Chapter 6

The Apoptosis Regulation Mechanisms in Hypothalamic Neurons in Physiological and Pathological (Overexpression of Oncogene HER-2/Neu) Aging

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Additional information is available at the end of the chapter

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Abstract

This study reveals the molecular regulation mechanisms of neurosecretory cell apoptosis in physiological and pathological (oncogene human epidermal growth factor receptor (HER)-2/Neu overexpression) aging. As we have shown previously, apoptosis level in hypothalamic neurosecretory centers increases in aging, and a low level of apoptosis in aged HER-2/Neu transgenic mice is associated with p53-dependent cascade suppression. In this chapter, we consider the participation of p53-regulating genes and p53 target genes in activation of this cascade during physiological aging, as well as suppression under HER-2/Neu overexpression. However, cell resistance to apoptosis may also be due to the activity of cytokine-dependent STAT-signaling pathway, including the high expression of survivin belonging to the family of inhibitors of apoptosis proteins (IAP). Also, another cytokine-dependent signaling, an extrinsic apoptosis pathway associated with the family of tumor necrosis factor (TNF) receptors, has been investigated. Thus, in the present work, three signaling cascades are considered: p53-dependent (the expression and interaction of apoptosis-associated proteins p53, WRN, pin1, p21, and caspase-3), STAT-mediated (STAT1, 3, 5, 6, and survivin), and TNF-dependent (CD95 (FAS), Fas-associated death domain (FADD), TNF receptor–associated death domain (TRADD), and caspase-8). These cascades are involved in both the activation of apoptosis and its suppression. This will reveal the general trends of regulation of neurosecretory cell apoptosis during aging.

Keywords: hypothalamus, neuron, aging, apoptosis, signal cascades

1. Introduction

This study reveals the molecular regulation mechanisms of neurosecretory cell apoptosis in physiological and pathological (oncogene human epidermal growth factor receptor (HER)-2/Neu overexpression) aging. In this chapter, we consider the participation of p53-regulating genes and p53 target genes in activation of this cascade during physiological aging, as well as suppression under HER-2/Neu overexpression. In addition, we consider cytokine-dependent pathways of apoptosis regulation (STAT-signaling pathway and tumor necrosis factor (TNF)-dependent pathway).

As shown at present, the process of apoptosis is an integral part of involutional tissue changes [1]. However, the mechanisms of senile apoptosis differ from the mechanisms of cell death in the early stages of ontogenesis. P53-mediated pathway of apoptosis plays a special role in aging, and its changes (excessive activation or suppression) cause severe pathologies, including neurodegenerative diseases and carcinogenesis. This is especially important for neurons and neurosecretory cells, which, as is known, are not capable of proliferation in the mass, so apoptosis is the only way to regulate their amount. Since the hypothalamic neurosecretory system is one of the main regulatory systems, the decrease in the amount of neurosecretory cells significantly changes the function of the hypothalamus and, consequently, of the target organs. Thus, the study of regulation of the p53-signaling path upon aging has particular importance.

DNA damage leads to the phosphorylation of p53 at the Ser/Thr-Pro sites, so the interaction of p53 with Ser/Thr-Pro sites of peptidyl-prolyl isomerase pin1 becomes possible [2–4]. Several studies have shown the suppressive role of prolyl isomerase pin1 in oxidative stress-induced apoptosis [5]. Pin1 involves in the stabilization of Mcl-1 and, further, in the prevention of apoptosis [6]. However, most authors report a high proapoptotic activity of pin1. Pin1 is necessary for the timely activation of p53, leading to apoptosis or cell arrest [4, 7–9]. It is known that pin1 participates in the realization of the cell cycle and mitosis in mammalian cells, transcription, and differentiation [10, 11]. In addition, overexpression of pin1 in various types of cancer tissue has been demonstrated [12, 13]. Pin1 plays an important role in cellular response to DNA damage [14], by conformational transformations participating in the transfer of the apoptotic stimulus and further initiation of the apoptotic cascade [15]. Pin1 isomerizes the bonds between molecules that are important for a variety of oncogenic and other signaling pathways in the cell, including Bcl-2, p53, c-Jun, beta-catenin, nuclear factor-kappa B (NF-kappaB), cyclin D1, c-Myc, and Raf-1. This can cause conformation changes leading to damage to catalytic activity, protein-protein interactions, subcellular localization, and protein stability. Similar changes have been shown to be associated with cell transformation and cancer development [3, 12, 13]. In addition, the absence of pin1 affects embryonic fibroblasts, leading to rapid genomic instability and the so-called immortality due to inactivation of p53 and further to aggressive transformation and carcinogenesis [16].

After attachment to pin1, p53 changes its transcriptional activity and increases the p21 transcription, which facilitates the cell arrest [3, 17]. Moreover, pin1 regulates the stability and transcriptional activity of p53 in relation to the p21 gene promoter [17]. P21 is known to be an inhibitor of cyclin-dependent kinases. Regulation of the cell cycle is an important part of development,

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differentiation, DNA repair, and apoptosis. P53-dependent expression of p21 due to DNA damage causes the arrest of the cell cycle in the late G1 phase [3, 18, 19]. In addition, p21 participates in cell aging [20, 21]. Besides, one of the genes, which play an important role in pathological involution process and take part in p53 regulation, is WRN, gene of Werner's syndrome.

However, neuron resistance to apoptosis may also be due to the activity of cytokine-dependent STAT-signaling pathway. Various molecules, including hormones, cytokines, and so on, participate in the regulation of apoptosis. As it is known, a significant change in the level of cell death is an important biological problem. Thus, a decrease in the level of apoptosis leads to oncogenesis, and an increase in the proportion of dying cells is the cause of diseases associated with tissue degeneration. Currently, it is considered a proven close connection between apoptosis and aging. To study the mechanisms of apoptosis associated with the aging, we used a line of transgenic mice with overexpression of the oncogene HER-2/Neu.

HER2, or ERBB2, belongs to the family of transmembrane tyrosine kinase receptors. After connecting to the ligand, members of this family form homo- or heterodimers and transmit the signal forward for activating a significant amount of cascades. Normally, ERBB receptors are involved in the processes of growth, differentiation, migration, and apoptosis. The extracellular domain of ERBB2 (HER2), unlike the HER1, 3, and 4 domains, has an open conformation and is normally capable of forming functionally active heterodimers with other HER receptors by carrying out and amplifying the signal, without preliminary binding to the ligand.

The HER-2/Neu overexpression in pathology promotes the formation of functionally active homo- and heterodimers, and, so, uncontrolled signal transduction [22] and it is characteristic of a number of tumors. The signal network, initiated by the interaction of ERBB family receptors with ligands, and its key elements regulating the direction and speed of signal transmission play an important role in the pathogenesis of tumor diseases [22].

We have previously shown that a low level of apoptosis of neurons upon aging in HER-2/Neu transgenic mice is associated with the suppression of the p53-dependent cascade. However, cell resistance to apoptosis may also be due to the activity of cytokine-dependent apoptosis-limiting systems (STAT-signaling pathway); these members are synthesized in various cell types, including neurons. At present, there is a report on the presence of a pro-inflammatory component in involutional changes of various tissues, including the brain tissues. It is shown that cellular stress and an inflammatory environment can trigger an immune response and provoke cell aging through epigenetic regulation involving STAT signaling [23]. However, there are almost no data characterizing age-related changes in the expression and activity of cytokines, the main mediators of inflammation. Some authors reported an increased pro-inflammatory reaction accompanying the involution processes, and other ones reported suppression of the inflammatory response at the cellular level, and about a decrease in neuroimmune interactions.

STAT proteins (signal transducers and activators of transcription) are transcription factors that conduct a signal from the cytoplasm to the nucleus. For the first time, proteins of this family were described in the 1990s of the twentieth century. Their usual ligands are cytokines, including interferons. In addition to cytokines, STAT are also activated by growth factors and growth factor receptors (a family of tyrosine kinase receptors, including HER-2) that stimulate STAT factors directly or indirectly through JAK kinases.

After activation, phosphorylation of the C-terminal domain of the STAT factors occurs, the homo- or heterodimers form, translocate into the nucleus, and activate DNA regions [24]. It is shown that STAT signaling is negatively regulated by two groups of proteins, one of them is suppressors of cytokine signaling (SOCs) and STAT-induced STAT inhibitors [25], or "proteins that inhibit activated STAT" [26]. The STAT family includes at least seven members—STAT1, 2, 3, 4, 5a, 5b, and 6 [27].

Although all members of the STAT family are structurally similar, they perform various biological roles, participating in such processes as embryonic development, inflammation, organogenesis, cell differentiation and control of cell growth [28, 29], regulation of immune processes, control of proliferation, and apoptosis (STAT3, 5) [30–32]. In many studies, it has been established that STAT proteins play a critical role in the activation of pro-inflammatory and antiproliferative processes (primarily, by the factor STAT1). Members of this family participate in the interferon gamma-induced response [33–35]. Information on the important role of STAT factors in the regulation of cell proliferation, differentiation, and survival suggests the active role of these proteins in malignancy.

However, most studies report about the participation of STAT factors in oncogenesis, but there are almost no data concerning the expression of STAT proteins in neurons with aging and their participation in the regulation of apoptosis. So, the purpose of the next part of the work was to reveal the role and molecular mechanisms of cytokine-dependent signaling in the mechanism of p53-dependent apoptosis suppression in the physiological and pathological (overexpression of the HER-2/Neu oncogene, epidermal growth factor receptor (EGFR)) aging and to investigate the causes of neuronal resistance to apoptosis with aging, possibly due to overexpression of HER-2/Neu.

Also, another cytokine-dependent signaling, an extrinsic apoptosis pathway associated with the family of tumor necrosis factor receptors, has been investigated.

Changes in cytokine expression are observed in various pathological conditions, so, TNF-alpha, a protein that plays a role in apoptosis, increases with oxidative stress [36].

As is known, tumor necrosis factor has a high antitumor and pro-inflammatory activity. Expression of the *tnf* gene activates the synthesis of the cytokine TNF-alpha (TNF-α), which regulates the processes of proliferation, apoptosis, immune cell activity, inflammation, embryo-, and carcinogenesis. Binding of TNF-α to cell death receptors leads to the activation of caspase-8, which initiates an apoptosis program. One of the receptors is the Fas-receptor (CD95, APO-1), whose main function is signaling to the development of apoptosis. In the case of Fas-dependent apoptosis, binding of the Fas ligand to the Fas receptor leads to conformational changes in the cytoplasmic domain of Fas receptor. This makes it possible to bind it to FADD-adapter molecule (Fas-associated death domain), and then to the same domain of the RIP protein (receptor-interacting protein). This complex activates caspase-8 (FLICE protease (FADD-like IL-1b-converting enzyme)), which means the development of apoptosis.

We supposed that the activity of a cytokine-dependent pathway associated with a family of tumor necrosis factor—the TNF-signaling pathway—can also be altered by aging. Only a few authors associate the TNF-dependent apoptotic pathway with aging or with the pathological

processes accompanying aging [37, 38]. So, it is reported that Fas signaling is activated in aged oocytes [39]; the extrinsic apoptotic pathway plays an important role in the development of macular degeneration in accelerated aged OXYS rats while the synthesis of proteins such as Fas, caspase-8, TRAIL increased [40], and in neurodegeneration in ischemia-reperfusion [41]. Apoptosis of the heart cells upon aging is induced via the Fas-FADD pathway, with a significantly suppressed survival signaling pathway which is associated with the insulin-like growth factor receptor (IGF1R), PI3K, and AKT kinases [42]. The senile neurodegeneration is an important clinical pathology, but there are almost no works devoted to the role of Fas-FADD and TNF-receptor-associated death domain (TRADD) pathway in neurons during physiological aging.

Thus, the aim of the present work is investigation of age-related changes of three signaling cascades, which are involved in both the activation of apoptosis and its suppression—p53-dependent (p53, WRN, pin1, p21, caspase-3), STAT-mediated (STAT1, 3, 5, 6, survivin), and TNF-dependent (FAS, FADD, TRADD, caspase-8). This will reveal the general trends of regulation of neurosecretory cell apoptosis during physiological and pathological aging.

2. Mechanisms of apoptosis regulation in ontogenesis: apoptosis signaling cascades in hypothalamic neurons in physiological and pathological (overexpression of oncogene HER-2/Neu) aging

2.1. Research methods

2.1.1. Animals

We studied a mouse model of accelerated aging, namely transgenic HER-2/Neu female mice, at the age of 2 and 10 months [43], obtained from the Italian National Research Center of Aging; the breeding is maintained at the Petrov Research Institute of Oncology (St. Petersburg, Russia). Outbred FVB/N female mice, descending from mice of the Swiss line (Rappolovo Nursery, Russian Academy of Medical Sciences, St. Petersburg, Russia), were 2 and 18 months of age (four to five mice in each group). The animal room was equipped with a 24-h light-dark cycle with 12:12 period.

The model of our study is transgenic mice with the overexpression of the transmembrane tyrosine kinase receptor HER-2/Neu, the wild type is the FVB/N line. The FVB/N line mice are often used to produce models of transgenic mice, since this line is characterized by high fertility and good survival of the embryo after injection. Overexpression of activated HER-2/Neu oncogene in transgenic female FVB/N mice leads to malignant transformation of mammary epithelial cells, followed by development of several breast adenocarcinomas, as well as hyperinsulinemia, hyperglycemia, and a decrease in the activity of the antioxidant system, which are biomarkers of the premature aging of transgenic mice (**Figure 1**). The lifespan of these mice is about 11–12 months. Thus, overexpression of HER-2/Neu causes hormone-metabolic changes, which are characteristic of accelerated aging, simultaneously with carcinogenesis, in this line of mice.

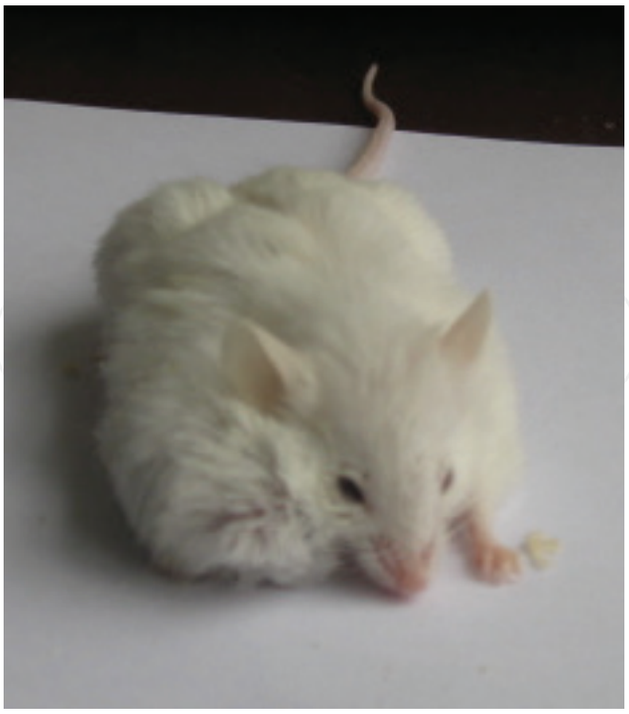


Figure 1. HER-2/Neu transgenic old mice.

2.1.2. Sample preparation

The brains from animals were fixed in 4% paraformaldehyde for morphological and immunohistochemical assays. Fresh-frozen brain sections containing supraoptic and paraventricular nuclei (SON and PVN) of the hypothalamus were prepared.

The fresh-frozen brain region containing SON and PVN were homogenized separately for further biochemical analysis.

2.1.3. TUNEL

The terminal deoxynucleotidyl transferase-biotin dUTP-nick end labeling (TUNEL) assay was used to detect 3′ hydroxyl ends in fragmented DNA in the hypothalamic neurosecretory cells. In brief, after rehydration, cryo-sections (5–7 mkm) were processed according to the manufacturer's instructions for the TUNEL assay using the detection kit (Sileks, Russia) and then stained with diaminobenzidine (DAB).

2.1.4. In situ hybridization

We identified the transcripts of genes involved in the signaling cascade of p53 protein (*p53*, *WRN*, *pin1*, *p21*) in fresh-frozen brain sections containing neurosecretory nuclei (SON and PVN) (in situ hybridization using a riboprobe labeled with digoxigenin). Riboprobes for the detection of mouse p53, WRN, pin1, and p21 mRNA were prepared by in vitro transcription method using a synthetic template, as recommended in the DIG-11-UTP guide. To mark the riboprobes, we

used modified NTP, DIG-11-UTP (Roche Applied Science, USA), which can be combined with growing T7 RNA polymerase transcripts and serve as a highly specific antigen with subsequent immunohistochemistry coloration [44]. We used a standard protocol published earlier [45] followed by densitometry to determine the level of expression of apoptosis-associated molecules (VideoTest Morphology).

2.1.5. Immunohistochemistry

The expression of WRN and survivin was examined immunohistochemically. WRN expression was detected for the determination of protein proportion in the nucleus or in the cytoplasm of cell (standard streptavidin-biotin-peroxidase method) [46]. The fresh-frozen brain sections containing SON and PVN of the hypothalamus cut sections were incubated with primary rabbit polyclonal antibody to WRN (NBP-23002, 1:100; Novus Biologicals, Inc., USA) and rabbit monoclonal antibody to survivin ([EPR17358], 1:500; Abcam, USA), and then with ABC elite complex (Vector Laboratories Inc., Peterborough, Cambridgeshire, UK). The peroxidase reaction of the avidin-biotin complex was revealed in the buffer containing 3,30 diaminobenzidine (0.05%) and hydrogen peroxide (0.01%). Additionally, reactions lacking primary antibodies were performed to ensure the specificity of the observed staining.

2.1.6. Western blot analysis

Tissues were homogenized in lysis buffer containing protease inhibitors and phosphatase inhibitor cocktail (both from Sigma-Aldrich, St. Louis, MO, USA). The total protein concentrations were determined by Bio-Rad protein assay (Bio-Rad Laboratories Inc., Hercules, CA, USA). Equal amounts of protein (15 lg per line) in sample buffer (Bio-Rad Laboratories Inc.) were denatured at 95°C for 5 min and separated on 10% acrylamide gel. The proteins from the gel were transferred to a nitrocellulose membrane. The membranes were incubated in 3% non-fat milk in Tris buffer (0.1% Tween 20, 0.2 mM Tris, 137 mM NaCl) for 30 min and then incubated overnight with primary antibodies against STAT1 (9172, 1:1000; Cell Signaling, USA); STAT3 (4904, 1:2000; Cell Signaling, USA); STAT5 (9363, 1:1000; Cell Signaling, USA); STAT6 (5397, 1:1000; Cell Signaling, USA); caspase-3 (4904, 1:2000; Cell Signaling, USA); FAS (ab82419, 1:1000; Abcam, USA), FADD (24,533, 4 mkg/ml; Abcam, USA), TRADD ([EPR3604] ab110644, 1:500; Abcam, USA), caspase-8 (25,901, 0.5 mkg/ml; Abcam, USA), (Abcam); p53 ([PAb 240] (ab26), 5 mkg/ml; Abcam, USA), and GAPDH (glyceraldehyde 3-phosphate dehydrogenase) (1:2000; Abcam, USA) or actin (1:1000; Abcam, USA). Subsequently, the membranes were incubated with secondary anti-rabbit (1:8000; Sigma-Aldrich, USA) or anti-mouse (1:80,000; Sigma-Aldrich, USA), followed by chemiluminescent detection by enhanced chemiluminescenceplus (Amersham, GE Healthcare, Little Chalfont, Buckinghamshire, UK).

2.1.7. Evaluation of sections and statistical analysis

The positive TUNEL staining cells in hypothalamic SON and PVN were counted under a high-power magnification (20×) field of light microscope (Zeiss Axiolab, Carl Zeiss Inc., Germany) [47–49]. At least five fields were sampled in a section and data were expressed as the number of TUNEL-positive counts.

The semi-quantitative analysis of protein amount in the histological slices was processed by measurement of optical density [50]. Five sections at the same level of the studied zones were analyzed from each animal. The relative optical density of immuno-positive substances in the SON and PVN of the hypothalamus was estimated, and the average and standard deviation were calculated. Optical density reflecting the content of immuno-positive substance was calculated as the 'gray level' (GL) of immunoreactive field of tissue minus background GL. Optical density of the background was estimated at the same slice in non-immunoreactive brain tissue field. Results are presented in relative units of optical density per lm2.

Immunohistochemical study showed that in the neurosecretory centers there are three types of cells—with WRN-immuno-positive cytoplasm, with WRN-immuno-positive nucleus, and cells where the immune reaction took place in the nucleus and cytoplasm simultaneously, and each type of cells was counted under a high-power magnification (20×) field of light microscope. At least five fields were sampled in a section.

The optical density of the bands (Western blotting) was measured and quantified by ImageJ.

Statistical analysis was carried out by Student's *t*-test ($p \leq 0.05$) (Microsoft Excel 5.0a), and values are expressed as mean SE for immunohistochemistry, TUNEL, in situ hybridization, and for Western blot analysis.

2.2. Expression of Werner syndrome genes in hypothalamic neurons in physiological and pathological (HER-2/Neu overexpression) aging

It is known that changes in the p53-mediated pathway of apoptosis with aging cause severe pathologies, including neurodegenerative diseases and cancer. DNA damage leads to the phosphorylation of the p53 protein and allows the interaction of p53 with the peptidylpropyl isomerase pin1. After being connected to pin1, the tumor suppressor p53 increases the expression of p21 (a cyclin-dependent kinase inhibitor), which helps stop the cell cycle [18, 19].

It was shown that p21 limits proliferation in cell culture and also this protein is an internal suppressor of neuronal regeneration in brain damage [51]. The process of differentiation of neuroendocrine cells is associated with up-regulation of the p21, p53, and activation of MAPK and STAT pathways [52]. Stable expression of p53 leads to the onset of p21 synthesis [53], and the role of p21 in p53-dependent cancer protection is shown. The absence of p21 significantly impairs the p53-mediated arrest of the cell cycle, without affecting apoptosis [19]. Other studies report an increase in p21 expression preceding TNF-induced necrosis-like cell death [54]. Most authors consider p21 (Waf1/Cip1) as one of the main mediators of p53 tumor suppressor [55].

According to other data, p21 acts as an oncogene, reducing the level of apoptosis in various tissues, including tumors [56]. Thus, a decrease in the level or the absence of p21 results in a significant increase in the lifespan of p53-deficient mice due to a decrease in the number of spontaneously occurring or induced tumors. It was shown that the reason for this is a higher apoptotic tissue index during the absence of p21 [55, 57]. The involvement of p21 in apoptosis depends on its interaction with the PCNA protein (proliferating cell nuclear antigen, the nuclear antigen of proliferating cells), which is an auxiliary factor in reparative DNA polymerase delta [58, 59].

According to the data of other authors, the p53-p21-dependent pathway determines the choice between apoptosis and cell aging [20]. As is known, the suppression of apoptosis significantly increases the number of aging cells.

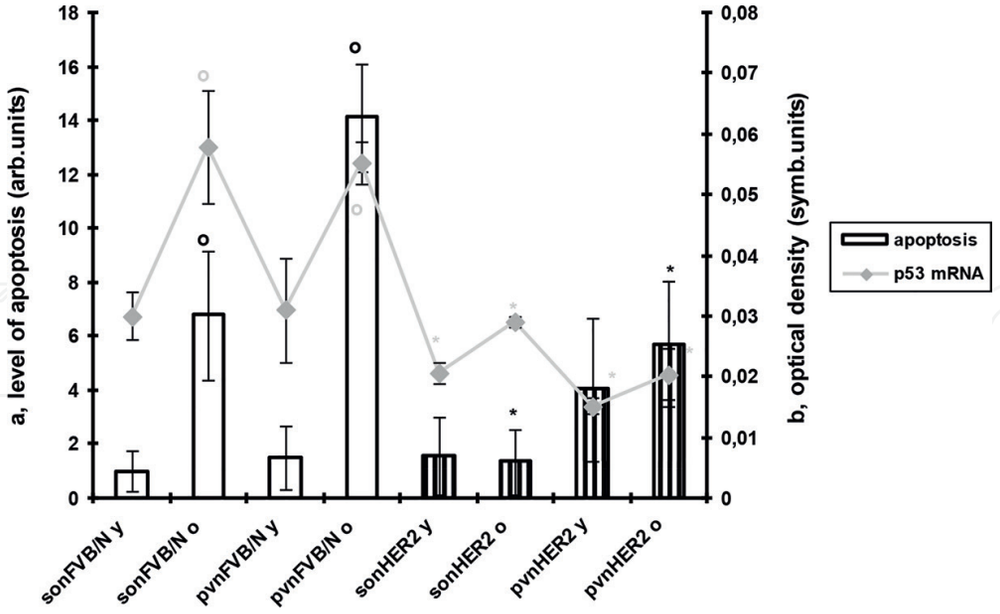
In addition, other genes participate in the aging process. Thus, the Werner syndrome gene (WRN) encodes DNA-helicases and endonucleases. WRN mutation causes Werner's syndrome, a progeria, when the characteristic signs of aging appear at an early age. It is known that aging triggers by two mechanisms—telomere shortening and DNA damage. These mechanisms are interrelated, shortening telomeres and their dysfunction can lead to DNA damage; accumulation of DNA damages leads to genomic instability and accelerated cellular aging. Both of these mechanisms are mainly dependent on p53 status [6]. The function of WRN is closely related to the p53 protein and its participation in apoptosis [60]. The destruction of the WRN gene or its mutation leads to spontaneous carcinogenesis, which is also characteristic of Werner's syndrome [61, 62]. Deficiency of the WRN protein reduces the phosphorylation of p53, as shown on the cell lines of normal fibroblasts and osteosarcoma cells [63]. The effects of p53 and WRN on each other are mutual. The absence of p53-WRN interaction can disrupt the signal for apoptosis and lead to genomic instability and carcinogenesis [64]. Many authors show depletion of p53-dependent apoptosis in the cell lines from patients with Werner's syndrome. The physical interaction between p53 and WRN was identified, that suggests functional interaction [65–68]. In addition, overexpression of WRN induces p53 expression, and further, p21, which indicates their overall involvement in premature aging and cancer processes [69]. However, the role of the WRN gene in the regulation of physiological aging is still unknown.

So, one of the aims of this study was determining the role of the *WRN* gene in the apoptosis in the physiological and pathological (HER-2/Neu overexpression) aging. One of the important regulatory systems of organism, the neuroendocrine system of the hypothalamus, was used as a model of the investigation.

The results of our studies showed that the level of apoptosis of the hypothalamus neurons in transgenic animals is low and does not increase with aging, in contrast to mice FVB/N (**Figure 2a** and **b**). Expression of p53 in young FVB/N mice is low and increases with aging (**Figure 2a**). In HER-2/Neu mice, the amount of p53 mRNA is significantly lower in both neurosecretory centers and does not change with aging (**Figure 2a**). Obviously, a decrease in p53 expression is the main reason for the low level of apoptosis in older transgenic mice.

According to our data, WRN is synthesized in the neurosecretory cells of the hypothalamus at a sufficiently low level, and there are no differences in the young animals FVB/N and HER-2/Neu (**Figure 3a**). With aging, WRN expression increases in SON and PVN in wild-type mice (**Figure 3b** and **c**), while overexpression of HER-2/Neu suppresses WRN expression (**Figure 3a**). A low level of WRN does not lead to the activation of p53 and thus suppresses the subsequent cascade of apoptosis. In addition, the WRN protein, which is synthesized in the neurosecretory cells of transgenic mice, is not functionally complete, as revealed in our experiments by immunohistochemistry (a cytoplasmic localization is characteristic of the defective protein [70]).

Immunohistochemical study showed that in the neurosecretory centers, there are three types of cells—with WRN-immuno-positive cytoplasm, with WRN-immuno-positive nucleus, and cells



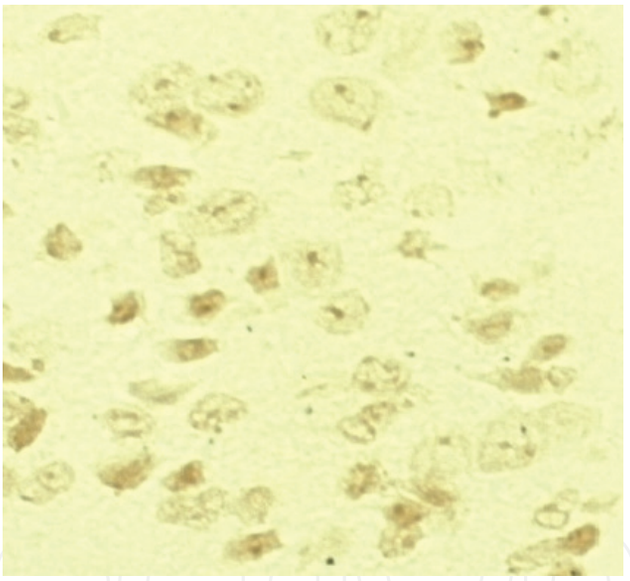
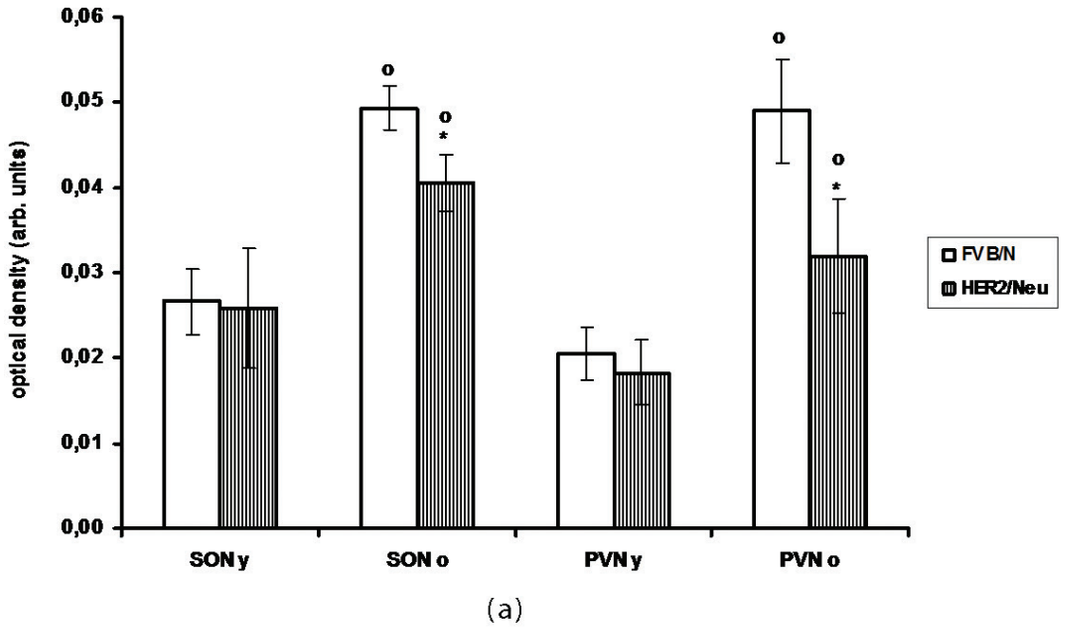


Figure 2. (a) The level of apoptosis and expression of p53 in neurosecretory centers (supraoptic (SON) and paraventricular (PVN) nuclei) in FVB/N and HER-2/Neu mice. The left ordinate axis shows the number of TUNEL-positive (apoptotic) cells ($M \pm m$, $n = 6$) in neurosecretory cells of SON and PVN of FVB/N and HER-2/Neu mice. Designations: (y)—young mice, (o)—old mouse. The right ordinate axis shows the content of p53 mRNA ($M \pm m$, $n = 6$) in the neurosecretory cells of SON and PVN of FVB/N and HER-2/Neu mice. Here and in other figures: optical density presented in arbitrary units. (o) is the significance of differences between the indices for young and old mice of the same group ($p \leq 0.05$), and (*) is the significance of differences between the indices of FVB/N and HER2/Neu mice of the same age ($p \leq 0.05$). (b) PVN of old FVB/N mice, cells with dark-stained nuclei are apoptotic (TUNEL).

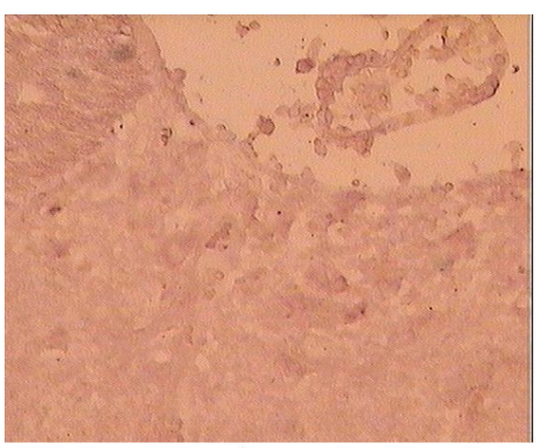
where the immune reaction took place in the nucleus and cytoplasm simultaneously. With aging, there are significant changes in the composition of immuno-positive cells. Thus, in the SON of FVB/N old mice the amount of cells with colored nuclei decreases with a simultaneous increase in the amount of cells with cytoplasmic coloring; in PVN, the amount of all three cell types increases. In old transgenic mice, the amount of neurosecretory cells with a nuclear-cytoplasmic

color increases, with an increase in the amount of cells with nuclear localization WRN. There were significant differences between wild-type mice and transgenic mice—a smaller amount of all types of cells in the HER-2/Neu mice, both young and old.

Thus, the results showed the activation of the p53-dependent pathway of apoptosis upon aging in the hypothalamic neurons of wild-type mice. Werner's syndrome gene was found to be involved in physiological aging. Previously, fibroblast culture showed that cell aging is associated with a decrease in the WRN protein [71]. The increase in WRN expression in the late stages of ontogenesis plays a decisive role in the induction of p53-mediated apoptosis of neurosecretory cells (**Figures 2a** and **3a**). The functional relationship of p53 and WRN found in our experiments can confirm the data obtained by other authors on cell cultures and on a model of mice with Werner's syndrome [66, 68, 72]. Increased expression of WRN in wild-type animals leads



(a)



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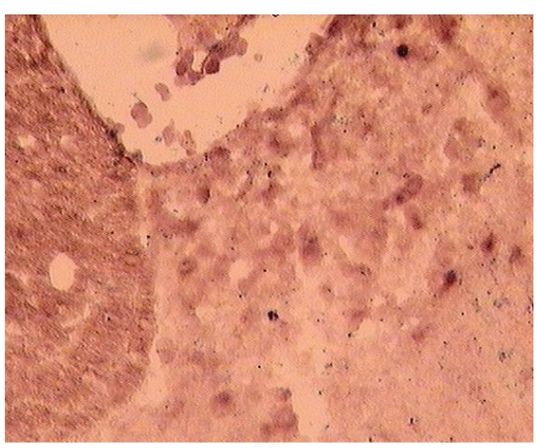

(c)

Figure 3. (a) Content of WRN mRNA in neurosecretory cells of the SON and PVN of FVB/N and HER-2/Neu mice of different ages. (b) SON of young FVB/N mice, immunostaining with antibody to WRN. (c) SON of old FVB/N mice, immunostaining with antibody to WRN.

to an increase not only in the synthesis of p53 but also in an increase in p53-dependent transcriptional activity and induction of pin1 expression [69], which is shown in our experiments.

It is known that p21 is the transcriptional target of the tumor suppressor p53, but these proteins mutually affect each other. There is evidence that p21 can induce cell arrest irrespective of the involvement of p53 [21]. In addition, p53 can act on the p21-independent mechanism [73]. P21 can both activate apoptosis and inhibit this process, depending on the involvement of other proteins, in particular PCNA [59]. In the works of many authors, the enhancement of the synthesis of p21 is shown in rest cells, that is, in highly differentiated cells, due to the ability of p21 to reduce proliferation and to participate in cellular aging [20, 51].

In our experiments, the level of the studied genes was higher in both age groups in FVB/N mice (p53, pin1, and p21) or only in older animals (WRN) compared to transgenic mice (**Figures 2a–4**). The high level of expression of p21 in young wild-type mice and its decrease in aging correlates with the level of apoptosis of neurosecretory cells.

The decrease in p21 synthesis in HER-2/Neu mice (as a result of low p53 level in these mice) indicates the possibility of maintaining a balance of cell death survival with the p53-p21 pathway; however, overexpression of the HER-2/Neu oncogene results in the suppression of the apoptotic cascade. Dynamics of the synthesis of pin1 corresponds to a change in the synthesis of p53, a significant level of pin1 expression in young FVB/N mice, increasing with aging, and a decreased content of pin1 mRNA without response to aging in HER-2/Neu mice. Increasing WRN expression leads to not only increased synthesis of p53 but also the induction of pin1 expression in FVB/N animals [69], as shown in our experiments.

Thus, the relatively high level of p21 mRNA that we detected in young wild-type mice is consistent with data of some investigators [55]. The simultaneous enhancement of p53 and p21 expression in aging in various tissues has been reported. The activation of the p53-mediated

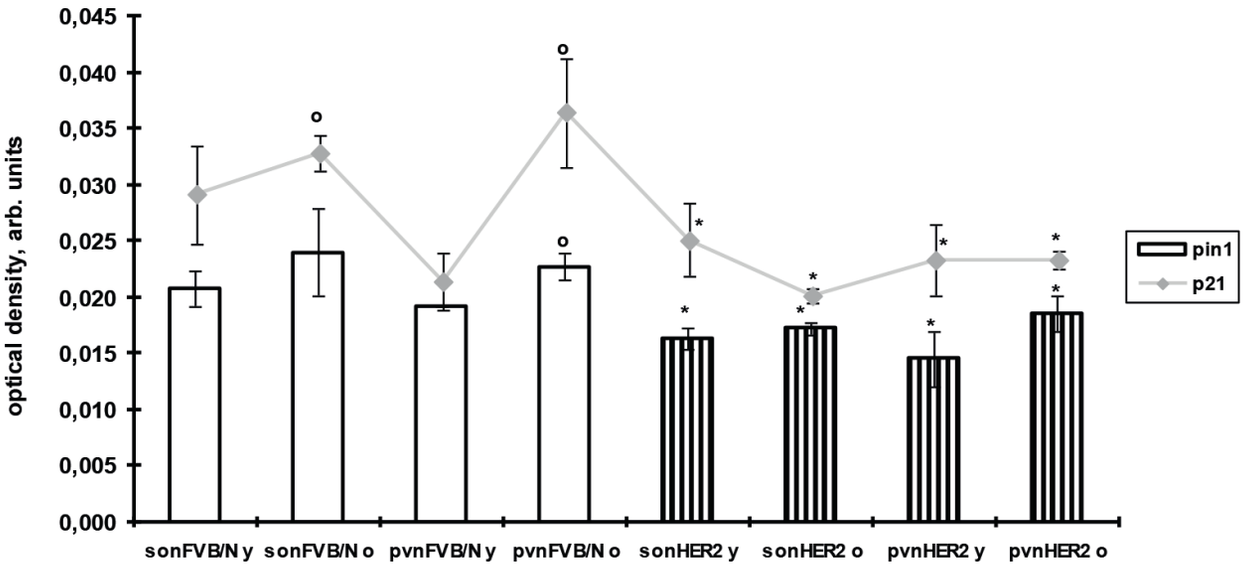

Figure 4. Content of pin1 mRNA and p21 mRNA in neurosecretory cells of the supraoptic and paraventricular nuclei of FVB/N and HER-2/Neu mice of different ages.

transcriptional program may be a common symptom of aging of different tissue types, but the expression pattern of p53-dependent genes is tissue-specific [74]. In our experiments, the level of the p21 protein, an inhibitor of cyclin-dependent kinases, necessary to lower the level of apoptosis [56], is high in neurons of the hypothalamus of young FVB/N mice and decreases with aging, inversely correlating with the expression of p53 and the level of apoptosis.

The data obtained in our experiments showed significant damage to the regulation of the p53-dependent cascade of apoptosis during overexpression of the HER-2/Neu oncogene. The expression of the studied p53-signaling genes (p53, p21, WRN, and pin1) in the hypothalamic neurons is significantly lower in HER-2/Neu transgenic mice, regardless of the ontogenesis stage, compared to wild-type mice (**Figures 2a–4**). An insufficient increase in WRN expression in transgenic mice results in a low synthesis of p53 and, correspondingly, a low level of programmed cellular cell death in aging. In addition, it was found that in HER-2/Neu mice irrespective of the ontogeny stage, the functionally defective WRN protein is synthesized, which is confirmed by immunohistochemistry. Changes in pin1 expression in transgenic mice correspond to the dynamics of p53 synthesis; it is a low level of p53 expression and no response to aging. It confirms the functional relationship of these genes, shown in the works of other authors [9], and supports the suppressive effect of the HER-2/Neu oncogene on various links of p53-mediated signaling.

P21 plays an important role in tumor suppression [19]. The decrease in p21 synthesis in HER-2/Neu mice (as a result of low p53 level in these mice) indicates attempts to maintain a balance of cell death survival with p53-p21 pathway; however, the expression of the HER-2/Neu oncogene results in the suppression of the apoptotic cascade.

2.2.1. Conclusion

This study for the first time revealed the involvement of the Werner syndrome gene in the physiological aging of neurons. The age-related increase in WRN expression promotes the activation of p53-dependent apoptosis of hypothalamic neurosecretory cells in wild-type mice (an increase in the expression of members in this cascade—pin1, p53, and, indirectly, p21). In transgenic animals with overexpression of the oncogene HER-2/Neu, the synthesis of pin1, p53, and p21 is low and there is no reaction to aging. The result of suppression of apoptosis in HER-2/Neu mice is increased carcinogenesis and reduced life expectancy.

Thus, it is shown that the WRN gene not only determines the pathological premature aging (Werner's syndrome) but also plays an important role in the mechanisms of physiological aging.

2.3. The role of STAT transcription factors in apoptosis regulation of hypothalamic neurons in aging in wild-type FVB/N mice and HER-2/neu transgenic mice

The interferon gamma is the main potential activator of STAT1, but STAT1 is also activated by growth factors and their receptors [75, 76]. According to some data, induction of STAT1 leads to a decrease in proliferation and an increase in p53-dependent apoptosis of cells of hepatocellular carcinoma [77]. The protein-protein interaction between p53 and STAT1 is shown. This interaction occurs via the C-terminal domain of STAT1, which is critical for the stress-induced apoptotic response. It was shown that the induction of expression of p53 and its target genes

in response to DNA damage is significantly reduced in STAT1-deficient cells [75]. As is known, Mdm2 is a key regulator of p53 expression. The stabilization of the p53 protein level is regulated by the Mdm2 protein, which interacts with the p53 protein and promotes its degradation by ubiquitination [78]. It is shown that the level of Mdm2 expression is increased in STAT1-deficient cells, and STAT1 represses the promoter of the gene encoding the Mdm2 factor, and STAT1 inhibits the p53-mediated activity of the Mdm2 promoter. Therefore, an elevated level of Mdm2 in STAT1-deficient cells may be responsible for a low level of p53 after DNA damage. These data indicate that STAT1-p53 interaction can have both positive and negative effects on various gene promoters. Evidence of this relationship is also the fact that tumors develop significantly faster in p53-STAT1-knockout mice than in mice knocked out by one of these genes [79]. In many tumors, the p53 function is reduced by 50%, in most cases due to overexpression of Mdm2 [80]. In experiments, it has been shown that the administration of interferon gamma restores p53-induced apoptosis by inhibiting the expression of Mdm2 via the STAT1-mediated mechanism, which is of great importance for the therapy of cancer. Thus, STAT1 is a checkpoint protein and also acts as an oncosuppressor.

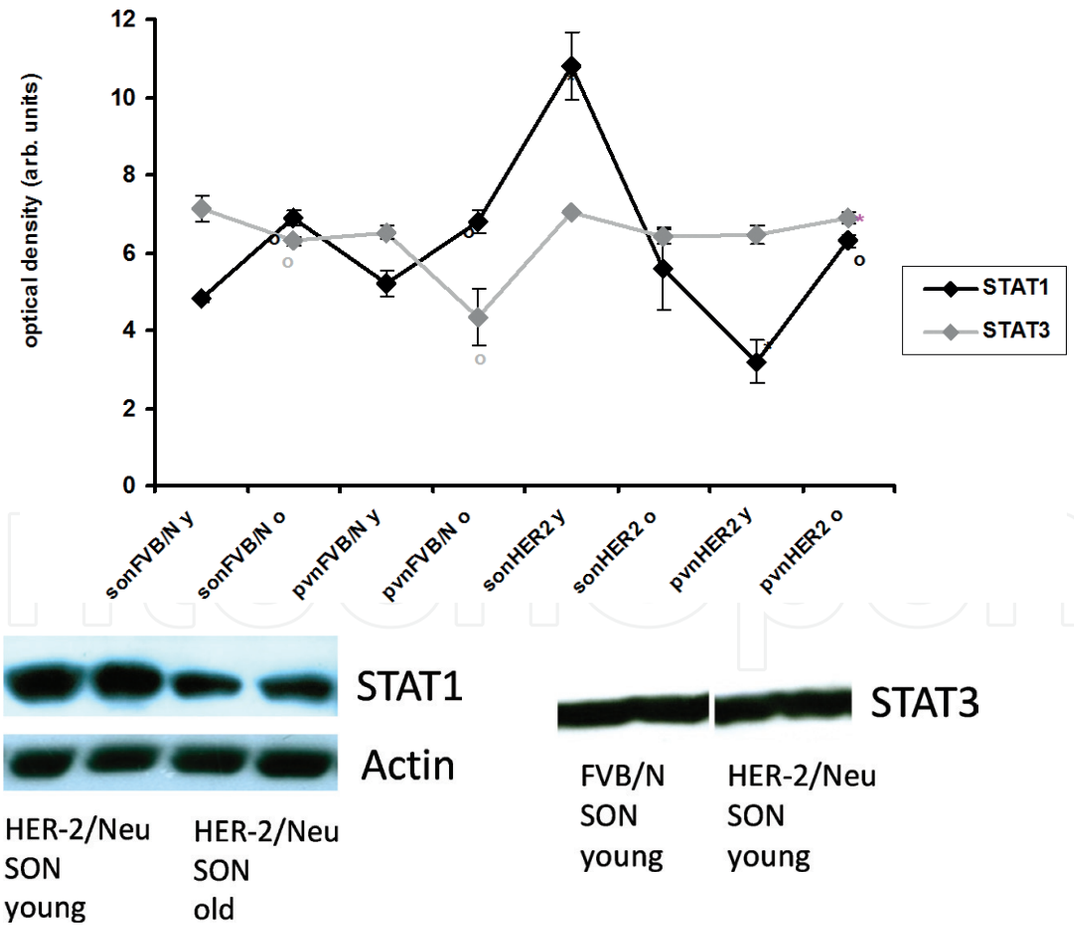

Figure 5. (a) The expression level of STAT1 and STAT3 in the neurosecretory cells of SON and PVN of FVB/N and HER-2/Neu mice of different ages. (b) STAT1 expression in SON of young and old HER-2/Neu mice (Western blotting). (c) STAT3 expression in SON of young FVB/N and HER-2/Neu mice (Western blotting).

In our experiment, the STAT1 level in young FVB/N mice is low, approximately the same in the SON and PVN (**Figure 5**). With aging, an increase in the expression of STAT1 in wild-type mice was found, which correlates with an increased level of neuronal apoptosis and overexpression of p53, caspase-3, and -8 (**Figures 2a**, **5a**, and **6**). The levels of expression of caspase-8 and caspase-3 are similar. The expression of caspases is low and the same in hypothalamic neurons in young mice of both lines (**Figure 8b**). In aging, we obtained the increase of caspases synthesis only in hypothalamus in wild-type mice (**Figure 6**). Some authors too reveal the proapoptotic function of this transcription factor. In young HER-2/Neu mice, STAT1 expression is high in SON and decreases with aging (**Figure 5b**), although some activation of synthesis is observed in the PVN (**Figure 5a**).

In young mice of both lines, STAT3 expression is approximately at the same level. With aging, the synthesis of this factor decreases only in mice FVB/N. In transgenic animals, there is no change in the synthesis of STAT3 in the late stages of ontogenesis (**Figure 5a**).

It was previously shown that STAT1 and STAT3 have the opposite effect on the apoptotic death of various cells [81]. STAT1-deficient cells are resistant to TNF-alpha-induced apoptotic death [82]. By contrast, STAT3 has oncogenic characteristics; its overexpression is observed in many types of tumors [83]. Significant activation of STAT3 is often observed in different types of cancer, including breast cancer. STAT3 plays a role in the progression of tumors and their resistance to anti-cancer treatment by regulating the survival of cancer cells [84]. Activation of STAT3 in glioblastoma multiforme correlates with malignancy and poor prognosis. The phosphorylating signal transducer JAK2 activates STAT3 in response to cytokines and growth factors [85].

It is known that overexpression of STAT1 induces the induction of apoptosis, for example, of cardiomyocytes in ischemia-reperfusion, while overexpression of STAT3 decreases STAT1-induced cell death [86].

One of the targets of STAT3 is survivin, an antiapoptotic protein belonging to the family of IAP (inhibitors of apoptotic proteins) [87]. It is known that HER-2 initiates oncogenic cascades. It has now been shown that HER-2 promotes the activation of STAT3 and, further, survivin [88]. Thus, it is obvious that members of the STAT family are involved in modulating the expression of apoptotic genes.

The problems of neuronal survival or death during aging are very important, since the regulation of the amount of neurons is carried out unilaterally (by cell death). The role of survivin and STAT factors in the regulation of apoptosis in aging is currently unclear. Survivin is almost not expressed in normal differentiated tissues. It is known that overexpression of survivin leads to an increase in proliferation in the hippocampus [89]. Recently, it has been shown that the synthesis of survivin decreases with aging and neurogenesis decreases. In addition, increased expression of survivin induces a significant reduction in β-galactosidase activity; thus, survivin allows cells to avoid aging [90].

It should be noted that in our experiment, STAT3 expression is the same in neurosecretory cells in FVB/N and HER-2/Neu mice (**Figure 5a** and **b**). The age-related changes in the expression of STAT1 and STAT3 are opposite—overexpression of STAT1 with a simultaneous decrease in the synthesis of proapoptotic factor STAT3 in aging in wild-type mice (**Figure 5a**). According to other investigations, STAT1-activated apoptosis proceeds via

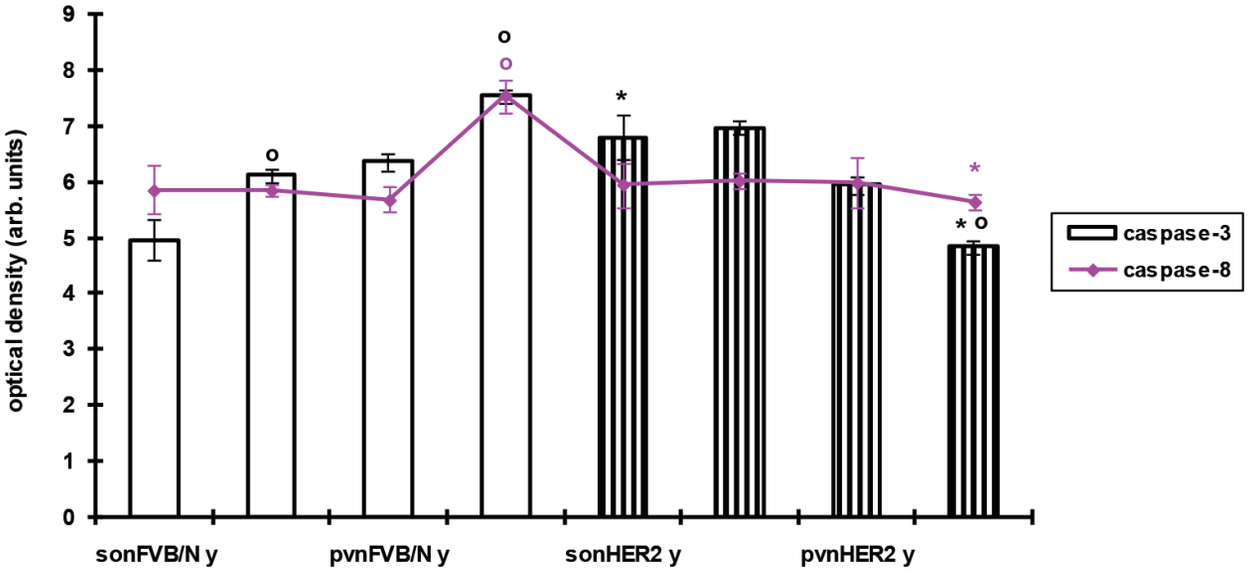

Figure 6. The expression level of caspase-3 and caspase-8 in the neurosecretory cells of SON and PVN of FVB/N and HER-2/Neu mice of different ages.

TNF- and p53-signaling pathways, with activation of caspase-3. Our results also showed an increase in the synthesis of p53 and caspase-3 (**Figures 2a** and **6**). Some authors report a suppression of STAT3 expression in an increased synthesis of STAT1; probably, this mechanism is present in our experience.

The level of survivin in hypothalamic neurons in young FVB/N mice was quite high in comparison with transgenic mice. With aging, survivin expression decreased in both nuclei in wild-type mice. In old HER-2/Neu mice, survivin synthesis did not change in SON and increased in PVN (**Figure 7**). These changes correspond to the dynamics of STAT3 expression (**Figure 5a**).

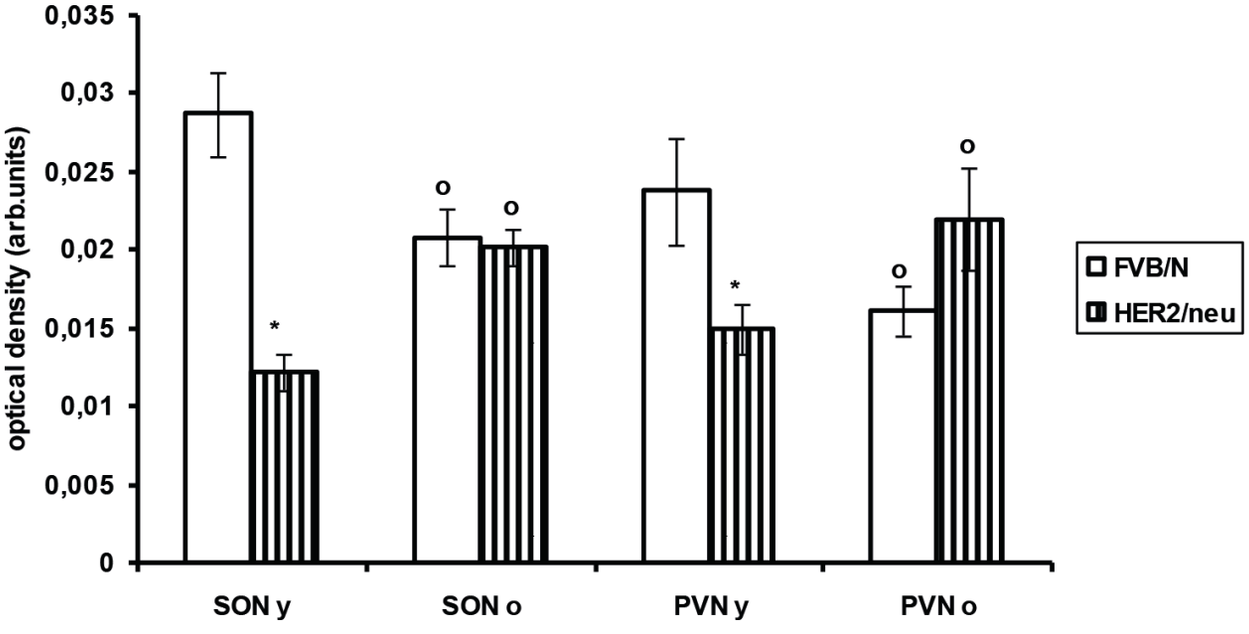

Figure 7. The expression level of survivin in the neurosecretory cells of SON and PVN of FVB/N and HER-2/Neu mice of different ages.

Other members of this family, STAT5 and 6, are involved in differentiation and cell survival process [91, 92]. STAT5 has pleiotropic functions for the regulation of cell proliferation, differentiation, and apoptosis. There is evidence of a pro-apoptotic activity of the JAK/STAT5 pathway in neurons [93], but most of the work reports an antiapoptotic orientation of STAT5 [94]. The STAT5 transcription factors are essential for both lymphocyte development and acute immune responses [95]. STAT5 is a regulator of cyclin D, Myc, and Bcl-2 in non-neuronal cells and thus is involved in the prevention of apoptosis [92]. It has been shown that STAT5 and STAT6 antiapoptotic cascades [91, 94], and antiapoptotic activity of the JAK/STAT5 pathway are carried out through Bcl-2 [96]. The problem of survival and death of neurons is especially important in aging. According to some studies, the Jak/STAT pathway is involved in the regulation of cytokine-dependent apoptosis and the activity of growth factors and their receptors.

Expression of STAT5 is approximately the same in young HER-2/Neu and FVB/N mice. With aging in FVB/N mice, this factor decreases in both neurosecretory centers (**Figure 8a**). In transgenic mice, in SON, there is no change in aging, and in PVN, age-dependent overexpression of STAT5 is observed (**Figure 8a** and **b**).

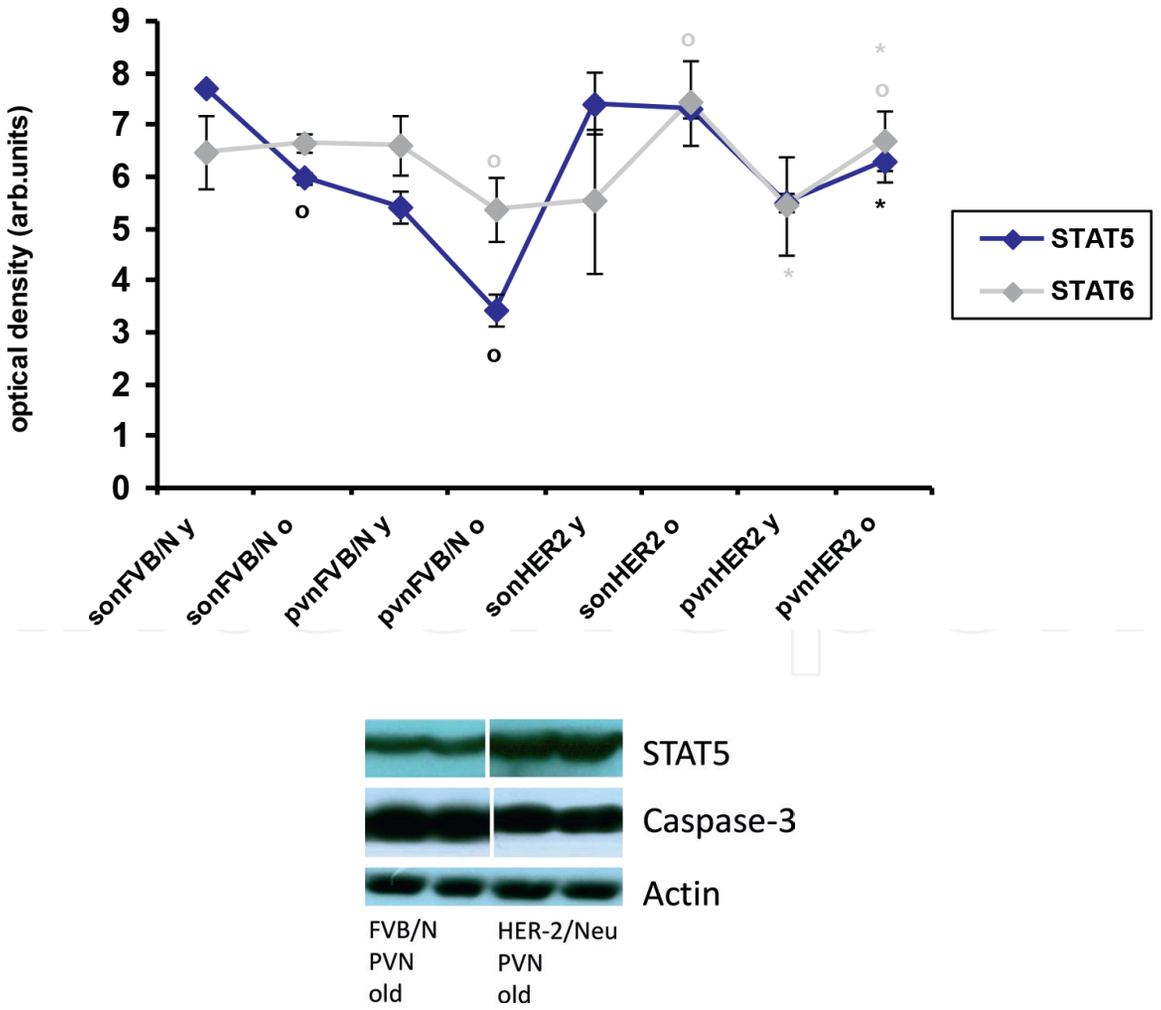

Figure 8. (a) The expression level of STAT5 and STAT6 in the neurosecretory cells of SON and PVN of FVB/N and HER-2/Neu mice of different ages. (b) STAT5 and caspase-3 expression in PVN of old FVB/N and HER-2/Neu mice (Western blotting).

Expression of STAT6 is almost the same in SON and PVN of young FVB/N mice. With aging in FVB/N mice, there are no changes in the SON, and a decrease in STAT6 synthesis is observed in the PVN (**Figure 8a**). In SON in transgenic mice there are no age-related changes, and the age-dependent overexpression of STAT6 is observed in the PVN (**Figure 8a**).

2.3.1. Conclusion

Thus, the synthesis of the studied transcription factors, which show antiapoptotic activity—STAT3, 5, 6, survivin—decreased in the late stages of ontogeny in the hypothalamic neurosecretory centers of wild-type mice. It can be concluded that the suppression of antiapoptotic factors STAT3, 5, and 6 and overexpression of the proapoptotic factor STAT1 is one of the reasons for the increase of the amount of dying neurons during physiological aging.

In young HER-2/Neu mice, the antiapoptotic factors STAT3, 5, and 6 are synthesized at a sufficiently high level. With aging, there is no change in the synthesis of STAT3 and an increase in STAT5, and 6 and survivin expression is observed. These factors are activated, in addition to cytokines, by growth factors and their receptors. Accordingly, overexpression of the HER-2/Neu receptor tyrosine kinase receptor results in cell survival by activating the STAT-signaling pathway, while suppressing the proapoptotic factor STAT1.

Thus, in this study, the participation of the STAT pathway in the regulation of neuronal apoptosis in physiological aging and in old mice with overexpression of the HER-2/Neu oncogene was studied for the first time. Active participation of this signaling pathway in the regulation of neuronal apoptosis during aging was observed.

2.4. The role of TNF-dependent way in apoptosis regulation of hypothalamic neurons in physiological and pathological (HER-2/Neu overexpression) aging

Most of the works is associated HER-2- and TNF-signaling in malignancy tissues [97, 98]. But some investigations demonstrate that HER-2 and TNF can interrelate in normal tissues. TNF-α, a pro-inflammatory and apoptosis-inducing cytokine, stimulates several intracellular signaling pathways. TNF-α can promote cell survival using activation of TAK1 kinase, which is especially important for cancer cells. As is shown, on the other hand, TNF-α induces apoptosis via formation of the death-inducing signaling complex (DISC), which consists of trimerized receptors, the death domain-containing adaptor protein FADD and caspase-8 [99]. HER is a member of the receptor tyrosine kinase family and plays a critical role in a wide variety of cellular functions, including proliferation, differentiation, and apoptosis. At present, the interaction of these factors (HER-2 and TNF) at various pathological conditions is described [100]. However, there is almost no data on the involvement of the TNF-signaling pathway in the regulation of age-related neuronal apoptosis during overexpression HER-2 in vivo.

So, we studied the role of a cytokine-dependent cascade—the TNF-mediated pathway in the regulation of apoptosis of neurosecretory cells of the hypothalamus in physiological aging and in old HER-2/Neu transgenic mice. Expression of the members of the TNF-dependent cascade was assessed at different levels: receptor perception of the apoptotic signal—expression of the Fas receptor (CD95), signaling—adapter expression: FADD and TRADD, and implementation:

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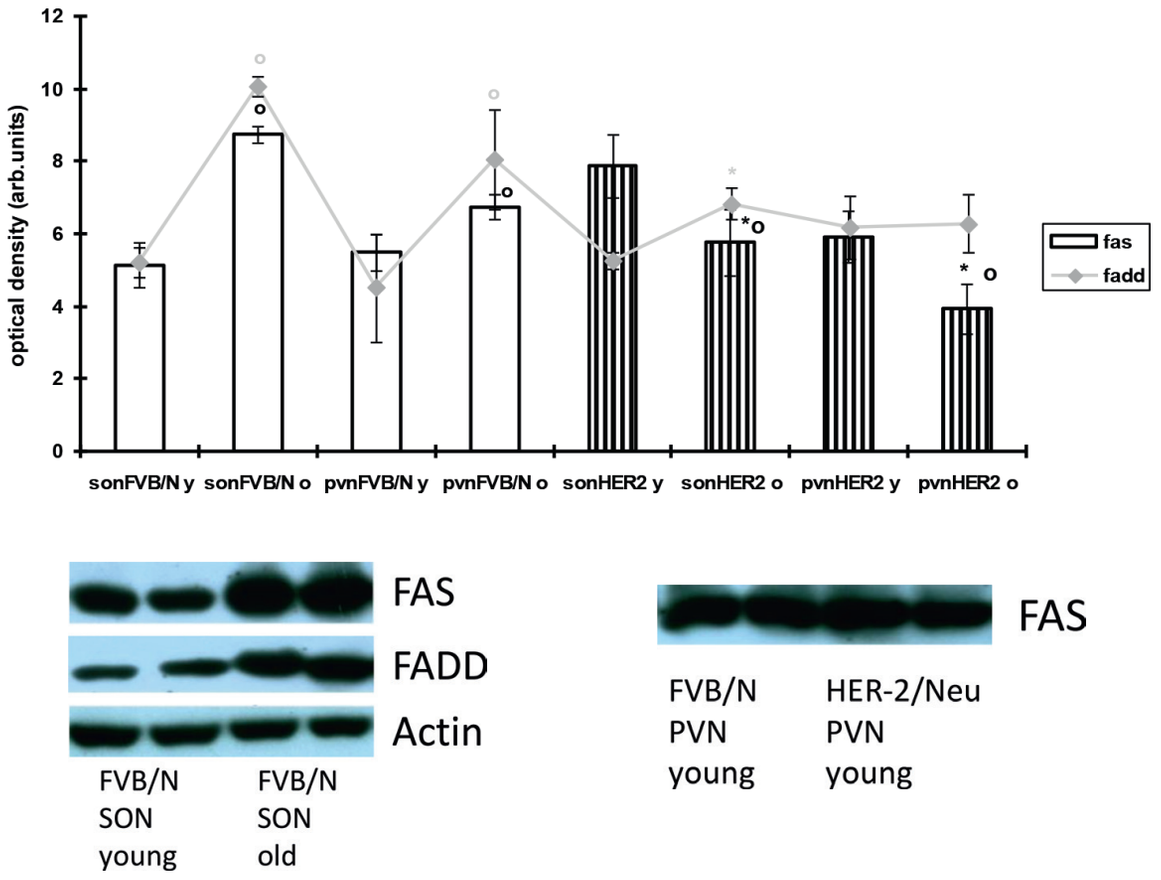

Figure 9. (a) The expression level of Fas and FADD in the neurosecretory cells of SON and PVN of FVB/N and HER-2/Neu mice of different ages. (b) Fas and FADD expression of SON in young and old FVB/N mice (Western blotting). (c) Fas expression in PVN of young FVB/N and HER-2/Neu mice (Western blotting).

the level of caspase-8 expression and, further, the level of neuronal apoptosis in the nuclei of young and old mice.

Fas (CD95) expression was increased significantly in both hypothalamic nuclei in old wild-type mice compared to young ones (**Figure 9a** and **b**). A high level of Fas is correlated with an increase of the synthesis of adapter proteins FADD and TRADD (**Figures 9a**, **b** and **10**). The expression of caspase-8 and -3 and the level of apoptosis were also increased in old wild-type mice (**Figures 2a** and **6**). Thus, in this case (in wild-type mice, i.e., in physiological aging), TNF signaling is an apoptosis-activating pathway and plays an important role in the mechanism of neuronal death.

Expression of the tyrosine kinase receptor HER-2/Neu causes significant changes in intracellular regulatory mechanisms, which is developed in the increased survival of cells, and it is the basis for possible oncogenesis. Our results show that HER-2/Neu expression at late stages of ontogenesis suppresses the main apoptotic cascades—p53- and TNF-dependent (**Figures 2a**, **9a**, and **10**). It was found that in young transgenic mice, the synthesis of the Fas receptor, the adapter proteins FADD and TRADD, is at the same level as in the young wild-type mice (**Figure 9c**) or exceeds it (in case of Fas and TRADD expression in SON). With aging, the expression of these proteins either decreases (Fas) or does not change (FADD, TRADD) in the SON and PVN in hypothalamic nuclei in transgenic mice. Accordingly, significant differences are noted between the studied

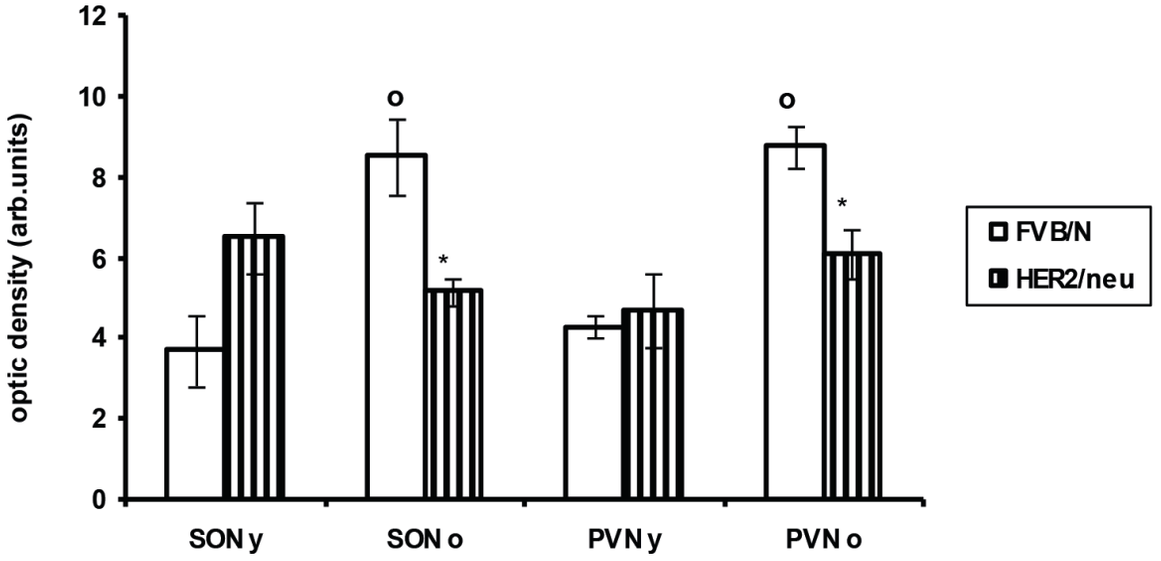

Figure 10. The expression level of TRADD in the neurosecretory cells of SON and PVN of FVB/N and HER-2/Neu mice of different ages.

protein levels in old wild-type and in old transgenic mice (**Figures 2a**, **9a**, and **10**). We show any age-related dynamics in the expression of initiator caspase-8 and effector caspase-3 in neurosecretory cells in young and old HER-2/Neu mice (**Figure 6**).

It can be concluded that one of the ways to ensure increased cell survival in aging in transgenic HER2 animals is the suppression of the TNF-dependent apoptotic cascade.

TNF was previously considered a proapoptotic factor. Recently, it has now been shown that the role of the TNF pathway in apoptosis is ambiguous—first, the canonical pathway of apoptosis activation involving caspase-8 and -3 [36, 99, 101–104] and the second, the anti-apoptotic effect of TNF are associated with the activation of independent survival ways—IkB (inhibitor of kappa-beta kinase-nuclear factor kappa B) pathway (signaling cascade associated with NF-kB) [103, 104] and MAPK-EGFR pathway [99, 100, 105], or the PI3K-AKT pathway [105].

These signaling pathways (PI3K-AKT and MAPK/ERK) are well known as survival. It is known that the main function of transcription factor NF-kB is the coordination of immune and pro-inflammatory cellular responses. However, it has now been shown that, in addition, members of the NF-kB family are factors in cell survival, and some data indicate the importance of NF-kB as a survival factor in the central nervous system. One of the key kinases that activate both NF-kB and MAPK pathways is the TAK1 kinase, which is capable of regulating the phosphorylation and endocytosis of EGFR, regardless of its tyrosine kinase activity. Some authors consider that the TRAIL factor (TNF-related apoptosis-inducing ligand), Fas ligand, Fas, and FADD are proapoptotic factors, whereas TNF-receptor 1 and TRADD may have an antiapoptotic effect, acting through the survival pathway is the NF-kB and the p38-MAPK-EGFR pathway [106].

A high level of Fas in hypothalamic neurons in young HER-2/Neu mice does not lead to an increase in the level of apoptosis, since FADD expression is low (**Figure 9a**). At the same time, increased expression of TRADD may further activate any of the above-mentioned survival

ways—NF-kB, MAPK/ERK, or PI3K-AKT cascade (**Figure 10**). Perhaps, HER-2/Neu expression and TNF receptor 1 and TRADD factors stimulate the phosphorylation of ERK and AKT cascades, which leads to an increase of cell survival. Possibly, these signaling pathways prevent proapoptotic cleavage of caspases mediated by the DISC [99]. A similar mechanism exists in epithelial non-transformed breast cells, when inhibition of EGFR signaling causes up-regulation of the inhibitor of caspase-8 FLICE-inhibiting protein (FLIP(L)) and makes cells more sensitive to TRAIL-induced apoptosis, and the ERK cascade played an important role [107]. In addition, it is known that TNF-α induces the formation of membrane-bound complexes, which include, among other components, IAP, which are triggers of the NF-kB cascade [104]. We have shown an increase of the expression of the antiapoptotic protein survivin, a member of the IAP family, in hypothalamic neurons in aged HER-2/Neu mice (**Figure 7**). We can suppose that the increase of survivin expression is caused by a high content of TNF-alpha in transgenic mice, and this probability will be investigated.

2.4.1. Conclusions

Thus, we showed the participation of the pro-inflammatory component in the aging process (**Figure 11**). Fas expression, adapter proteins associated with the death domain (FADD and TRADD), and caspase-8 expression are activated in the hypothalamic neurons in FVB/N line mice (wild type) during aging. It correlates with an increase of caspase-3 expression and an increase of the apoptosis level of the hypothalamic neurons (**Figure 11**). It can be assumed that

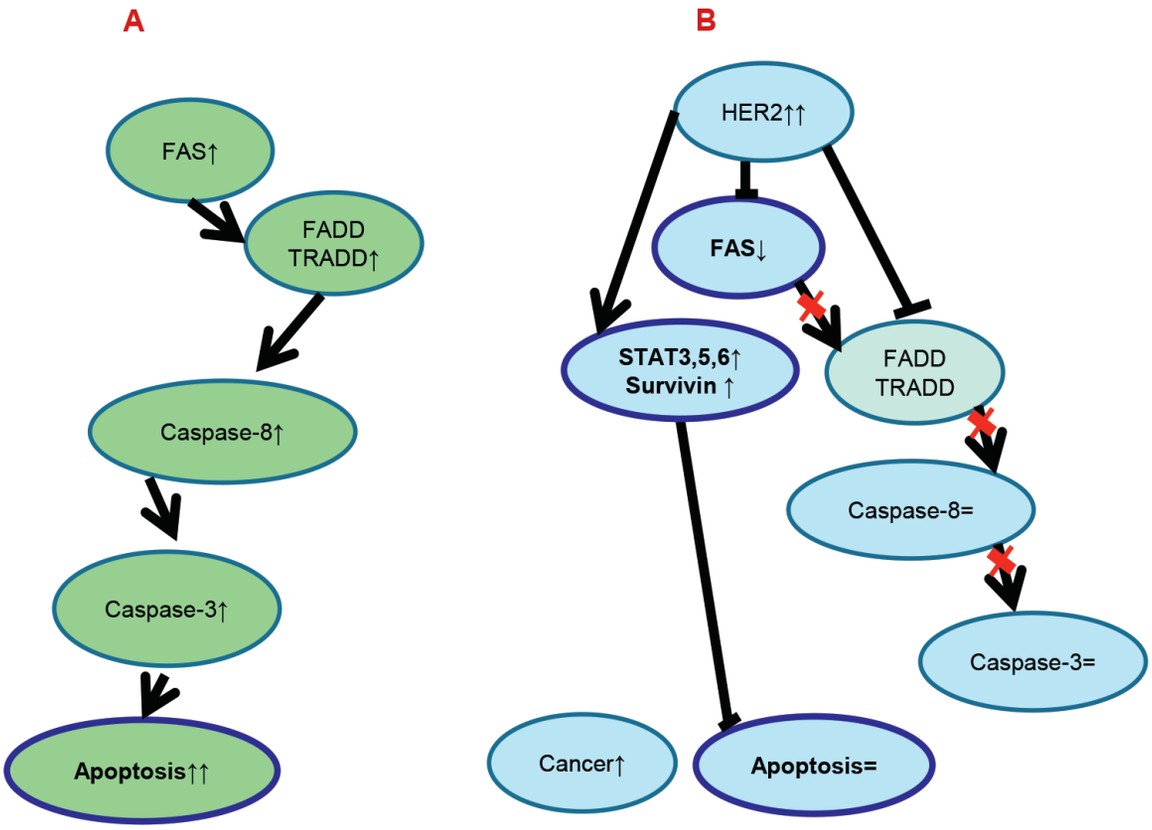

Figure 11. TNF-signal cascade of apoptosis in physiological aging (A), in aged HER-2/Neu transgenic mice (B).

one of the reasons for this is a possible suppression of the survival ways (AKT and ERK cascades) or an increased content of proapoptotic factors, for example, FasL in physiological aging.

HER-2/Neu expression causes suppression of the extrinsic pathway of apoptosis (TNF-dependent). In this case, both the reception of an apoptotic signal (Fas receptor expression) and its further carrying out (FADD and TRADD expression) are suppressed. However, in young transgenic mice, the increased TRADD expression may activate one of the survival ways—NF-kB, MAPK/ERK, or PI3K-AKT cascade (**Figure 11**). Thus, HER-2/Neu tyrosine kinase receptor plays an important role in the mechanism of cell resistance to age-dependent apoptosis, and TNF-signaling pathway is one of the targets of HER-2/Neu.

3. Conclusions

The aging process remains one of the most intriguing problems of biology and medicine. Recent advances in molecular biology make it possible to achieve an understanding of the fundamental foundations of this complex process. An intensive study of time- and tissue-specific gene expression is a tool that should lead us to a tangible control over age-dependent lesions.

Hypothalamic neurosecretory centers have been an object of deep interest since its role in the regulation of many body functions: adaptation, stress response, food and sexual behavior, emotions, thermoregulation, cognitive processes, and circadian rhythms have been discovered.

Aging disrupts vital activity, as noted in many cases. The first sign of aging, discovered by those who studied aging in vivo, is a disruption in the regulation of functions in almost all body systems. We know that the regulation of all processes is at least duplicated, so, the cell has not only an internal genome-dependent development program but also a subject to the influence of the nervous and endocrine systems. So, it can be concluded that the age-dependent changes are found in the central part of endocrine system, that is, in the hypothalamic neurosecretory centers. Indeed, our preliminary study showed that in old mice there is a significant loss of hypothalamic neurosecretory cells by apoptosis [1]. Obviously, a reduced amount of neurosecretory cells cannot maintain the previous level of functional regulation for a long time. This functional stress in the hypothalamic neurosecretory cells can be the cause of avalanche-like morphofunctional changes in the body caused by aging.

So, the results obtained in our studies allow to propose a possible scheme of apoptosis regulation of the hypothalamus neurons in physiological aging and in aged transgenic mice with HER-2/Neu overexpression (**Figure 12**). Thus, in the late stages of ontogeny, we observe an increase in the synthesis of proteins involved in the induction of apoptosis, only in a group of wild-type mice. At physiological aging, we observed increased level of hypothalamic neuron apoptosis mediated by the p53- and Fas-dependent pathways, with caspase-8 and -3 activation. As we have shown, the WRN gene also participates in the regulation of physiological aging. The synthesis of

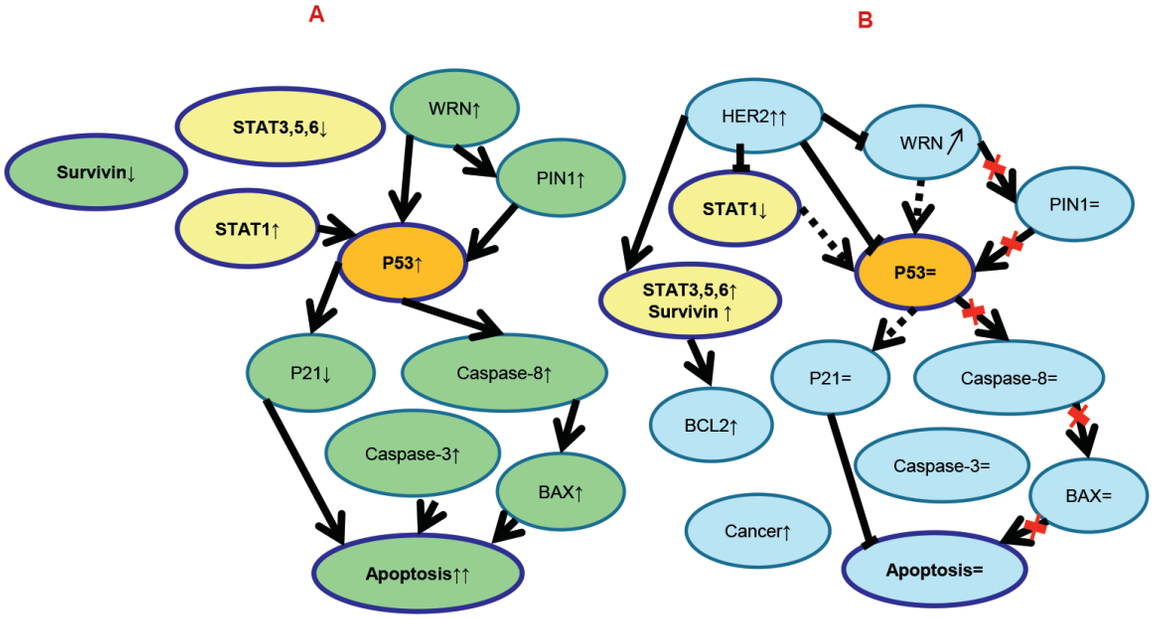

Figure 12. The apoptosis regulation in neurons in aging. (A) Signal cascade of apoptosis in physiological aging; (B) signal cascade of apoptosis in aged HER-2/Neu transgenic mice.

WRN and pin1 proteins increases and stimulates the expression of p53. The p21 protein requires for stable low level of apoptosis. The level of p21 protein is high in young FVB/N mice and decreases with aging. In transgenic animals with overexpression of the oncogene HER-2/Neu, the synthesis of these proteins is low, and there is no reaction to aging (**Figure 12**).

Thus, overexpression of HER2 blocks the signal pathway of p53, affecting both the p53 regulating proteins and the targets of p53.

In addition, we investigated age-related changes of STAT-signaling pathway. We revealed that in hypothalamus of wild-type mice, the synthesis of STAT1 increases and activates p53-mediated way. The expression of antiapoptotic factors STAT3, 5, 6, and survivin decreases in the studied neurosecretory centers. By contrast, in aged HER-2/Neu mice the expression of these factors increases, and STAT1 synthesis was low (**Figure 12**).

We showed the involvement of cytokine-dependent pathways in the mechanisms of apoptosis during aging. The realization of TNF-dependent apoptosis in hypothalamic neurons during physiological aging shows an increase of Fas receptor expression and expression of adapter proteins associated with the death domain (FADD and TRADD). In older transgenic animals, the expression of HER-2/Neu causes suppression of the extrinsic pathway of apoptosis—the TNF-dependent pathway (**Figure 12**). In other side, in the hypothalamus of young transgenic mice, the high TRADD expression may activate one of the survival ways (NF-kB, MAPK/ERK, or PI3K-AKT). So, HER-2/Neu tyrosine kinase receptor plays an important role in the mechanism of cell resistance to apoptosis in aging, and one of the targets of HER-2/Neu is TNF-signaling cascade.

The result of such suppression of the apoptotic cascade in transgenic mice is increased carcinogenesis and a half-reduced life expectancy, compared to the control.

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