Proteomic Markers in the Muscles and Brain of Pigs Recovered from Hemorrhagic Stroke

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Abstract: (1) Background: Stroke is the leading cause of serious long-term disability. Walking dysfunction and paresis of the upper extremities occurs in more than 80% of people who have had a stroke. (2) Methods: We studied post-genomic markers in biosamples of muscle and brain tissue from animals that underwent intracerebral hematoma and recovered after 42 days. Our purpose was to understand the biological mechanisms associated with recovery from hemorrhagic stroke. We analyzed the peptides formed after trypsinolysis of samples by HPLC-MS, and the results were processed by bioinformatics methods, including the establishment of biochemical relationships (gene to gene) using topological omics databases such as Reactome and KEGG. (3) Results: In the pig brain, unique compounds were identified which are expressed during the recovery period after traumatic injury. These are molecular factors of activated microglia, and they contribute to the functional recovery of neurons and reduce instances of hematoma, edema, and oxidative stress. Complexes of the main binding factors of the neurotrophins involved in the differentiation and survival of nerve cells were found in muscles. (4) Conclusions: A network of gene interactions has been constructed for proteins involved in the regulation of synaptic transmission, in particular presynaptic vesicular and endocytic processes. The presence of transmitters and transporters associated with stimulation of NMDA receptors at neuromuscular junctions shows the relationship between upper motor neurons and neuromuscular junctions.

Keywords: proteomics; intracerebral hematoma; protein–protein network interaction; bioinformatics

1. Introduction

Among the numerous consequences of stroke, movement disorders are the main cause of long-term disability in the adult population. Limb paralysis, gait dysfunction, and paresis of the upper extremities persist in more than 80% of people who have had a hemorrhagic stroke [1]. The prognosis of recovery from movement disorders is difficult, and is based on a subjective clinical assessment. Six months after a stroke, two-thirds of survivors are unable to perform daily activities, and often do not return to work [2].

It is known that brain damage accompanied by a large cascade of reactions, including damage to the upper motor neurons and their descending corticospinal tracts, leads to subsequent muscle weakness [1,3,4]. The greatest effectiveness of motor rehabilitation is achieved within a three-month time window, although recovery may continue at a slower pace in subsequent months and years [5].

At present, the interaction of many biological mechanisms that limit recovery, including the motor system, after a stroke remains poorly understood. Various biomodels have been used to understand the biochemical pathways that affect recovery time after stroke. The use of pigs as a model for studying stroke is due to the similarity of their neurophysiological processes with humans [6]. Using pigs as an example, we sought to
apply an exploratory approach to identifying molecular pathways of recovery, including moto-sensory functions, after stroke, since they are not well understood. The study of postgenomic markers of the organism by proteomic methods reflects both rapid changes in the acute period of stroke and molecular pathways of recovery after stroke, and changes in the long term [7].

The aim of our study was to identify proteins and genes encoding their expression, as well as to establish biochemical relationships in order to understand the biological mechanisms associated with recovery after hemorrhagic stroke.

2. Materials and Methods

As objects of study, biosubstrates were used—fragments of the brain of female pigs (hybrids of the Vietnamese Pot-bellied × Wiesenau, n = 9, 280–300 days old and weighing 44.7 ± 4.3 kg), divided into control, sham-operated, and reconvalescents—which were recovered after reproduction of intracerebral hematoma.

A model of left-sided intracerebral hemorrhage (IH) was used, stereotaxically (coordinates: A:3; H:10; L:12 [8]) inserted mandrel-wire knife destroyed appropriate brain structures (tissues and local blood vessels in the area of Capsule interna (CI)) for four rotations. Further autologous venous blood (from the ear vein of the same pig, 300 mkl) was injected into the damaged area (Figure 1a). This surgery allowed us to obtain a reproducible model of intracerebral hemorrhage with necessary precision, as was described in [9]. Sham surgery was performed without damaging the brain structures.

Full recovery after surgery was noted on day 42, according to the results of health monitoring (control of physiological parameters included body weight, physical activity, motor and cognitive functions, biochemicals, hematological blood parameters, distribution of leukocytes in the blood, and brain histology).

Brain samples (0.7 × 0.5 cm) were obtained from the left hemisphere in the IH zone (IP, ipsilateral) and right (CL, contralateral) hemispheres (Figure 1b). Similar samples were
taken from 3 control (CON) and sham-operated (SHAM) pigs. Muscle tissue samples (biceps femoris) were also taken from IH and CON animals.

Figure 2 shows protein separation of the samples using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [10]. For proteomic analysis, protein fractions were selected in the range of molecular weights of 50–40 kDa, 35–30 kDa, and 20 kDa for the brain and 25 kDa, 20 kDa, and 15 kDa for muscle tissues, because of the difference among the analyzed groups of samples. Thus, 5 protein fractions for brain samples and 3 protein fractions for muscle samples were selected for further proteomic analysis.

**Figure 2.** SDS-PAGE of pig brain and muscle biosubstrates: IH, convalescent pigs; SHAM, sham operated; CON, intact pigs; IpL, ipsilateral side; CL—contralateral side. Fractions selected for mass spectrometric studies are marked with yellow rectangles.

The HPLC-MS/MS analysis of the protein bands was carried out using equipment of the “Human Proteome” Core Facility Center (Institute of Biomedical Chemistry, Moscow). Protein hydrolysis with trypsin in a polyacrylamide gel was performed according to the protocol described earlier [11]. Peptides were analyzed using an Ultimate 3000 RSLCnano high-performance liquid chromatography (HPLC) system (Thermo Scientific, Waltham, MA, USA) connected to a Q-Exactive HF mass spectrometer (Thermo Scientific, USA), according to the procedure described in [12].

Proteins were identified by the MaxQuant v.1.6.17.0 program using the Andromeda search algorithm [13]. Proteins were identified using the Uniprot database, with a restriction on the species specificity of the studied organism Sus scrofa (UP8227 S_scrofa). The following search parameters were set: the cleaving enzyme trypsin, the accuracy of mass determination of monoisotopic peptides of ±5 ppm, the mass accuracy in tandem mass spectrometry (MS/MS) spectra of ±0.01 Da, and the possibility of skipping two trypsin cleavage sites. Oxidation of methionines and modification of cysteine by propionamide were considered possible modifications of the peptides. For validation of comparisons (pairing) of PSM (Peptide–Spectrum Matches) spectra and peptides, identification of peptides, and identification of proteins, an FDR (False Discovery Rate) value of no more than 0.01 (FDR < 0.01) was used. Proteins were considered to be reliably identified if at least two peptides were found for them. Label-free quantification of protein content was based on the empirical indicator Ibaq.

The PANTHER classification system was used to categorize and analyze the gene ontology (GO) of the identified proteins [14]. The Reactome pathways and reactions database (including data from NCBI, Ensembl, UniProt, and KEGG) were used to analyze the unique brain proteins of convalescent pigs [15]. Visual Paradigm (Hong Kong, China) was used for visualization.
The study was conducted in accordance with the Helsinki Declaration, and approved by the Ethics Committee of the V.M. Gorbatov Federal Research Center for Food Systems of the Russian Academy of Sciences (protocol #3/2019, dated 19 January 2019).

3. Results

Proteomic analysis made it possible to identify a total of 514 compounds for control brain samples, 402 for sham-operated, and 1114 for IH. At the same time, 294 proteins were present in all samples; 25 were common for the control and SHAM, 154 for the control and IH, and 46 for SHAM and IH. A total of 41 proteins were identified only in control samples, and 37 proteins were identified only in SHAM samples. For the IH samples, 620 compounds not found in other samples were identified, of which 195 compounds were common to the ipsi- and contralateral hemispheres, 216 proteins were characteristic of the ipsilateral hemisphere, and 209 proteins were characteristic of the contralateral hemisphere (Figure 3).

Figure 3. Venn diagrams showing the total number of porcine brain proteins identified and their distribution along the ipsilateral and contralateral sides for pigs with IH.

In order to understand the function of the identified proteins, analyses of the GO and REACTOME pathways were performed. An analysis of the distribution of proteins by cellular components did not reveal any differences: 43–45% accounted for cellular anatomical entity (GO: 0110165: 44.92% of proteins for CON, 44.42% for SHAM, and 43.65% for IH); 15% for protein-containing complex (GO:0032991: 14.92%, 14.88%, and 14.88%, respectively); 40–41% for intracellular anatomical structure components (GO:0005622: 40.17%, 40.70%, and 41.47%, respectively).

When distributing proteins according to molecular functions (Figure 4A), the main proportion of compounds in all samples belonged to proteins with binding and catalytic activities. In IH samples, compounds with molecular transducer activity and structural molecule activity increased. In samples IH and SHAM, relative to CON, a decrease in the amount of activity between the compounds and the translation regulator was revealed.
An analysis of the distribution of proteins by biological processes (Figure 4B) revealed that the main proportion of compounds in brain samples are involved in cellular, biological and metabolic processes, and localization. At the same time, the number of compounds involved in biological adhesion increased in the IH samples and immune processes. In IH samples, there was a decrease in the compounds involved in multi-organ processes, response to stimulus, signal processes, and developmental processes, which are processes occurring at the level of a multicellular organism. For IH samples, a unique protein, IGLL1 (GO:0044419), was identified.

When classifying the identified porcine brain compounds (Figure 4C), it was found that a significant proportion of the compounds belonged to metabolite interconversion enzymes, protein-modifying enzymes, cytoskeletal proteins, and protein-binding activity modulators. In IH samples, relative to CON and SHAM, an increase was found in the following: transport proteins, scaffold/adapter proteins, gene-specific transcription regulators, translational proteins, membrane transport proteins, chaperones, and transmembrane signaling receptors. At the same time, in IH samples, relative to CON and SHAM, a decrease in extracellular matrix proteins was detected by the intercellular signal molecule. In

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\text{Figure 4. The PANTHER classification of the identified proteins in the brain by molecular function (A), biological processes (B), and class of proteins (C).}
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the IH and SHAM samples, relative to CON, the amount of nucleic acid binding proteins increased.

The analysis of REACTOME pathways showed that 136 out of 184 genes corresponding to differentially expressed proteins characteristic of both hemispheres of the IH brain were associated with a total of 755 biological pathways (Figure 5). The most representative pathways included the following categories: cell cycle (checkpoint G2/M—16 genes, \( p = 8.3 \times 10^3 \); regulation of APC/C activators between G1/S and early anaphase—11 genes, \( p = 3.48 \times 10^3 \); G1/S checkpoint of DNA destruction—10 genes, \( p = 9.62 \times 10^3 \); ubiquitin-dependent degradation of cyclin D—9 genes, \( p = 9.38 \times 10^3 \)), RNA metabolism (regulation of mRNA stability by gold-binding proteins—13 genes, \( p = 1.03 \times 10^3 \); AUFI (hnRNP) mRNA binding and destabilization—10 genes, \( p = 9.45 \times 10^3 \)), programmed cell death (18 genes, \( p = 9.74 \times 10^3 \); including apoptosis—17 genes, \( p = 2.6 \times 10^3 \)), metabolism (regulation of ornithine decarboxylase—10 genes, \( p = 3.94 \times 10^3 \)), signal transduction (degradation of GLI1 by the proteasome—10 genes, \( p = 2.43 \times 10^3 \); degradation of GLI2 by the proteasome—10 genes, \( p = 2.43 \times 10^3 \); conversion of GLI3 by the proteasome into GLI3R—10 genes, \( p = 2.43 \times 10^3 \)), and immune system (cross-presentation of soluble exogenous antigens—9 genes, \( p = 8.02 \times 10^3 \)).

![Figure 5. Genes of identified porcine brain proteins in the corresponding biological processes in the REACTOME: proteins—proteins unique for IH brain samples; IpL, proteins unique to the ipsilateral hemisphere; CL, proteins unique to the contralateral hemisphere; GIpL, co-proteins common to the ipsilateral and contralateral hemispheres (Co are genes common to both hemispheres), for which a match was found in the REACTOME system.](image)

Of those identified in the ipsilateral hemisphere, 165 out of 189 genes for differentially expressed proteins were associated with 813 biological pathways. The key factors were: biological development (axonal guidance—31 genes, \( p = 6.80 \times 10^3 \), cellular response to the stimulus (cellular response to heat stress—18 genes, \( p = 1.27 \times 10^3 \)), protein metabolism (cooperation of prefoldin and TriC/CCT in actin and tubulin folding—9 genes,
$p = 1.26 \times 10^3$; association of TriC/CCT with target proteins during biosynthesis—9 genes, $p = 2.44 \times 10^3$; cooperation of PDCL (PhLP1) and TriC/CCT in the beta-fold of the G-protein—8 genes, $p = 4.29 \times 10^3$; signal transduction (neurotrophin retrograde signaling—7 genes, $p = 2.33 \times 10^3$; PCP/CE pathway—8 genes, $p = 2.25 \times 10^3$); vesicle-mediated transport (clathrin-mediated endocytosis, 14 genes, $p = 4.57 \times 10^3$; gap junction degradation, 5 genes, $p = 1.78 \times 10^3$); immune system (representation of MHC class II antigen—13 genes, $p = 1.14 \times 10^3$); and transport of small molecules (plasma clearance of lipoproteins—5 genes, $p = 6.34 \times 10^3$).

In the contralateral hemisphere of the brain, 199 genes for differentially expressed proteins were identified, of which 136 are associated with 529 biological processes. The most significant of these were processes related to the immune system (IL12—signaling pathway—12 genes, $p = 7.1 \times 10^3$), protein metabolism (initiation of eukaryotic translation—11 genes, $p = 4.89 \times 10^3$; termination of eukaryotic translation—8 genes, $p = 2.10 \times 10^3$; SRP-dependent co-translational protein targeted to the membrane—8 genes, $p = 4.51 \times 10^3$; mitochondrial translation—7 genes, $p = 9.05 \times 10^3$), cellular response to stimulus (EIF2AK4 (GCN2) response to amino acid deficiency—9 genes, $p = 6.48 \times 10^3$), metabolism (selenocysteine synthesis—8 genes, $p = 3.03 \times 10^3$), protein metabolism (peptide chain elongation—8 genes, $1.16 \times 10^3$), RNA metabolism (nonsense-mediated decay—8 genes, $p = 5.89 \times 10^3$), muscle contraction (striated muscle contraction—5 genes, $3.58 \times 10^3$).

Figure 6 compares the biological responses with the genes involved in them for the identified proteins unique to the brain of convalescent pigs.

**Figure 6.** Unique proteins identified for the cerebral hemispheres of convalescent pigs according to biological responses: ChMPF—chaperonin-mediated protein folding; CLME, clathrin-mediated endocytosis; CRHS—cellular response to heat stress; HSP90 and SHR—HSP90 chaperone cycle for steroid hormone receptors with ligand present; MTR—mitochondrial translation; IL12 FS, interleukin 12 signaling pathway; ETRI translation initiation; MHCMAPP—antigen processing and presentation mediated by MHC class I; SHH—Hedgehog signaling pathway; DNAChG1/S—G1/S DNA damage checkpoints.
Next, we carried out a comparative analysis of the protein composition of the muscle tissues of pigs IH and CON. In total, 201 compounds were identified for CON muscle samples, 219 for IH, and 152 proteins were present in all samples. There were 49 proteins identified only in control samples, and 66 proteins identified only in IH samples.

No differences were revealed in the samples with regard to the distribution of proteins by cellular components: cellular anatomical entity (GO:0110165) was found to account for 44.71% of the proteins in CON and 43.86% in IH; protein-containing complexes (GO:0032991) accounted for 16.86% and 16.14%, and intracellular components (GO:0005622) accounted for 40–41%; 38.43%, and 40.00%, respectively.

According to molecular functions (Figure 7A), the main proportion of compounds in all samples belonged to proteins with binding and catalytic activities. In IH samples, compounds with molecular transduction activity and structural molecule function increased. In IH samples, relative to CON, a decrease in the number of compounds and translational regulators was revealed. When distributing proteins according to seven molecular functions, an increase was found in IH muscle samples relative to CON compounds, translational regulators, molecular adapter activity, structural function, and transport activity, with a decrease in molecular function regulator proteins.

According to biological functions (Figure 7B), an increase in IH was noted relative to CON compounds involved in biological and signaling processes, and reactions to irritants, with a decrease in proteins involved in developmental processes and multi-organ processes. In addition, the proteins involved in the processes of biological adhesion were found in CON, but were not detected in IH muscle samples.

An analysis of the distribution by 18 classes of identified protein compounds (Figure 7C) showed that in the muscle samples of IH pigs, relative to CON, an increase was found in gene-specific regulators of transcription, translation, and membrane transport, as well as a decrease in extracellular matrix proteins, scaffold/adapter proteins, intracellular signal molecules, and immune proteins.

Over the course of further study, the identified proteins of the muscle tissue of convalescent pigs were subjected to a comparative analysis in order to exclude compounds which were common with CON samples and proteins identified in all brain samples. They were also compared with data from the literature, which had been previously identified for the muscle tissues of pigs. Filtration revealed 13 proteins unique to IH muscles (Table 1).

The identified proteins are involved in the processes of mitochondrial translation (DR1, MRPL18), biogenesis, and functioning of organelles (MRPL18, TMEM11, CBFB, MRPL58). EIF4E is a signaling protein that regulates cell growth and proliferation [16], and H-FABP is a muscle-specific small heat shock protein.

An analysis of protein-coding genes revealed that 6 out of 13 genes encode proteins and control various processes, including cell adhesion, formation of intercellular connections and polarity of cells, intercellular and cell-matrix interactions, and regulating the path of MAPK and PLC. It is interesting to note here that the regulation of intracellular signaling cascades of these pathways may indirectly indicate that proteins (RAP1B; MSRB3; HSPB3; 4EBP1) are part of the complex of the main binding factors of the neurotrophins involved in the nerve cells’ differentiation and survival.
Figure 7. The PANTHER classification of the identified proteins in porcine muscle by molecular function (A), biological processes (B) and class of proteins (C).
Table 1. Unique proteins identified in the muscle tissue of convalescent pigs.

| №  | Uniprot ID | Protein                                                                 | MW, kDa | Intensity     |
|----|------------|-------------------------------------------------------------------------|---------|---------------|
| 1  | Q9BG57     | Translation initiation factor 4E binding protein 1 (Fragment) EIF4E     | 10,697  | 125,890,000   |
| 2  | H6UI30     | Heart fatty acid-binding protein H-FABP                                | 14,761  | 243,990,000   |
| 3  | A0A287A271 | SHSP domain-containing protein HSPB3                                    | 16,808  | 189,480,000   |
| 4  | A0A287B931 | Uncharacterized protein RAP1B                                          | 18,778  | 749,450,000   |
| 5  | F1S534     | Down-regulator of transcription 1 (Negative cofactor 2-beta) DR1        | 19,245  | 15,008,000    |
| 6  | A0A286ZNK1 | Mitochondrial ribosomal protein L18 MRPL18                              | 19,919  | 194,620,000   |
| 7  | K7GP19     | 39S ribosomal protein L13, mitochondrial MRPL13                         | 2291    | 74,807,000    |
| 8  | A0A286ZJR2 | Mitochondrial ribosomal protein L58 MRPL58                              | 23,503  | 35,632,000    |
| 9  | A0A121VUJ8 | Peptide-methionine (R)-S-oxide reductase (EC 1.8.4.12) MSRB3           | 19,931  | 56,872,000    |
| 10 | A0A287B4D9 | GTPase HRas isoform 1 HRAS                                              | 21,223  | 281,550,000   |
| 11 | A0A287ARV8 | Mitochondrial ribosomal protein L12 MRPL12                              | 21,237  | 76,581,000    |
| 12 | A0A286ZWB1 | Transmembrane protein 11 TMEM11                                         | 21,287  | 128,060,000   |
| 13 | M3V828     | Core-binding factor subunit beta isoform X2 CBFB                        | 21,478  | 69,361,000    |

4. Discussion

In the brains of pigs which had recovered from intracerebral hematoma, unique proteins were identified to be involved in the biological reactions of proteostasis—autophagy (chaperonin-mediated and mitochondrial), protein biosynthesis (folding, formation of ensembles, and translocation of proteins), apoptosis, and endocytosis. Similar specific compounds expressed during recovery after traumatic injury may be molecular factors of activated microglia. In the acute period, activated microglia increases the production of pro-inflammatory, and potentially neurotoxic, mediators that damage neurons, and in the “secondary injury” phase, it promotes the resorption of hematoma and edema, reduces oxidative stress, and contributes to the functional recovery of neurons, which is reflected in the works of other researchers [17–19] and was indirectly confirmed in our experiment.

A significant portion of the IH proteins identified in the brain (>200) is involved in these processes, and modulates the folding, formation of ensembles, translocation of proteins, and protein quality control, maintaining cellular protein homeostasis. Thus, the identified proteins of the chaperone family (HSP60; Hsp70/HSPA1A; HSPA12A; HSP4; CALR/A0A287B0P6; HSP90AB1; TRIC/D0G0C8; F1RP17; A0A287AUX8; F1SQN1; L7PBE6; I3L9J4; I3LCA2) are responsible for the regulation of synthesized cellular processes, including novo proteins, refolding and degradation of misfolded proteins, disaggregation, membrane translocation, and endocytosis [15,20]. The components of the 20S proteasome complex (F1SSL6; A0A287BIV4; F2Z5K2; A0A287AT18; A0A287B088; A0A287B5Q7; A0A286ZLP7; Q5JC42) contribute to the removal of misfolded or damaged proteins [21,22].

The components of the translation initiation factor complex of the mRNA subset (F1RW03; F1RME2; I3LU08; A0A287BLN5; F1SRT0) are involved in cell proliferation, including the cell cycle, differentiation, and apoptosis [23,24]. The adapter protein complex (AP-2/I3LK24; K9J6K8; I3LY6Y) belongs to the components of the vesicle envelope, and is involved in clathrin-dependent endocytosis. Axonemal and cytoplasmic dyneins (A0A287B9W3; A0A286ZWC7) are involved in intracellular motility, including retrograde axonal transport, protein sorting, organelle movement, and spindle dynamics. Proteins which are regulators of neurotransmitter levels, including glutamate (GLS/F1SN47), as well as transporters/carriers of excitatory amino acids (SLC1A2/A0A287B6T1), have also been
identified. The presence of multifunctional proteins (EPB41L/A0A287BLI2; A0A287ACK8; A0A287ASJ1; A0A286ZL32) was noted; these are able to bind with and stabilize dopamine receptors on the plasma membrane of neurons, impart stability and plasticity to the neuron membrane, and also affect the polarity of neurons and axon growth (A0A287B9D0). In addition, representatives of the family of cytoskeletal proteins (A0A287AMR4; A0A287B749; A0A286ZJ54; Q9N0Y9; F1RU49), essential scaffold proteins that also stabilize the plasma membrane and organize intracellular organelles, have been found.

The data obtained have been confirmed by earlier studies [9], where it was found that in reconvalescent pigs, after 24 h and 60 days, an increase in the expression of proteins involved in the prevention of unwanted protein aggregation and apoptosis, the release of neurotransmitters, and the assembly of the cytoskeleton was revealed.

The network of gene interactions which we have discovered can be considered in relation to proteins involved in the regulation of synaptic transmission, as well as in particular presynaptic vesicular and endocytic processes. The presence of transmitters and transporters indirectly indicates the secretion of glutamate precursors from synaptic vesicles and subsequent stimulation of NMDA receptors in neuromuscular synapses. Such processes are a humoral link between upper motor neurons and neuromuscular synapses [25].

Unique proteins identified in the muscle tissue of reconvalescent pigs are involved in several brain functions, including emotion, learning, and memory. Fatty acid binding proteins (FABPs) play a critical role as their cellular shuttles [26]. EIF4E is a signaling protein that regulates cell growth and proliferation, and can be activated in dendrites due to the activation of neurons [27]. MSRB3 is involved in protein–protein interactions, including protein folding, neuroprotection, and cell survival [28]. HSPB3 is a specialized chaperone involved in muscle cell differentiation, cytoskeletal remodeling, and protein degradation, and HSPB3 dysregulation causes neuromuscular diseases [29–31]. RAP1B has not been characterized, but the signaling pathway plays a dominant role in the control of intercellular and cell-matrix interactions, regulating the function of integrins, trophic factors, and other adhesion molecules [32]. Mitochondrial ribosomal proteins that we have identified in muscle tissue may be responsible for the degradation of polypeptides, induction of apoptosis, cells proliferation, and the reuse of broken ribosomes [33,34].

Together with the synaptic activation proteins identified in the brain, the detection of proteins in the brain and muscle tissues which are involved in the functioning and biogenesis of mitochondria, as well as signaling pathways of mitochondrial regulation, indicates the activity of mitochondria in distal axonal synapses and dendrites, which are key regulators of neuronal survival.

5. Conclusions

The signaling pathways which we identified mediate increased growth and branching of axons, an increase in the laminarity of neural structures and intercellular communications, a neuroprotective effect with increased remyelination, and an improvement in the functions of the motor cortex and its descending corticospinal tracts. The data obtained regarding the molecular mechanisms of recovery after hemorrhagic stroke can supplement our existing knowledge about molecular targets in the treatment, and can help to develop a prognostic system for assessing the recovery of patients after a stroke.

Author Contributions: Conceptualization, L.F. and A.M.; data curation, O.T.; formal analysis, G.T.; funding acquisition, L.F.; investigation, L.K.; methodology, O.T.; project administration, A.M.; resources, L.K.; supervision, E.V.; visualization, L.F. and E.V.; writing—original draft, L.F. and E.V.; writing—review and editing, L.F. and E.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Russian Science Foundation, grant number 19-76-10034.

Institutional Review Board Statement: The study was conducted in accordance with the Helsinki Declaration and approved by the Ethics Committee of the V.M. Gorbatov Federal Research Center for Food Systems of the Russian Academy of Sciences (protocol #3/2019, dated 19 January 2019).
Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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