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Features of Intracellular Signal Transduction in Neural Stem Cells under the Influence of Alkaloid Songorine, an Agonist of Fibroblast Growth Factor Receptors

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We performed a comparative *in vitro* study of the involvement of NF-κB, PI3K, cAMP, ERK1/2, p38, JAKs, STAT3, JNK, and p53-dependent intracellular signaling in the functioning of neural stem cells (NSC) under the influence of basic fibroblast growth factor (FGF) and FGF receptor agonist, diterpene alkaloid songorine. The significant differences in FGFR-mediated intracellular signaling in NSC were revealed for these ligands. In both cases, stimulation of progenitor cell proliferation occurs with the participation of NF-κB, PI3K, ERK1/2, JAKs, and STAT3, while JNK and p53, on the contrary, inhibit cell cycle progression. However, under the influence of songorin, cAMP- and p38-mediated cascades are additionally involved in the transmission of the NSC division-activating signal. In addition, unlike FGF, the alkaloid stimulates progenitor cell differentiation by activating ERK1/2, p38, JNK, p53, and STAT3.

Key Words: *songorine; fibroblast growth factor; neural stem cells; regenerative medicine; neuroprotective agents*

Neurodegenerative diseases apart from the death and dysfunction of mature nerve cells are associated with impaired plasticity and dysfunction of the cell renewal systems in the nervous tissue [1-3]. Despite numerous attempts at developing the methods of treating neurodegenerative diseases with transplantation of various stem cells (SC), no serious encouraging results were achieved in this direction so far [4-6]. This can be explained by impossibility of solving a number of problems: potential tumorigenicity of SC products (in particular, based on allogeneic pluripotent SC); the lack of technologies to ensure the development of transplanted cells into the required elements, expression of the major histocompatibility complex antigens by allogeneic SC during their specialization; mutations of autologous SC during their culturing at the stage of preparation of cellular material for transplantation [7], *etc.*

It is known that the efficiency of neurogenesis depends primarily on the activity of resident progenitor cells in special areas of the CNS (primarily, the subventricular zone, hippocampus, and olfactory bulbs) [6,7]. In recent decades, the fundamental possibility of solving the problems of regenerative medicine with the help of pharmacological agents that imitate the activity of natural regulatory systems has been demonstrated [7-10]. One of the most promising direc-

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tions in the development of drugs that stimulate the realization of the growth potential of progenitor cells is the search for compounds of plant origin (primarily alkaloids) that can act as ligands for cytokine receptors or other surface regulatory cellular structures involved in the regulation of SC functions [7,11].

Specific neuroprotective and neuroregenerative activity of the diterpene alkaloid songorine was demonstrated in our previous studies [12-14]. It was found that the therapeutic action of songorine is based on stimulation of the functions of neural SC (NSC) through activation of fibroblast growth factor receptors (FGFR) expressed on their surface [15]. In *in vitro* experiments, songorin had a significantly more pronounced stimulating effect on NSC than fibroblast growth factor (FGF) in equimolar concentrations [12,15].

It is known that the implementation of cell functions is carried out due to the activity of the system of intracellular signal transduction [7,10]. At the same time, in some cases, structural features of different ligands interacting with receptors can determine some specificity of intracellular signaling, and, as a result, the cellular response to a stimulus [7,9,11].

The aim of this work was a comparative study of the role of the main signaling cascades in the implementation of NSC functions under the influence of FGF and alkaloid songorine.

MATERIALS AND METHODS

The studies were carried out on male C57BL/6 mice (n=30; age 2-2.5 months, body weight 20-22 g). Animals (1st category conventional mice) were obtained from the Department of Experimental Biological Models of E. D. Goldberg Research Institute of Pharmacology and Regenerative Medicine. The study was conducted in compliance with the principles of humane treatment of experimental animals with the permission of the Local Ethical Committee (Protocol GRIPh&RM-2022-01/12 of December 1, 2022).

We studied the effect of selective inhibitors of NF-κB (aurothimalate, 50 μM), PI3K (LY294002, 50 μM), ERK1/2 (PD98059, 100 μM), p38 (SB203580, 10 μM), adenylate cyclase (2',5'-dideoxyadenosine, 30 μM), JNK (SP600125, 10 μM), p53 (Pifithrin-α, Cyclic, 5 μM), JAKs (Pan JAK Inhibitor Ruxolitinib, 200 nM), and STAT3 (STAT3 Inhibitor XIV, LLL12, 5 μM) (all produced by Calbiochem) on the realization of the growth potential of NSC under the influence of basic FGF (bFGF; 2 nM, Calbiochem) and songorin (2 nM, isolated from aerial parts of *Aconitum baicalense* at the E. D. Goldberg Research Institute of Pharmacology and Regenerative Medicine [13]). The working concentrations of inhibitors, which indicate their sufficient selectivity for the corresponding signaling molecules, were determined based on the manufacturer's instructions for information on the concentration of half-maximal inhibition. The cell cultures with bFGF and songorine, respectively, without inhibitors of signaling molecules, served as controls. The effect of bFGF and songorine on the functioning of progenitor cells was assessed in comparison with an intact control (the cell culture in a medium without these factors).

The progenitor cells were obtained from the subventricular zone of the cerebral hemispheres. To this end, the nervous tissue around the lateral ventricles of the cerebral hemispheres at a distance of 1 mm around the ventricular cavity was taken with microtweezers under a binocular loupe. NSC were studied by culturing unfractionated cells of the subventricular zone of the cerebral hemispheres. To this end, cells at a concentration of 10^5/ml were incubated in MACS Neuro Medium (Miltenyi Biotec) for 5 days in a CO_2 incubator at 37°C, 5% CO_2, and 100% air humidity. After incubation, the content of clonogenic cells, their mitotic activity, and intensity of specialization were evaluated. The number of NSC was determined by the yield of CFU-N (neurospheres containing more than 100 cells). The proliferative activity of progenitor cells was assessed by the hydroxyurea cell suicide method. The intensity of specialization (differentiation/maturation) of progenitor elements was determined by calculating the ratio of cluster-forming elements (ClFU, neurospheres consisting of 30-100 cells) to CFU (differentiation index) [3,8].

The obtained results were processed by the method of variation statistics in the Statistica 6.0 software (StatSoft, Inc.) using the nonparametric Mann–Whitney U test (mean value of the indicator, significance of differences in indicators between groups at p<0.05).

RESULTS

Addition of bFGF to the culture of nervous tissue cells was accompanied by a significant increase in the yield of CFU-N and their proliferative activity to 276.5 and 252.0% of the initial levels, respectively (Figs. 1 and 2) and a decrease in the intensity of specialization of progenitor cells (up to 56.2% of the same parameter in the medium without FGF) (Fig. 3). Alkaloid songorine caused more pronounced changes in the colony-forming ability of neural precursor cells. Thus, the number of CFU-N and their mitotic activity in the medium with songorine reached 319.8 and 302.3% of the background values. The alkaloid, in contrast to bFGF, did not affect maturation of progenitor cells (Fig. 3).

These results corresponded to our previous reports on more pronounced stimulating activity of songorine compared to FGF, implemented in both cases through FGFR [12,15].

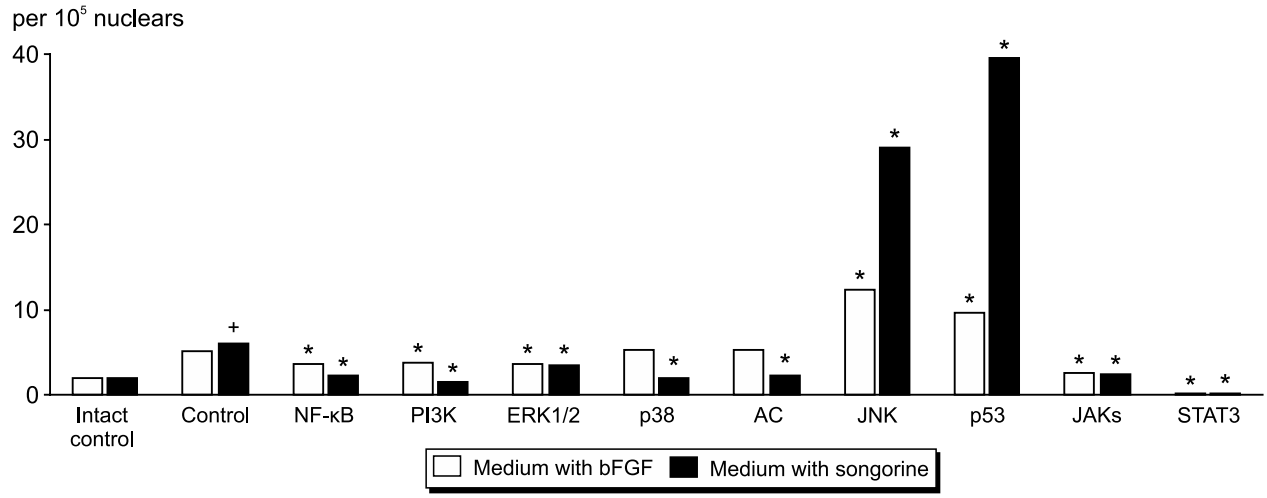

Fig. 1. The number of CFU-N in the culture of nervous tissue cells when the studied inhibitors were added *in vitro*. AC: adenylate cyclase. $p<0.05$ in comparison with *the corresponding control (medium without inhibitors), +the medium with bFGF in the control.

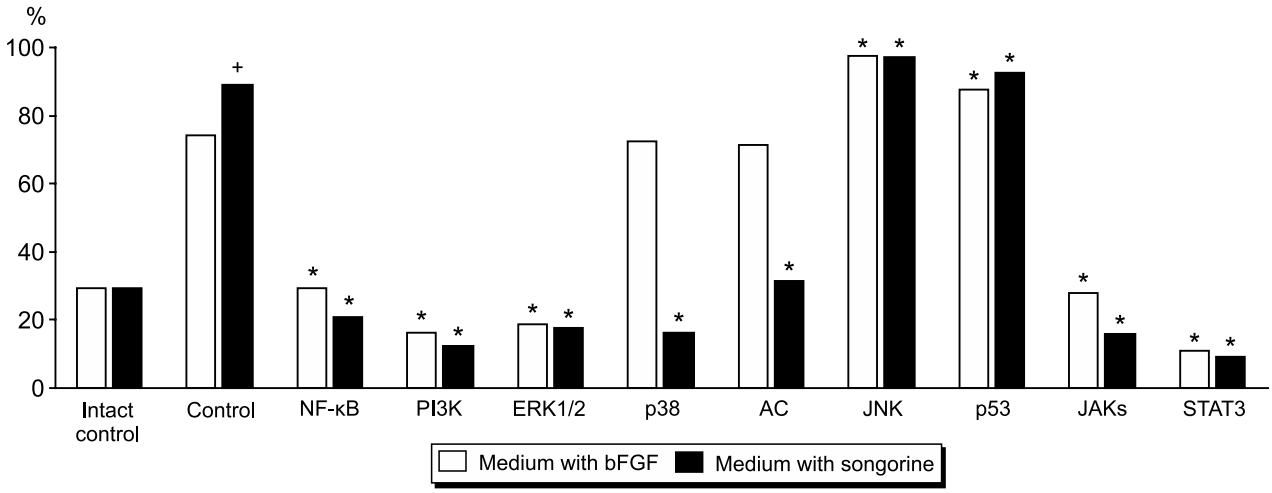

Fig. 2. Proliferative activity of NSC (% S-phase CFU-N) when the studied inhibitors were added *in vitro*. AC: adenylate cyclase. $p<0.05$ in comparison with *the corresponding control (medium without inhibitors), +the medium with bFGF in the control.

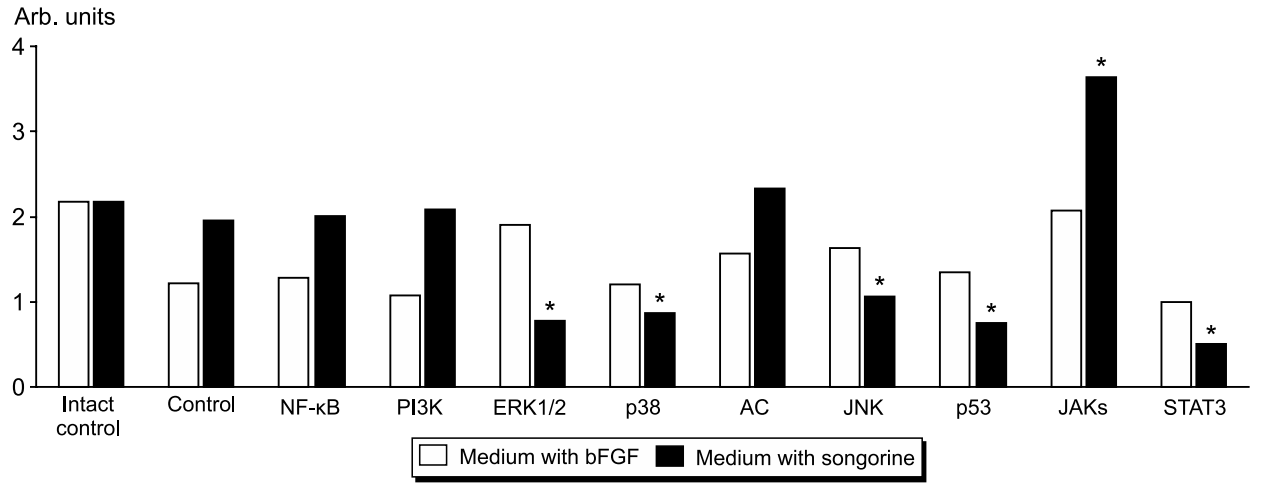

Fig. 3. The intensity of NSC differentiation (CIFU-N/CFU-N) when the studied inhibitors were added *in vitro*. AC: adenylate cyclase. $p<0.05$ in comparison with *the corresponding control (medium without inhibitors), +the medium with bFGF in the control.

The study of the participation of individual signaling pathways in the realization of the proliferative-differentiating potential of neural progenitors under the influence of FGF and songorine revealed a number of features of intracellular signaling for the plant-derived FGFR agonist. The selective blockade of NF-κB, PI3K, ERK1/2, JAKs, and STAT3 under conditions of stimulation of the realization of the growth potential of progenitor cells by bFGF was accompanied by a significant decrease in the yield of CFU-N and their proliferative activity (Figs. 1 and 2). At the same time, the JNK and p53 inhibitors, on the contrary, increased the level of neurosphere formation in the medium containing bFGF, and also increased their mitotic ability (to 131.1 and 117.9% of the same indicator in the medium with FGF without inhibitors of signaling molecules). The inactivation of NF-κB and ERK1/2 did not affect the realization of the growth potential of NSC. In addition, none of the inhibitors of signaling molecules affected the intensity of specialization (differentiation) of neural progenitors (Fig. 3).

In many respects, other regularities were recorded during the blockade of individual signaling molecules under conditions of stimulation of NSC functions with songorine. The blockade of NF-κB, PI3K, ERK1/2, JAKs, and STAT3 signaling was accompanied by changes in neurosphere formation and proliferation of NSC, similar to those when bFGF was added to the culture medium. The JNK and p53 inhibitors also (as in the case of exposure to bFGF) stimulated the release of CFU-N when the alkaloid was added to the medium. However, the severity of the increase in this indicator was significantly higher (by 2.4 and 4.1 times compared with the corresponding parameters when NSC functions were stimulated by FGF). In addition, the fundamental difference between intracellular signaling under the influence of songorine was the involvement of p38 and cAMP in the regulation of the NSC cell cycle. Inactivation of p38 and adenylate cyclase led to a significant decrease in the number of CFU-N and their mitotically active forms in the culture of nervous tissue cells (Figs. 1 and 2).

Another significant feature of the participation of individual signaling molecules in the regulation of NSC functions under the influence of songorine was their effect on the intensity of specialization of progenitor cells. The blockade of ERK1/2, p38, JNK, p53, and STAT3 caused a drop in the CFU-N differentiation index (minimal values were observed after STAT3 inactivation: to 25.2% of the initial level). At the same time, the JAKs inhibitor, on the contrary, significantly accelerated NSC maturation (to 185.7% of the initial level) (Fig. 3).

In general, the obtained results indicate a significant specificity of FGFR-mediated intracellular NSC signaling under the influence of songorine alkaloid in relation to that of the endogenous ligand (FGF) [15]. Stimulation of the functions of neural progenitors under the influence of the plant-derived FGFR agonist, in contrast to FGF, occurs with the participation not only of NF-κB, PI3K, ERK1/2, JAKs and STAT3 signaling, but also as a result of activation of an alternative MAP-kinase (p38-dependent [7]) and cAMP-dependent pathways. Obviously, it is this circumstance that determines the more significant stimulating activity of songorine in relation to the growth potential of NSC [12,15]. In addition, under the influence of the alkaloid, the pattern of regulation of the cell cycle of neural progenitors is characterized by the involvement of a number of signaling molecules (ERK1/2, p38, JNK, p53, JAKs, and STAT3) in controlling their differentiation. The role of ERK1/2, p38, JNK, p53, and STAT3 is confined to an increase in the intensity of their specialization, which, under certain conditions of CNS damage, can also contribute to accelerated recovery of the nervous tissue [1,10].

Thus, the intracellular molecular mechanisms of the cell cycle regulation of neural precursors by plant-derived FGF mimetic songorine have significant specificity compared to those of the endogenous FGFR ligand. The identified features of FGFR-mediated intracellular signaling in the presence of songorine explain its more pronounced stimulatory activity against NSC [12,15] and attest to great prospect of developing a cerebroprotective drug with neuroregenerative activity on its basis [12-14].

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Conflict of interest. The authors have no conflicts of interest to declare.

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