negative cells. Nucleic Acids Res. 2004; 32: 3743-51.
35.
36. 35. Jaiswal RK, Kumar P, Yadava PK. Telomerase and its extracurricular activities. Cell. Mol. Biol. Lett. 18, 538–54 (2013).
37. 36. Parkinson EK, Fitchett C, Cereser B. Dissecting the non-canonical functions of telomerase. Cytogenet. Genome Res. ;122:273-80.
38. 37. Chang S, DePinho R. Telomerase extracurricular activities. Proc Natl Acad Sci U S A. 2002; 99:12520-2.

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