Luiza Stanaszek
NeuroRepair Department
Mossakowski Medical Research Centre PAS

Regulation of endogenous neurogenesis processes after forebrain ischemia: the involvement of matrix metalloproteinases in a signal transduction from extracellular matrix

Adviser: Teresa Zalewska, Prof.

ABSTRACT

Many recent studies have noted that ischemia resembles other brain injuries in producing enhanced neurogenesis in neuroproliferative regions of the adult rodent brain, including the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of dentate gyrus of the hippocampus. The discovery of neurogenic responses subsequent to ischemic injury has led to the hypothesis that the expansion of the pool of endogenous progenitors could augment the regenerative capacity of the damaged areas. Therefore, the identification of mechanisms that promote the proliferation of progenitors, migration toward injured brain areas and differentiation into the phenotype of lost neuronal cells has become particularly relevant to the development of stem cell-based therapies. It is hypothesized that following ischemic insult, neurogenesis proceeds as it does during embryonic development, involving the concerted action of cell surface receptors and extracellular matrix molecules (ECM), thereby providing an environment which may be instructive or permissive to neurogenesis-associated processes. In this context, enzymes that modify the extracellular matrix and cell adhesion molecules are particularly interesting. The matrix metalloproteinases -2 and -9 (MMP-2, MMP-9) are one of such group of proteinases known to play important roles in the ECM remodelling required for developmental processes. Cleavage of matrix components by proteolysis affects the interaction between the ECM and intracellular signalling pathways that would determine further cell fate. Despite ever-growing information concerning the involvement of MMPs in neurogenesis-associated processes *in vitro* and *ex vivo* in experimental stroke models the proof of relevance *in vivo* after transient forebrain ischemia is still missing.

This prompted us to investigate the role of signalling from extracellular matrix in the endogenous neurogenesis after forebrain ischemia in the adult gerbil. We employed a well defined model of 5 min. ischemia in Mongolian gerbils, which leads to neuronal death restricted to the CA1 region of the hippocampus. Our attention was primarily focused on the temporal and spatial relationship between the proliferation of neural stem/progenitor cells and /or further differentiation with activity of metalloproteinases – MMP-2 and MMP-9. In an effort to further elucidate the involvement of MMPs in neurogenesis-associated processes, we have also tested the effect of MMPs inhibitors on the development of a neural stem cell line derived from human umbilical cord blood (HUCB-NSCs). The last stage of investigation comprised the evaluation of expression and activity of non-receptor tyrosine kinases (FAK, PYK2, Src, ERK, JNK, Akt) engaged in signal transduction from extracellular matrix.

The results showed that adult neuronal progenitors proliferate *in situ* in response to ischemia. At the prolonged time of reperfusion (14 and 28 days) numerous progenitor cells relocate into the granular cell layer and become mature granular neurons.

In contrast, in the damaged CA1 pyramidal layer only a small number of proliferating cells was observed. Moreover, they did not express mature neuronal antigen, suggesting they undergo programmed cell death before attaining maturity. There was also no evidence of SGZ neural stem cells migration into the CA1 to replace neurons lost after ischemia. From the above it follows that in the present experimental conditions, the expected endogenous regenerative capacity fails as a source of meaningful compensation for lost neuronal circuits.

One of the most interesting findings obtained in the current work is that ischemia elicits contrasting effects on the spatial pattern of MMP activity that matches the progression of proliferation in the DG across time and correlates well with the process of differentiation of stem/progenitor cells into mature neurons. Such a spatio-temporal relationship between activation of MMPs and neurogenesis may suggest a casual link between these processes. This finds strong support in our cell culture experiments showing that inhibition of endogenous activity in the presence of SB-3CT significantly reduced both their proliferation and their differentiation toward the neuronal lineage. Simultaneously the number of oligodendrocytes and astrocytes augmented compared to the control, probably due to increased proliferation. These data support our *in vivo* results relative to the involvement of MMPs in the development of progenitors. Further support to stress the importance of MMPs in neurogenesis as compared with other proteinases stems from the failure of serine proteinase and furin inhibitors (Pefabloc and Dec-RVKR-CMK) to modulate this process.

In contrast, in the ischemia-damaged pyramidal cell layer the activity of MMPs dropped below the control levels during reperfusion. At comparable time points incipient activation of MMPs was found in adjacent brain areas – *stratum oriens* and *stratum radiatum.* In these regions increased MMPs activity may facilitate delayed tissue remodelling at the periphery of the lesion.

Focal adhesion kinase (FAK) and proline-rich calcium dependent kinase (PYK2) are thought to pay a major role in transduction of extracellular matrix-derived signal into the cells. Therefore, the last purpose of the current work was to check if the MMPs activity differentially affects the FAK- and/or PYK2-coupled signalling in investigated hippocampal structures as well as in the HUCB-NSCs culture. However, the obtained results do not allow to confirm the participation of the FAK- or PYK2-dependent pathway in post-ischemic neurogenesis in gerbil hippocampus.

In conclusion:

- global ischemia of a gerbil brain stimulates neurogenesis in the subgranular zone of the dentate gyrus
- spatio-temporal correlation between the neurogenesis and metalloproteinases activity in the dentate gyrus indicates the involvement of these enzymes in the development of stem/progenitor cells
- enhancement of post-ischemic endogenous neurogenesis does not enable compensation for lost neuronal circuits
- activity of metalloproteinases in the CA1 regions – *stratum oriens* and *stratum radiatum* may facilitate tissue remodelling
- on the basis of the obtained results it is not possible to state whether investigated non-receptor intracellular protein kinases – mediators of signal from ECM, participate in postischemic neurogenesis in gerbil hippocampus.