EXPERIMENTAL BIOLOGY

Comparative Analysis of Expression of Human Telomerase Catalytic Subunit at the Transcription Level in Cell Cultures of Different Origin

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The expression of human telomerase catalytic subunit in HL-60 and HT-1080 malignant transformed cells and telomerized fibroblasts was studied by quantitative PCR. It was found that the number of transcripts of human telomerase catalytic subunit per cell in telomerized fibroblasts could be hundreds of times higher than in HL-60 and HT-1080 cells. Telomerized fibroblast cultures are suggested as experimental systems for selection of basal compounds for creation of anticancer drug prototypes, the molecular target of which is human telomerase catalytic subunit. The effects of human telomerase catalytic subunit expression on the fibroblast proteome are analyzed.

Key Words: *telomerase; expression; telomerized fibroblasts; malignant cells*

Telomerase reactivation in somatic cells is closely associated with their malignant transformation (telomerase activity is detected in about 90% malignant tumors [12]). This fact makes the enzyme a promising target for testing of active anticancer drugs [3,12]. The search for and initial selection of basal compounds for creation of drug prototypes is carried out on cell cultures. Standardization of experimental conditions of this selection implies the use of well-characterized cell cultures optimized for the tasks of research [6]. The most important characteristics of cultures to be used for selection of basal compounds capable of inhibiting telomerase activity are the level of expression of the catalytic subunit of human telomerase (human telomerase reverse transcriptase, hTERT) in cells and its capacity to lengthen the specific substrate *in vitro* (telomerase repeat amplification protocol, TRAP) [8]. The latest TRAP modifications suggest quantitative evaluation of telomerase activity [13], but evaluation of the level of hTERT transcripts in "immortal" cells of different origins by quantitative PCR (with appropriate standards and absolute count) seems to be a more reliable quantitative characteristic of these cells by the present time. The systematic comparative analysis of ectopic expression of hTERT in cultured immortal cells of different origin has never been carried out.

We previously derived human fibroblast cultures telomerized by insertion of hTERT gene by plasmid construct or lentivirus vector [1]. Here we carried out a comparative quantitative analysis of hTERT transcripts expression in telomerized fibroblasts and cultured malignant transformed cells.

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MATERIALS AND METHODS

Derivation of human telomerized fibroblast culture was described previously [1]. By the present study, telomerized fibroblasts survived more than 300 passages. The cells were cultured under standard conditions: DMEM/F-12 (control and telomerized fibroblasts, HT-1080) or RPMI-1640 (HL-60) with 10% FCS (HyClone) and 40 U/ml gentamicin in a CO_2 incubator at 5% CO_2. For quantitative PCR (qPCR), adherent cells were collected after attaining 80% confluence, HL-60 cells in the logarithmic growth phase. Total RNA was isolated using commercial kit (Agilent) according to manufacturer's instruction. The quality of RNA was evaluated on an Agilent 2100 bioanalyzer (Agilent) according to manufacturer's instruction. RNA specimens with RIN≥9.2 were used. Quantitative PCR was carried out with Brilliant II SYBR Green QPCR Master Mix kit (Stratagene) on a Stratagene MX 3005P amplifier according to manufacturer's instruction. The data were mathematically processed and presented using MxPro™ software (QPCR Software) supplied with Stratagene MX 3005P. The optimal ("cutting off") level of the fluorescent signal shown in Figure 1 by a horizontal line and used for comparison of gene expression in different cells was selected automatically by the MxPro™ software. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene commonly used in qPCR served as the standardizing gene [9]. The expression of this protein, as we showed previously [1], was not changed in telomerized fibroblasts and in other fibroblast model systems [14,15]. Primer sequence for GAPDH: forward: gccttccgtgtccccactgc; reverse: cctccgacgcctgcttcacc. Primer sequence for hTERT: forward: accggaagagtgtctggagc; reverse: cagactccgcttcatcccc. The primers were synthesized as described previously [2].

RESULTS

The typical real-time amplification curves for the analyzed cell cultures are presented in Figure 1. The number of amplification cycles needed for attaining the same fluorescence level corresponding to equal quantity of the amplification product was significantly lower for telomerized fibroblasts and HL-60 and HT-1080 cells (curves *4*-*7*) than for fibroblasts (curve *3*). Curves *1* and *2* present the fluorescent signal of GAPDH transcripts cDNA amplification products for fibroblasts and other cells, respectively (they in fact coincide in the latter case). Amplification curves for fibroblasts were obtained with more diluted samples, which was shown by a shift of the GAPDH amplification curve towards higher values indicating the number of cycles (Fig. 1). However, the use of more

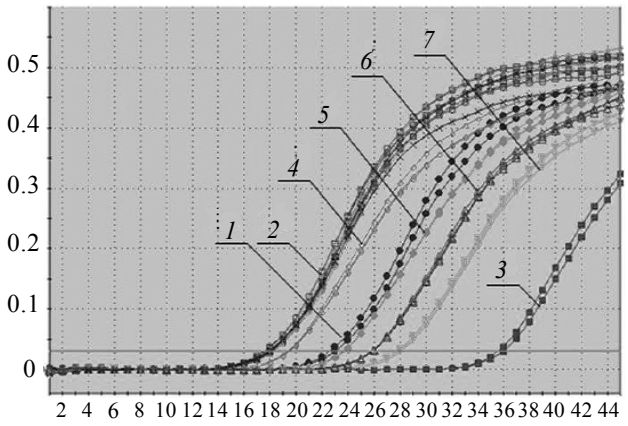

Fig. 1. Typical amplification profiles obtained by qPCR. Ordinate: fluorescent signal (in arbitrary units). Abscissa: number of cycles. Curve *1* and curve group *2*: GAPDH expression in fibroblasts and the rest cell types, respectively; curves *3*-*7*: hTERT expression in fibroblasts (*3*); fibroblasts telomerized by lentivirus and plasmid constructs (*4* and *5*, respectively); HL-60 (*6*); HT-1080 (*7*). Expression of hTERT and GAPDH transcripts in cells of each type is presented by two curves corresponding to repeats.

concentrated samples did not result in reduction of the number of cycles corresponding to the "cutting off" value of the fluorescent signal (horizontal line in Fig. 1) below 35. As this number of cycles was commonly regarded as the threshold value above which nonspecific PCR products could appear [9], we could not say for sure that hTERT transcripts were present in fibroblasts.

The expression in this methodological approach was expressed as the difference in the numbers of cycles N needed for attaining equal amounts of the PCR product (with consideration for standardization); the differences in the matrix quantities (in that case reflecting the differences in the quantity of hTERT transcripts) could be evaluated as 2^N. Figure 2 presents

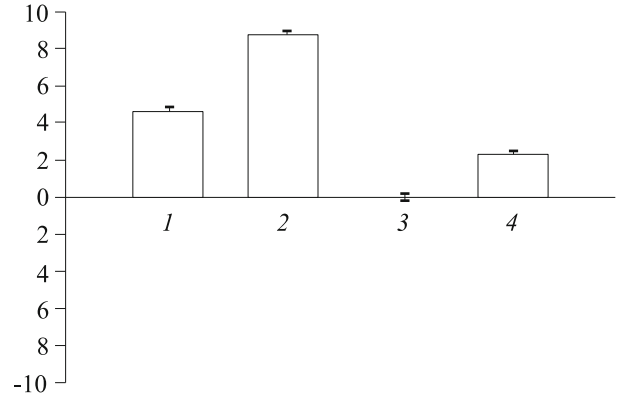

Fig. 2. Expression of hTERT at the transcript level in different cells. Ordinate: difference between the numbers of cycles needed for attaining the "cutting off" level of fluorescent signal with consideration for GAPDH normalization for HT-1080 cells (calibrator) and other cell types. *1*, *2*) fibroblasts telomerized by plasmid construct and lentivirus vector, respectively; *3*) HT-1080; *4*) HL-60.

the level of hTERT expression in analyzed cells as *N*. HT-1080 cells containing the least level of hTERT transcripts were selected as the calibrator (cells serving as the reference point for evaluating the expression of hTERT gene in other cells). The number of hTERT transcripts in HL-60 cells slightly (5-fold) surpassed their content in HT-1080. On the other hand, fibroblasts telomerized by the plasmid construct and lentivirus vector exhibited a significantly higher (20-25 times and 300-400 times, respectively) expression of hTERT in comparison with HT-1080. The number of transcripts per cell in the fibroblasts telomerized by lentivirus was 16-fold higher than in fibroblasts telomerized by plasmid construct. Presumably, cultured fibroblasts telomerized by lentivirus construct would become the most useful experimental cell model for search and selection of basal compounds for creation of anticancer drug prototypes with hTERT for the molecular target.

As was shown previously, fibroblast telomerization also led to increase of expression of many proteins [1] which, according to previously published data, could be regarded as tumor markers. These were EEF1D (eukaryotic translation elongation factor 1 delta isoform 2), nucleophosmin, PCNA (proliferating cells nuclear antigen), G3BP1 (ras-GTPase-activating protein SH3-domain-binding protein), RHOA (ras homolog gene family member A), STMN1 (stathmin 1), and others [1]. For example, enhanced expression of nucleophosmin in cancer cells has been described in 1989 [5] and at present this protein is regarded as cancer biomarker [7] and a potential oncogene modulating the genes involved in cell proliferation, cancer, and cell cycle [4]. High expression of STMN1 was detected in thyroid cancer and acute leukemia [10,11]. High expression of genes for the above proteins in telomerized fibroblasts, which, despite high level of hTERT expression (tens and hundreds times surpassing the level of its expression in cultured malignant cells), retain the normal mechanism of proliferation regulation [1] and hence, are not malignant transformed cells, makes doubtful their potential use as specific protein markers of tumors.

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