# BIOGERONTOLOGY

# Effect of Bioregulatory Tripeptides on the Culture of Skin Cells from Young and Old Rats

**M. A.Voicekhovskaya[1], N. I. Chalisova[2], E. A. Kontsevaya [1], and G. A. Ryzhak[1]**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 152, No. 9, pp. 327-329, September, 2011
Original article submitted May 18, 2010

We studied the effects of synthesized tripeptides T-32 (Glu-Asp-Ala), T-33 (Glu-Asp-Arg), T-34 (Glu-Asp-Gly), T-36 (Glu-Asp-Pro), and T-38 (Lys-Glu-Asp) on organotypic skin cultures of young and old rats. In skin explants from young rats, all peptides except T-34 produced a stimulating effect on cell proliferation. In skin explants from old rats, tripeptide T-38 produced a marked stimulatory effect on proliferation. Immunocytochemical study of the proapoptotic p53 protein expression showed that cell proliferation increased due to less pronounced apoptosis. The capacity of the studied tripeptides to promote cell proliferation in the skin tissues of young and old animals provides the basis for further study of these substances as preparations boosting the regenerative processes in the skin, including those at age-associated pathology.

**Key Words:** *skin cell culture; bioregulatory short peptides; aging*

The most important functions of the skin are homeostasis maintenance and protection of the body against adverse environmental factors. In aging skin, deceleration of cell renewal during aging leads to reduction of its elasticity, appearance of cosmetic defects, and impaired wound healing. Reduced proliferative activity of skin epithelium accompanied by activation of apoptotic processes is a cause of age-related deceleration of reparation processes. It is known that skin aging is accompanied by changes in the expression levels of several signaling molecules including proapoptotic protein p53 [9,11,14]. In light of this, activation of cell proliferation in the skin and modulation of the expression of signaling molecules, including those regulating proliferation and apoptosis, are important problems of gerontology and gerontocosmetology.

Peptide bioregulators are of great importance for the maintenance and coordination of organism functioning during aging. Regulatory peptides affect differentiation, proliferation, apoptosis and cell-cell interactions. They are widespread in living organisms and are produced by various cells and tissues as endocrine and autocrine carriers of information about local functions of the organ or tissue [4,12]. Bioregulatory peptides also affect reparative processes in tissues via stimulation of cell proliferation or deceleration of this process during apoptosis activation [1,3,7]. Studied at St. Petersburg Institute of Bioregulation and Gerontology, the Russian Academy of Medical Sciences, developed the technology of short peptide synthesis based on the analysis of amino acid composition of extracts from various tissues. Organotypic cultures

[1]St. Petersburg Institute of Bioregulation and Gerontology of the North-Western Division of the Russian Academy of Medical Sciences; [2] Pavlov Institute of Physiology of Russian Academy of Sciences, St. Petersburg, Russia. ***Address for correspondence:*** ni_chalisova@mail.ru. N. I. Chalisova

are the most promising models for studying biological activity of various drugs and their effects on the structural and functional homeostasis of cell populations. The advantage of this method is the absence of nervous, humoral, and other regulatory influences in the tissue culture. At the same time, exact dosing of the test substances according to the rules of controlled experiment is possible [6,8,10]. Biological activity is primarily assessed by changes in cell number in the tissue culture.

Here we studied the effects of synthesized tripeptides on cell proliferation in skin organotypic cultures from rats of different age.

## MATERIALS AND METHODS

Organotypic tissue culturing was carried out by the method described previously [6,10]. Experiments were performed on 900 skin explants from the lower part of the abdomen of 3-month-old (young) and 24-month-old (old) Wistar rats. Skin fragments isolated under sterile conditions were divided into smaller pieces (~1 $mm^3$) and transferred to collagen-coated Petri dishes. Growth medium consisted of 35% Eagle's medium, 35% Hanks solution, 25% FBS, with addition of 0.6% glucose and 100 U/ml gentamicin. To find effective concentrations, the peptides were added to the culture medium in different concentrations (from 0.01 to 10 ng/ml). Culture medium (3 ml) with or without the examined peptides was added to Petri dishes with experimental and control explants, respectively. The volume of the medium for experimental and control explants was equal. The dishes were placed in an incubator at 37°C with constant supply of 5% $CO_2$ and after 3 days examined under a phase contrast microscope.

Area index (AI) was calculated as the ratio of the total explant area (together with the zone of outgrowing cells) to the area of the central zone of the explant. The explants were visualized using micro teleconverter for microscope (Series 10, MTN-13 Alfa Telecom). AI of explants was calculated using PhotoM 1.2. software. For each substance, 20-25 experimental explants and 20-25 control explants were analyzed. The significance of differences in AI between the control and experimental explants was assessed using Student's *t* test.

Immunocytochemical detection of proapoptotic protein p53 was performed using monoclonal antibodies to p53 protein (1:75, Novocastra). A universal kit containing biotinylated anti-mouse and anti-rabbit immunoglobulins was used as secondary antibodies. Staining was visualized using the avidin complex with biotinylated peroxidase (ABC-kit) followed by the development of horseradish peroxidase with diaminobenzidine (all reagents were from Novocastra). Morphometriy was carried out using a computer image analysis system consisting of Nikon Eclipse E400 microscope, digital camera Nikon DXM1200, and Intel Pentium 4 computer with Morphology–Videotest 5.0 software. In each case, at least 10 fields of view were analyzed at ×400. The area of p53 expression was determined as the ratio of the area occupied by immunopositive cells to the total area of cells in the field of view.

## RESULTS

On day 1 of culturing, the explants were spread on a collagen substrate. Outgrowth of proliferating and migrating epithelial cells and a small number of fibroblasts was observed. Structurally, peripheral growth zone (measure of AI) and explant capsule presented by 1-2-layers of fibroblasts not forming a continuous layer were most noticeable in the peripheral area of the explants. The capsule had large gaps through which some cells migrated out of the explant, proliferated, and formed a growth zone.

In organotypic culture of the skin tissue the following synthesized tripeptides were studied: T-32 (Glu-Asp-Ala), T-33 (Glu-Asp-Arg), T-34 (Glu-Asp-Gly), T-36 (Glu-Asp-Pro), and T-38 (Lys-Glu-Asp). Titration of tripeptides in concentration range from 0.01 to 100 ng/ml showed that they promote proliferation in a concentration of 0.05 ng/ml, *i.e*. this concentration was effective.

When tripeptides in effective concentrations were added to the culture medium of skin explants from young rats, AI increased by 16-62% compared to AI of control explants. Tripeptide T-32 added to the culture medium increased AI by 16±3% ($n$=23; $p$<0.05) in comparison with the control ($n$=21) and reduced p53 expression by 21±7%. Tripeptide T-33 significantly increased AI by 18±3% ($n$=24; $p$<0.05) in comparison with the control ($n$=21) and reduced p53 expression by 25±5%in comparison with the control explants. Tripeptide T-36 significantly increased AI by 20±3% ($n$=20; $p$<0.05) in comparison with the control ($n$=23) and reduced p53 expression by 27±5% in comparison with the control. Tripeptide T-38 increased AI by 62±9% ($n$=23; $p$<0.05) in comparison with the control ($n$=22) and decreased p53 expression by 35±7%. Tripeptide T-34 had no effect on the development of skin explants and AI; p53 expression remained at the control level.

After addition of effective tripeptide concentrations to the culture medium of skin explants from old rats, the amount of active peptides decreased. Statistically insignificant trend toward an increase in AI was detected under the action of T-36 tripep-

tide. However, T-38 tripeptide markedly stimulated cell proliferation, and AI increased by 54±7% ($n$=21; $p$<0.05) in comparison with the control ($n$=23). In this case, expression of p53 decreased by 20±3%, which attests to stimulation of cell proliferation due to inhibition of apoptosis.

Thus, the examined synthesized short peptides can enhance cell proliferation and regeneration processes by reducing the intensity of apoptosis in the model of skin organotypic culture from both young and old rats. Short bioregulatory peptides can be used at the treatment of skin diseases. The capacity of the investigated tripeptides to promote cell proliferation in the skin from young and old animals proves their further study as agents boosting the regenerative processes in the skin including those at age-related diseases. Tripeptide T-38 considerably stimulating cell proliferation in skin explants from old animals is particularly promising as a geroprotective peptide.

## REFERENCES


1. N. S. Lynkova, V. O. Polyakova, and I. M Kvetnoy, *Byull. Eksp. Biol. Med.*, **151**, No. 4, 442-444 (2011).
2. G. A. Ryzhak, E. V. Voytan, and N. I. Chalisova, *Uspekhi Gerontol*., **15**, Issue 20, 56-58 (2007).
3. V. Kh. Khavinson, *Peptide Regulation of Aging* [in Russian], St. Petersburg (2009).
4. V. Kh. Khavinson, N. S. Lynkova, V. O. Polyakova, *et al*., *Byull. Eksp. Biol. Med.*, **151**, No. 5, 569-572 (2011).
5. V. Kh. Khavinson, N. I. Chalisova, V. V. Malinin, and E. I. Grygoryev, *Uspekhi Gerontol*., **10**, Issue 9, 95-100 (2002).
6. N. I. Chalisova, N. M. Bykov, and P. N. Zezulin, *Uspekhi Gerontol*., **11**, Issue 10, 104-108 (2003).
7. N. I. Chalisova, I.V. Knyazkin, and I. M. Kvetnoy, *Neuroimmunoendocrine Mechanisms of Action of Peptides and Amino Acids in Tissue Cultures* [in Russian], St. Petersburg (2005).
8. N. I. Chalisova, V. Kh. Khavinson, V. G. Morozov, and V. B. Okulov, *Tsitologia*, **39**, No. 1, 571-578 (1997).
9. M. A. Barnadas., L. Colomo, R. Curell, *et al*., *Acta. Derm. Venerol.*, **76**, No. 3, 203-204 (1996).
10. N. I. Chalisova and A. A. Zakutzkii, *Cell Biol. Int*., **32**, No. 2, 1574-1577 (2008).
11. M. El-Domiaty, S. Attia, F. Saleh, *et al*., *Exp. Dermatol*., **11**, No. 5, 398-405 (2002).
12. V. Kh. Khavinson, L. I. Fedoreeva, and B. F.Vanyshin, *Biochem. Biophys. Mol. Biol.*, **437**, No. 1, 124-127 (2011).
13. V. Kh. Khavinson and V. V. Malinin, *Gerontological aspects of genome peptide regulation*, Basel (2005).
14. I. M. Kvetnoy, V. V. Yuzhakov, A. K. Sandvik, and H. L. Waldum, *J. Pineal Res*., **22**, No. 3, 169-170 (1997).