Peptides Tissue-Specifically Stimulate Cell Differentiation during Their Aging

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We verified expression of CXCL12 and Hoxa3 transcription factors of differentiation in cultures of human embryonic pancreatic and bronchial cells and CXCL12 and WEGC1 factors in a culture of human prostatic fibroblasts. Reduced expression of these differentiation markers was detected in late-passage (aging) cultures. The expression of differentiation factors CXCL12, Hoxa3, and WEGC1 was tissue-specifically stimulated by short peptides: pancragen (Lys-Glu-Asp-Trp) in pancreatic cells, bronchogen (Ala-Glu-Asp-Leu) in bronchial epithelial cells, and vesugen (Lys-Glu-Asp) in fibroblasts. The inducing effect of peptides on the expression of differentiation factors was more pronounced in aged cultures, which can serve as a mechanism of their geroprotective effect.

Key Words: *peptides; differentiation; acinar cells; bronchial epithelial cells; fibroblasts*

Expression of genes participating in cell differentiation is regulated by transcription factors. They stimulate or suppress the synthesis of regulatory proteins through interaction with specific nucleotide sequences of the gene. Synthesis of proteins (including differentiation factors) in cells decreases with age and under pathological conditions, which leads to dysregulation of gene expression and a decrease in functional activity of organs and tissues [1,2,4].

Peptides pancragen (Lys-Glu-Asp-Trp), bronchogen (Ala-Glu-Asp-Leu), and vesugen (Lys-Glu-Asp) created at St. Petersburg Institute of Bioregulation and Gerontology promote functional recovery of the pancreas and organs of the respiratory and cardiovascular systems, respectively. However, their effect on expression of differentiation factors has never been studied. Pancragen reduces glucose concentration in the blood [3,5]. It is demonstrated that pancragen significantly reduced glycemia (by 35%) in rats with alloxan-induced diabetes mellitus, which correlated with decrease in the animal mortality [3]. Clinical studies of pancragen demonstrated its efficiency in individuals with diabetes mellitus [9]. Bronchogen attenuated the inflammatory reaction in the lungs of rats with bleomycin-induced fibrosis: fibrotic changes in the lungs, hemodynamic disturbances in the lesser circulation, and myocardial hypertrophy were less pronounced [6]. Vesugen improves metabolism in the vascular wall [7]. The use of these peptides is important for gerontology, because diabetes mellitus and bronchial and vascular pathologies often develop in elderly and senile individuals.

In cell of the bronchial epithelium, pancreatic islets, and fibroblasts of the prostatic gland, WEDC1, CXCL12 (SDF-1), and Hoxa3 proteins act as differentiation factors. Factors WEGC1 and CXCL12 are expressed by human prostate fibroblasts and their synthesis decreases with age, which is one of the causes of hyperplastic processes [7]. Increased concentration of chemokine CXCL12 in the plasma and alveolar fluid and migration of cells carrying CXCR4 receptors selectively binding CXCL12 into the lungs were observed in rats with lung injury [11]. Protein Hoxa3 is

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transcribed by tissue-specific homeotic gene HOXA3 participating in tissue differentiation via modulation of the expression of various genes [8,10].

Here we studied the effects of pancragen, bronchogen, and vesugen on the expression of transcription factors WEDC1, Hoxa3, and CXCL12 in cultures of human lung and pancreatic cells and fibroblasts during aging.

MATERIALS AND METHODS

Experiments were performed on embryonic cultures of acinar cells of the pancreas MIA PaCa-2 (Institute of Cytology, Russian Academy of Sciences) and bronchial epithelium FLECH (Research Institute of Influenza, Ministry of Health and Social Development) of passages 1, 7, and 14. Cultures of passages 1, 7, and 14 were considered as young, mature, and aged cultures, respectively (according to recommendations of International Association of Cell Culture Studies, San Francisco, USA, 2007). We also used culture of human prostate fibroblasts (clone CX3CR1, Cambrex Bioscience) passing 1, 4, and 7 passages, which corresponded to 1, 7, and 14 passages for embryonic cultures. Physiological saline or one of the test peptides was added to control and experimental cultures respectively: bronchogen to culture of bronchial epithelial cells, pancragen to culture of acinar cells of the pancreas, and vesugen to fibroblast cultures. Preliminary experiments showed that peptides not specific for the tissue (bronchogen for acinar cells, pancragen for bronchial epithelial cells, and cardagen for fibroblasts) do not affect the expression of transcription factors.

Acinar cells of the pancreas were cultured in 25-cm^2 flasks (JetBiofil) in 5 ml DMEM supplemented with L-glutamine (Biolot), 15% fetal calf serum SC-BIOL (Biolot), and 1% penicillin-streptomycin at 37°C. Bronchial epithelial cells were cultured in MEM supplemented with L-glutamine (Biolot), 10% fetal calf serum SC-BIOL (Biolot), and 1% penicillin-streptomycin. Fibroblasts were cultured in 24-well plates (Costar) at 37°C and 5% CO_2 in RPMI-1640 (Flow) supplemented with 10% ECS (Serva), 300 μg/ml L-glutamine (Flow), 0.02 M HEPES buffer (Sigma), and 100 μg/ml gentamicin (Pharmakhim). Initial concentration was 10^6 cell/ml.

For immunocytochemical study, primary monoclonal antibodies WEDC1 (1:150, Vectorlab), CXCL12 (1:200, Vectorlab), and Hoxa3 (1:150, "Vectorlab") and secondary antibodies, biotinylated anti-mouse immunoglobulins (Novocastra) were used. Permeabilization was performed with 0.1% Triton-X100. The reaction was visualized with horseradish peroxidase and diaminobenzidine (EnVision Detection System, Peroxidase/DAB, Rabbit, Mouse).

The results of immunocytochemical analysis were evaluated morphometrically using a computer-assisted microscopic image analysis system consisting of Nikon Eclipse E400 microscope, Nikon DXM1200 digital camera, and Videotest-Morphology 5.0 software. In each case, at least 5 fields of view were analyzed at ×200. The area of expression was calculated as the ratio of the area occupied by immunopositive cells to the total area of cells in the field of view and expressed in percents. This parameter characterizes the intensity of the synthesis of the studied transcription factors in cells.

Immunofluorescent confocal microscopy was performed on non-fixed cell suspensions. Cell smears were treated with primary antibodies (Torrey Pines Biolabs) to CXCL12 and WEDC1 proteins. Expression of signal molecules was visualized using Vector Red kits (Vector Lab) for immunofluorescent visualization of alkaline phosphatase. Levamisole (1.25 mM) was added during incubation with alkaline phosphatase of the kit for blockade of endogenous enzyme. The preparations were examined under Leica TCS SP5 confocal microscope at ×400 and ×1000 using an MRC-1024 system equipped with LaserSharp 5.0 software (Bio-Rad) for confocal image analysis. In each case, the area of expression was analyzed in 10 fields of view at ×400. Statistica 7.0 software was used for comparison and evaluation of between-group differences.

RESULTS

Expression of CXCL12 and Hoxa3 markers was verified in control cultures of pancreatic acinar cells and bronchial epithelial cells and CXCL12 and WEDC1 markers in fibroblast culture. In culture of pancreatic acinar cells, the area of Hoxa3 expression significantly decreased with age. In young cultures, the area of Hoxa3 expression was 1.2- and 2-fold larger than in mature and aged cultures (Table 1). The area of CXCL12 expression decreased by 22 and 12% in mature and aged cultures, respectively. Area of WEDC1 expression in fibroblast cultures of passages 4 and 7 was reduced by 60 and 81% in comparison with passage 1 (Table 2).

Addition of pancragen to the culture of pancreatic acinar cells reduced the area of expression of differentiation factor CXCL12 by 21% in young cultures and by 86 and 72% in mature and aged cultures, respectively (Table 1, Fig. 1, *a*, *b*). Expression of Hoxa3 significantly increased after addition of pancragen: by 60% in young cultures and by 90% in mature cultures. The most pronounced effect was observed in aged cultures: Hoxa3 expression area increased by 2.8 times in comparison with the control. Enhanced

TABLE 1. Effect of Pancragen on Expression of Pancreatic Acinar Cell Differentiation Factors (*M±m*; %)

Factor	Expression area					
	passage 1 (young cultures)		passage 7 (mature cultures)		passage 14 (aged cultures)	
	control	pancragen	control	pancragen	control	pancragen
CXCL12	8.3±1.1	10.1±1.3	6.5±0.9	12.1±3.1*	7.3±1.2	12.6±1.7*
Hoxa3	5.4±0.8	8.6±0.7*	4.7±0.6	8.9±0.9*	2.7±0.3	10.2±2.1*

Note. Here and in Tables 2, 3: *$p<0.05$ in comparison with the control.

TABLE 2. Effect of Vesugen on Expression of Fibroblast Differentiation Factors (*M±m*; %)

Factor	Expression area					
	passage 1 (young cultures)		passage 4 (mature cultures)		passage 7 (aged cultures)	
	control	vesugen	control	vesugen	control	vesugen
CXCL12	2.22±0.08	2.72±0.05	1.14±0.04	9.13±1.72*	0.37±0.01*	2.81±0.2*
WEDC1	1.72±0.06	2.66±0.09*	0.70±0.03*	5.73±0.16*	0.32±0.01*	5.23±0.21*

TABLE 3. Effect of Bronchogen on Expression of Bronchial Epithelium Differentiation Factors (*M±m*; %)

Factor	Expression area					
	passage 1 (young cultures)		passage 7 (mature cultures)		passage 14 (aged cultures)	
	control	bronchogen	control	bronchogen	control	bronchogen
CXCL12	6.2±1.3	6.3±1.4	5.4±0.9	6.0±0.7	5.0±0.6	5.4±0.9
Hoxa3	4.0±0.8	6.5±1.2*	5.2±1.7	12.7±3.4*	5.2±1.9	14.2±4.1*

Hoxa3 expression was also observed after addition of bronchogen to the culture of bronchial epithelial cells: by 1.7, 1.4, and 1.7 times in young, mature, and aged cultures (Table 3). Bronchogen had no effect of the expression of differentiation factor CXCL12 in all studied passages of bronchial epithelial cells (Table 3).

Addition of vesugen to fibroblast culture increased expression of differentiation factors CXCL12 and WEDC1: in young cultures by 1.2 and 1.5 times, respectively, in mature cultures by 8 and 8.2 times, and in aged cultures by 7.6 and 16 times (Fig. 2, *a-c*; Table. 2).

Aging of the culture of bronchial epithelium, pancreatic acinar cells, and prostate fibroblasts is associated with a decrease in the expression of differentiation factors CXCL12, Hoxa3, and WEDC1, which attests to reduced differentiation capacity of these cells and can be a cause of reduced functional activity of the pancreas, bronchi, and prostate during aging.

Stimulating effects of peptides pancragen, bronchogen, and vesugen on the glandular and endothelial tissues was reported previously. In our experiments, these peptides induced the expression of differentiation factors CXCL12, Hoxa3, and WEDC1 in cells of the pancreas, bronchi, and prostate fibroblasts. Since these differentiation factors participate in transcription, we can hypothesize that stimulation of cell differentiation by short peptides is mediated by an epigenetic mechanism underlying peptide regulation of aging processes.

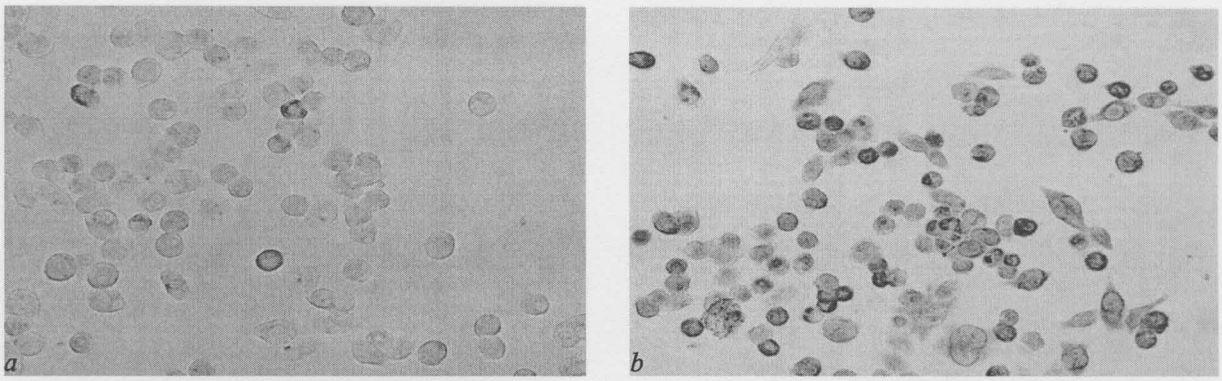

Fig. 1. Expression of differentiation factor CXCL12 in culture of pancreatic acinar cells: passage 14 (×200). *a*) control; *b*) addition of pancragen, immunocytochemistry.

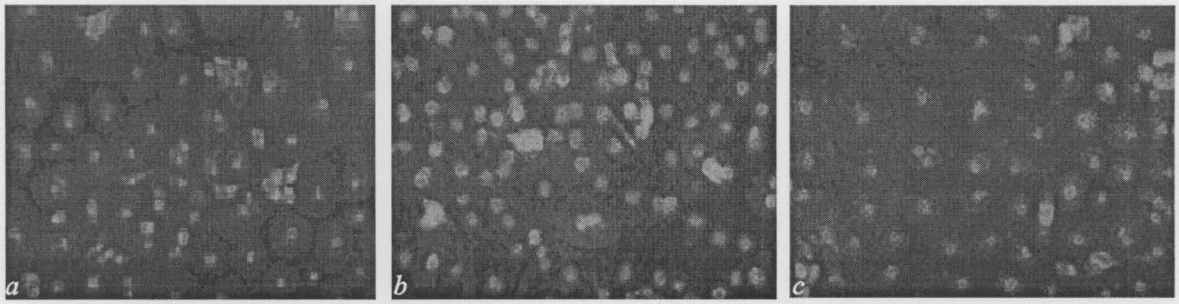

Fig. 2. Expression of WEDC1 protein (green fluorescence) in prostate fibroblasts. Confocal microscopy. *a*) passage 1; *b*) passage 7; *c*) passage 7 in the presence of T-38. Magnification: ×1000 (*a*, *b*) and ×1600 (*c*).

REFERENCES

1. V. N. Anisimov and V. Kh. Khavinson, *Biogerontology,* **11**, No. 2, 139-149 (2010).
2. V. Kh. Khavinson, L. I. Fedoreeva, and B. F. Vanyushin, *Dokl. Biochem. Biophys.*, **437**, 64-67 (2011).
3. V. Kh. Khavinson, M. M. G. Gapparov, N. E. Sharanova, *et al., Bull. Exp. Biol. Med.*, **149**, No. 3, 351-353 (2010).
4. V. Kh. Khavinson and V. V. Malinin, *Gerontological Aspects of Genome Peptide Regulation.* Basel (2005).
5. V. Kh. Khavinson, V. V. Malinin, E. I. Grigoriev, and G.A. Ryzhak, *Tetrapeptide Regulating Blood Glucose Level in Diabetes Mellitus*. European patent EP 1697401, 06.01.2010.
6. V. Kh. Khavinson, G.A. Ryzhak, E. I. Grigoriev, and I. Yu. Ryadnova, *Peptide Substance Restoring Respiratory Organs Function*. USA Panent US 7,625,870, 01.12.2009.
7. O. V. Kheyfets, V. O. Polyakova, and I. M. Kvetnoy, *Adv. Gerontol.*, **23**, No. 1, 68-70 (2010).
8. N. Mizusawa, T. Hasegawa, I. Ohigashi, *et al., Gene*, **331**, 53-63 (2004).
9. A. P. Ryzhak and I. N. Kostjuchek, *Adv. Gerontol.*, **20**, No. 3, 97-100 (2007).
10. D. P. Tan, X. Shao, L. Pu, *et al., Proc. Natl. Acad. Sci. USA.*, **93**, No. 16, 8247-8252 (1996).
11. J. Xu, A. Mora, H. Shim, *et al., Am. J. Respir. Cell Mol. Biol.*, **37**, No. 3, 291-299 (2007).