BIOGERONTOLOGY

Effects of Pancragen on The Differentiation of Pancreatic Cells During Their Ageing

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 154, No. 10, pp. 498-501, October, 2012
Original article submitted April 17, 2011

Expression of differentiation markers was found to be reduced during ageing of pancreatic cells. Tetrapeptide pancragen stimulates the expression of differentiation factors of acinar (Pdx1, Ptfla) and islet of Langerhans (Pdx1, Pax6, Pax4, Foxa2, NKx2.2) cells in "young" and "aged" cultures. Differentiation of acinar and islet pancreatic cells induced by pancragen can be a mechanism underlying its anti-diabetic and anti-inflammatory effects. Thus, transcription factors that regulate differentiation of pancreatic cells are a pharmacological target for pancragen, which allows considering it as an effective tool in the treatment of diabetes mellitus and pancreatitis.

Key Words: *tetrapeptide; differentiation; pancreas; cellular senescence*

The number of elderly patients with diabetes and pancreatitis considerably increases worldwide during the last decade [1]. Tetrapeptide pancragen is known to enhance insulin expression by β-cells and regulates glucagons release by α-cells, and also normalizes functional activity of exocrine cells of the pancreatic gland (PG) [6-8]. It was found that pancragen penetrates through the membrane into the cell nucleus and nucleolus [4]. Its geroprotective effect on PG is probably related to the regulation of transcription of genes encoding differentiation factors [4]. Ptf1a, Pdx1, Pax6, Foxa2, Nkx2.2, and Pax4 are the most important factors of PG cell differentiation [5]. Pdx1 protein regulates proliferation and differentiation of all types of PG cells. Ptf1a protein is involved in the differentiation of pancreatic acinar cells and regulates gene expression of enzymes in PG exocrine cells including elastase 1 and amylase. Tumor cells of PG cancer show reduced expression of Ptf1a gene [2,10]. Pax6, Foxa2, Nkx2.2, and Pax4 proteins activate gene expression in insulin-producing β-cells, glucagon-producing α-cells and pancreatic polypeptide-secreting cells in the islets of Langerhans [3,9,12]. Pax6 and Pax4 expression was also found in somatostatin-containing δ-cells [11]. Mutations in Pax6, Foxa2, and Nkx2.2 genes are known to be responsible for diabetes caused by impaired insulin synthesis in β-cells [5]. Ageing reduces Ptf1a synthesis in acinar cells leading to a decrease in functional activity of the cells and promotes the development of chronic pancreatitis. Type 2 diabetes mellitus is the basic pathology that develops under conditions of insufficient synthesis of Pdx1, Pax6, Foxa2, Nkx2.2, and Pax4 in PG islet cells. In light of this, the study of pancragen effect on the total expression of the above transcription factors of differentiation is essential for understanding of the molecular mechanisms of its geroprotective and pancreas-protective action.

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0007-4888/13/1544-0501

Here we studied the molecular mechanisms underlying pancragen effects on the differentiation of pancreatic acinar and islet cells.

MATERIALS AND METHODS

Experiments were performed on embryonic cultures of pancreatic acinar cells MIA PaCa-2 of passages 1 and 14 obtained at Institute of Cytology (St. Petersburg). Passages 1 and 14 were considered "young" and "aged" cultures, respectively, according to recommendations of International Association of Cell Culture Studies, San Francisco, USA, 2007. The cultures were subdivided into 3 groups. Saline was added to group 1 culture (control); bronchogen (20 ng/ml) as control peptide was added to group 2 and pancragen (20 ng/ml) to group 3. The cells 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 (initial concentration 10^6 cell/ml). For immunocytochemical study, primary monoclonal antibodies Ptf1a, Pdx1, Pax6, Foxa2, NKx2.2, and Pax4 (1:50, Abcam) and secondary biotinylated anti-mouse antibodies (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, 5 fields of view were analyzed at ×200. The area of expression was calculated as the percentage of the area occupied by immunopositive cells compared to the total area of cells in the field of view. This parameter characterizes the number of cells expressing the investigated differentiation factor.

The data were statistically processed with Statistica 7.0 software. To compare and evaluate inter-group differences, non-parametric Mann–Whitney U test was used, which is the most accurate method for comparing samples including about 10-15 cases. The differences were considered statistically significant at $p<0.05$.

RESULTS

In group 1 and 2, we found weak positive reaction for all the studied markers in cells of passage 14 indicating low rate of synthesis in the cells. Pancragen increased staining intensity, which attested to activation of the synthesis of the studied molecules (Figs. 1 and 2).

The expression area for all markers except Pax4 decreased in the control group during culture ageing

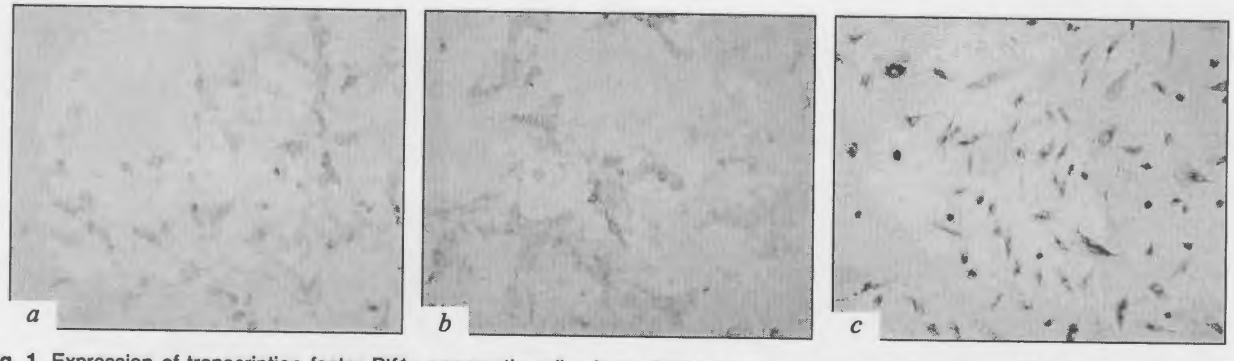

Fig. 1. Expression of transcription factor Ptf1a pancreatic cell cultures (passage 14). Immunocytochemistry, ×200. Here and in Fig. 2: *a)* control; *b)* addition of bronchogen; *c)* addition of pancragen.

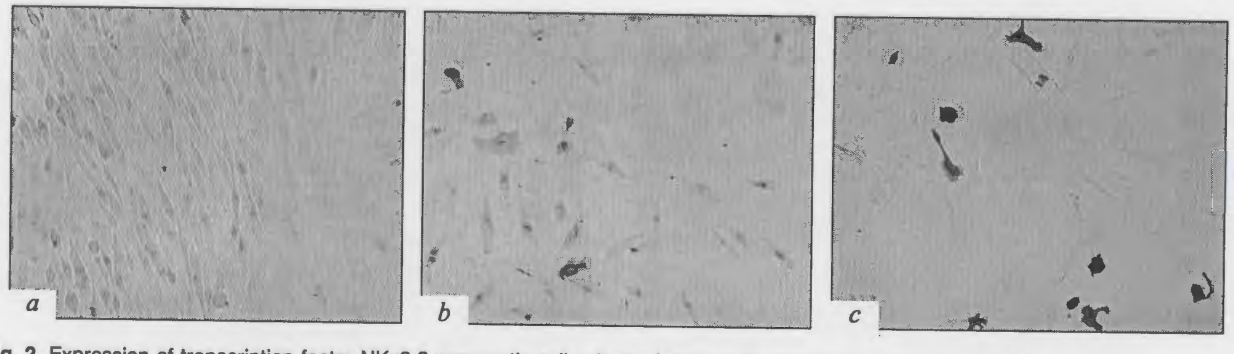

Fig. 2. Expression of transcription factor NKx2.2 pancreatic cell cultures (passage 3). Immunocytochemistry, ×200.

TABLE 1. Effects of Peptides on the Expression Area PG Cell Differentiation Factors (*M±m*; %)

Differentiation factors	Control		Bronchogen		Pancragen	
	young cultures	aged cultures	young cultures	aged cultures	young cultures	aged cultures
Ptf1a	1.48±0.08	0.32±0.03	1.65±0.12	0.45±0.01*	2.04±0.11*	1.95±0.05*
Pdx1	2.84±0.08	1.05±0.03	3.05±0.11*	1.15±0.03*	3.63±0.22*	2.75±0.14*
Pax6	2.32±0.07	1.15±0.02	2.29±0.20	1.35±0.04*	2.96±0.11*	2.64±0.05*
Foxa2	1.55±0.14	0.45±0.02	1.63±0.05	0.36±0.06	1.66±0.14	0.75±0.01*
Nkx2.2	1.32±0.04	0.34±0.03	1.45±0.06*	0.40±0.03	1.74±0.06*	0.68±0.05*
Pax4	0.96±0.06	1.19±0.03	1.16±0.07*	1.38±0.03*	1.26±0.11*	1.74±0.03*

Note. *$p<0.05$ compared with controls.

(Table 1). Ptf1a marker, differentiation factor of acinar cells, showed the most pronounced decrease in the expression. The expression area decreased by 4.6 times in aged cultures compared to young ones. Bronchogen did not significantly change the expression of Ptf1a marker in young and aged PG cell cultures (Table 1). Under the action of pancragen, the area of Ptf1a expression increased by 1.5 and 6 times in young and old cultures, respectively. Differentiation factor Pdx1 is a key maturation marker of all PG cell types. The area of Pdx1 expression decreased by 2.72 times in aging cultures compared to young ones. Bronchogen administration increased Pdx1 expression in young and aged cultures by 10% compared to the controls. Pancragen expanded the area of Pdx1 expression in young cultures 1.3-fold, in old ones, 2.6-fold. Thus, pancragen has a much more pronounced stimulatory effect on Pdx1 expression compared with bronchogen.

Differentiation factors Pax6, Foxa2, and Nkx2.2B are involved in the maturation of α-, β-, δ-, and PP-cells in PG. The area of their expression reduced 2-4-fold in old cultures compared with young ones (Table 1).

Pancragen did not change significantly the area of expression of Foxa2 marker in young cultures and increased 1.3 times that of Pax6 and Nkx2. Under the action of pancragen, the area of Foxa2, Nkx2.2, and Pax6 expression increased by 1.6, 2, and 2.3 times respectively in aged cultures compared to the control. Bronchogen had no effect on the area of Foxa2 marker expression in young and aged cultures. At the same time, bronchogen had weak stimulatory effect on the on the area of expression of Nkx2.2 marker in young cultures and of Pax6, in aged cultures.

Cell ageing was found to dramatically reduce the concentration of Pdx1 and Ptf1a proteins inducing the maturation of acinar and islet cells of PG. It is known that decreased Ptf1a expression was registered in tumor cells during PG cancer, and the lack of Pdx1 protein or reduction in its concentration inhibits the development of PG cells from pluripotent cells [2,10]. Pancragen up-regulated Pdx1 and Ptf1a expression in aged cultures to their levels in young ones. Thus, pancragen increases the number of $Ptf1a^+$ early precursors of PG acinar cells.

In addition, pancragen increased marker expression in precursors of Pdx1, Pax6, Pax4, Foxa2, and Nkx2.2. Consequently, pancragen stimulates differentiation of various PG islet cells, thereby restoring the synthesis of insulin, glucagon, somatostatin, and pancreatic polypeptide. The area of marker Pax4 expression δ-cell precursors remained practically unchanged in ageing cultures and after pancragen introduction, which is consistent with the findings of other researchers [11].

Thus, pancragen peptide promotes multidirectional differentiation of PG cells. Genes and encoded transcription and differentiation factors involved in the development of different types of PG cells and regulating their enzymatic and hormonal activity are pharmacological targets for pancragen. The inductive action of pancragen on the differentiation and functional activity in acinar and islet cells of different allows us to consider it as a promising tool for the treatment of metabolic abnormalities including diabetes mellitus and pancreatitis.

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