

## **hTERT GENE EXPRESSION LEVELS AND TELOMERASE ACTIVITY IN DRUG RESISTANT MCF-7 CELLS**

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**Aim:** Cancer cells and some highly proliferative normal cells can stabilize telomere lengths by telomerase, which adds hexameric repeats to the ends of linear chromosomes. In this study, the activity of telomerase reverse transcriptase (*hTERT*) and its gene expression levels were investigated in paclitaxel, docetaxel, vincristine and doxorubicin resistant human MCF-7 breast adenocarcinoma cells. **Materials and Methods:** Resistant cell lines were developed by stepwise selection of cells (MCF-7/S) in increasing doses of paclitaxel (MCF-7/Pac), docetaxel (MCF-7/Doc), vincristine (MCF-7/Vinc) and doxorubicin (MCF-7/Dox). Antiproliferative effects of anticancer drugs were evaluated by XTT assay and  $IC_{50}$  values for different drugs were determined from cell proliferation curves. Expression levels of *hTERT* gene in sensitive and resistant cells were analyzed by RT-PCR. TRAP-Silver Staining assay was used to evaluate telomerase activities in these cells. **Results:** When drug resistant and sensitive MCF-7 cells were compared no significant differences were observed in *hTERT* expression levels and telomerase enzyme activities. **Conclusion:** This report demonstrates that drug resistance developed against paclitaxel, docetaxel, vincristine and doxorubicin in MCF-7 cells is independent of the expression of *hTERT* gene and telomerase activity. **Key Words:** *hTERT*, telomerase activity, multidrug resistance, MCF-7.

Eukaryotic chromosome ends have highly specialized structures called telomeres, which shorten with each cell division and reach to a critical size resulting in senescence and eventually cell death [1]. In cancer cells, this shortening of telomeres is prevented by a special reverse transcriptase enzyme, telomerase. Human telomerase is a ribonucleoprotein complex consisting of a catalytic subunit (*hTERT*) and RNA component (*hTR*), and adds telomeric repeats (TTAGGG) to the 3' ends of chromosomes [2, 3]. Telomerase activity is upregulated in most carcinomas but not in adjacent normal tissues [4] and these cells overcome the limitation of small telomere length and entering senescence or apoptosis due to genomic instability [5]. In addition *hTERT* is upregulated in 95% of breast carcinomas [6]. Chemotherapeutic treatment of breast carcinomas results in acquirement of drug resistance by tumor cells. Resistance of tumor cells to various cytotoxic drugs is defined as multidrug resistance (MDR). Increased drug efflux that results from up-regulation of ATP binding cassette (ABC) transporters [7, 8], mutations in genes encoding drug target proteins [9], differential expressions of drug target proteins and anti/proapoptotic proteins [10] are some mechanisms of MDR. Although there have been many studies concerning the role of telomerase in cancer, its involvement in multidrug resistance remains unclear. Krunaga *et al.* [11] suggested that high telomerase activity and elongation of telomeres both appear to help maintain and/or increase drug resistance in colorectal cancer cells. Cancer cells with long telomeres and a high proliferative activity may thus be able to survive upon exposure to anti-cancer drugs. Cereno *et al.* [6] showed that telomerase inhibition enhances response to anticancer drug treatment in breast cancer however they have also concluded that

presence of shorter telomeres may impair the ability of cells to recover from drug treatment.

The aim of the present study is to investigate a possible correlation between MDR and telomerase in paclitaxel (MCF-7/Pac), docetaxel (MCF-7/Doc), vincristine (MCF-7/Vinc) and doxorubicin (MCF-7/Dox) resistant MCF-7 cells. For this purpose, the expression levels of the catalytic component of telomerase (*hTERT*) and telomerase activity in sensitive MCF-7 and drug resistant sublines were determined.

### **MATERIALS AND METHODS**

**Cell lines.** Drug resistant MCF-7 cell lines, which were models for drug resistant human mammary carcinoma, were developed in the laboratory. The features and growth conditions of the parental and resistant sublines were described previously by Kars *et al.* [12]. The sublines resistant to 400 nM paclitaxel (MCF-7/Pac), 120 nM docetaxel (MCF-7/Doc), 120 nM vincristine (MCF-7/Vinc) and 1000 nM doxorubicin (MCF-7/Dox) were used to test the antiproliferative effects of anticancer agents.

**Assay for cell proliferation.** Antiproliferative effects of anticancer drugs (paclitaxel, docetaxel, vincristine and doxorubicin) on sensitive and resistant cell lines were determined by means of a XTT based Cell Proliferation Kit (Biological Industries, Israel).  $IC_{50}$  values were found from percent cell proliferation versus log (anticancer drug concentration) curves and resistance indices of developed cell lines were evaluated as previously described [13].

**RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR).** RNA isolation was performed according to guanidium thiocyanide/phenol-chloroform single-step RNA isolation method [14]. cDNA synthesis reaction was performed with 5  $\mu$ g of total RNA and cDNA was used as template for PCR reactions. PCR conditions for *hTERT* and internal standard *beta2-microglobulin* ( $\beta_2$ -m) was previously described [2, 12]. PCR products were examined by native agarose (2% w/v) gel electrophoresis and visualized by ethidium

Received: December 3, 2007.

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**Abbreviation used:** *hTERT* – telomerase reverse transcriptase;  
RTA – relative telomerase activity.

bromide staining. Densitometric measurements of band intensities were performed using Scion Image Software (Scion Corporation, Maryland, USA).

**Assay for telomerase activity.** TRAPeZe® telomerase detection kit (Chemicon, MA, USA) was used according to manufacturer’s instructions with modifications to assess telomerase activity of sensitive and resistant MCF-7 cells. In brief, 1 x 10<sup>5</sup> cells were resuspended in 200 µL of ice-cold CHAPS lysis buffer and incubated for 30 min on ice. After centrifugation of lysate at 12,000 x g for 30 min at 4 °C, the resulting supernatant was stored at –80 °C. The protein concentration of each extract was determined with Bradford assay [15]. Two microliters of diluted extract (200 ng/µL) was added to 48 µL of reaction mixture containing 10X TRAP buffer (5 µL), 50X deoxynucleotide triphosphates mix (1 µL), TS primer (1 µL), TRAP primer mix (1 µL), 5 units/µL Taq polymerase (0.4 µL), and distilled H<sub>2</sub>O (39.6 µL). Each analysis included a positive control obtained from telomerase positive cell extract (provided with the kit) and a negative control containing CHAPS lysis buffer. The tubes were incubated in thermocycler at 30 °C for 30 min for the elongation of TS primer by telomerase and followed by 33 cycles of PCR amplification (94 °C for 30 s, 59 °C for 30 s and 72 °C for 60 s). The products were examined on non-denaturing polyacrylamide gel (10% w/v) and visualized by a silver staining. A sample was considered as positive for telomerase activity when characteristic ladder of TRAP products (TP) with 6 bp increments and a 36-bp internal control (IC) were observed and negative when only internal control was present. Densitometric analysis of the gels was carried out by Image J Software and relative telomerase activity (RTA) was calculated by the following formula:

$$RTA = \frac{(X_{TP}/X_{IC})}{(Tel_{TP}/Tel_{IC})} \quad \text{(Equation 1)}$$

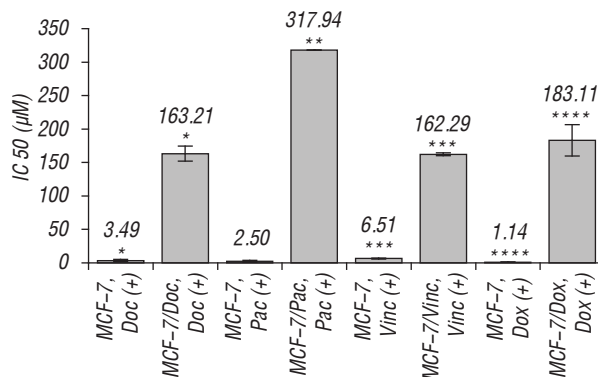
where densitometric band intensities were designated by X and Tel for the samples and telomerase positive control, respectively.

**Statistical analysis.** The results of RT-PCR and RTA were subjected to two-tailed *t*-test by using SPSS Software (SPSS Inc., Illinois, USA) to determine significant difference between means of groups ( $\alpha = 0.05$ ).

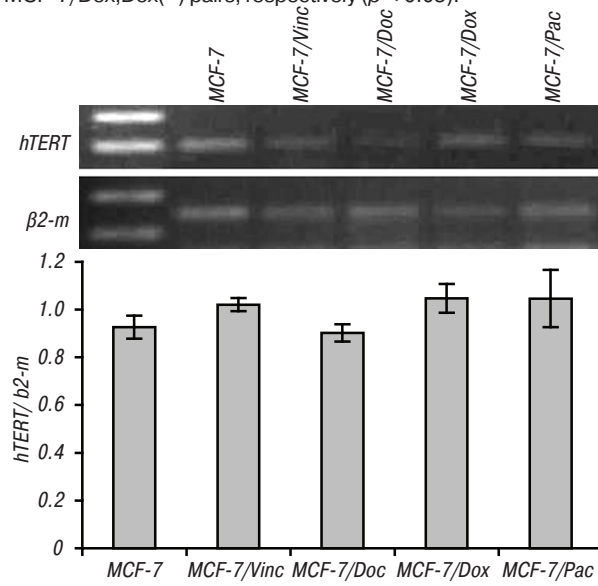
**RESULTS**

**Determination of drug resistance.** According to results of cell proliferation assays (Fig. 1), developed sublines were acquired resistance to their selective drugs. Calculated IC<sub>50</sub> values of sensitive and resistant cells were significantly different and the resistance indices showed that MCF-7/Pac, MCF-7/Doc, MCF-7/Vinc and MCF-7/Dox cells were 150-, 47-, 30- and 160-folds resistant to these drugs, respectively compared to the original sensitive MCF-7 cells.

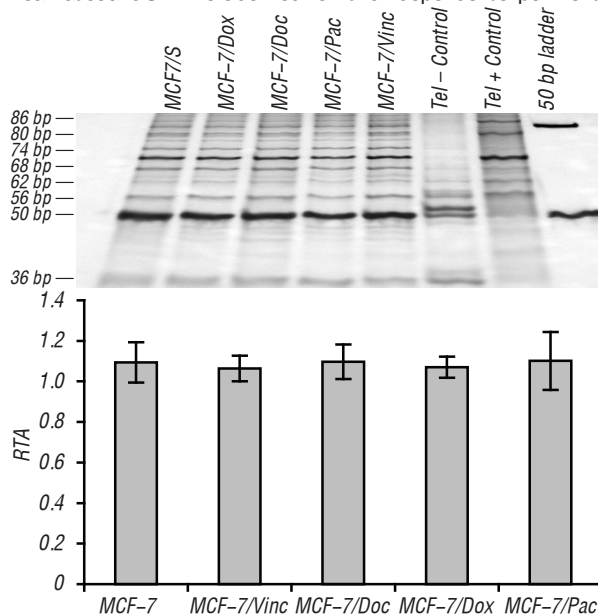
**RT-PCR.** RT-PCR results demonstrated that sensitive and all resistant cells had intrinsic expression of *hTERT* (Fig. 2, a). According to band intensity ratios (*hTERT*/ $\beta$ 2-*m*), difference in *hTERT* expression levels of sensitive and resistant cell lines was not found statistically significant (Fig. 2, b).



**Fig. 1.** Effects of anticancer drugs on sensitive and resistant MCF-7 cell lines. Mean IC<sub>50</sub> and standard error of the means (SEM) were derived from three independent experiments \* , \*\* , \*\*\* , \*\*\*\* marks on bars represent significant differences between MCF-7,Doc(+)-MCF-7/Doc,Doc(+); MCF-7,Pac(+)-MCF-7/Pac,Pac(+); MCF-7,Vinc(+)-MCF-7/Vinc,Vinc(+); MCF-7,Dox(+)-MCF-7/Dox,Dox(+) pairs, respectively ( $p < 0.05$ ).



**Fig. 2.** RT-PCR results: a — *hTERT* and 122 bp  $\beta$  microglobulin RT-PCR products on 2% agarose gel, b — Relative *hTERT* expression levels. Mean ratios and SEM were derived from two independent experiments



**Fig. 3.** TRAP assay: a — TRAP products on 10% polyacrylamide gel; b — illustration of RTA levels in sensitive and resistant cell lines. Mean RTA and SEM were derived from two independent experiments

**Telomerase activity.** As it was determined by TRAP — Silver Staining assay it was clear that all of the cell lines showed telomerase activity regardless of their resistance (Fig. 3, a). There was no statistically significant difference in telomerase activity levels of sensitive and drug resistant cell lines (Fig. 3, b).

## DISCUSSION

Human tumors develop resistance to chemotherapeutic drugs with an approximate outcome of 40% [16] and overcoming this phenomenon requires the understanding of the resistance mechanisms. Even though some molecular mechanism of multidrug resistance is well known, the idea that telomerase might be involved in this process is under investigation. Hoos *et al.* [17] previously showed that telomerase activity was decreased in all chemotherapy-treated tumors compared to untreated tumors in breast cancer patients. In concordance, telomerase activity was shown to decline in tumor cell cultures after treatment with antineoplastic agents [18]. In this study, the expression levels of the catalytic component of telomerase enzyme, *hTERT*, and telomerase activity were investigated in paclitaxel, docetaxel, vincristine and doxorubicin resistant MCF-7 sublines and their sensitive parental line. Proliferation assays confirmed development of resistance to these drugs in selected cells. These sublines also overexpressed resistance related *MDR1* gene (data not shown). According to RT-PCR results, there were no significant differences in mRNA levels of *hTERT* in sensitive and resistant MCF-7 cells. Similarly, study of telomerase activities did not demonstrate significant differences in relative levels of resistant MCF-7 sublines when compared to sensitive MCF-7 cells. In conclusion, the data provided here suggest that drug resistance developed against paclitaxel, docetaxel, vincristine and doxorubicin in MCF-7 cells does not seem to have direct association with expression levels of *hTERT* and telomerase activity levels.

## ACKNOWLEDGEMENTS

We gratefully acknowledge Dr. Aykut İnan İşeri for his contribution in statistical analysis. This study was supported by TUBITAK (SBAG 3297), Turkey.

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## ЭКСПРЕССИЯ ГЕНА *hTERT* И ТЕЛОМЕРАЗНАЯ АКТИВНОСТЬ В КЛЕТКАХ MCF-7, ОБЛАДАЮЩИХ ЛЕКАРСТВЕННОЙ РЕЗИСТЕНТНОСТЬЮ

В опухолевых клетках, а также некоторых нормальных клетках с высоким пролиферативным потенциалом длина теломеров может стабилизироваться за счет фермента теломеразы, добавляющего гексамерные повторы к концам линейных хромосом. *Цель:* проанализировать активность обратной транскриптазы теломеразного комплекса и экспрессию гена теломеразы в клетках MCF-7 аденокарциномы молочной железы человека, устойчивых к паклитакселу, доцетакселу, винкристину и доксорубину. *Материалы и методы:* сублинии клеток MCF-7, обладающих лекарственной резистентностью, были получены путем селекции исходных клеток при культивировании их в присутствии возрастающих доз паклитаксела (MCF-7/Pac), доцетаксела (MCF-7/Doc), винкристина (MCF-7/Vinc) и доксорубина (MCF-7/Dox). Антипролиферативный эффект противоопухолевых препаратов определяли в ХТТ-тесте. Величины  $IC_{50}$  для различных препаратов определяли по кривым пролиферации клеток. Уровень экспрессии гена *hTERT* в чувствительных и резистентных клетках анализировали методом ОТ-ПЦР. Теломеразную активность определяли с помощью набора TRAPeze. *Результаты:* проанализированные резистентные линии клеток MCF-7 не отличались от исходной линии ни по уровню экспрессии гена *hTERT*, ни по уровню теломеразной активности. *Выводы:* развитие лекарственной резистентности к паклитакселу, доцетакселу, винкристину и доксорубину в клетках MCF-7 не связано с изменениями экспрессии гена *hTERT* или уровня теломеразной активности в них. *Ключевые слова:* *hTERT*, теломеразная активность, лекарственная резистентность, MCF-7.