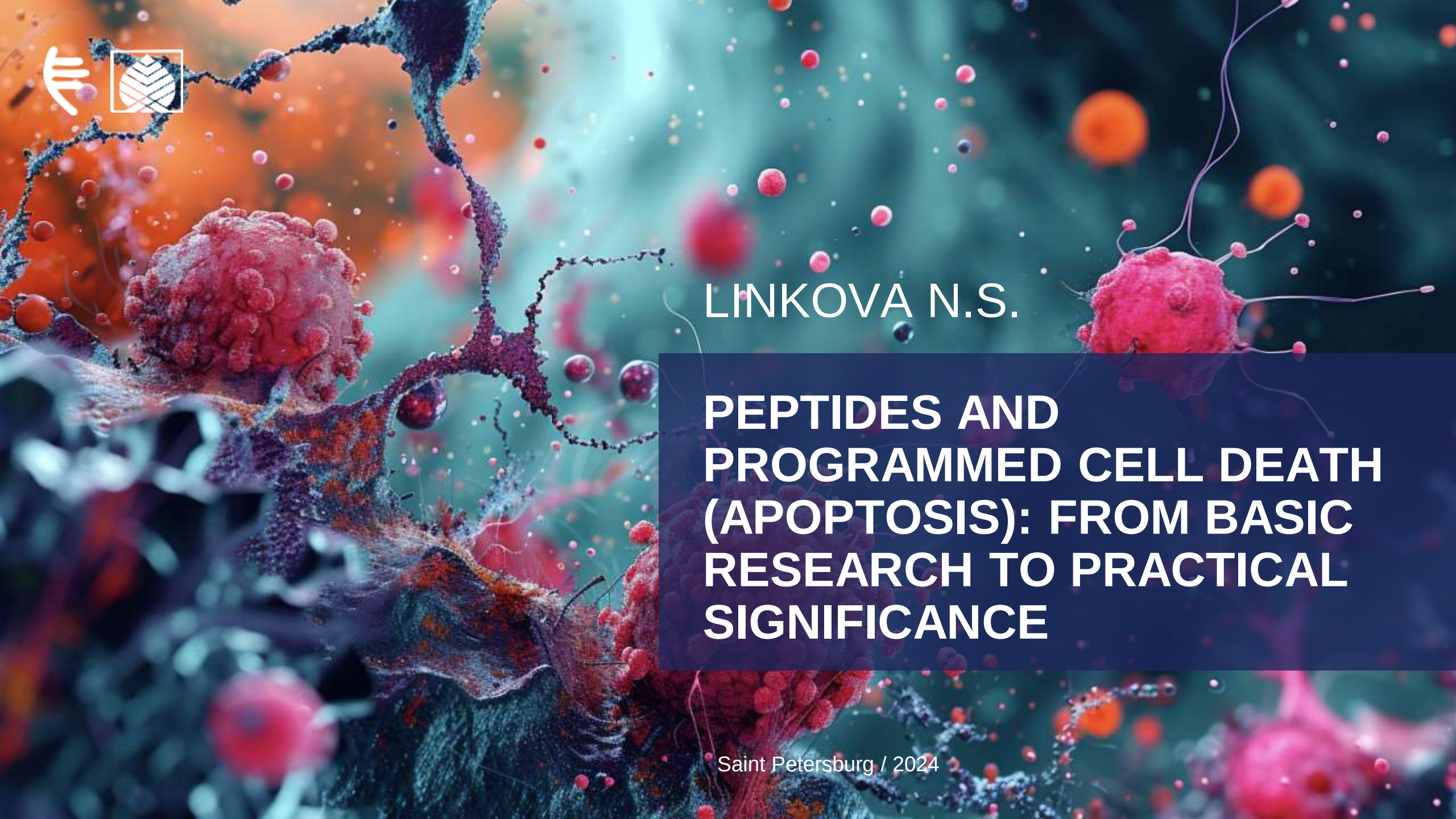


LINKOVA N.S.

# PEPTIDES AND PROGRAMMED CELL DEATH (APOPTOSIS): FROM BASIC RESEARCH TO PRACTICAL SIGNIFICANCE

Saint Petersburg / 2024

Saint Petersburg Institute of Bioregulation and Gerontology

# PEPTIDES AND PROGRAMMED CELL DEATH (APOPTOSIS): FROM BASIC RESEARCH TO PRACTICAL SIGNIFICANCE

Professor Natalia Linkova

Saint Petersburg / 2024

# APOPTOSIS

**APOPTOSIS** (from the ancient Greek word meaning "leaf fall") is a physiologically programmed cell death.

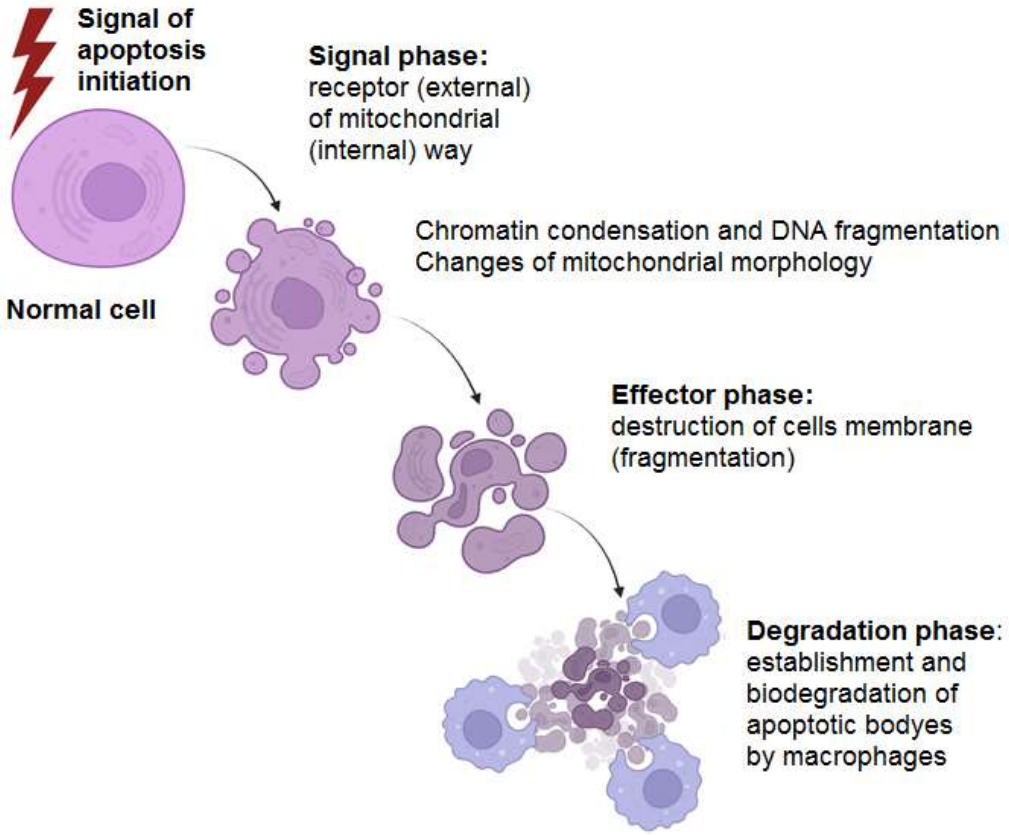
- John Kerr described the process of lymphocyte death under the action of glucocorticoids and called it apoptosis in 1972.
- Sidney Brenner, Robert Horwitz and John Sulston were awarded the Nobel Prize for their research on apoptosis in 2002.

**Is apoptosis always a bad thing and what biological meaning does this phenomenon have?**

**The positive effects of apoptosis** include protection from the formation of cancer cells, the replacement of aging, functionally defective cells with more "young" cells that perform their functions well.

**The negative effects of apoptosis** include cell death in pathological processes and during aging. There are not replaced by a sufficient number of more "young" and functionally active cells in these cases.

# Apoptosis stages



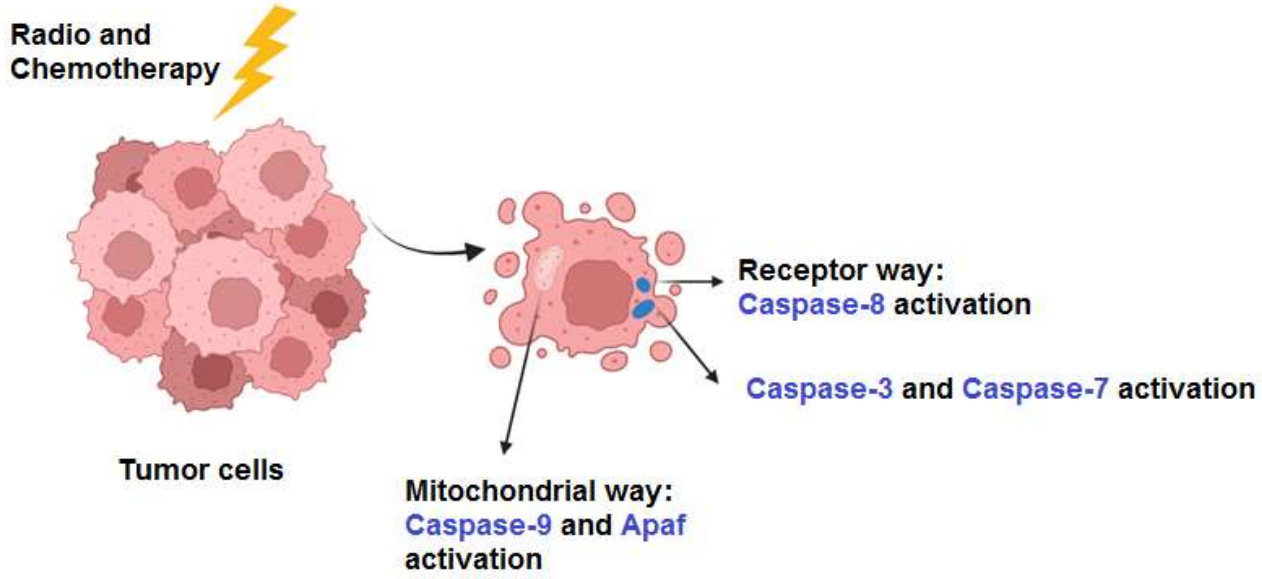

- First, cell receives an intracellular or extracellular signal to trigger apoptosis. Extracellular signals include glucocorticoid hormones, TNFa and Fas ligand receptors.
- Intracellular signals of apoptosis include DNA damage that cannot be repaired and damage of cell membranes. Damage of DNA and cell membranes can be caused by the action of chemical poisons, ionizing radiation, and various pathological processes.
- At the beginning, the signaling phase of apoptosis develops. It includes the receptor (external) or mitochondrial (internal) way of apoptosis.
- These signal ways lead to chromatin condensation, DNA fragmentation, and disruption of mitochondrial structure. Next, the effector phase of apoptosis is observed – the destruction of the cell membrane.
- The final stage is the degradation phase – the formation of apoptotic bodies and their destruction by macrophages.



Diatlova A.S., Dudkov A.V., Linkova N.S., **Khavinson V.Kh.** Molecular Markers of Caspase-Dependent and Mitochondrial Apoptosis: Role in the Development of Pathology and Cellular Senescence. Biol Bull Rev. 2018; 8: 472–481.

# Receptor (external) apoptosis way

The signal for the onset of receptor (external) apoptosis may be hypoxia, exposure to physical or chemical factors, disruption of cell cycle in various diseases and aging. Activation of apoptosis with the participation of cell death receptors can be carried out by immune cells.

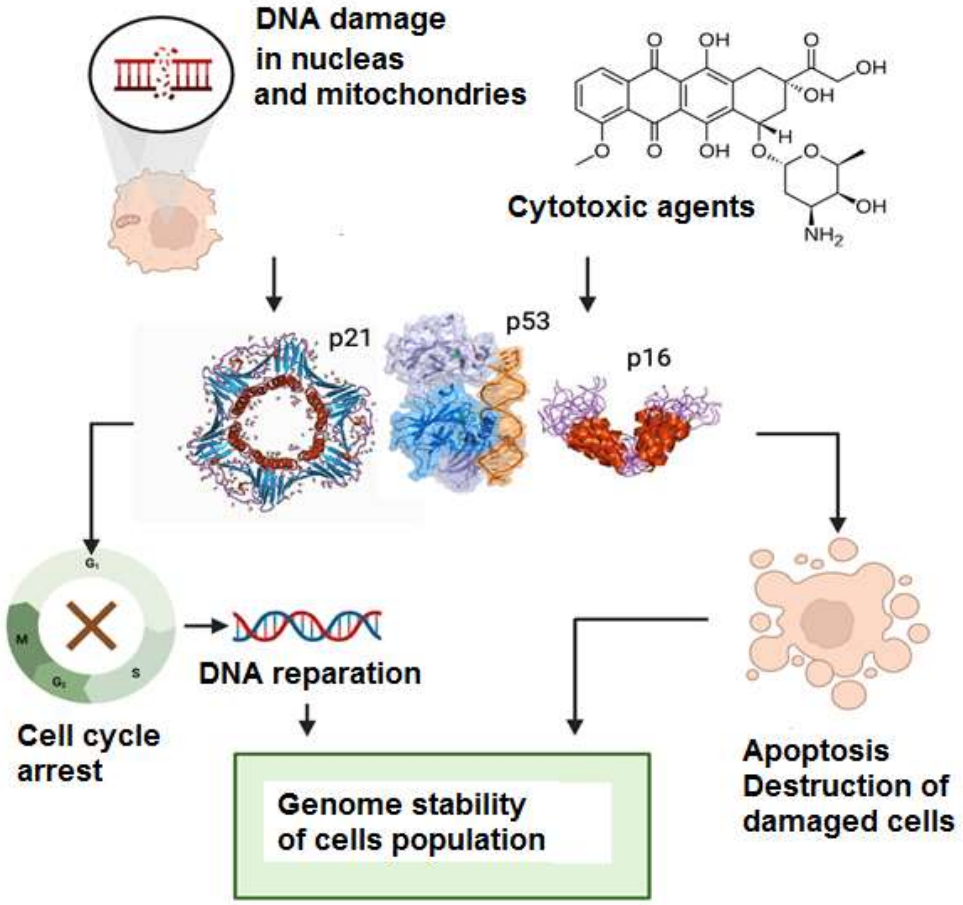



One example of triggering receptor apoptosis is chemo- and radiotherapy aimed at destroying cancer cells. **Key molecules of apoptosis** are proteins - **Caspases – 3,-7** and **-8**.


The figure was adapted from Huang Q., Li F., Liu X., et al. Caspase 3-mediated stimulation of tumor cell repopulation during cancer radiotherapy. Nat Med. 2011;17(7):860-866.

Diatlova A.S., Dudkov A.V., Linkova N.S., **Khavinson V.Kh**. Molecular Markers of Caspase-Dependent and Mitochondrial Apoptosis: Role in the Development of Pathology and Cellular Senescence. Biol Bull Rev. 2018; 8: 472–481.

# Mitochondrial (external) apoptosis way




- DNA damage in the nucleus and mitochondria under the influence of pathological processes or chemicals leads to the activation of **p53, p16 and p21 proteins**.
- P53 activates proteins of the Bcl-2 family - regulators of the permeability in the membrane of mitochondria.
- After that, cytochrome C, procaspases-2, -3, -9 and AIF protein (apoptosis inducing factor) are released from the intermembrane space of mitochondria inside cell.
- The next step in forming of apoptosome. This stage characterized by **Caspase-9** activation.

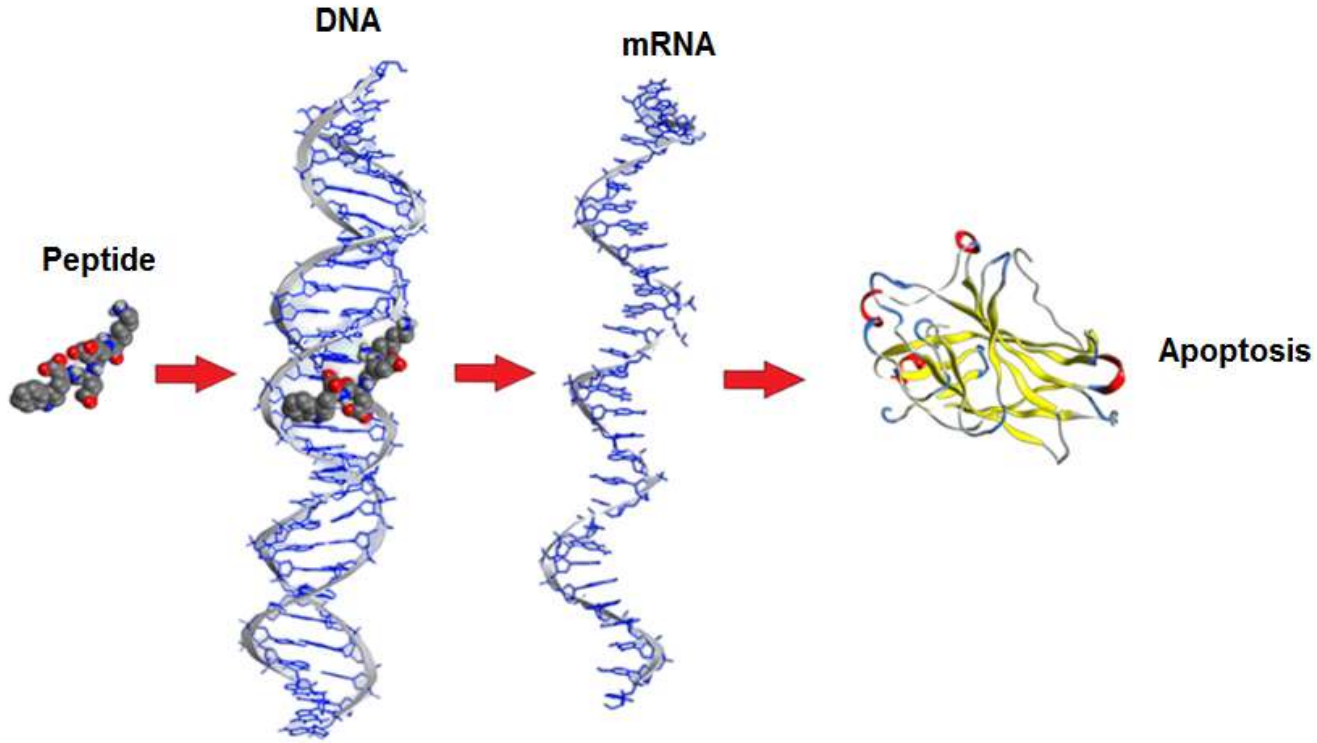
Diatlova A.S., Dudkov A.V., Linkova N.S., **Khavinson V.Kh**. Molecular Markers of Caspase-Dependent and Mitochondrial Apoptosis: Role in the Development of Pathology and Cellular Senescence. Biol Bull Rev. 2018; 8: 472–481.

# Apoptosis peptide regulation

Short peptides consisting of 2, 3 and 4 amino acid residues (Chonluten, Vesugen, Epithalon, Thymogen) can interact with the trigger zones (promoters) of apoptosis genes in DNA. This interaction changes synthesis of matrix RNA (mRNA) and synthesis of apoptosis proteins.

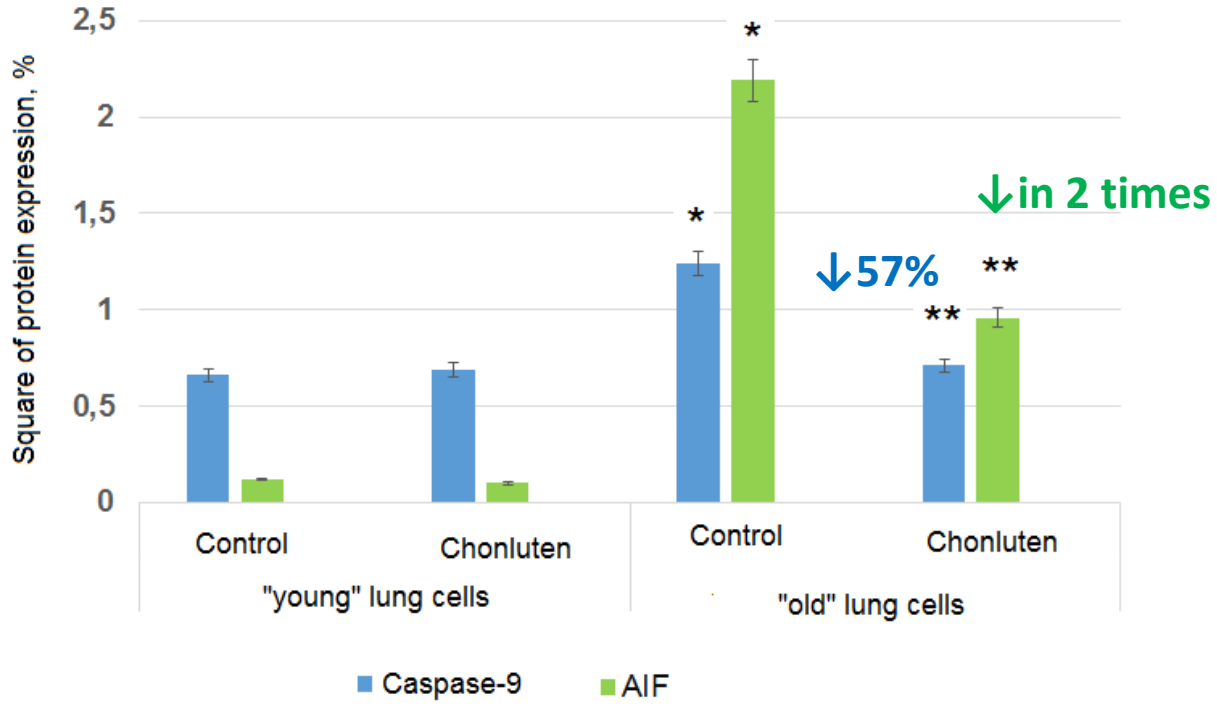
Chonluten, Vesugen, Epithalon and Thymogen regulate apoptosis protein synthesis in various types of cell cultures:

- p16
- p21
- p53
- Caspase-8
- Caspase-9
- AIF



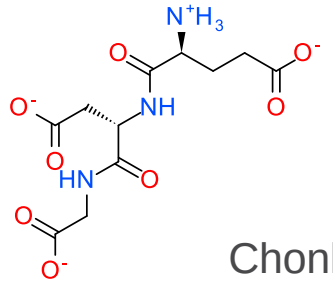
# Chonluten reduces synthesis of mitochondrial apoptosis proteins in human bronchial epithelial cell culture during aging

Human bronchial epithelial cells of the FLECH line were obtained from the Russian Collection of vertebrate cell cultures of the Institute of Cytology of the Russian Academy of Sciences (St. Petersburg). Cells were grown up to the 3rd passage ("young" cells) and up to the 14th passage ("old" cells).




* – p<0.05 in comparison with he control in "young" cells
** – p<0.05 in comparison with he control in "old" cells

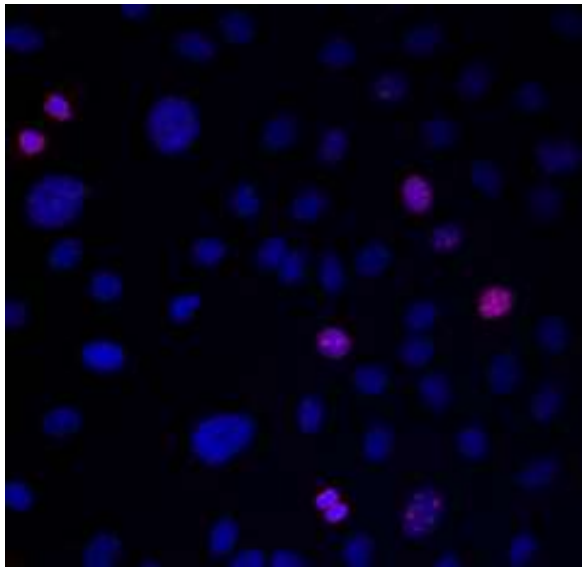
- Synthesis of Caspase-9 increased by 2 times and synthesis of AIF increased by more than 10 times during aging of human bronchial epithelial cells.
- Chonluten reduced the synthesis of Caspase-9 by 57% and AIF - by 2 times in "old" human lung cells.



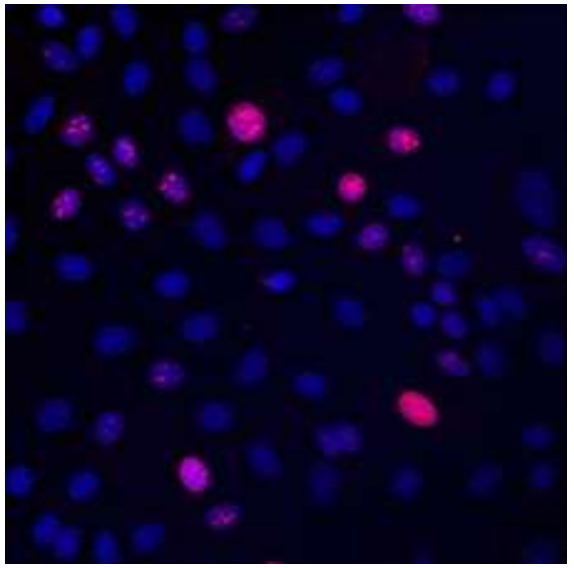

Chonluten (EDG tripeptide)

Dudkov A.V. Peptide regulation of caspase-dependent apoptosis in cellular aging. Modern problems of science and education (In Russian). 2019; 2: 1-11. http://www.science-education.ru/article/view?id=28701.

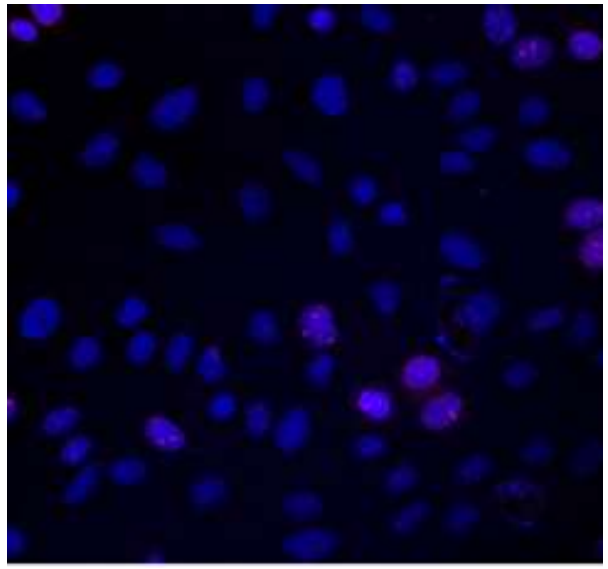
# Chonluten decreases Caspase-9 synthesis in human bronchial epithelium cells during aging



Control, «young» cells: weakly expressed synthesis of Caspase-9



Control, «old» cells:
strong expressed synthesis of Caspase-9

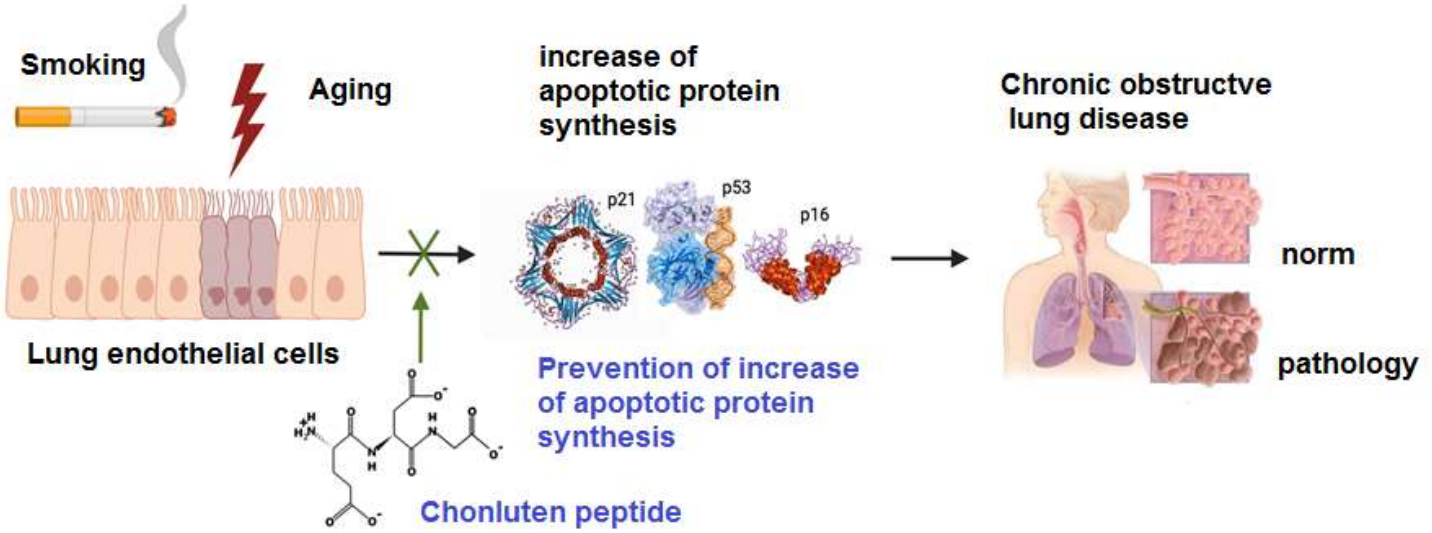


Chonluten, «old» cells:
weakly expressed synthesis of Caspase-9 (on the control level)

Immunofluorescence confocal microscopy of human bronchial epithelial cell culture (FLECH line), x200. The 3rd passage – "young" cells; the 14th passage – "old" cells. The nuclei of the cells are dark blue fluorescence, the expression of caspase-9 is pink fluorescence.

# Chonluten: regulation of apoptosis and prevention of lung diseases

- Aging of lung tissue (similar to the senescence of cells in culture) is characterized by an increase of synthesis of proapoptotic proteins in epithelial cells – Caspase-9 and AIF.
- Chonluten reduces synthesis of these proteins, preventing apoptosis of lung cells. Smoking leads to activation of mitochondrial apoptosis in lung tissue and the development of various pulmonary pathologies [Künzi L. et al., 2019].
- Chonluten, prevents the development of lung diseases by reducing synthesis of AIF apoptosis protein. Chronic obstructive pulmonary disease (COPD) is characterized by increased synthesis of Caspases in the molecular level [Pandey K.C. et al., 2017]. Chonluten helps to prevent the development of COPD by reducing synthesis of Caspase-9.



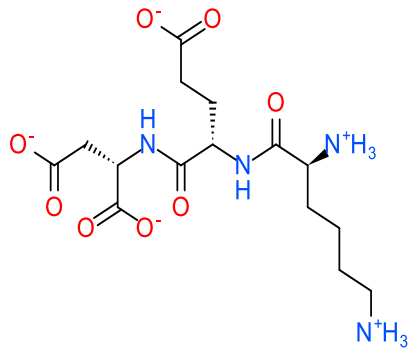

Pandey K.C., De S., Mishra P.K. Role of Proteases in Chronic Obstructive Pulmonary Disease. Front. Pharmacol. 2017. 8: 512.

Künzi L., Holt G.E. Cigarette smoke activates the parthanatos pathway of cell death in human bronchial epithelial cells. Cell Death Discov. 2019. 5:127.

# Vasoprotective KED tripeptide Vesugen reduced synthesis of apoptosis proteins in the culture of rat vascular cells (endotheliocytes) during aging

Cells were grown up to the 3rd passage ("young" cells) and up to the 14th passage ("old" cells). All cultures were divided into 2 groups: control and addition of KED peptide at the concentration of 100 ng/ml. The area of expression of p16, p21 and p53 proteins increased by 1.3-3.5 times during aging of vascular endothelial cells. The KED peptide reduced the synthesis of these proteins almost to the level of "young" cell cultures.

| Apoptotic protein | The area of apoptotic protein expression, % | | | |
|---|---|---|---|---|
| | «Young» cells | | «Old» cells | |
| | Control | KED peptide | Control | KED peptide |
| p16 | 0.78±0.12 | 0.70±0.11 | 2.75±0.34*<br>**↑ in 3.5 times** | 1,12±0,10**<br>**↓ in 2.5 times** |
| p21 | 0.98±0.16 | 0.77±0.10 | 2,13±0,32*<br>**↑ in 2.2 times** | 1,69±0,16**<br>**↓ in 1.3 times** |
| p53 | 1.18±0.20 | 1.04±0.09 | 2,02±0,14*<br>**↑ in 1.7 times** | 1.18±0.14**<br>**↓ in 1.7 times** |



Vesugen
(KED tripeptide)

* – p<0.05 in comparison with he control in "young" cells
** – p<0.05 in comparison with he control in "old" cells

Dudkov A.V. Peptide regulation of caspase-dependent apoptosis in cellular aging. Modern problems of science and education (In Russian). 2019; 2: 1-11. http://www.science-education.ru/article/view?id=28701.

# Vesugen: regulation of apoptosis and prevention of vascular diseases

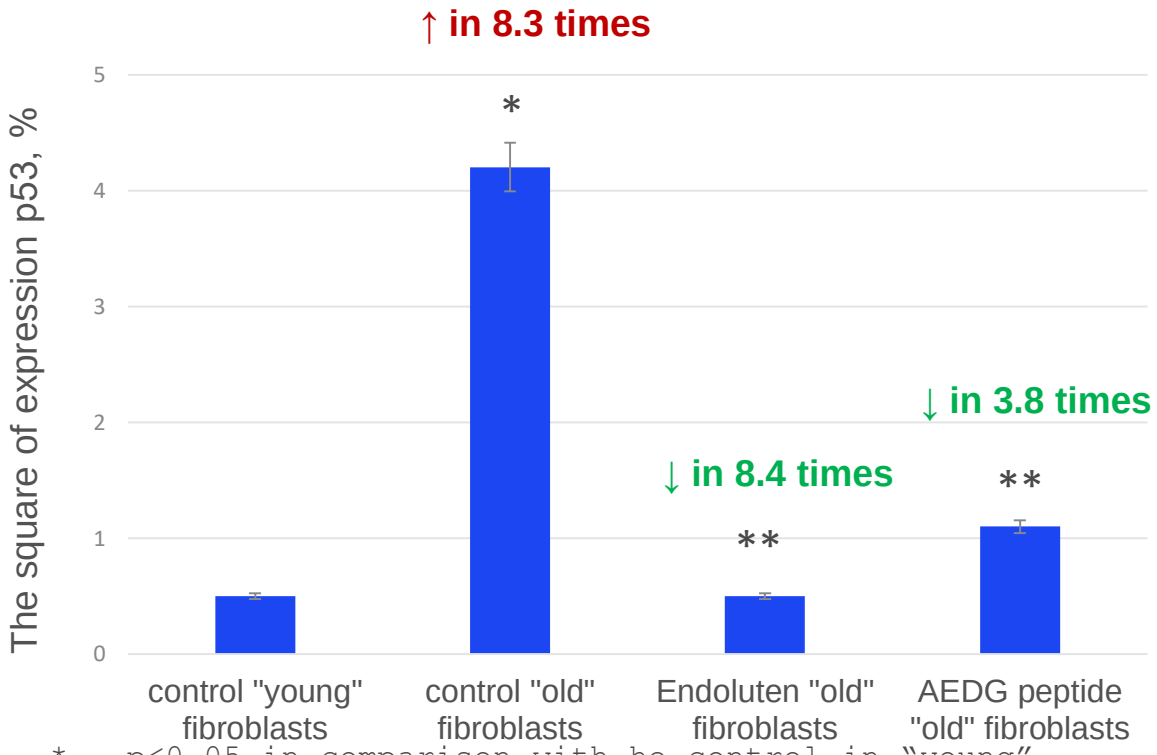
- It is known that synthesis of p16, p21 and p53 apoptosis proteins increases with accelerated aging of vascular cells. This can lead to the development of atherosclerosis and other vascular diseases. The KED peptide reduces synthesis of apoptosis proteins. In this regard, the KED peptide can be used to prevent the development of atherosclerosis and its consequences – coronary heart disease and myocardial infarction. This information was described in the review article in the highly rated Journal «Cells» by MDPI Publishing House.

- The articles by K.V. Kitachev and co-authors present data of the successful clinical use of Vesugen in capsules in 41 men aged 65-80 years with lower limb ischemia and erectile dysfunction. These diseases can occur due to vascular endotheliocyte apoptosis. The decrease of apoptosis in endotheliocyte under the action of the KED peptide is a molecular mechanism of its vasoprotective effect in vascular diseases.


Kitachev K.V., Sazonov A.B., Kozlov K.L., Petrov K.Iu., Sliusarev A.S., **Khavinson V.Kh**. The efficacy of peptide bioregulators of vessels in lower limbs chronic arterial insufficiency treatment in old and elderly people. Adv Gerontol. 2013; 26(2): 292-296.

Kitachev K.V., Sazonov A.B., Kozlov K.L., Petrov K.Iu., Sliusarev A.S., **Khavinson V.Kh.** The effectiveness of the peptide vascular bioregulator in the complex therapy of vasculogenic erectile dysfunction in the elderly and senile. Adv Gerontol. 2014; 27(1): 156-159.

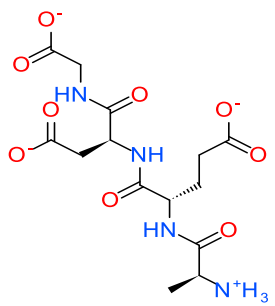

# The AEDG peptide and Endoluten decrease p53 apoptotic protein synthesis in skin fibroblasts during aging



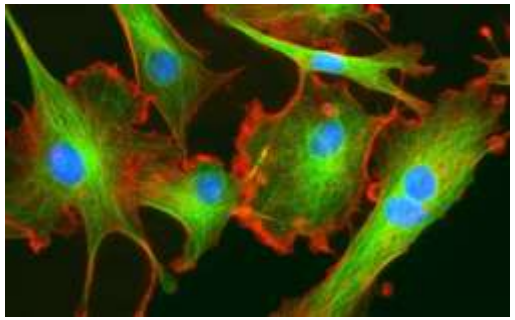

* – p<0.05 in comparison with he control in "young" cells

** – p<0.05 in comparison with he control in "old" cells

- The pineal gland polypeptide complex Endoluten and the AEDG tetrapeptide Epithalon in its compound reduced synthesis of p53 apoptosis protein in the culture of dermal rat (cutaneous) fibroblasts during aging.
- The more pronounced effect of Endoluten compared to the AEDG peptide is due to the fact that in addition to the AEDG peptide, it contains a large number of other short peptides and amino acids.




AEDG tetrapeptide



Skin fibroblasts

Lin'kova N.S., Drobintseva A.O., Orlova O.A., Kuznetsova E.P., Polyakova V.O., Kvetnoy I.M., **Khavinson V.Kh.** Peptide Regulation of Skin Fibroblast Functions during Their Aging In Vitro. Bull. Exp. Biol. Med. 2016; 161(1): 175-178.

# Endoluten: regulation of apoptosis and prevention of skin aging

- The applying of Endoluten capsules in combination with tripeptides increased the thickness of the epidermis and dermis and improved the appearance of the skin according of the clinical study in 30 middle-aged and elderly women.
- This effect of Endoluten is explained by a decrease of dermal fibroblasts apoptosis during their aging, revealed in experiments on cell cultures.
- The applying of the pineal gland polypeptide complex (Endoluten in capsules or Revilab evolution series cream) will prevent age-related skin changes.


Fridman N., Bojko L., Trofimova S. Peptide bioregulators applying perspectives for skin structure restoration in middle aged women. Vrach. 2020. Vol. 31, N 9. P. 63–67 (In Russian).

Fridman N.V., Linkova N.S., Bojko L.V., Kacheli M.A. The influence of peptide bioregulators on the structural and functional specific of face skin in elderly women. Molekulyarnaya meditsina. 2021. Vol. 19, N 4. P. 42–46 (in Russian).

# EW peptide, as the part of the polypeptide complex of thymus Vladonix, reduces synthesis of apoptosis proteins in human blood lymphocytes during aging

It has been established that the EW peptide (Thymogen drug), which is part of the polypeptide complex of thymus Vladonix, reduces synthesis of apoptosis proteins in human blood lymphocytes during aging. Lymphocytes were grown up to the 3rd passage ("young" cells) and up to the 14th passage ("old" cells).

| Apoptotic protein | The area of apoptotic protein expression, % | | |
|---|---|---|---|
| | «Young» cells | «Old» cells | |
| | Control | Control | EW peptide |
| p16 | 0.31±0.05 | 3.12±0.23*<br>↑ in 10.1 times | 2.18±0.11**<br>↓ in 1.4 times |
| p21 | 0.29±0.07 | 2.20±0.15*<br>↑ in 7.6 times | 1.53±0.12**<br>↓ in 1.4 times |
| p53 | 0.39±0.06 | 2.88±0.25*<br>↑ in 7.4 times | 0.95±0.14**<br>↓ in 3 times |
| Caspase-8 | 0.25±0.04 | 0.99±0.09*<br>↑ in 4 times | 0.60±0.07**<br>↓ in 1.7 times |

* – p<0.05 in comparison with he control in “young” cells
** – p<0.05 in comparison with he control in “old” cells

The expression area of p16, p21, p53 and Caspase–8 apoptosis proteins was assessed in cells by immunocytochemistry method.

The expression area of p16, p21, p53 and Caspase-8 proteins increased in 4-10 times during aging of blood lymphocytes.

The EW peptide reduced synthesis of these proteins in "old" blood lymphocytes by 1.4 – 3 times.

Dudkov A.V. Peptide regulation of caspase-dependent apoptosis in cellular aging. Modern problems of science and education (In Russian). 2019; 2: 1-11. http://www.science-education.ru/article/view?id=28701.

# The use of Chonluten for the prevention and treatment of lung diseases

- Chonluten is recommended to use 1-2 capsules in the morning before 30 minutes of meals during 1 month for the prevention of chronic obstructive pulmonary disease (COPD), including smoking. Chonluten is recommended to use in drops before 10-15 minutes of meals, 6-12 drops 1-2 times a day for the prevention of COPD. It is advisable to repeat the prophylactic course 2-3 time in the year.

- Chonluten is recommended to use 1-2 capsules 2 times a day before 30 minutes of meals during 2-3 months in the complex treatment of COPD. Chonluten is recommended to use in drops before 10-15 minutes of meals, 6-12 drops 3-4 times a day in the complex treatment of COPD. It is advisable to repeat the course after 1-2 months.

# The use of Vesugen for the prevention and treatment of vascular diseases

- Vesugen is recommended to use 1-2 capsules before 30 minutes of meals in the morning during 1 month for the prevention of atherosclerosis of blood vessels, coronary heart disease and erectile dysfunction. Vesugen is recommended to use in drops before 10-15 minutes of meals in the morning, 6-12 drops for the prevention of these diseases.  It is advisable to repeat the prophylactic course 2-3 time in the year.

- Vesugen  is recommended to use 1-2 capsules 2 times a day before 30 minutes of meals during 2-3 months in the complex treatment of atherosclerosis of blood vessels, coronary heart disease and erectile dysfunction. Vesugen is recommended to use in drops before 10-15 minutes of meals, 6-12 drops 3-4 times a day in the complex treatment of these diseases. It is advisable to repeat the course after 1-2 months.

# The use of Endoluten and Revilab Evolution series of creams for the prevention of facial skin aging

- It is recommended daily use of the Peptide revitalizer face cream (morning and evening) in combination with Revilab Anti-A.G.E. serum No. 5 (morning) and Eye-line filler cream for the skin around the eyes (evening).
- It is recommended to use Endoluten 1 capsule 1 time every 3 days before 30 minutes of meals in the morning during 1 month. The course of Endoluten can be repeated in addition to cosmetics after 4-6 months.



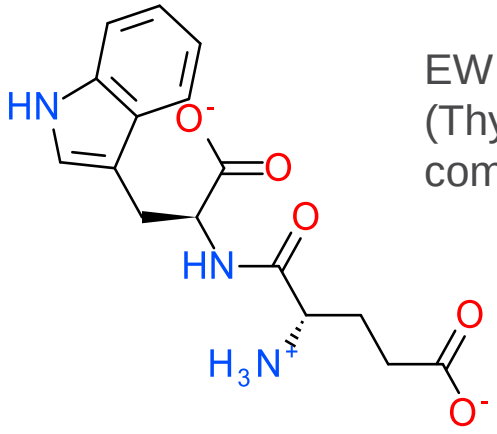

# The use of Vladonix for the prevention and treatment of diseases of the immune system

- Vladonix is recommended to use 1-2 capsules before 30 minutes of meals in the morning during
1 month for the prevention of infectious diseases. Vladonix is recommended to use in drops before 10-15 minutes of meals in the morning, 6-12 drops for the prevention of these diseases. It is advisable to repeat the prophylactic course 2-3 time in the year.

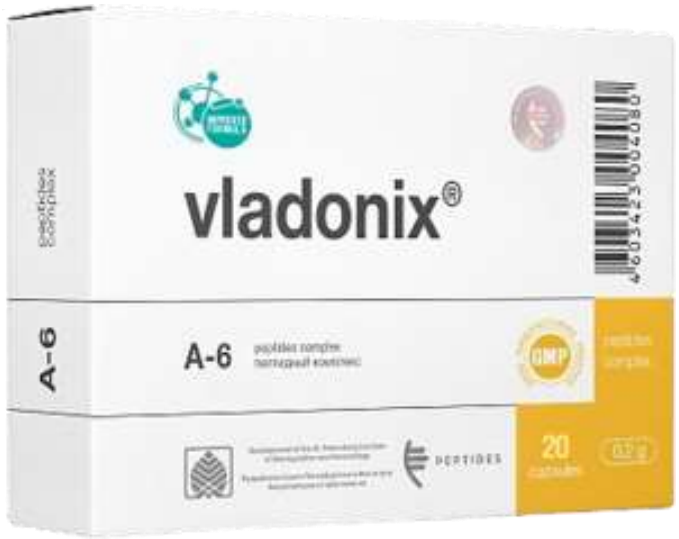
- Vladonix  is recommended to use 1-2 capsules 2 times a day before 30 minutes of meals during 2-3 months in the complex treatment of infectious diseases. Vladonix is recommended to use in drops before 10-15 minutes of meals, 6-12 drops 3-4 times a day in the complex treatment of these diseases. It is advisable to repeat the course after 1-2 months.

# Vladonix: regulation of apoptosis and normalization of immunity

Vladonix is applied for viral and bacterial infections, after courses of chemical and radiation therapy in cancer patients and during accelerated aging of the immune system. The active component of Vladonix is the drug Thymogen, EW dipeptide. The EW peptide in Vladonix compound reduces synthesis of p16, p21, p53 and Caspase-8 apoptosis proteins in blood lymphocytes during it aging. It can be concluded that this mechanism underlies the immunoprotective action of this polypeptide complex.




EW dipeptide (Thymogen) in the compound of Vladonix



# Conclusions

- Apoptosis is the programmed cell death in response to its aging or damage caused by a pathological process. Short peptides, by interacting with the promoter (trigger) zones of genes, regulate synthesis of apoptosis proteins. ***Peptide regulation of apoptosis is one of the most important mechanisms of the effectiveness of peptide bioregulators.***

- Chonluten (EDG peptide) reduces synthesis of apoptosis proteins (Caspase-9 and AIF) in lung cells. This explains the effectiveness of Chonluten in the prevention and treatment of chronic obstructive pulmonary disease.

- Vesugen (KED peptide) reduces synthesis of apoptosis proteins (p16, p21, p53) in vascular endothelial cells. This explains the effectiveness of Vesugen in the prevention and treatment of atherosclerosis.

- Endoluten (pineal gland polypeptide complex) and its active component, AEDG peptide, reduce the synthesis of p53 apoptosis protein in skin fibroblasts. This explains the effectiveness of Endoluten in capsule form and as the part of cosmetics to slow down aging and improve the appearance of the skin.

- Vladonix (thymus polypeptide complex) and its active component, EW peptide, reduce synthesis of apoptosis proteins (p16, p21, p53, Caspase-8) in blood lymphocytes. This explains the effectiveness of Vladonix in infectious diseases.

# Thank you for your attention!

Санкт-Петербург / 2023