BIOGERONTOLOGY

Peptide Promotes Overcoming of the Division Limit in Human Somatic Cell

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 5, pp. 573-577, May, 2004
Original article submitted January 24, 2004

We previously showed that treatment of normal human diploid cells with Epithalon (Ala-Glu-Asp-Gly) induced expression of telomerase catalytic subunit, its enzymatic activity, and elongation of telomeres. Here we studied the effect of this peptide on proliferative potential of human fetal fibroblasts. Primary pulmonary fibroblasts derived from a 24-week fetus lost the proliferative potential at the 34th passage. The mean size of telomeres in these cells was appreciably lower than during early passages (passage 10). Addition of Epithalon to aging cells in culture induced elongation of telomeres to the size comparable to their length during early passages. Peptide-treated cells with elongated telomeres made 10 extra divisions (44 passages) in comparison with the control and continued dividing. Hence, Epithalon prolonged the vital cycle of normal human cells due to overcoming the Heyflick limit.

Key Words: *peptide; Epithalon; telomeres; fibroblasts; aging*

Epithalon peptide (Ala-Glu-Asp-Gly) was designed and synthesized on the basis of the analysis of amino acid composition of Epithalamin, a complex peptide preparation isolated from animal pineal gland [10]. Epithalon restores impaired neuroendocrine regulation in old monkeys [11], induces activation of ribosomal genes [1]. Addition of the tetrapeptide to cultured lymphocytes isolated from elderly and senile humans promotes decondensation of pericentromer heterochromatin and activation of genes repressed as a result of age-associated condensation of euchromatin regions [4]. Epithalon prolonged the mean life span in mice [5] and decreases the incidence of chromosome aberrations in senescence-accelerated SAM mice [2]. It is noteworthy that Epithalon prolongs the mean and maximum life span without increasing the incidence of malignant tumors in these animals [5-7]. It is now known that the life span of human diploid cells is limited [9]. The telomerase theory of aging attributes the age-associated decrease of the tissue proliferative potential to critical shortening of telomeres in the course of cell division [14]. In humans expression of protein component of telomerase and the relevant enzyme activity are observed in the majority of malignant, sex, early embryonic, and probably, stem cells. Human somatic cells are devoid of telomerase activity [12]. Treatment of normal human diploid cells with Epithalon induces the expression of telomerase catalytic subunit, its enzymatic activity, and elongation of telomeres [3]. Here we studied the effect of Epithalon on proliferative potential of human fetal fibroblasts.

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MATERIALS AND METHODS

Primary culture of human fetal lung fibroblasts (strain 602/17, 24 weeks) was obtained from Laboratory of Cell Cultures, Institute of Influenza, Russian Academy of Medical Sciences. The cells were cultured in Eagle's medium without antibiotics, containing 10% fetal calf serum and 2 mM L-glutamine; the cells were subcultured 1:2 once a week until passage 34, after which

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the fibroblast division ceased. In order to compare simultaneously the cells of different passages, they were periodically frozen and stored in liquid nitrogen. Epithalon in a concentration of 0.05 μg/ml was added to some flasks with fetal fibroblasts starting from passage 28 [4]. The mean length of telomeres in individual cells was measured by fluorescent *in situ* hybridization with flow cytofluorometry (flow-FISH) [13]. To this end, the cells were washed twice with buffered saline (BS) with 0.2% bovine serum albumin (BSA) and resuspended in solution for hybridization of the following composition: 70% formamide, 1.0% BSA, 20 mM Tris-HCl (pH 7.0, Sigma), and 0.3 μg/ml FITC-labeled C_3TA_2 telomere-specific peptide-nucleotide sample (PerSeptive Biosystems). The samples were subjected to thermal denaturing at 80°C for 10 min followed by 2-h hybridization at 20°C in the dark. Then cell suspension was centrifuged, the supernatants were discarded, and the cells were washed twice in a buffer containing 70% formamide, 10 mM Tris-HCl (pH 7.0), 0.1% BSA, 0.1% Tween-20 (Sigma), and once in BS containing 0.1% BSA and 0.1% Tween-20. After washout the cells were resuspended in BS containing 0.1% BSA, 10 μg/ml RNase A (Sigma), 0.06 μg/ml propidium iodide (Sigma), and incubated at 20°C in the dark for 2 h. Control hybridization (without peptide-nucleotide sample) was carried out in order to detect basal autofluorescence and calculate telomere-specific fluorescence in arbitrary units (arb. units). A total of 10^4 cells were analyzed by flow cytofluorometry on a Beckman-Coulter device. The data were statistically processed using CellQuest and WinList 4.0 software.

RESULTS

Primary lung fibroblasts (strain 602/17) exhibited loss of the proliferative potential and cessation of cell growth during passage 34. Flow-FISH method demonstrated decreased mean and maximum length of telomeres in

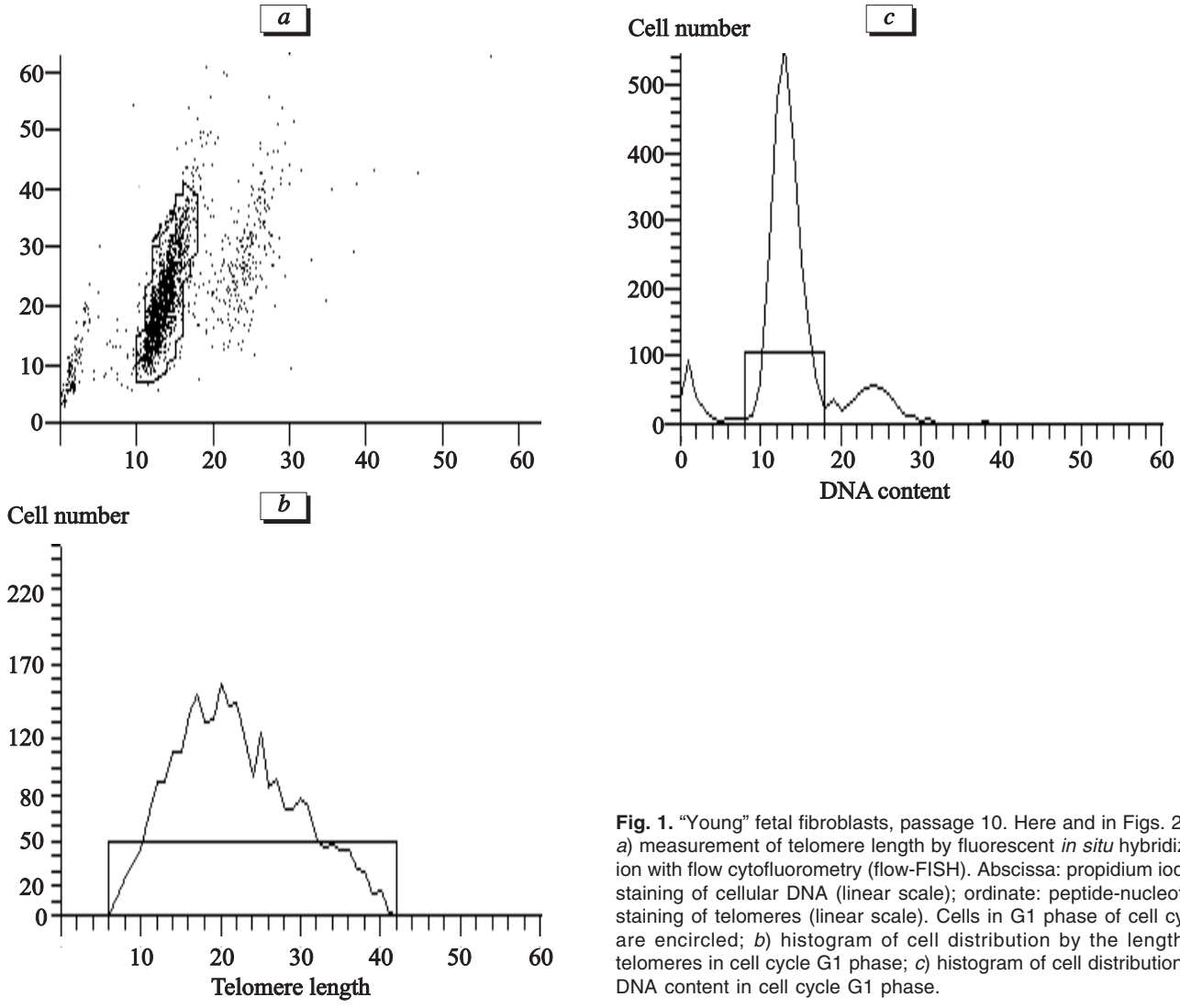

Fig. 1. "Young" fetal fibroblasts, passage 10. Here and in Figs. 2, 3: *a*) measurement of telomere length by fluorescent *in situ* hybridization with flow cytofluorometry (flow-FISH). Abscissa: propidium iodide staining of cellular DNA (linear scale); ordinate: peptide-nucleotide staining of telomeres (linear scale). Cells in G1 phase of cell cycle are encircled; *b*) histogram of cell distribution by the length of telomeres in cell cycle G1 phase; *c*) histogram of cell distribution by DNA content in cell cycle G1 phase.

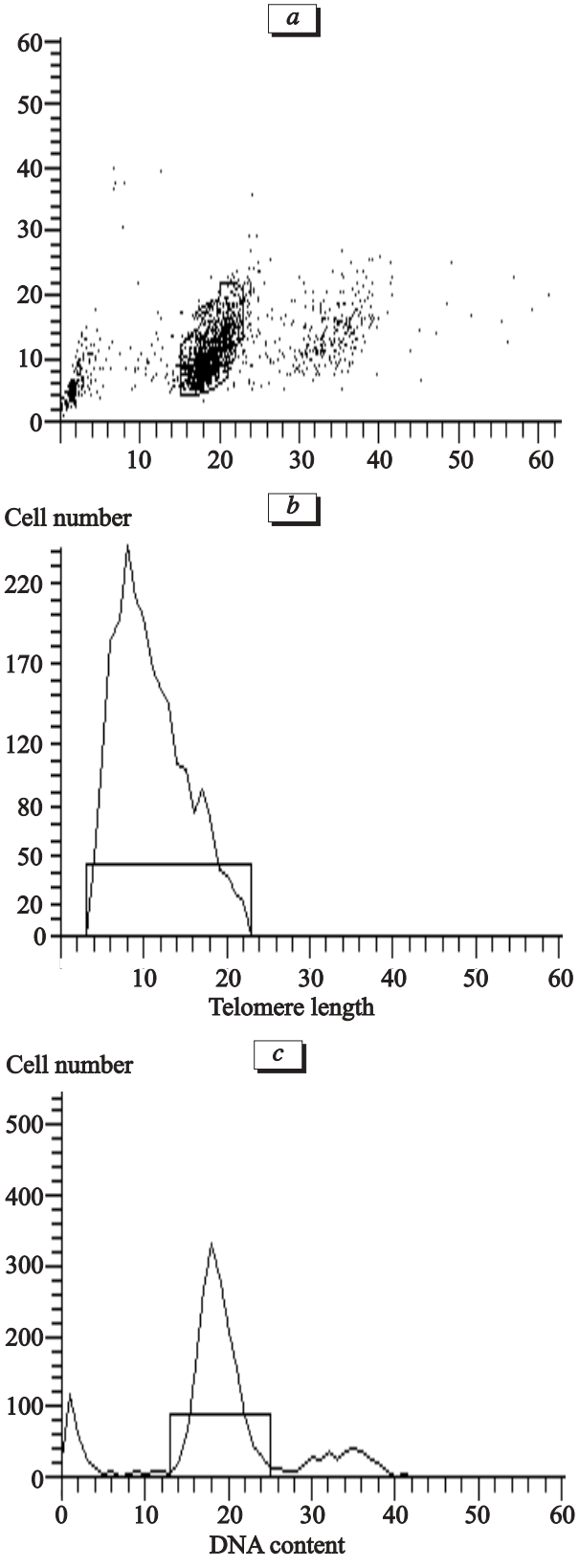

Fig. 2. "Old" fetal fibroblasts, passage 32.

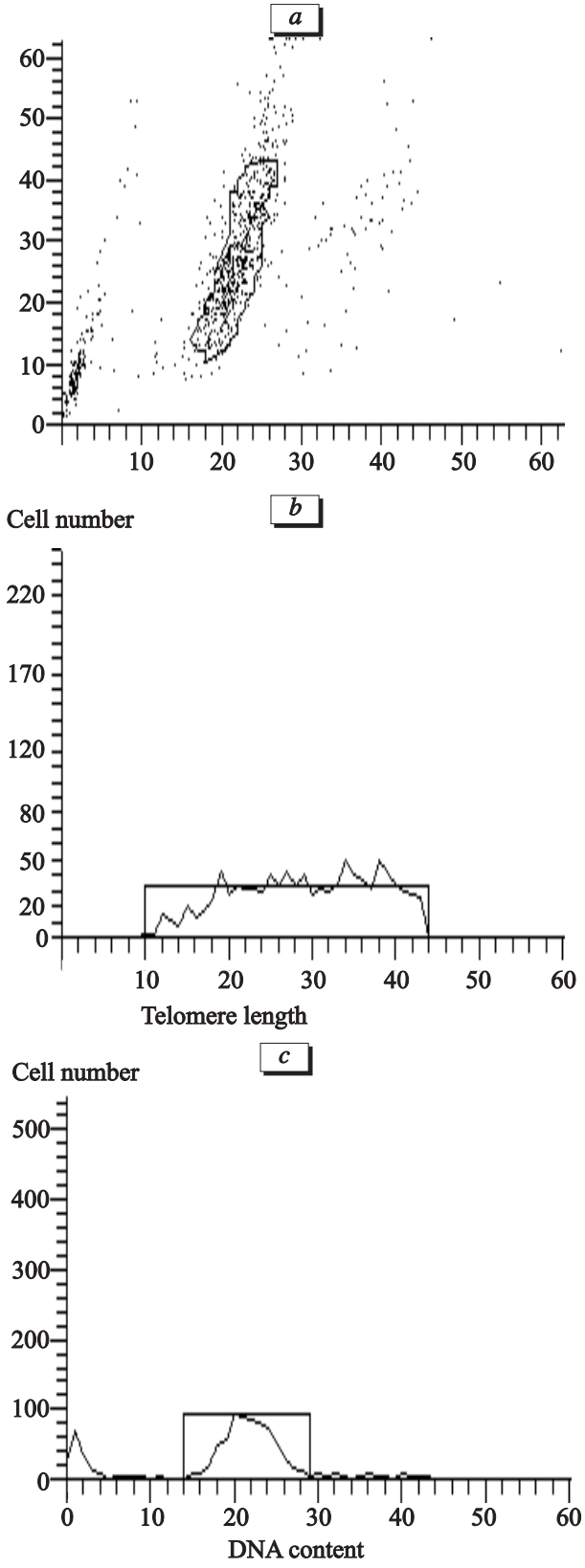

Fig. 3. Epithalon-treated fetal fibroblasts, passage 38.

the G1 cell cycle in "old" fetal fibroblasts (strain 602/17) after passage 32 (Fig. 2, *a*, *b*) in comparison with "young" cells after 10 passages (Fig. 1, *a*, *b*). The mean length of telomeres in the G1 cell cycle phase after 10 passages (control) was 20.9±6.6 arb. units (Fig. 1, *a*, *b*). Long-term culturing (32 passages) led to shortening of telomeres to 10.9±4.3 arb. units ($p<0.05$; Fig. 2, *a*, *b*). Staining of "young" cells with propidium iodide showed that the mean content of DNA in fibroblasts was 13.2±1.5 arb. units (Fig. 1, *a*, *c*) *vs.* 18.6±1.9 arb. units in "old" cells ($p<0.05$; Fig. 2, *a*, *c*). This fact seems to be due to overall chromosome aberrations caused, in turn, by shortening of telomeres to a critical length, when they were no longer able to prevent chromosome fusion [8]. According to flow-FISH analysis, Epithalon added to the culture medium increased in the mean length of telomeres in the G1 cell cycle phase of "old" fibroblasts (passage 38) to 26.2±7.9 arb. units (Fig. 3, *a*, *b*), which significantly (2.5 times, $p<0.05$) surpassed the mean length of telomeres in passage 32 cells (Fig. 2, *a*, *b*). In the presence of Epithalon in the culture medium fibroblasts underwent 44 divisions and dividion continued. It is noteworthy that the content of DNA in cells did not change after elongation of telomeres and remained the same as in the cells after many passages (Fig. 2, *a*, *c*; Fig. 3, *a*, *c*). This seems to be explained by irreversible chromosome aberrations, which took place by that time [7].

Hence, Epithalon peptide prolonged the vital cycle of human diploid cells by overcoming the Heyflick limit. These data explain the geroprotective effect of Epithalon in different experimental models [10].

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