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General Characteristics of Brain Immunohistochemical Markers

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Abstract

Neurons and neuroglia are important objects of medical and biological studies of the brain in normal and pathological conditions. The complexity of the cyto- and myeloarchitectonic of the central nervous system requires adequate methods for studying it. Immunohistochemical methods are among the most highly informative in establishing the morphological and functional characteristics of the nervous system, including during ontogenesis.The purpose of this review is to summarize and systematize literature data on immunohistochemical markers of neuro- and gliogenesis, proliferation, differentiation, and functional activity of nerve cells.

Key Words: *neurons, neuroglia, immunohistochemical markers*

Classification of markers of nerve cells

To identify nerve cells, several types of immunohistochemical markers can be distinguished:

1. markers of neuro- and gliogenesis
2. proliferation markers
3. differentiation markers
4. markers of synaptic activity
5. glia markers
6. astrocyte markers
7. oligodendrocyte markers
8. microgliocyte markers

Markers of neuro- and gliogenesis

Neurogenesis is a complex process that includes the proliferation of neuroblasts, their migration, differentiation, and integration of nerve cells into the neuronal network.

The sources for newly formed neurons and gliocytes are the same cells - neuronal stem cells (NSCs). They are present in all parts of the developing brain during embryogenesis, where they look like radial gliocytes.

There are a number of proteins, the presence of which in a cell with a high probability indicates its belonging to the NSC. Among the potential NSC markers, several groups can be distinguished: intermediate filament proteins (nestin, vimentin), transcription factors (Sox2, Pax6), proteins involved in Notch, want and Shh signaling pathways, and other regulatory proteins Msi-1, CDL33, Bmil [1, 20, 24].

Intermediate filament proteins

The best-known of the NSC molecular markers is nestin, which is involved in the formation of intermediate filaments along with vimentin and neurofilaments. Usually, nestin is found in poorly differentiated cells and plays an important role in neuropil growth. The expression of nestin is determined at the early stages of embryonic development in several cell types: radial gliocytes, multipotent neuronal progenitor cells, and polypotent progenitor cells. The cessation of proliferation of nestin-immunopositive cells is accompanied by a rapid decrease in the concentration of nestin mRNA and, accordingly, a decrease in the population of nestin-immunopositive cells [1, 4].

Vimentin is a protein of intermediate filaments of connective tissue and tissues of mesodermal origin. During embryogenesis, vimentin is found in the precursors of the main CNS cell types, radial lipocytes. In the mature brain, vimentin is found in endotheliocytes, ependymocytes, astrocytes, and meningocytes. [1, 20, 22].

Transcription factors

The second group of neuroblast and glioblast markers is the transcription factors Sox2 and Pax6. The Sox2 protein is encoded by the SRY gene (sex-

determining region Y) and, by binding to certain DNA regions, regulates the level of expression of individual genes. During neurogenesis, Sox2 maintains the pluripotency of CNS stem cells. The lack of Sox2 causes high embryonic lethality and leads to the appearance of various anomalies in mature neurons. The expression level of Sox2 decreases with cell differentiation, however, a significant amount of Sox2 is retained in neurons at early stages of postnatal ontogenesis. In the brain of adult animals, Sox2 is expressed in neurons of various parts of the brain (neocortex, striatum, thalamus, subependymal region, hippocampus) and is involved in the regulation of the expression of the survivin protein, which inhibits the mitochondrial pathway of apoptosis by inhibiting caspase 9 [16].

Pax6 (Pairę box gene 6) – transcription factor, a member of the Pax family, plays an important role in the embryonic differentiation of cells of the central nervous system. Pax6 regulates the expression of other transcription factors: Sox2, cell adhesion proteins, and a number of other substances necessary for the proliferation, migration, and differentiation of nerve cells. In embryogenesis, Pax6 immunoreactivity is determined in the ventricular zone of the neural tube, eye primordia, pituitary gland, and olfactory placodes. In postnatal embryogenesis, Pax6 is expressed in the dentate gyrus and hippocampus [5, 19, 23].

Notch signaling pathway

Notch is a family of transmembrane proteins involved in neurogenesis. The Notch signaling pathway regulates the processes of intracellular and intercellular integration. Its launch is carried out according to the juxtacrine mechanism with direct physical contact of two cells, one of which carries the ligand, and the other the receptor corresponding to it). In neurogenesis, the Notch signaling pathway plays an important role in synaptic transmission, learning, and memory [2, 3].

Prominin-1 is a transmembrane glycoprotein involved in the topology of cell membranes. Its expression in postnatal ontogenesis is observed in hippocampal neurons and ependymocytes [22].

Proliferation markers

Currently, immunocytochemical labeling of cells passing through various phases of the cell cycle is used. The methods are based on the immunohistochemical detection of proteins and nucleosides, which are involved in the preparation of cells for mitosis. Labeled nucleosides and nuclear proteins involved in DNA metabolism or cell cycle regulation are used as markers [1, 20].

Thymidine is a nucleoside involved in the formation of the polynucleotide structure of DNA, it is absent in RNA.

Bromodeoxyuridine is a synthetic nucleotide, a non-radioactive analog of thymidine, is a marker of neuroblast proliferation, and can be detected in mature neurons during DNA repair synthesis and gene duplication.

Ethinyldeoxyuridine is an analogue of thymidine. DNA labeling using ethinyldeoxyuridine and bromodeoxyuridine is used to study the cell cycle of neuroproliferative cells in the brain.

Proliferating cell nuclear antigen (PCNA) – takes part in the processes of DNA repair and replication by interacting with the chromatin assembly factor. The concentration of PCNA in the nuclei of neuroblasts is highest in the G1 and S phases of the cell cycle and decreases in the G2 phase.

Minichromosome maintenance protein 2 (MCM2) protein is involved in the initiation of DNA replication and elongation. It stabilizes histones and helps prepare the cell for mitosis. The concentration of MCM2 is high during the entire cell cycle of maturing nerve cells.

The Ki-67 protein is found only in proliferating cells and is present in the nuclei of neurons in the G1, S, G2 phases and during mitosis. Upon transition to the G0 phase, the Ki-67 protein is destroyed.

Cyclin-dependent kinases (CDKs) are the main regulators of the cell cycle, their action is to phosphorylate certain target proteins in accordance with the phase of the cell cycle.

With damage to the central nervous system and the development of neurodegenerative diseases, reactivation of the cell cycle can occur in neurons, leading to polyploidy but not accompanied by division. At the same time, markers of proliferation can be detected in the nuclei of neurons [1, 20].

Differentiation markers

To assess the differentiation of brain stem cells, methods of immunocytochemical detection of marker proteins synthesized by them, whose synthesis is controlled by microRNA, are currently widely used. These neuromarkers include doublecortin and neuronal nuclear protein NeuN [1, 20, 24].

Doublecortin (Doublecortin, DCX) – a protein associated with microtubules is used to determine the degree of maturity of neurons; it is expressed almost exclusively by immature cortical neurons. Neuronal progenitor cells in embryogenesis begin to produce

DCX shortly after entering the cell cycle, with expression fading after 2–3 weeks, by the time of the final transformation into developed neurons, which corresponds to the second stage of ontogenesis of the rat brain cortex. Doublecortin is required for normal radial and tangential migration of differentiating neuroblasts in the developing cerebral cortex. The protein is expressed during the migration of neurons to the corresponding layers of the cortex. When the expression of doublecortin is impaired, heterotopias of neurons occur. However, data have been obtained in studies on the possible synthesis of doublecortin in neurons after the completion of their migration, which can be used to identify neurogenic activity in the brain of an adult animal. Expression of doublecortin in differentiated neurons may be associated with the ability of nerve cells to reorganize microtubules, and grow and regenerate axons. Doublecortin is localized in the neuron perikaryon (associated with polyribosomes), dendrites, and the initial segment of the axon and provides morphological stability of neurons, growth, and branching of dendrites [13, 24].

The NeuN protein (neuronal nuclear antigen) is located in the nuclei and perinuclear cytoplasm of only mature brain neurons. It is believed that NeuN appears at the early stages of embryonic development in postmitotic neuroblasts and is retained in differentiating and differentiated neurons throughout subsequent ontogeny. The binding of antibodies to the NeuN protein is noted mainly in the cell nuclei and, to a lesser extent, in the perinuclear region of the cytoplasm. In the nucleus, NeuN is located mainly in areas with low chromatin density and is absent in areas with dense DNA packing. Most intranuclear NeuN is associated with the nuclear matrix. The data from chromatography of nuclear proteins of the brain indicate the ability of the NeuN protein to bind to DNA. The fact that NeuN expression is associated with neuronal differentiation, which persists throughout the life of the cell, points to NeuN as a regulator of neuronal phenotype [21, 24].

Detection of synaptic activity of neurons

Synaptophysin is a glycoprotein found in the synaptic vesicles of brain neurons, the retina, and the adrenal medulla. Synaptophysin provides contact between the synaptic vesicle and the plasma membrane and is involved in the process of mediator exo- and endocytosis in synaptic transmission. It is used as a specific marker of synapses. Synaptophysin is used to assess the differentiation of neural stem cells in vitro. With the help of an immunohistochemical reaction to synaptophysin, synaptogenesis, the density of synapses is assessed, and the innervation of internal organs is studied. Mutation of synaptophysin in the protein gene leads to the fact that neurons lose the ability to package and transport neurotransmitters and cease to effectively transmit a nerve impulse. Without synaptophysin, vesicles with signal molecules freely eject their contents into the synaptic cleft, but the supply of vesicles in the neuron is not renewed. A nerve cell in the absence of this protein can transmit a nerve impulse a limited number of times until the entire supply of synaptic vesicles is exhausted. Mutations in the synaptophysin gene are often detected in mental retardation since the basis of cognitive processes is the repeated conduction of a nerve impulse. Synaptophysin is actively formed in maturing rat neurons from the 7th day after birth during the period of synapse formation; its production continues throughout the life of the animal [12, 24].

Identification of catecholaminergic neurons

Tyrosine hydroxylase is an enzymatic marker of neurons in the catecholaminergic systems of the brain and sympathetic ganglia. Catecholaminergic neurons play an important role in the regulation of such physiological processes and behavioral acts as stress, sleep and wakefulness, learning, attention, memory, respiration, nociception and analgesia, sexual activity, aggressiveness, emotions, as well as in the regulation of hormonal activity and in the pathogenesis of many neurological and mental diseases, which include Parkinson's disease, attention deficit hyperactivity disorder, depression, schizophrenia, Alzheimer's disease, drug addiction.

The presence of tyrosine hydroxylase in the cytoplasm of a neuron indicates the ability to synthesize catecholamines. Two forms of tyrosine hydroxylase are known - cytosolic and membrane-bound. Cytosolic is located mainly in the somatodendritic region of the cytoplasm, and membrane-bound - in axon terminals [1, 14].

Identification of cholinergic neurons of the central nervous system

As the main neurotransmitter, cholinergic neurons of the CNS synthesize acetylcholine (Ach), which activates nicotinic and muscarinic cholinergic receptors. ACh plays an important role in regulating the functions of both the central and peripheral nervous systems, participates in the processes of learning, and memory, and provides motor and sensory functions.

Morphological studies of cholinergic neurons are performed using immunohistochemical detection of the protein of the vesicular ACh transporter or the

enzyme choline acetyltransferase. This stains the cytoplasm, cholinergic terminals, and axodendritic and axosomatic synapses [1, 18].

Immunohistochemical markers of astrocytes

Astroglia is a heterogeneous population of cells that perform various functions. The most significant markers of astrocytes are enzymes, cytoplasmic, and transport proteins. They are localized mainly in the perinuclear cytoplasm and processes.

Glial fibrillary acidic protein refers to intermediate filaments. The level of its expression correlates with the morphological differentiation of astrocytes. The protein is found in pathological conditions of the central nervous system, accompanied by the proliferation and activation of astrocytes (ischemia, traumatic injury, inflammation, epilepsy, neurodegenerative diseases).

Glutamine synthetase is an enzyme highly specific for astrocytes. Astrocytes capture 80% of the glutamate released during synaptic transmission, under the influence of glutamine synthetase, glutamate is converted into glutamine and again released into the intercellular space, where it is used by glutamatergic neurons for the synthesis of glutamate.

Deiodinase type 2 (DIO-2) is an enzyme that catalyzes the conversion of thyroxine to triiodothyronine, and also ensures the conversion of triiodothyronine to diiodothyronine in the CNS. Only protoplasmic astroglia are capable of synthesizing DIO-2. The expression of this enzyme is significantly increased in cerebral ischemia and traumatic brain injury.

Aldehyde dehydrogenase – the immunoreactivity of this enzyme is determined in the perikarya and processes of astroglia, increasing with neurodegenerative processes [1, 9, 10, 11, 20].

Immunohistochemical markers of oligodendrocytes

Most often, myelin components are used as markers of oligodendrocytes: proteolipid protein, myelin basic protein, myelin-associated glycoprotein, 2,3-cyclic nucleotide-3-phosphodiesterase, basic protein of oligodendrocytes associated with myelin, myelin glycoprotein of oligodendrocytes.

Proteolipid protein is an integral protein of the plasma membrane, its expression is determined in the perikarya of oligodendrocytes, their processes, and myelin sheaths.

Myelin basic protein – is involved in the formation of multilayer membrane structures, their stabilization, and intercellular signaling. It is localized in the membrane of oligodendrocytes, their processes, and myelin.

The main protein of oligodendrocytes associated with myelin – contributes to the compaction and stabilization of myelin. Determined in myelin sheaths.

2,3-cyclic nucleotide 3-phosphodiesterase – is necessary for the hydrolysis of toxic cAMP into non-toxic AMP, which is then converted into neuroprotective adenosine. Immunoreactivity is determined in the bodies and processes of oligodendrocytes, and myelin sheaths.

Myelin-associated glycoprotein – is an integral protein of the plasma membrane of oligodendrocytes, is involved in the processes of myelination and intercellular signaling. Present in the periaxonal membrane.

The Myelin glycoprotein of oligodendrocytes is a myelin stabilizer. Antibodies to it allow the labeling of oligodendrocytes and myelin sheaths [6, 7, 8].

Immunohistochemical markers of microgliocytes

The Iba-1 protein is a calcium-binding protein involved in the reorganization of the cytoskeleton and cytoplasmic membrane necessary for phagocytosis. It is determined by the cytoplasm and processes of microglia.

The CD68 protein is a transmembrane protein involved in microglia activation and modulation of immune responses.

The CDIIb protein is the alpha subunit of the integrin type 3 complement receptor (CR3). It is used to label activated microglia.

Class II histocompatibility proteins are also used to mark microglia; their expression level significantly increases during pro-inflammatory cell activation and neurodegenerative processes. [15, 17].

Conclusion

Thus, the use of neuronal and glial markers makes it possible to evaluate the proliferation and differentiation of nerve cells and to study their morphological and functional features at the molecular level, which contributes to deepening knowledge about the pathogenesis of cerebral pathology and can serve as a fundamental for the prevention and correction of diseases of the nervous system.

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