## Role of brain pericytes in the formation of central nervous system metastases

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Final report

Brain metastases are life threatening pathologies with limited therapeutic options, representing a major cause of death. Therefore, understanding key mechanisms involved in their formation is of primordial importance for the development of future therapies.

In the framework of the present project, we have been following the process of breast cancer brain metastasis formation starting from the transmigration of the tumour cells through the blood-brain barrier (BBB) until proliferation in the brain parenchyma (Figueira *et al*. Cancers 2021, DOI: 10.3390/cancers13040910), with particular focus on the interaction with pericytes. Our results underlined our theory that cells of the neurovascular unit have a Janus-faced attitude towards brain metastatic cells, being both destructive and protective (Wilhelm *et al.* J Cereb Blood Flow Metab 2018, DOI: 10.1177/0271678X17732025).

According to our *in vivo* and *ex vivo* observations, before extravasation into the brain, malignant cells induced vasoconstriction and development of intraluminal endothelial plugs, which isolated invading cells from the circulation (Haskó *et al.* Acta Neuropathol Commun 2019, DOI: 10.1186/s40478-019-0788-1). In addition, we were the first to show that breast cancer cells – in contrast to melanoma cells – were able to utilize the transcellular pathway (through individual endothelial cells, and not by opening the intercellular junctions) when migrating through the BBB (Herman *et al.* J Cell Mol Med 2019, DOI: 10.1111/jcmm.14156). During diapedesis, metastatic cells induced formation of multiluminal vessels and claudin-5-positive endothelial blebs. However, even severe endothelial blebbing could be reversed and the vessel morphology was restored shortly after the tumour cells completed transendothelial migration (Haskó *et al.* Acta Neuropathol Commun 2019, DOI: 10.1186/s40478-019-0788-1).

Similar to neuroinflammatory leukocytes, tumour cells migrated not only through the endothelial layer, but through the *glia limitans perivascularis* as well. Nevertheless, along with the growth of the metastatic lesions, astrocytes and astrocyte endfeet were gradually expelled from the vessels to the border of the tumour (Haskó *et al.* Acta Neuropathol Commun 2019, DOI: 10.1186/s40478-

019-0788-1). Peritumoral astrocytes became reactive and expressed MEF2C (myocyte enhancer factor 2C), presumably as a consequence of downregulated expression of miR-802-5p and miR-194-5p in mice bearing breast cancer brain tumours (Sereno *et al.* Mol Oncol 2020, DOI: 10.1002/1878-0261.12632). Indeed, miRNAs and extracellular vesicles are important elements of pre-metastatic and metastatic niche formation (Sereno *et al.* Cells 2020, DOI: 10.3390/cells9081790; Fazakas *et al.* Colloids Surf B Biointerfaces 2021, DOI: 10.1016/j.colsurfb.2021.111810) and distinctive biomarkers in precocious and advanced stages of breast cancer brain metastasis development (Figueira *et al.* Int J Mol Sci 2021, DOI: 10.3390/ijms22105214).

In the brain parenchyma, metastatic breast cancer cells grew by cooption of pre-existing capillaries. During this process, cancer cells attached to the extraluminal surface of the vessels. In this process, breast cancer cell-secreted nephronectin (Magnussen *et al.* Sci Rep 2020, DOI: 10.1038/s41598-020-69242-1) and pericyte-produced extracellular matrix proteins proved to be crucial.

Most importantly, by using *in vivo* and *in vitro* techniques, as well as human samples, we have shown that pericytes play a crucial role in the development of metastatic brain tumors by directly influencing key steps of the development of the disease (Molnár *et al.* Mol Oncol 2020, DOI: 10.1002/1878-0261.12752), as follows:

Brain pericytes had a prompt chemoattractant effect on breast cancer cells and established direct contacts with them. By secreting high amounts of extracellular matrix proteins, pericytes enhanced adhesion of both melanoma and triple negative cancer cells, which might be particularly important in the exclusive perivascular growth of these tumor cells.

In addition, pericyte-secreted factors had a very significant pro-proliferative effect on mammary carcinoma, but not on melanoma cells. Exosomes were not involved in this process. Interestingly, in response to pericyte-secreted factors, breast cancer cells – although higher in number – appeared to have fewer contact points with each other and became more dissociated. In line with this, E-cadherin expression decreased significantly in breast cancer cells exposed to pericyte-secreted factors. By inhibiting formation of intercellular adhesions and expression of E-cadherin, pericytes endowed breast cancer cells with a migratory, invasive phenotype, characteristic of cells undergoing epithelial-to-mesenchymal transition (EMT).

Among the pericyte-secreted factors responsible for tumour cell proliferation in the brain, using *in silico* and experimental tools, we identified insulin-like growth factor 2 (IGF2), highly and specifically expressed in pericytes among brain cells. By inhibiting IGF2 signaling using silencing or picropodophyllin (PPP), we could block the proliferation increasing effect of pericytes on breast cancer cells. Administration of PPP (a BBB-permeable substance) significantly decreased the size of brain tumors in mice inoculated with triple negative breast cancer cells.

Taken together, our results are the first to indicate that brain pericytes have significant pro-metastatic features, especially in breast cancer. Our study underlines the importance of targeting pericytes and the IGF axis as potential strategies in brain metastatic diseases.

Altogether, we published 21 peer-reviewed papers during the project period (with a cumulative IF of 122.72, 6 D1 and 12 Q1), with one first-author and six last-/co-last-author papers of the PI. The report is based on the first-author and three last-author papers of the PI and six co-authored articles strongly related to the project topic. The key paper of the project is Molnár *et al.* Mol Oncol 2020, DOI: 10.1002/1878-0261.12752, the results of which were presented in a plenary lecture of the PI in the 22$^{nd}$ International Symposium on Signal Transduction at the Blood-Brain Barriers (September 2019, Würzburg, Germany). The present grant has also substantiated the PhD thesis of Kinga Molnár, defended in 2021.