# BIOGERONTOLOGY

# Peptides Regulate Expression of Signaling Molecules in Kidney Cell Cultures during *In Vitro* Aging

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 157, No. 2, pp. 227-230, February, 2014
Original article submitted September 3, 2013

We studied the effect of polypeptide complex isolated from calf kidney and short peptides T-31 (AED) and T-35 (EDL) on the expression of signaling molecules, markers of cell renewal (Ki-67, p53), remodeling of the extracellular matrix (MMP-14), and immune response (IL-8) in primary kidney cell cultures during aging. The complex of renal polypeptides and T-31 peptide activate cell renewal processes during aging of the renal epithelium, while gelatinase MMP-14 is the target of T-35 peptide.

**Key Words:** *peptides; kidneys; signaling molecules; pathology; cellular senescence*

The incidence of renal diseases is about 6% in humans above 60 years. More than 60% of them develop renal pathology in young or middle age [2]. Renal disease in elderly and senile patients is featured by increasing prevalence of angionephrosclerosis, pyelonephritis, and combined lesions of the kidneys that constitute about $^{4}/_{5}$ of the total renal pathology. All this promotes chronic and acute renal failure that reduces quality of life and increases mortality in individuals over 60 years [5]. Antibiotics and chemotherapy drugs constitute ~64% of drugs prescribed to patients with renal disease [3]. Most of them are nephrotoxic and cannot be recommended for individuals in older age groups. Peptide bioregulators of natural and synthetic origin having a tissue-specific action are a new promising class of drugs [2,6,12].

The technology of isolation of the polypeptide complex from calf kidney was developed at St. Petersburg Institute of Bioregulation and Gerontology for correction and prevention of various renal diseases [13]. Moreover, preliminary experiments have demonstrated that the polypeptide complex and short peptides T-31 (AED) and T-35 (EDL) stimulated the growth of organotypic cultures of kidney tissue from young and old animals. It was also found that the peptide complex isolated from pig kidney and its synthetic analogues (PEKDLRK, PEKDSRK, PEKDDRL) exhibit biological activity in autoimmune nephritis in animals [3]. The studied short peptides showed different nephroprotective activity. The peptide complex isolated from pig kidney reduced the urine oxalate level, amount of calcium deposits in the kidney tissue, and intensity of free radical oxidation in animal model of experimental nephrolithiasis [1]. Thus, a comparative study of the molecular mechanisms underlying the action of peptide bioregulators of natural and synthetic origin is an important aspect to identify their targets in the development of renal disease.

Here we compared the influence of the peptide complex isolated from calf kidney and peptides T-31 and T-35 on the expression of signaling molecules,

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markers of renal pathology, in aging cultures of kidney cells.

## MATERIALS AND METHODS

Primary cells cultures were isolated from the tissue of kidneys of young 3-month-old Wistar rats. Slices of kidney tissue were embedded into 10 ml of 0.2% collagenase II. The resulting cell suspension was transferred to Petri dishes treated with gelatin solution (BioloT). Culturing was performed in 50-ml precoated flasks (JetBiofil, BioloT). The cells were cultured in 5 ml of culture medium (DMEM+15% FCS+1.5% HEPES-buffer+antibiotics: 50,000 U/flask of penicillin G and 50 mg/flask of streptomycin+L-glutamine) per flask and in 3 ml of culture medium per Petri dish 3.5 cm in diameter. Trypsin-versene solution in proportion of 1:3 (BioloT) was used to reseed the cells. Cell concentration at seeding was $5\times10^3$ per flask. Cells were passaged after attaining 80% confluence on day 4 after seeding. Solution with peptides was added to the growth medium at each reseeding. Cells were cultured until passage 14, when the immunocytochemical staining was performed. Cultures of passage 14 were considered as aged cultures according to recommendations of International Association of Cell Culture Studies (San Francisco, USA, 2007). The cultures were divided into four groups. Saline was added to control cultures (group 1); peptide T-31 (AED), to cultures in group 2; peptide T-35 (EDL), to group 3, and the polypeptide complex isolated from calf kidney, to group 4. Peptides T-31 and T-35 were added to the culture medium at the concentration of 20 ng/ml, and polypeptide complex, at 100 ng/ml.

For immunocytochemical staining of the complex of renal polypeptides, primary monoclonal antibodies to Ki-67 (1:50, Novocastra), p53 (1:50, Novocastra), IL-8 (1:60, Novocastra), and MMP-14 (1:75, Novocastra) were used because these signaling molecules play an important role in the development of renal pathology. The expression of proliferotropic protein Ki-67 decreases with renal pathology (urethral obstruction) and increases after the successful treatment [14,15]. Urine level of cytokine IL-8 increases 5-10 times in patients with kidney disease, hence, this parameter can serve as a predictor of renal pathology [4,9]. Expression of proapoptotic factor p53 increases during acute renal failure, and this process is expressed most intensely during aging [10,11]. The expression of MMP, including MMP-14 in the glomeruli and renal interstitium is increased at the stage of nephropathy preceding tubulointerstitial fibrosis and decreases with the further development of the disease [15].

The data were morphometrically evaluated with the system of computer analysis of microscopic images including Nikon Eclipse E400 microscope, Nikon DXM1200 digital camera, and software VideoTest Morphology 5.2. The relative area of expression was calculated as the percentage of the ratio of the area occupied by immunopositive cells to the total area of cells in the field of view.

Statistical processing of the data including calculation of the arithmetic mean, standard deviation from the mean and the confidence interval for each sample was carried out using Statistica 7.0 soft. For the analysis of the distribution and checking the null hypothesis, Shapiro–Wilk test was used. nonparametric ANOVA procedure (Kruskal–Wallis test) was used to assess the statistical homogeneity of multiple samples. Differences between the groups were considered statistically significant at $p<0.05$.

## RESULTS

The complex of renal polypeptides increased expression of Ki-67 twice in aging kidney cell cultures, short peptides T-31 and T-35 did not exert such an effect (Fig. 1).

The synthetic T-31 peptide and the complex of renal polypeptides reduced p53 expression 1.33- and 1.42-fold, respectively, in aged cell cultures (Figs. 1 and 2). IL-8 expression was not changed after peptides were added to the culture. Altered expression of this chemokine is usually associated with severe inflammatory reactions in kidney. Therefore, it is logical that changes in expression of IL-8 cannot be detected in cultures of normal kidney epithelial cells. T-35 peptide increased the expression of gelatinase MMP-14 1.52 times in aging kidney cell cultures, wherein T-31 peptide and the complex of renal polypeptides had no such an effect (Fig. 1).

Thus, all tested peptides affected the expression of signaling molecules, which level of synthesis

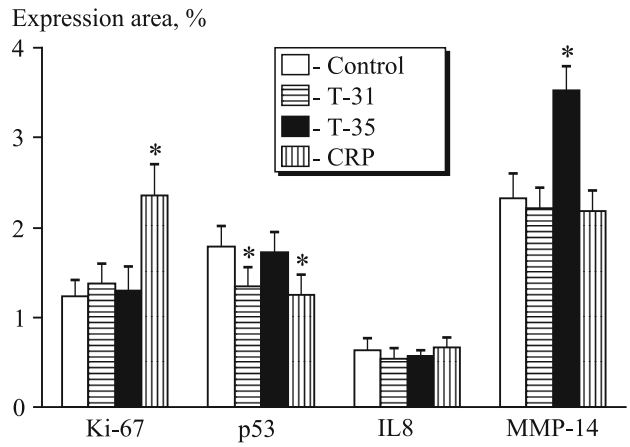


**Fig. 1.** Effect of peptides on the expression of signaling molecules in dissociated kidney cell cultures at the 14th passage (aged cell cultures). CRP, complex of renal polypeptides.

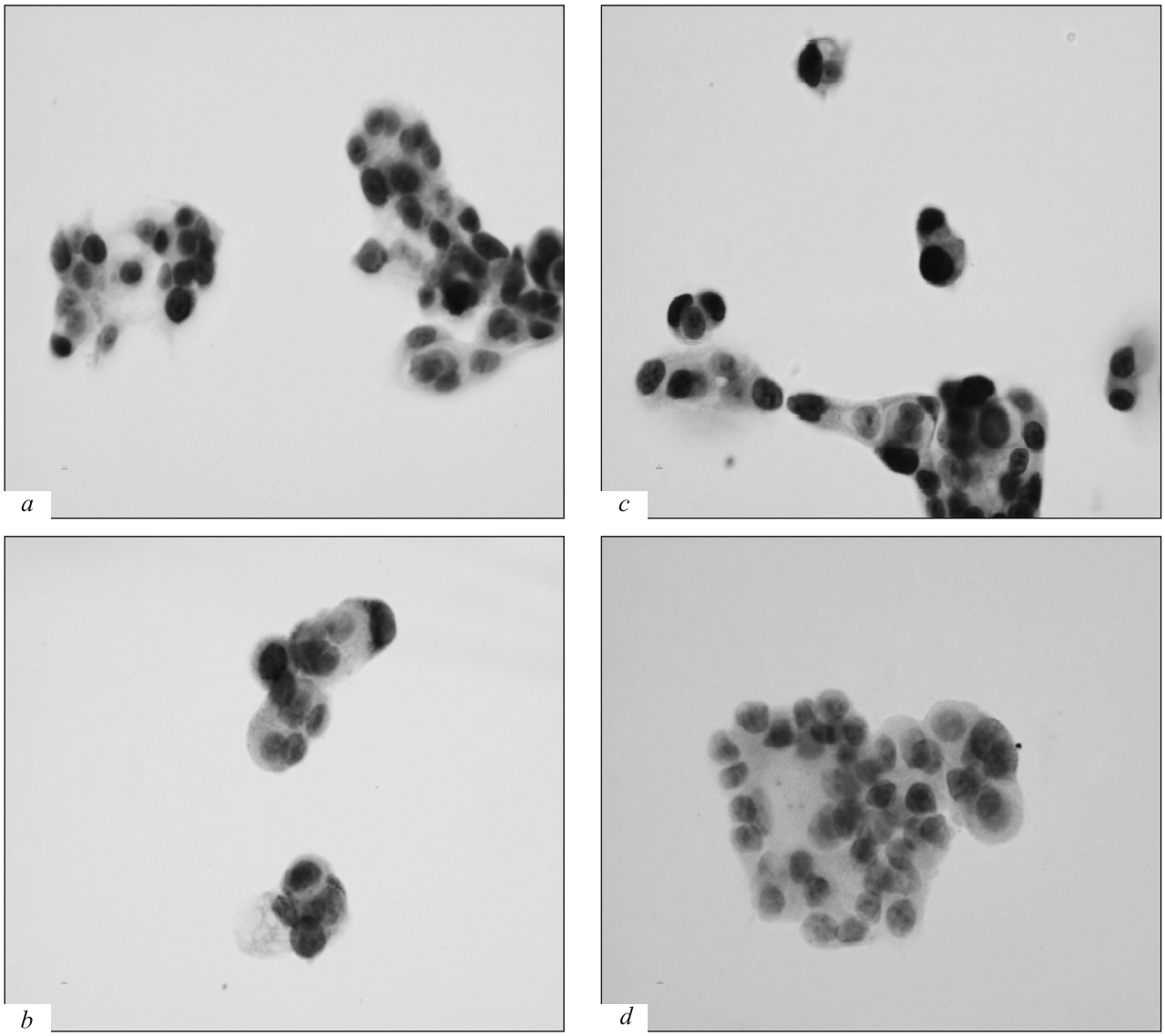


**Fig. 2.** Effect of peptides on p53 expression of in kidney cell cultures at the 14th passage (aged cell cultures). Immunocytochemical staining, ×200. *a*) Control; *b*) T-31 peptide; *c*) T-35 peptide; *d*) complex of renal polypeptides.

changes in renal pathology. However, these peptides have different molecular targets. Thus, the complex of renal polypeptides and T-31 peptide are unidirectional, increasing the proliferative capacity by almost 2 times and lowering the level of apoptosis in the aging cells of the renal epithelium by 1.5 times. Gelatinase MMP-14 is the target of T-35 peptide. Its expression increased 1.5-fold in renal epithelia under the action of this peptide. In this, T-35 peptide did not affect the proliferation and apoptosis in kidney cell cultures.

The obtained data indicate that the complex of renal polypeptides and T-31 peptide activating cell renewal may be considered as potential hero protective drugs for correction of age-related pathology of the kidneys. T-35 peptide, which targets are MMP and probably their inhibitor TIMP [7,8], may be investigated as potential medication for the treatment of tubulointerstitial fibrosis.

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