THE ROLE OF EXTRACELLULAR MATRIX MOLECULES IN THE REGENERATION OF FROG OPTIC NERVE

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INTRODUCTION

The central nervous system, and the cranial and spinal nerves, which belong to the peripheral nervous system, are often injured during accidents. In mammals, only peripheral nerves can regenerate if the cut edges are reunited by surgery, while the central nervous system cannot regenerate after injury and the peripheral nerves cannot grow into the central nervous system. The causes of the absence of regeneration can be the glial scar, the absence of the basal lamina and the non-permissive molecular environment of the injured nerve.

In fish, amphibians and reptiles the central and peripheral nervous systems are able to regenerate. After cutting the optic nerves, axons of the retina can grow into the optic centers and the animal can see again as physiological research has proved. After the injury of the vestibulocochlear nerve regeneration can be seen histologically. The cut and reunited dorsal horn of the spinal cord can penetrate the spinal cord, where the terminals make functional connections. These results show that the microenvironment of the regenerating axon in lower vertebrates is different from the non-permissive environment in mammals.

In the last decades it was found that different molecular mechanisms help the regenerating axons to reach their targets. The extracellular matrix (ECM) macromolecules participate in these regenerating processes as they interact with the cell adhesion molecules (CAM) on the growth cone. They can regulate different signaling processes through the cytoskeleton and may modify gene expression. As the consequence of these processes, ECM macromolecules can play permissive or non-permissive roles in nerve regeneration, development and maturation of the nerve system. In spite of numerous data from *in vitro* experiments, the role of ECM macromolecules in nerve regeneration is still poorly understood.

The optic system of the frog is a good model to study the role of the ECM macromolecules in nerve regeneration. The time course of the regeneration is well known from physiological, behavioral and neuronal labeling experiments, therefore the changes of the ECM expression probably correlate with the regenerating process.

AIMS OF THE STUDY

To investigate the permissive or non-permissive role of the ECM macromolecules in nerve regeneration, we have to know the distribution pattern of each molecule in different structures of the nerve system. With these distribution maps it will be possible to follow the changes in the expression pattern of the ECM.

In accordance with the hypothesis presented above, our aims were the following:

1. The study of the distribution pattern of hyaluronic acid, tenascin C and fibronectin in the nervous system of the frog.
2. The quantitative study of the distribution of hyaluronic acid and tenascin C in the termination areas of the optic nerve.
3. The quantitative and qualitative changes of hyaluronic acid and tenascin C during optic nerve regeneration.

MATERIALS AND METHODS

The experiments were performed on adult common water frogs (*Rana esculenta*) in accordance with state regulations and with the approval of the University Animal Care Committee (No: 22/2001.). For detecting hyaluronic acid/ hyaluronan (HA), tenascin C and fibronectin we used specimens from three frogs. For nerve regeneration experiments we used 42 frogs. The animals were anesthetized with 0.1% MS-222 (3-aminobenzoic acid ethyl esther, Sigma, St. Louis, MO) and transcardially perfused with isotonic saline for 2-3 minutes. The whole central nervous system was removed and cut into blocks of telencephalon, diencephalon, brainstem with the attached cranial nerves, and spinal cord with dorsal and ventral roots fixed by immersion in Sainte-Marie's fixative (99% absolute ethanol and 1% concentrated acetic acid) overnight. The specimens were embedded into paraffin and sections were cut with a Reichert microtome at a thickness of 10 μm.

Detection of hyaluronan

Following deparaffination the sections were treated with 2% hydrogen peroxide in PBS for 30 minutes and with 1% BSA in PBS for 30 minutes. For the specific detection of HA we used a biotinylated hyaluronan binding probe (bHABC) in a final dilution of 1:10 overnight at 4 °C (kindly provided by Raija Tammi and Markku Tammi, Dept. Anat. Univ. of Kuopio, Kuopio, Finland). The sections were incubated by using avidin-biotin peroxidase complex for 1 hour and the reaction was completed with a DAB (diamino-benzidine) chromogen reaction.

We checked the specificity of the reaction by two methods. The negative controls included sections incubated without bHABC or with bHABC but after treating the sections with *Streptomyces* hyaluronidase (Calbiochem, California) in a final dilution of 4 mg/ml overnight. For positive controls we used hyaline cartilage from frog sternal cartilage.

Detection of tenascin C

For detecting tenascin C first we deparaffinated and then rehydrated the specimens. For immunoreactions we used monoclonal mouse tenascin C antibody (Sigma). After rehydration we rinsed specimens with 0.1 M PBS, then specimens were incubated with the primer antibody in final dilution 1:4000 overnight at room temperature. The negative control was treated with PBS only. The positive control was the kidney of the frog. On the

following day specimens were rinsed with 0.1 M PBS, then incubated with biotinylated anti-mouse secondary antibody for an hour. After that we rinsed with 0.1 M PBS, then the sections were incubated by using avidin-biotin peroxidase complex for one hour. Then the sections were incubated first with 0.1 M PBS, then with PB, and finally with .05 M pH 7.4 TRIS. The reaction was completed with a DAB chromogene reaction. Sections were washed with PB, then dehydrated and covered.

Detection of fibronectin

For detecting fibronectin first we deparaffinated and then rehydrated the specimens. After rehydration we blocked specimens with 20% normal horse serum overnight at 4 °C. For immunoreactions we used monoclonal mouse antibody (Sigma). After rehydration we rinsed specimens with 0.1 M PBS, then specimens were incubated with the primer antibody in final dilution 1:400 overnight at room temperature. The negative control was treated with PBS only. The positive control was the kidney and skin of the frog. Next day specimens were rinsed with 0.1 M PBS, then incubated with biotinylated anti-mouse antibody for an hour. After that we rinsed with 0.1 M PBS, then the sections were incubated by using avidin-biotin peroxidase complex for one hour. Then the sections were incubated first with 0.1 M PBS, then with PB and finally with .05 M pH 7.4 TRIS and the reaction was completed with a DAB chromogene reaction. Sections were washed with PB, then dehydrated and covered.

Semi quantitative and quantitative analysis

The HA, tenascin C and fibronectin positive areas from the representative sections of frog brain and spinal cord of were drawn with the aid of camera lucida attachment with a final magnification x80. Based on that, we represented the HA, tenascin C and fibronectin distribution pattern with five degrees of intensity. The terminology used for the different parts of the central nervous system was based on the comprehensive work of ten Donkelaar (1998). Photographs and digitized images were taken with a Nikon Eclipse 800 microscope using a Spot RT digital camera. Computer assisted image analysis was made with Scion Image (NIH analyze system). We measured the optical density of the HA and tenascin C reaction in the optic nerve and its termination areas because later we intended to study their role in optic nerve regeneration. From each section two randomly selected pictures were shot. Twenty measurements were carried out on each picture. The area of the

region of interest (ROI) for all measurements was 400 μm^2 and the area integrated mean grey values (AIMV) were calculated for each ROI, and those data were used for presentation of the results. Within each group, the mean and standard deviation (SD) of the AIMV were calculated.

Optic nerve regeneration experiments

The animals were anesthetized as described above, and then we opened their mouths and palatine mucosa. After cutting the oculomotor muscles, we transsected the right optic nerve near the eye. After operation we closed mucosa. We used 42 frogs for experiments, which survived after operation for 3 and 5 days, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 weeks. After the surviving period animals were anesthetized and transcardially perfused with isotonic saline for 2-3 minutes. The telencephalon, diencephalon and brainstem with the attached cranial nerves were removed, fixed by immersion in Sainte-Marie‘s fixative (99% absolute ethanol and 1% concentric acetic acid) overnight. The specimens were embedded into paraffin and sections were cut with a Reichert microtome at a thickness of 10 μm. We carried out HA probe and tenascin C reaction on the specimens. Photographs and digitized images were taken with a Nikon Eclipse 800 microscope using a Spot RT digital camera. Computer assisted image analysis was made with Scion Image (NIH analyze system). We measured the optical density of the HA and tenascin C reaction in the optic nerve and its termination areas at different survival periods.

RESULTS AND DISCUSSION

In the first part of our study we observed the quantitative distribution of HA and tenascin C, as well as the qualitative distribution of HA, tenascin C and fibronectin in the nervous system of the frog with specific histochemical reactions. During our studies we found that these ECM macromolecules showed inhomogenous distribution patterns in the gray and white matter. In the second part we showed the changes of the HA and tenascin-C distribution in the terminals of the optic nerve in the diencephalon and mesencephalon during optic nerve regeneration.

Distribution of HA reaction in the central nervous system of the frog

HA positive areas can be found from the telencephalon to the caudalmost part of the spinal cord. In the telencephalon the most intense reactions were found in the amygdala pars medialis and preoptic area and in the internal granular and molecular layers of the olfactory bulb. The same level of intensity was found in the diencephalon in the nucleus of Bellonci (nB), the optic nerve, the optic chiasm and the optic tract, in the mesencephalon B, D, F, 5 and 3 layers of the optic tectum, the basal optic nucleus, the lateral part and the neuropil of the isthmic nucleus and the perineuronal net (PN) around the nucleus of oculomotor nerve. The Purkinje cell layer in the cerebellum, the PN around cochlear and lateral vestibular nucleus and the transitional zone of the cranial nerves in the rhombencephalon showed similar intense reactions. In the spinal cord the PN around motoneurons and the transitional zone of the dorsal root were intensely stained. The PN around the perikaryon of the lateral geniculate body (LGB) in the diencephalon, G, E, C, 6, 7 and 8 layers of the optic tectum in mesencephalon, the molecular and granular layers in the cerebellum, the medial and median zones of the reticular formation in the rhombencephalon and the transitional zone of the ventral root in the spinal cord were less intensely stained. The A, 1, 2 and 4 layers of the optic tectum and the peripheral parts of the cranial nerves and spinal roots were faintly stained.

The optical density measurements in the optic centers showed that in normal frog in the diencephalon the highest optical density was in the nucleus of Bellonci. The optical density was higher in the lateral geniculate body and preoptic area than in the magnocellular preoptic nucleus. In the optic tectum of mesencephalon in the B layer we found the highest density, and in the A, 1, 2 and 4 layers we found the lowest.

Compared to earlier experiments we found certain overlap in the HA pattern of the vestibular system and reticular nucleus in frog, rat and human brain. Besides similarity, some differences can be detected: the optic tectum is rich in HA, while there was no reaction in the rat and human superior colliculus, which is homologous of the optic tectum of the frog. We cannot explain this difference, but it may be related to the fact that the optic nerve does not regenerate in mammals.

In the gray matter the HA can be found as a component of the perineuronal net. In accordance with literature data the HA molecule can take part in at least two interactions in the perineuronal net. It can connect with the HA receptors, with CD44 or RHAMM (receptor for hyaluronan-mediated motility) and the HA can bind different types of proteoglycans from the lectican family. Since the HA receptors and proteoglycans have not yet been studied in the frog, we cannot state for the moment whether both molecular groups could participate in frog HA binding.

The distribution of tenascin C in the central nervous system of the frog

As with HA, the tenascin C positive areas can be found from the telencephalon to the caudalmost part of the spinal cord in the central nervous system of the frog. Intense tenascin C immunoreaction (IR) appeared in the optic nerve, optic chiasm and optic tract of the diencephalon, in the G, F, 8 and 7 layers of the optic tectum of the mesencephalon, and in the Purkinje cell layer of the cerebellum. In the rhombencephalon the PN around the cochlear and lateral vestibular nucleus, the transitional zone of the motor and sensory cranial nerves, and the PN around motoneurons of the spinal cord and the transitional zone of the dorsal root were intensely stained. The PN around the perikaryon of the amygdala pars medialis, anterior commissure in the telencephalon, the PN around lateral geniculate body in the diencephalon, 5, 6, B, C, D and E layers of the optic tectum and basal optic nucleus in the mesencephalon were stained less intensively. In the rhombencephalon the superior olive and the medial and median zone of the reticular formation, and the transitional zone of the ventral root in spinal cord were less intensely stained. The A, 1, 2, 3 and 4 layers of the optic tectum, the molecular and granular layers of the cerebellum, and the peripheral part of the cranial nerves and spinal roots were faintly stained.

In contrast with our results in adult mouse and chicken, it was found that tenascin C expression is low in the central nervous system and is expressed in those parts of the nervous system where the neuronal plasticity is intense.

The distribution of fibronectin in the central nervous system of the frog

In our study the fibronectin reaction was positive in the gray and white matter in the brain and spinal cord of the frog. In the gray matter only the neuropil and cytoplasm of the neurons were intensely stained; the PN did not show a positive reaction. In the telencephalon the accessory olfactory bulb, anterior and pallial commissure, and in the diencephalon the optic nerve, optic chiasm and optic tract showed the most intensive fibronectin reactions. The isthmic nucleus in the mesencephalon, the Purkinje cell layer in the cerebellum, the cytoplasm of the motoneurons of the trigeminal nerve in the rhombencephalon, the transitional zone of the motor and sensory cranial nerves, the anterior commissure in the spinal cord, and the cytoplasm of the motoneurons showed intense fibronectin reactions. The basal optic nucleus was faintly stained.

The peripheral part of the cranial nerves and spinal roots were darkly stained. We do not know the cause of the inhomogenous distribution of the fibronectin in the white matter of the central nervous system. One reason can be that the glia cells which produce fibronectin are distributed inhomogenously in the white matter.

According to literature data, fibronectin is secreted by glia, while in our study we found fibronectin in the cytoplasm of the neurons. We do not know its cause. Possibly in frog the motoneurons can also secrete fibronectin. To prove this hypothesis, further investigations are needed to detect mRNA with the aid of *in situ* hybridization in neurons.

Comparison of the HA, tenascin C and fibronectin distribution patterns in the frog nervous system

Our research showed that the distribution pattern of HA, tenascin C and fibronectin is inhomogenous in the central nervous system of the frog. There were no similar studies in frogs and other lower vertebrates and there are scant data in higher vertebrates about the distribution pattern of ECM macromolecules in the central nervous system. We detected all those three molecules in the area of the gray and white matter, but we did not find a distribution related to any specific structure. In the gray matter the most important difference was that the HA and tenascin C can be found in the PN around the perikaryon and in dendrites, while fibronectin is detected in the cytoplasm of neurons. The neuropil showed a positive reaction in all of the ECM molecules investigated. The cause of the

similarity between distributions can be that the HA connects to the tenascin C in the PN. The most important similarities and differences in each area of the central nervous system are the following:

Telencephalon

The accessory olfactory bulb showed an intense fibronectin reaction, while for HA and tenascin C the reactions were much weaker. The magnocellular region of the preoptic area was intensely stained for HA. The PN can be seen around perikarya and proximal dendrites. In the tenascin C and fibronectin reaction it was weakly stained.

In the pallium we found intense reaction for HA while the tenascin C and fibronectin IR were weak. The anterior commissure was darkly stained for tenascin C reaction, while the pallial commissure was also stained by the fibronectin reaction. The lateral forebrain bundle was weakly stained with all of the three reactions.

Diencephalon

The nucleus of Bellonci was darkly stained for HA and the perineuronal net was not clearly distinguished in the nucleus of Bellonci on account of the heavily stained neuropil, while the same structure was moderately and inhomogenously stained for tenascin C and fibronectin. The lateral geniculate body showed positive reactions in all three cases, whereas the PN was easily recognizable in the lateral geniculate body with HA and tenascin C reactions.

The dorsal habenula was intensely stained with HA reaction, while the ventral habenula was faintly stained by all three reactions. The hypothalamus displayed an inhomogeneous HA reaction having darkly stained subependymal and subpial areas, however it faintly stained for tenascin C and fibronectin. The intensity of all three reactions was strong in the optic nerve, in the optic chiasm and in the optic tract.

Mesencephalon

In the optic tectum the HA and tenascin C reactions overlapped in several areas. Layers B, D, F, G, 5 and 3 more intensely stained for HA than other layers. There was a moderate HA reaction in layers C, F, G, 8, 7 and 6 whereas in layers A, 4, 2 and 1 we could find a very weak HA reaction. In layers F and 8 the darkly stained perineuronal net was recognizable. Layers G, F, 8 and 7 were more intensely stained for tenascin C than other layers. There was a moderate tenascin C reaction in layers 5, 6, B, D and E whereas in layers A, 1, 2, 3 and 4 we could find a very weak HA reaction. In layers 7 and F the

darkly stained perineuronal net was recognizable. In the termination areas of the contralateral primer afferent optics the reaction was intense in the F and G layers, while in the B and D layers the HA reaction was more intense than the tenascin C reaction. The intense HA and tenascin-C reaction in the primary afferent optic termination areas of the optic tectum may indicate that these molecules play an important role in the changes during optic nerve regeneration. From those layers which do not receive primary afferent optic fibers HA and tenascin C reactions were intense in layers 7 and 8 and the PN was recognizable. Of these two layers, layer 8 connects with the isthmic nucleus. Presumably after transecting the optic nerve, the isthmotectal connections change, and HA and tenascin C may play important roles in signaling processes. For fibronectin, we did not find significant differences between the staining of different tectal layers.

In the mesencephalic tegmentum the gray matter was intensely stained for HA and tenascin C, and moderately stained for fibronectin, while the anterodorsal and anteroventral tegmental nuclei were faintly stained for all ECM molecules. In the PN around the nucleus of the oculomotor and trochlear nerves we found intense HA and tenascin C reactions. Literature data clearly show that when there is a strong PN presence around neurons, those neurons are less vulnerable, to certain alterations (for example, Alzheimer's disease and Parkinson's disease). Around neurons of the oculomotor nerve the intense HA and tenascin C reactions show that these neurons are intact, or only slightly injured in diseases of the somatomotor neurons (amyotrophic lateral sclerosis). The basal optic nucleus was darkly stained for HA and tenascin C, while lightly stained for fibronectin reaction. In the nucleus isthmi the rim layer was faintly stained only for fibronectin, while in the neuropil intense staining was detected only for HA.

Cerebellum

All three molecules can be detected in all layers of the cerebellum, but the intensity was different.

Rhombencephalon

All nuclei of the motor cranial nerves were darkly stained for HA and tenascin C due to the presence of the perineuronal net around the motoneurons. This staining is not as intense as in the oculomotor nucleus. The nuclei of the sensory cranial nerves, the cochlear and the lateral vestibular (Deiters) nucleus showed intense HA and tenascin C reactions around neurons. The cytoplasm of the motoneurons intensely stained for fibronectin reaction.

The different parts of the reticular formation were differently stained with the three reactions; the large cells were surrounded with a very intensely stained perineuronal net. All three reactions were also positive in the superior olive. In the rhombencephalic white matter the intensity of the three reactions was variable.

We detected very strong reactions for all three ECM molecules in the transitional zone of the motor and sensory cranial nerves at their entrance to the neuraxis. The peripheral part of the cranial nerves was faintly stained for HA and tenascin C and darkly stained for fibronectin.

Spinal cord

All of the reactions were detected both in the gray and white matter of the spinal cord. In the ventral horn a very intense perineuronal net was discernable around the perikarya and proximal dendrites of the motoneurons with HA and tenascin C reactions, while the cytoplasm of the motoneurons was positive for fibronectin reaction. The anterior commissure was darkly stained for HA and tenascin C. In the white matter the most intense reaction was detected in the subpial area of the lateral funiculus. The transitional zone of the dorsal root was intensely stained with all reactions, whereas the transitional zone of the ventral root was less intensely stained. The ventral and dorsal roots were lightly stained with HA and tenascin C reactions and darkly stained for fibronectin.

Study of optic nerve regeneration

The changes of the HA reaction in the optic center of the frog during optic nerve regeneration

Diencephalon

In general from the fifth postoperative day, the intensity of the reaction decreased on both sides in the nB and LGB. Till the third postoperative week the decrease was less in both nuclei on the side receiving the intact optic fibers. From the third week the optical density increased on both sides and the increase was higher in the regenerated fibers on the receiving side. In all stages the changes were less intense in the nB than in the LGB and this difference could be seen n both sides.

The disintegration of the PN on the injured side in the lateral geniculate body started in the second week and could be seen until the fifth week. From this time the disintegration of the PN was gradually diminished. The disintegration of the PN and decrease of the HA reaction may be important, because it shows the forming of new

synaptic connections that can be stabilized by the reintegrated PN. The disintegration of the PN in the lateral geniculate body can be seen on the injured side from the second to the fifth weeks, which corresponds to the time when the regenerated optic nerve fibers reach the diencephalon.

Mesencephalon

The optical density measurements in different survival periods showed the following: on the fifth postoperative day we did not find differences from the normal frog. On the second and third week the D layer was less intensely stained, than in the normal frog, and in this time we found changes in the LGB, as well. In the other layers similar HA staining could be seen. From the fifth postoperative week we found changes in the D, B and F layers. In the regenerating fibers on the receiving side the reaction was homogenous in the D, B and F layers. In layers F and 8 the PN could be seen in all survival periods, while in the case of the tenascin C reaction, the PN in layers 7 and F could be seen only on the sixth week.

Until the fifth postoperative week the basal optic nucleus showed a positive HA reaction on both sides. On the fifth postoperative week we found a faint HA reaction on the regenerating fibers of the receiving side and a positive HA reaction could be seen only from the ninth week.

During optic nerve regeneration the order of arrival to the diencephalic and mesencephalic targets by the regenerating optic nerve fibers has been demonstrated in the literature. On the fifth to eighth week the reaction in the optic tectum and BON decreased, which is in accordance with the observation of Stelzner et al., 1981, so the regenerating optic fibers reach the mesencephalic center on the eighth week. We found that HA reaction is higher in those layers where the primary afferent optic fibers terminate. The role of HA in nerve regeneration is not well known, however, its permissive role has been described in the peripheral nerve system of the rat and non-permissive role has been described in cell migration and fiber growth in the central nervous system. Its effect is influenced by the molecular weight of HA and by the connecting proteoglycans and cell surface receptors that can take part in signaling processes, and can modify its permissive or non-permissive role.

The changes of the tenascin C reaction in the optic centers of the frog during optic nerve regeneration

Diencephalon

In the area of the nucleus of Bellonci we found a weak tenascin C reaction in all cases. On the second to third postoperative week the perineuronal net became disintegrated on the operated side in the lateral geniculate body, in contrast with the intact side, when the PN reintegrated on the fourth week. From the second week the intensity of tenascin C reaction decreased in the lateral geniculate body on both sides. From the ninth week the optical density of tenascin C reaction increased and on the eleventh week it approached the intensity values detected in normal frog.

Mesencephalon

In the optic tectum the intensity of the tenascin C reaction was highest in the G, F, 8 and 7 layers and lowest in the A,1 ,2, 3 and 4 layers just as in the normal frog.

Between the third and sixth survival weeks we found a more intense reaction than in the normal frog in all layers on both sides. The PN can only be seen in layers 7 and F on the sixth week. The optical density was highest between the third to sixth weeks, decreased on both sides, and then it became the lowest by the eighth to ninth weeks. By the twelfth week the optical density of the tenascin C reaction approached the values of the normal frog on both sides. In the BON, the changes were similar to those found in the HA, but degree of the changes was less.

Comparing the changes of the HA and tenascin C reactions, the disintegration of the PN in the LGB can be seen on both sides between the second and third weeks. In the optic tectum the changes were less intense for both reactions than in the diencephalon.

According to the literature, tenascin C plays an important role in nerve regeneration of the peripheral and central nervous system after nerve injury, but the exact role is not clearly understood. The changes in expression patterns during optic nerve regeneration are similar in other frog species, while in triton the changes are opposite. All literature data show that in contrast to higher vertebrates, tenascin C expression does not decrease in different brain areas during development of the amphibian central nervous system. This pedomorphism may explain why the optic nerve regenerates in amphibians.

The possible role of HA and tenascin C in optic nerve regeneration

Based on our investigations we demonstrated that HA and tenascin C distributions change during optic nerve regeneration in the diencephalic and mesencephalic optic centers. We cannot draw conclusions about the permissive or non-permissive roles of HA and tenascin C in optic nerve regeneration based on changes in the distribution patterns and intensity of reactions. The effect of the ECM molecules can be influenced by numerous conditions: such as the molecular weight in the case of HA and the expression of the different splice-variants in the case of tenascin C. The connection to different receptors and the type of enzymatic degradation may also play important roles.

Nowadays many data suggest the hypothesis that the ECM macromolecules take part in a signaling complex in the synaptic gap. In this complex the ECM macromolecules connect to receptors on pre-and postsynaptic membranes, CAMs, ion channels, neurotransmitter receptors and receptor tyrosine kinase and thus may coordinate the pre- and postsynaptic events. The signaling complex influences the release of neurotransmitters through the cytoskeleton, and different functions of the cell can be modified by changing gene expression. As a result of all of these processes, different macromolecules of the ECM can play permissive or non-permissive role in nerve regeneration and plasticity. During nerve injury, deafferentation of cells can cause changes in the signaling complexes, which can promote such intracellular processes that lead to changes in the composition of the ECM around neurons. The cause of the different regeneration potentials of the nervous system at distinct developmental stages in different species can be the different distribution of the ECM in the nervous system.

SUMMARY

In the first part of our study we observed, with the aid of specific histochemical reactions, the distribution patterns of hyaluronan (HA), tenascin C and fibronectin, which are extracellular matrix (ECM) molecules in the central nervous system of the frog. We determined that all of the three molecules were present in the gray and white matter in the telencephalon, diencephalon, mesencephalon, cerebellum, rhombencephalon and spinal cord, but we did not find a distribution that could be connected to specific structures. In the gray matter the most important difference was that the HA and tenascin C reaction can be found in the perineuronal net (PN) around perikaryon, while the fibronectin reaction was found in the cytoplasm of neurons. The cause of the similarities between the distribution of HA and tenascin C may be that HA is connected to tenascin C in the PN. We showed the differences of the HA and tenascin C reaction in the termination areas of the optic nerve by measuring their optical density.

In the second part of our study we examined the changes of HA and tenascin C distribution after transecting the optic nerve. From the fifth survival day the intensity of the reaction decreased in the nucleus of Bellonci (nB) and the lateral geniculate body (LGB) on both sides. Till the third postoperative week a lower decrease can be found in the intact optic nerve fibers of the receiving side in both nuclei. The intensity of the reaction in the nB decreased to a lesser degree than in the LGB at all investigated stages and this difference could be observed on both sides. With both of the reactions we found disintegration of the PN in the LGB on the regenerating optic nerve fibers of the receiving side between the second and fourth weeks. This finding indicates that HA and tenascin C are important for the initiation of regeneration. The increase in reaction intensity in later reintegration stages shows that reexpression of HA and tenascin C may contribute to the stabilization of new synaptic connections in the reintegrating PN. The degree of changes was less in the layers of the optic tectum than in the diencephalic centers. According to our results it can be established that optic nerve regeneration is accompanied by qualitative and quantitative changes of HA and tenascin C in the optic center of the frog.

PUBLICATIONS

IN EXTENSO PUBLICATIONS:

1. **Szigeti ZM**, Matesz C, Szekely G, Felszeghy S, Bacskai T, Halasi G, Mészár Z, Modis L: Distribution of hyaluronic acid in the central nervous system of the frog. J Comp Neurol. 496:819-831. 2006 **IF:3.400**

2. Matesz C, Módis L, Halasi G, **Szigeti ZM**, Felszeghy S, Bacskai T, Székely G: Extracellular matrix molecules and their possible roles in the regeneration of frog nervous system. Brain Res Bull. 66:526-31. 2005 **IF:2.283**

ANOTHER IN EXTENS PUBLICATIONS:

1. Halasi G, Bácskai T, Wolf E, Módis L, Székely G, Mészár Z, **Szigeti ZM**, Matesz C: Vestibular lesion-induced changes in the expression of the hyaluronan in the frog, *Rana esculenta*. In preparation. Exp Brain Res.

CONGRESS ABSTRACTS:

1. Matesz C, Módis L, **Szigeti ZM**, Felszeghy S, Bácskai T, Halasi G, Székely G: Extracellular matrix molecules in the nervous system of the frog. Magyar Idegtudományi Társaság Kongresszusa, Balatonfüred, 2003. Clinical Neurosci. 56: 2. 57.

2. **Szigeti ZM**, Matesz C, Bácskai T, Halasi G, Matesz C: Hyaluronan distribution pattern in the nervous system of the frog. Magyar Idegtudományi Társaság Kongresszusa, Balatonfüred, 2003. Clinical Neurosci. 56: 2. 86-87.

3. **Szigeti ZM**, Matesz C, Bácskai T, Halasi G, Székely G, Módis L: Distribution of tenascin-C and fibronectin in the nervous system of the frog. IBRO International Workshop, Budapest, 2004. Clinical Neurosci. 57: 1. 65.

4. Matesz C, Módis L, Halasi G, **Szigeti ZM**, Felszeghy S, Bácskai T, Székely G: Extracellular matrix molecules and their possible role in the regeneration of the frog nervous system. Congerss Book of 4th ECCN. Oxford. 2004.

5. **Szigeti ZM**, Mészár Z, Matesz C, Módis L, Székely G: Expression of extracellular matrix molecules during optic nerve regeneration in the frog. Magyar Idegtudományi Társaság Kongresszusa, Pécs, 2005. Clinical Neurosci. 58: 1. 62.

6. Mészár Z, Matesz C, **Szigeti ZM**, Székely G, Felszeghy Sz, Módis L: Expression of hyaluronan and hyaluronan binding proteins during spinal cord development of chicken

embryos. Magyar Idegtudományi Társaság Kongresszusa, Pécs, 2005. Clinical Neurosci. 58: 1. 66-67.

7. **Szigeti ZM**, Mészár Z, Matesz K, Bácskai T, Székely G, Módis L: Distribution of the extracellular matrix molecules in the visual system of the frog. IBRO International Workshop, Budapest, 2006. Clinical Neurosci.