Tetrapeptide H-Ala-Glu-Asp-Arg-OH Stimulates Expression of Cytoskeletal and Nuclear Matrix Proteins

V. Kh. Khavinson[1,2], N. S. Lin'kova[2], V. O. Polyakova[3], I. M. Kvetnoy[3], V. V. Benberin[2], M. M. D'yakonov[2], and Yu. S. Titkov[2]

Translated from *Kletochnye Tekhnologii v Biologii i Meditsine*, No. 2, pp. 88-91, April, 2012
Original article submitted February 8, 2012

Tetrapeptide H-Ala-Glu-Asp-Arg-OH enhances the expression of cytoskeletal (actin, tubulin, vimentin) and nuclear matrix proteins (lamin A, lamin C) in cultured mouse embryonic fibroblasts by 2-5 and 2-3 times, respectively. Thus, the previously reported cardioprotective activity of this tetrapeptide is determined by its capacity to activate synthesis of cytoskeletal and nuclear matrix proteins, which stimulates cell proliferation and reduces apoptosis.

Key Words: *cytoskeleton; fibroblasts; tetrapeptide*

Cytoskeleton is a protein framework involved in cell motion and attachment to the substrate. The most important components of the cytoskeleton are actin, vimentin, and tubulin forming microfilaments, intermediate filaments, and microtubules [2, 11].

The most important components of nuclear framework, lamin A and lamin C, ensure stability of the cell nucleus and heterochromatin spiralization and act as endogenous antiapoptotic factors [6].

Cyto- and karyoskeleton remodeling is an essential condition of eukaryotic cell passage through the cell cycle; it is an obligatory stage of mitosis, apoptosis, and differentiation of cells [5,12]. Thus, regulation of expression of cytoskeleton and nuclear matrix proteins is an important factor determining cell functions.

At the St. Petersburg Institute of Bioregulation and Gerontology, some short di-, tri-, and tetrapepties were synthesized [1,8,9] that belong to a group of cell penetrating peptides and exhibit high biological activity [3]. One of them, tetrapeptide H-Ala-Glu-Asp-Arg-OH, produces a pronounced reparative effect on the heart [10]. It was found that FITC-labeled tetrapeptide penetrates into the cytoplasm, nucleus, and nucleolus of HeLa cells [4]. Moreover, this peptide can inhibit hydrolysis of DNA fragments with endonucleases [7]. In light of this, we studied the effect of this tetrapeptide on the expression of cytoskeleton and nuclear matrix proteins.

MATERIALS AND METHODS

Mouse embryonic fibroblasts (MEF+/+, knockout LMNA mice) were cultured (37°C, 5% CO_2) in a humid atmosphere in DMEM (ICN Biomedicals) supplemented with 10% embryonic calf serum. For subculturing, the cells were diluted 1:3-1:5 using 0.125% trypsin, 0.02 M EDTA, and 0.02% glucose in phosphate buffered saline. The cells were grown in the culture medium for 5 days and then divided into 2 groups: group 1 cultures were intact (control), while group 2 cultures were incubated with 10 ng/ml H-Ala-Glu-Asp-Arg-OH for 30 min.

For immunofluorescent confocal laser microscopy, the cells were placed onto sterile slides and permeabilized in 0.1% Triton X-100 for 10 min at room temperature. Then the cells were fixed in 4% neutral buffered formalin (pH 7.2) for 15 min and incubated with primary monoclonal mouse antibodies to actin (Mubio Products B.V.; 1:50) vimentin (Mubio Pro-

[1]I. P. Pavlov Institute of Physiology, Russian Academy of Sciences; [2]St. Petersburg Institute of Bioregulation and Gerontology, Northwestern Division of the Russian Academy of Medical Sciences; [3]D. O. Ott Institute of Obstetrics and Gynecology, Russian Academy of Medical Sciences, St. Petersburg, Russia. ***Address for correspondence:*** miayy@yandex.ru. N. S. Linkova

0007-4888/12/1534-0559

ducts B.V.; 1:50), tubulin (clone E7, Developmental Studies Hybridoma Bank; 1:50), lamin A (ab26300, Abcam; 1:100), and lamin C (ab8981, Abcam; 1:100) for 1 h.

After washout in phosphate buffer, the cell monolayer was incubated with second antibodies, rabbit antimouse FITC-conjugated Ig (DAKO; 1:100). After washout in phosphate buffer, a medium containing 90% glycerin, 0.02 M Tris-HCl (pH 8.0), 0.8% NaN_3, and 2% 1,4-di-azabicyclo-(2,2,2)-octan (Merc) was added to the cells.

Optical density was measured. The preparations were examined under a Leica TCS SP5 confocal microscope using an MRC-1024 system equipped with LaserSharp 5.0 software (Bio-Rad) for confocal image analysis. Significance of differences in optical density between the groups was evaluated using two-sample Wilcoxon rank-sum test. The data were processed statistically using Statistica 7.0 software.

RESULTS

In the control group, the monolayer was uniform and retained its integrity after 1 day, fibroblasts had usual shape and size. Cell density in the monolayer was 774 cell/mm^2, doubling time 24 h. The general picture of the monolayer and cell structure did not differ from those in normal cell culture over 5 days of observation. The monolayer density on day 3 was 1519 cell/mm^2. On day 5 of the experiment, the cell attained optimum density 2020 cell/mm^2, which led to cell transition to the stationary growth phase. During the stationary phase, the fibroblasts had normal elongated shape with 2-4 processes, the cell and nuclear membranes were clearly seen. The cytoplasm was homogeneous and the following levels of cytoskeletal protein expression were recorded: 1.5±0.3 arb. units actin, 1.8±0.3 arb. units vimentin, and 1.1±0.2 arb. units tubulin (Fig. 1).

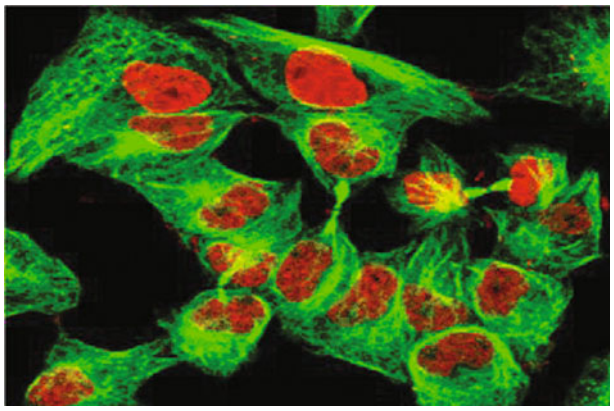

Fig. 1. Expression of vimentin (green fluorescence) in the cytoplasm and lamin A (red fluorescence) in nuclei of fibroblasts: control, day 5 in culture. Here and in Figs. 2-4: immunofluorescent confocal laser microscopy (×600).

Most cells had one centrally located nucleus of regular round shape with 1 or 2 nucleoli. The intensity of nuclear protein expression was medium: 1.4±0.3 arb. units lamin A and 1.3±0.4 arb. units lamin C (Fig. 1).

In cultures incubated with H-Ala-Glu-Asp-Arg-OH, the expression of cytoplasmic proteins actin, vimentin, and tubulin increased by 2-5 times (to 6.2±0.5,

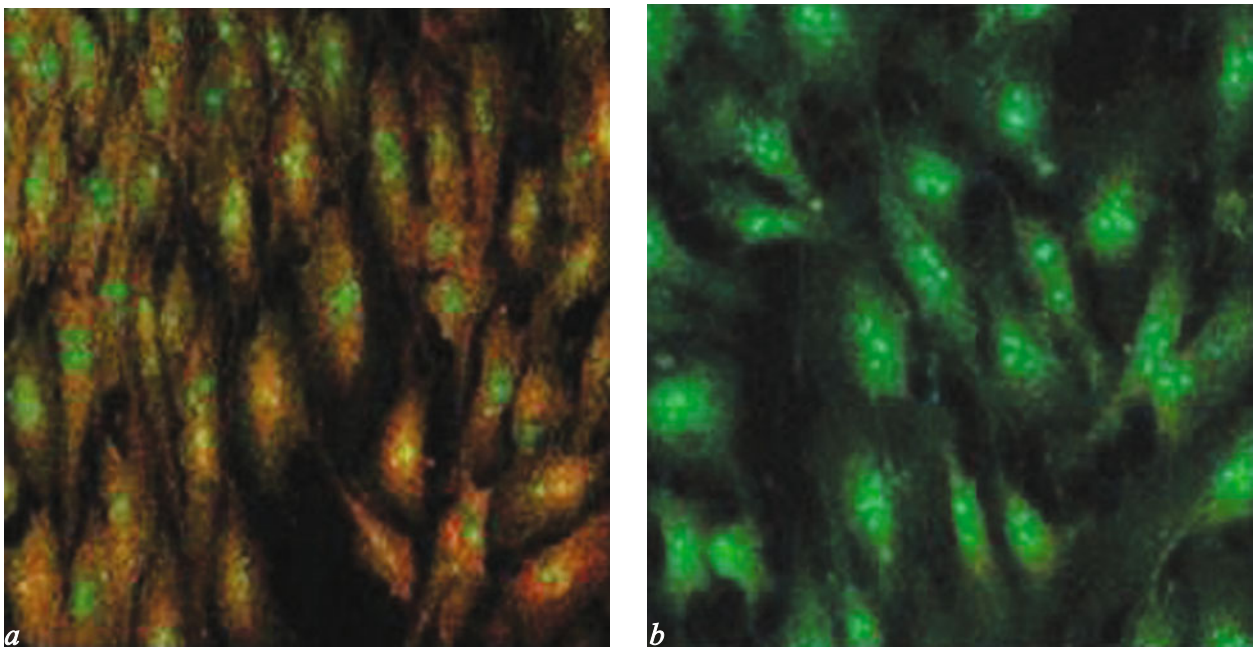

Fig. 2. Expression of vimentin in fibroblast cytoplasm (green fluorescence) on day 5 of culturing. Here and in Figs. 3, 4: *a*) control; *b*) tetrapeptide.

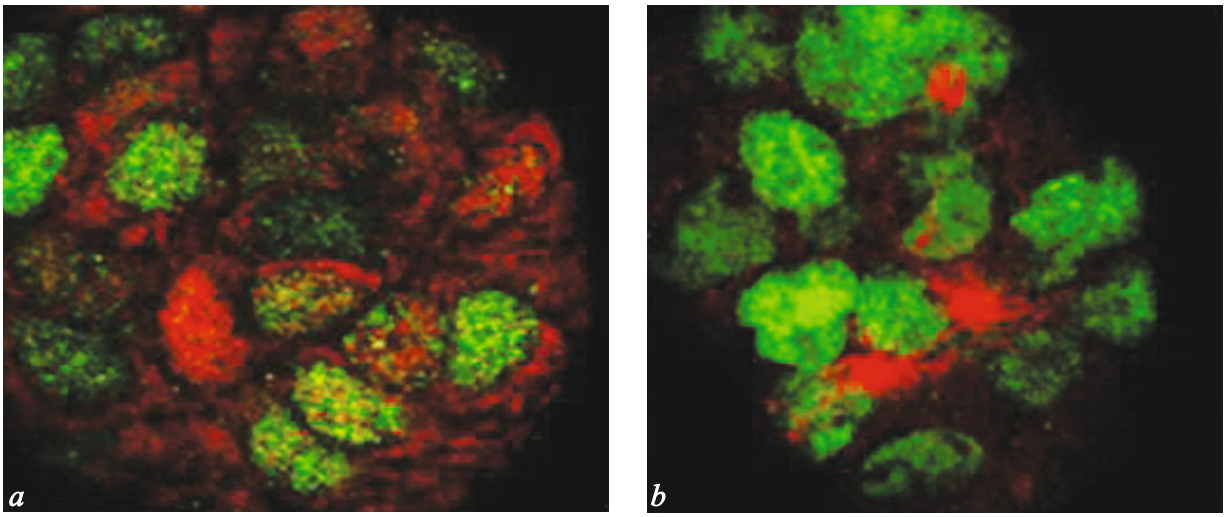

Fig. 3. Expression of tubulin (green fluorescence) in fibroblast cytoplasm on day 5 of culturing. Nuclei are poststained with rhodamin A and porous substrate with acridine orange.

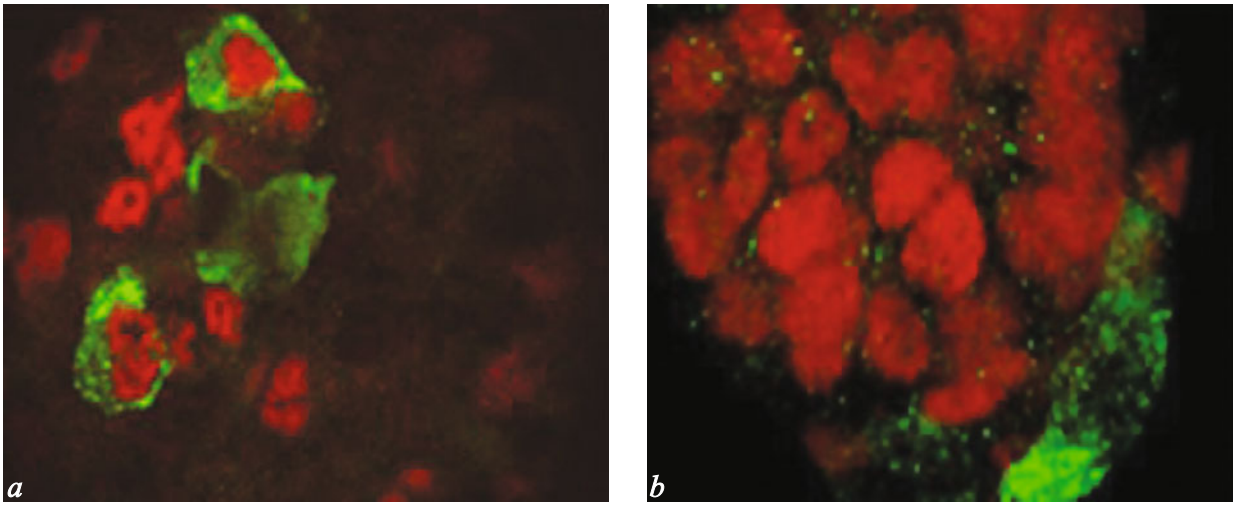

Fig. 4. Expression of lamin A (red fluorescence) in fibroblast cytoplasm on day 5 of culturing.

4.2±0.4, and 4.0±0.5 arb. units, respectively; Fig. 2, 3) and nuclear matrix proteins lamin A and lamin C by 2-3 times (to 3.9±0.2 and 4.2±0.3 arb. units, respectively) in comparison with the control (p<0,05; Fig. 4).

These results suggest that H-Ala-Glu-Asp-Arg-OH activates expression of cytoskeletal proteins actin, vimentin, and tubulin and nuclear matrix proteins lamin A and C.

It can be hypothesized that this peptide via regulation of DNA-associated proteins (enzymes and transcription factors) improves accessibility of genes encoding cytoskeletal proteins for transcription, which leads to activation of intracellular metabolism and induction of cell proliferation and differentiation. Enhanced production of lamin A and lamin C can attest to antiapoptotic effect of the tetrapeptide.

These findings suggest that this tetrapeptide can act as a bioactive substance stimulating expression of cytoskeletal and nuclear matrix proteins, which can be used in the therapy and prevention of pathologies related to myocardial damage.

REFERENCES

1. V. N. Anisimov and V. Kh. Khavinson, *Biogerontology,* **11**, 139-149 (2010).
2. M. Anitei and B. Hoflack, *Nat. Cell. Biol*., **14**, No. 1, 11-19 (2011).
3. A. Chung, F. Eudes, and Y. S. Shim, *IUBMB Life.,* **62**, No. 3, 183-193 (2010).
4. L. I. Fedoreyeva, I. I. Kireev, V. Kh. Khavinson, and B. F. Vanyushin, *Biochemistry* (Mosc.), **76**, No. 11, 1210-1219 (2011).
5. R. A. Figueroa, S. Gudise, and E. Hallberg, *Biochem. Soc. Trans*., **39**, No. 6, 1786-1789 (2011).
6. A. Gross, J. M. McDonnell, and S. J. Korsmeyer, *Genes Dev.*, **13**, No. 15, 1899-1911 (1999).

7. V. Kh. Khavinson, L. I. Fedoreeva, and B. F. Vanyushin, *Dokl. Biochem. Biophys*., **437**, 64-67 (2011).
8. V. Kh. Khavinson, N. S. Linkova, A. V. Trofimov, *et al., Biol. Bull. Rev.,* **1**, No. 4, 389-393 (2011).
9. V. Kh. Khavinson and V. V. Malinin, *Gerontological Aspects of Genome Peptide Regulation.* Basel (2005).
10. V. Kh. Khavinson, G. A. Ryzhak, E. I. Grigoriev, I. Yu. Ryadnova, *Peptide Substance Restoring Myocardial Function*. USA Patent. US 7,622,789, 16.02.2010.
11. S. Trabulo, A. L. Cardoso, and M. Mano, *Pharmaceuticals,* **3**, 961-993 (2010).
12. H. C. Tseng, H. W. Kao, M. R. Ho, *et al., Anticancer Res*., **31**, No. 12, 4239-4249 (2011).