INVOLVEMENT OF THE NEUROVASCULAR UNIT IN THE FORMATION OF BRAIN METASTASES

Ph.D. thesis

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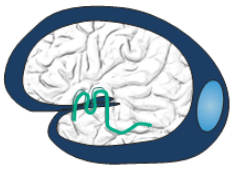

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1. Introduction

Cerebral metastases are frequent and dismal complications of a few tumour cell types, including lung cancer, breast cancer and melanoma. In breast cancer, which is the second most frequent cause of central nervous system (CNS) metastases, brain lesions primarily occur in triple negative (oestrogen receptor-, progesterone receptor- and Her2-negative) subtype. Triple negative breast cancer (TNBC) has a particularly dismal prognosis, many patients developing and eventually dying of brain metastasis. Prognosis of brain metastases is extremely poor; therefore, understanding the mechanisms involved in this process has a great importance. Presence of the blood-brain barrier (BBB), tumour heterogeneity and plasticity and unique molecular features of brain metastatic cells all contribute to the poor outcome of cerebral secondary tumours and the limited effectiveness of current treatments. The neurovascular unit (NVU) is a relatively new concept in neuroscience that describes the close relationship between brain cells and their blood vessels. It is formed by cerebral endothelial cells (CECs) conjoined by cellular junctions, together with pericytes, glial cells and neurons. Besides cellular elements, the basement membrane is also an integral part of the NVU, which contributes to the integrity of the BBB. To form metastasis, tumour cells have to complete a defined set of events, such as disseminating from the primary tumour as individual cancer cells and multi-cellular clusters, entering the blood or lymphatic vessels (intravasation), invading the target organ and surviving in the metastatic tumour microenvironment. Initial steps of brain metastasis formation are common with the development of non-cerebral metastases; however, the complex interaction of metastatic cells with the NVU confers very unique aspects to brain tumours.

2. Aims

In our experiments, we aimed at understanding reactions of the cellular elements of the NVU to the presence of metastatic tumour cells. We brought pericytes in the focus because the molecular details of the interactions of brain pericytes with metastatic tumour cells are largely unknown. We were primarily interested in breast cancer, especially TNBC since it is the most aggressive and successful brain metastasizing breast cancer subtype and one of the leading causes of brain metastases. We had previous results showing that melanoma cells utilize the paracellular migration during their invasion into the brain parenchyma. For this reason, we used these cells as a comparison.

Using *in vitro* and *in vivo* methods and human surgical samples as well, our specific aims were the following:

- To determine the possible transmigration routes of breast cancer cells: whether they use the paracellular or the transcellular way.
- To understand the reactions of CECs to invading TNBC cells.
- To explore whether metastasizing TNBC cells breach the *glia limitans perivascularis* and to find new mechanisms of interaction between cancer cells and astrocytes.
- To assess the role of pericytes in adhesion and proliferation of tumour cells *in vitro* and *in vivo.*
- To decipher the role of pericyte-secreted insulin-like growth factors (IGFs) in the proliferation of TNBC cells in the brain.

3. Results

3.1. Interactions of metastasizing breast cancer cells with CECs

Development of brain metastases is largely dependent on the ability of tumour cells to migrate through the tightest endothelium of the organism, which forms the BBB. Involvement of CECs in extravasation of cancer cells into the CNS is largely uncharacterized. We were primarily interested in understanding whether the tumour cells utilize only the paracellular transmigration pathway (through interendothelial junctions), as we have previously observed for melanoma cells, or transcellular migration (through the endothelial cell body) is also a possibility for the tumour cells to breach the brain endothelium.

We addressed this question using our *in vitro* transmigration experimental setup, where we plated cancer cells onto confluent layers of CECs and compared diapedesis of melanoma and TNBC cells using transmission electron microscopy (TEM). After 8 hours of co-culturing cancer cells with CECs, we observed several tumour cells attached to brain endothelial cells in close proximity to the interendothelial junctions. In the neighbourhood of the tumour cells, filopodia-like endothelial protrusions could be seen, partially engulfing the invading cells. We have also detected several breast cancer cells completely covered by endothelial processes, incorporating the tumour cell into the monolayer.

Melanoma cells transmigrated only paracellularly, through the tight and adherens junctions between endothelial cells, in accordance with our previous

confocal microscopy results. The same transmigration path was used by breast cancer cells as well; however, much less frequently. In addition, mammary carcinoma cells were able to also exploit the transcellular pathway—through individual endothelial cells—during their migration from the apical to the basolateral side of CECs **(Figure 1)**.

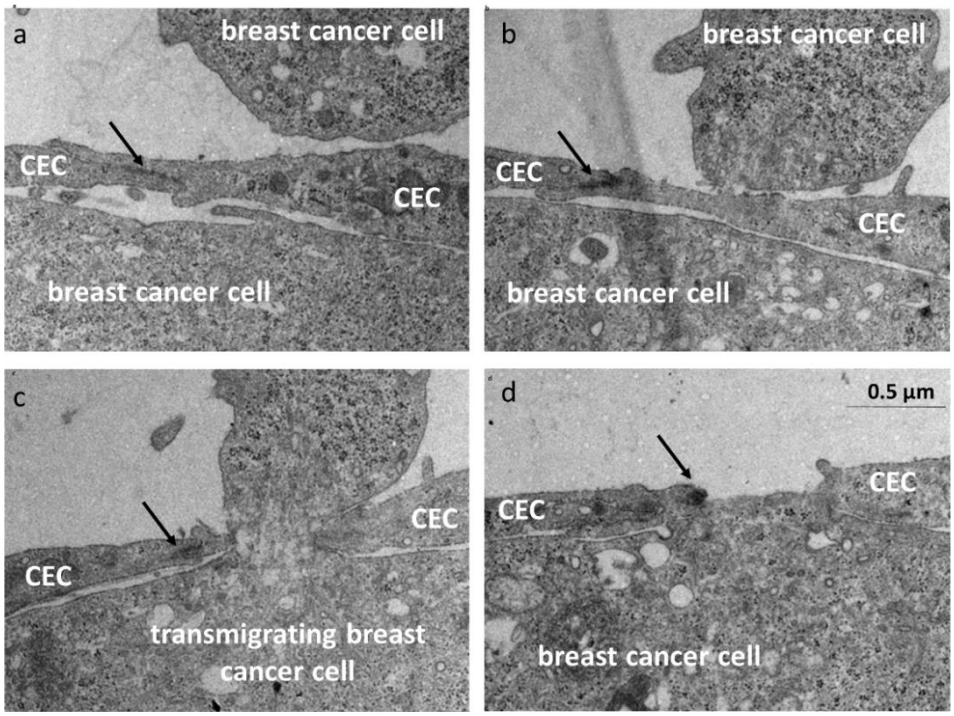

Figure 1: Transcellular migration of a breast cancer cells through a CEC. MDA-MB-231 cells were seeded onto a confluent monolayer of RBECs and left for 8 hours. Images presented are electron micrograph series of a transmigrating breast cancer cell. Arrows indicate interendothelial junctions.

In line with our *in vitro* results, we observed transcellular migration of breast cancer cells through cerebral capillaries of mice injected with tumour cells *in vivo*. We showed that breast cancer cells arrested in brain microvessels left the junctions intact during extravasation, as indicated by continuous claudin-5 immunostaining.

We observed several other endothelial changes before and during the transmigration process of TNBC cells, including formation of apical endothelial protrusions *in vitro* and development of basolateral claudin-5-positive blebs in initial phases of the transmigration process. In addition, after 24-48 hours spent in the lumen of parenchymal vessels, mammary carcinoma cells induced vasoconstriction and formation of endothelial plugs, which protruded into the lumen both upstream and downstream of the cancer cells to isolate the tumour cells from the circulation. Confocal microscopy images indicated that plugs were

formed by the nuclei of endothelial cells, which protruded into and obstructed the vessel lumen. Interestingly, these plugs were formed as a result of endothelial reorganization and not cell division. In addition, in some cases, tumour cell-containing capillaries were obstructed by vasoconstriction. Isolation of tumour cells from the circulating blood may have several consequences on the metastatic process, primarily protection from shear stress and immune attacks.

Migration of the tumour cells through the cerebral endothelium was frequently accompanied by blebbing of metastatic and/or endothelial cells. Ranging from a few extraluminal blebs to moderate or intensive vacuolization, blebbing of CECs was always linked to the presence of the tumour cells. Interestingly, even severe endothelial blebbing could be reversed and the vessels were restored in 1-2 days after the tumour cells completed transendothelial migration.

Furthermore, CECs, which were involved in plug formation, were found to overexpress N-cadherin. Interestingly, CECs not involved in plug formation and transmigrating breast cancer cells were all N-cadherin negative. In contrast, N-cadherin was shown to mediate interactions between melanoma and endothelial cells both in the brain and the periphery. Similarly, when melanoma cells were seeded upon a confluent monolayer of CECs *in vitro*, tumour cells tended to rapidly intercalate among CECs. We observed the appearance of N-cadherin in the melanoma-melanoma and melanoma-endothelial contact regions. However, almost no N-cadherin was detected in endothelial-breast cancer cell co-cultures, suggesting that N-cadherin is not involved in the transmigration process itself of breast cancer cells.

3.2. Interactions of metastasizing breast cancer cells with astrocytes

Beside CECs, astrocytes are the most active players in immediately responding to and continuously associating with invading tumour cells. Our aim was to understand whether—similarly to immune cells during neuroinflammation—breast cancer cells are able to penetrate the *glia limitans perivascularis* (formed by astrocytic end-feet) or immediately dissociate them from the vessels after migration through the endothelium.

Using mice inoculated with tdTomato-4T1 cells, we observed that the extravasated part of breast cancer cells passed through the *glia limitans perivascularis* right after breaching the endothelial wall, as indicated by localization of aquaporin 4 (AQP4)-marked astrocyte end-feet between the

endothelium and tumour cells. As metastatic lesions were growing, astrocytes were expelled from the tumour mass **(Figure 2A)**. In parallel, reactive astrocytes surrounding metastatic lesions gradually retracted their end-feet from the vessels to the parenchymal border of the tumour, which gained a discontinuous end-foot coverage **(Figure 2A)**.

Peri-tumoural astrocytes become activated upon metastasis formation. Through seeking for new biomarkers for early detection of brain metastases, we found a new element of astrocyte reaction in TNBC brain metastasis, namely myocyte enhancer factor 2C (MEF2C). We performed a high-throughput NGS analysis from plasma samples of TNBC cell-inoculated mice, followed by a qPCR validation, and we found downregulation of miR-802-5p and miR-194-5p as an early event in brain metastatic breast cancer in mice. By using miRNA target prediction, we showed that MEF2C is a specific target of both of these miRNAs, and is negatively regulated by them. MEF2C is a transcription factor initially described to be activated during embryogenesis to regulate tissue-specific gene expression and promote organ development. Subsequently, MEF2C was recognized to be also expressed during adult life in many types of cells, including neuronal and endothelial cells. In our model, in addition to being expressed in metastatic cells, among cells of the NVU, MEF2C expression was restricted to astrocytes in the neighbourhood of breast cancer cells, pointing to a role of MEF2C in the crosstalk between tumour cells and astrocytes **(Figure 2B)**.

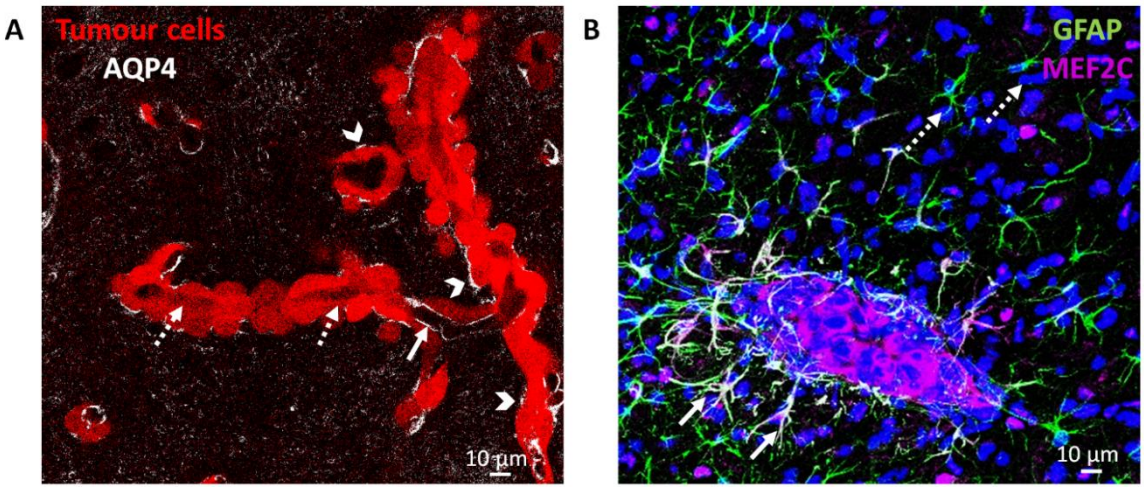

Figure 2: Interaction of breast cancer cells with astrocytes. (A) AQP4-positive astrocyte end-feet gradually retracted from the capillary wall to the surface of the metastatic tumour. Tdtomato-4T1 mouse TNBC cells (red) were injected into mice. Confocal z-projection of metastatic lesions from the brain of an animal on day 10. Arrows show vascular AQP4 staining, dotted arrows indicate absence of vascular AQP4 staining, while arrowheads point to AQP4 staining on the surface of the tumour. **(B)** MEF2C expression in the peri-tumoural astrocytes. Brain sections from 4T1-injected mice were analysed with confocal microscopy (z-projection). Arrows show MEF2C-positive peri-tumoural astrocytes, while dotted arrows indicate distant, MEF2C-negative astrocytes.

3.3. Direct interaction of brain metastatic tumour cells with pericytes *in vivo* and *in vitro*

Cells belonging to the NVU are known to interact with malignant cells after the transmigration through the BBB, but much less is known about the role of pericytes. Since we observed that tumour co-opted endothelial cells maintained tight junctions in the absence of astrocyte end-foot coverage, we hypothesized that pericytes—which also help to maintain BBB properties of CECs—remained in close contact with the vessels (*i.e.* were co-opted together with the endothelium). Indeed, we found that platelet-derived growth factor receptor β (PDGFRβ)-positive pericytes were localized to capillaries inside metastatic lesions in the mouse brain 7 days after inoculation with TNBC cells. In human TNBC brain metastases, PDGFRβ-positive perivascular cells were found in the stroma, in single or multiple layers. In addition, we also detected single pericyte-like cells expressing the pericyte-specific markers PDGFRβ and CD13, scattered among the tumour cells, especially in less cell-dense areas, probably in the proximity of necrotic zones.

All these data prompted us to hypothesize that direct interactions between tumour cells and pericytes might influence brain metastasis development. Therefore, we first modelled contacts between metastatic cells and pericytes using an *in vitro* setup, by plating the tumour cells onto sparse cultures of brain pericytes and other cells of the NVU. Breast cancer cells preferentially gathered onto the top of pericytes, avoiding the cell-free culture surface, and this was independent of the coating of the culture dish. In contrast, when breast cancer cells were co-cultured with CECs, they seldom adhered onto endothelial cells, but rather into gaps among them. In co-culture with astrocytes, direct contacts of the tumour cells were more frequent than with endothelial cells, but less preferred than interaction with pericytes. In contrast to breast cancer cells, the highest number of melanoma cells attached to free surfaces among the cells, independent of the cell type they were co-cultured with.

After identifying the ability of TNBC cells to preferentially form contacts with brain pericytes, we explored whether they could actively migrate in the direction of brain pericytes. In our *in vitro* wound healing assay, carcinoma cells readily migrated towards pericytes, covering a significantly larger distance on their way to brain pericytes than to endothelial cells.

When leaving a much longer distance (more than 1 cm) between tumour cells and the two different brain cells (endothelial cells and pericytes, respectively),

breast carcinoma cells preferentially migrated in the direction of pericytes. After a few days, several breast cancer cells were detected in the initially cell-free area between tumour cells and pericytes and large breast cancer cell colonies were formed among pericytes. In contrast, only a few scattered mammary carcinoma cells were observed in the direction of and among endothelial cells.

These results indicate that pericytes might communicate with tumour cells through secreted factors. Therefore, we conditioned culture medium on brain pericytes to characterize in details its effects on neoplastic cells.

3.4. Effects of pericytes on tumour cell adhesion

First, we seeded breast cancer and melanoma cells in control and pericyte-conditioned media and performed an adhesion assay. Both human and mouse breast cancer cells and melanoma cells as well attached to the culture dish surface and elongated more rapidly in pericyte-conditioned media, than in control conditions. The difference between cells seeded in control and conditioned media was very high and significant.

A feasible explanation for the prominent adhesion enhancing effect of conditioned media is that pericytes—similarly to astrocytes—secreted high amounts of collagen type IV and fibronectin. In addition, we observed increased levels of phosphorylated FAK (focal adhesion kinase) and Src focal adhesion proteins in breast cancer and melanoma cells seeded in pericyte-conditioned media. The adhesion enhancing effect of pericytes could be eliminated by using the specific Src inhibitor PP2.

3.5. Effects of pericytes on tumour cell proliferation

As a next step, we aimed to understand how brain pericytes influence tumour growth. Therefore, we performed a tumour cell proliferation assay in the presence and absence of factors released by pericytes. Four days after plating, the number of breast cancer cells was substantially higher in pericyte-conditioned media than in the control **(Figure 3)**. This was clearly observed in both human and mouse models. On the other hand, melanoma cells did not respond with increased proliferation to the presence of pericyte-secreted factors.

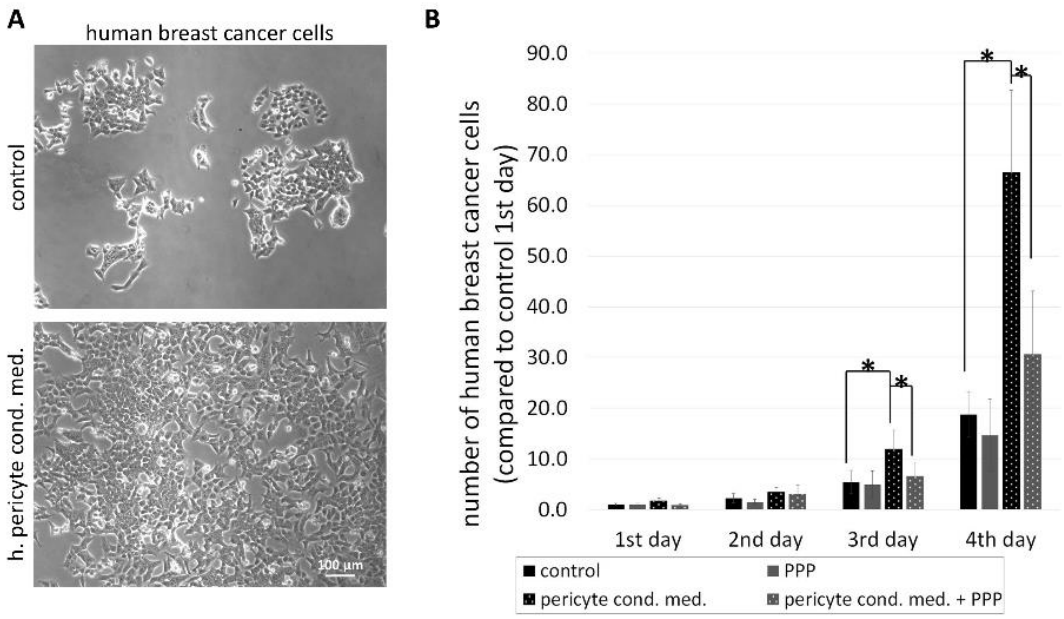

Figure 3: Role of pericyte-secreted factors in tumour cell proliferation. (A) Representative phase-contrast images of tumour cells grown in control or pericyte-conditioned medium for 4 days. **(B)** Quantification of MDA cell growth in control or pericyte-conditioned media in the presence or absence of PPP. Cells were counted in phase-contrast micrographs. N = 5, average +/- SD, * $P < 0.05$ (ANOVA and Bonferroni's post-hoc test).

In parallel, there was a significant increase in the expression of cyclin D1 in human and mouse breast cancer cells, but not in melanoma cells cultured in pericyte-conditioned media.

Apart from soluble factors, conditioned media may contain extracellular vesicles (EVs), such as microvesicles and exosomes. In order to distinguish between effects of EVs and soluble factors, we prepared EV-depleted conditioned medium and compared it with complete conditioned medium in a proliferation assay. EV depletion did not influence the proliferation-increasing effect of pericyte-conditioned medium; therefore, soluble factors released by pericytes are responsible for the observed phenomenon.

Interestingly, breast cancer cells in the conditioned media, 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. This suggests that while enhancing adhesion to the substrate, pericytes inhibit intercellular adhesions and expression of E-cadherin, conferring breast cancer cells a migratory, invasive phenotype, characteristic of cells undergoing epithelial-to-mesenchymal transition (EMT).

3.6. Role of brain pericyte-secreted IGFs in proliferation of TNBC cells

In order to identify which cerebral pericyte-secreted factors might be involved in enhancing tumour cell proliferation, we first performed a database search. In the http://betsholtzlab.org/VascularSingleCells/database.html collection we found Igf2 mRNA having the highest expression in pericytes among cells of the NVU. Igf2 mRNA expression level was 487.25-times higher in pericytes than in endothelial cells, and 86.62-times more in pericytes than in astrocytes in the mouse brain. In our human and mouse pericytes, not only Igf2, but Igf1 mRNA was also expressed. More Igf2 than Igf1 mRNA was found in both human and mouse pericytes; however, the ratio was higher in human cells. Mouse astrocytes expressed very low amounts of Igf1 and Igf2 mRNA, while in human astrocytes both Igf1 and Igf2 mRNA levels were higher than in tumour cells, but still lower than in pericytes.

These data were confirmed at the protein level using ELISA. Human pericytes secreted more than 1,000 pg/ml IGF2 corresponding to almost 100 pg/100,000 cells, while in astrocytes we detected significantly less IGF2 (< 200 pg/ml, < 40 pg/100,000 cells). IGF1 levels in both pericyte- and astrocyte-conditioned media were below the detection limit of 1.95 ng/ml.

By using immunofluorescence, we found both IGF1 and IGF2 to be expressed in CD13-positive pericytes in the normal human brain. IGF1 was also detected in a few CD13-negative cells, probably in astrocyte end-feet, while IGF2 was mainly expressed in pericytes. In human TNBC brain metastatic lesions, IGF1 and IGF2 expression were highly restricted to perivascular cells **(Figure 4)**.

By binding to the type 1 insulin-like growth factor receptor (IGF1R), IGFs are involved in growth and survival of both normal and neoplastic cells. Therefore, we next tested whether pericyte-secreted IGFs are responsible for the increased proliferation rate of breast cancer cells. For this purpose, we used a selective inhibitor of IGF1R, picropodophyllin (PPP), which efficiently blocks IGF1R without inhibiting the insulin receptor, and has low toxicity in rodents. Addition of PPP to pericyte-conditioned medium reduced proliferation-inducing effect of pericytes to almost control levels in both human and mouse breast cancer cells **(Figure 3B)**.

In addition, we silenced Igf2 gene in pericytes, collected conditioned media and performed a proliferation assay. Proliferation rate of breast cancer cells in

conditioned media of Igf2-silenced pericytes was similar to control cells, or cells cultured in pericyte-conditioned media in the presence of PPP.

In contrast to breast cancer cells, proliferation of melanoma cells was not affected by PPP. Resistance of melanoma cells to IGFs might be partly explained by their increased Igf2R expression compared to breast cancer cells, because IGF2R directs IGF2 to lysosomes to attenuate signalling.

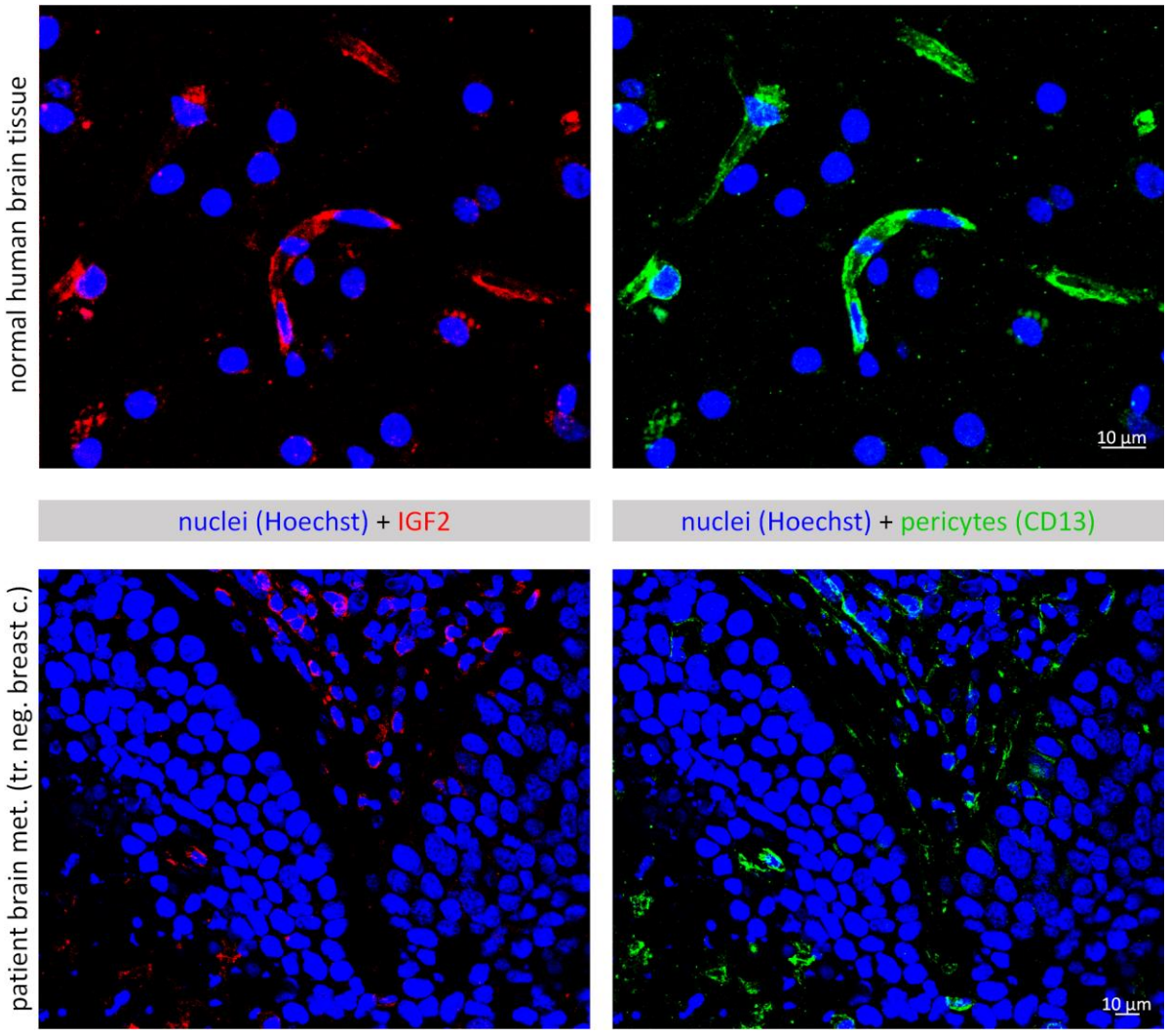

Figure 4: Distribution of IGF2 in normal or metastatic human brain samples. (A) Representative immunofluorescence staining showing co-localization of IGF2 and CD13 in normal human brain tissue. **(B)** Representative immunofluorescence staining showing co-localization of IGF2 and CD13 in human TNBC brain metastatic tissue.

Since PPP can cross the BBB, we next designed an *in vivo* setup to test the role of IGFs in breast cancer brain metastasis development. In order to test metastatic cell proliferation in the brain parenchyma, PPP was administered on days 5 and 6 after inoculation of tumour cells, when majority of cells have completed

extravasation from cerebral capillaries. Our results clearly showed that PPP inhibited proliferation of breast cancer cells in the brains of mice. The brain area covered by tumour cells became significantly, more than 2.5-times smaller in animals treated with the IGF1R inhibitor **(Figure 5)**.

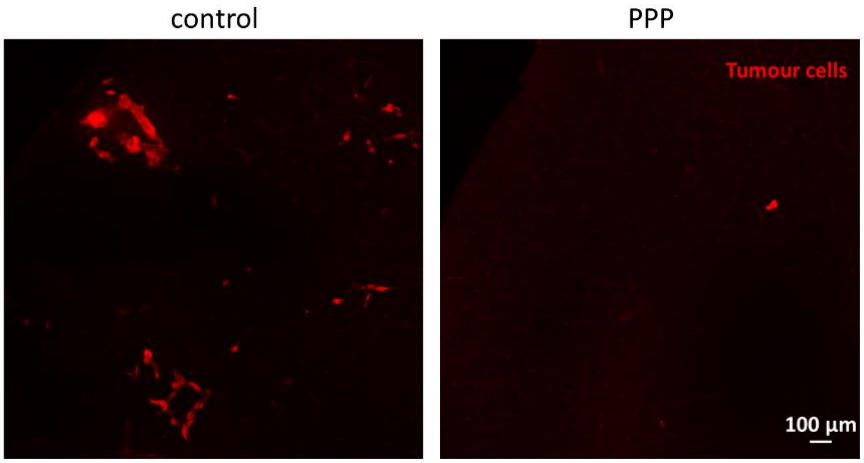

Figure 5: Role of IGFs in breast cancer cell proliferation *in vivo*. Representative confocal micrographs of parietal brain sections of control mice and animals treated with PPP, 8 days after inoculation of tdTomato-4T1 cells.

IGF signalling has been implicated in the growth and survival of both normal and malignant cells. IGF1R expression and activity increase in several tumour types, including breast cancer, resulting in enhanced proliferation rate, metastatic capacity and resistance to chemotherapy. Therefore, targeting of the IGF axis has been in the focus of therapeutic approach developments in numerous malignant diseases. However, since many clinical trials failed and further studies have been discontinued due to toxicity or low efficacy of the tested compounds, identification of predictive biomarkers has been urged to define potentially responsive patient subgroups. Nevertheless, brain metastatic disease has not been specifically investigated in IGF2-targeting clinical trials, although inhibition of IGF1R has been shown to reduce breast cancer brain metastasis development in experimental models. Moreover, a clinical phase I study has been completed with PPP (AXL1717) in patients with primary brain tumours, showing that the drug is well tolerated with no major side effects. Our study underlines the importance of IGF axis inhibition as a potential strategy in brain metastases, especially as there exists a compound (PPP/AXL1717) with high selectivity on IGF1R that not only has low toxicity, but is also BBB permeable. This is a great advantage, since the BBB represents the highest obstacle in the development of drugs targeting the CNS.

4. Summary

Brain metastases are life-threatening complications of breast cancer, melanoma and a few other tumour types. Poor outcome of cerebral malignancies largely depends on the microenvironment formed by cells of the NVU. Despite the unquestionable clinical significance, important aspects of the development of secondary tumours of the CNS are largely uncharacterized. By using *in vivo* and *in vitro* techniques and human samples, here we identified novel mechanisms related to CECs, astrocytes and brain pericytes, involved in brain metastasis development.

Our results indicate that the microvascular endothelium is directly involved in extravasation of tumour cells into the brain. CECs extend filopodia-like membrane protrusions towards the tumour cells to engulf them. Moreover, endothelial cells isolate invading cells from the circulation by vessel obstruction, before tumour cells proceed to extravasation. We also show that melanoma cells primarily utilize the paracellular route of transendothelial migration, while breast cancer cells are able to transcellularly migrate through the brain endothelium.

After overcoming the endothelium, mammary carcinoma cells breach the *glia limitans perivascularis* formed by astrocytic end-feet. As breast cancer cells start to proliferate in the brain, they co-opt the vessels, including endothelial cells and pericytes, and exclude astrocytes, which retract their foot processes onto the surface of the tumour. We also show that during breast cancer brain metastasis formation, circulating miR-802 and miR-194 are deregulated in a time-dependent manner. Along with this, we observed overexpression of MEF2C, a target of both miR-802-5p and miR-194-5p, in both metastatic cells and peri-tumoural astrocytes, underlining the interplay between glial and malignant cells.

Finally, we show for the first time that cerebral pericytes have significant pro-metastatic roles. Brain pericytes have a prompt chemoattractant effect on breast cancer cells and establish direct contacts with them. By secreting high amounts of extracellular matrix proteins, pericytes enhance adhesion of both melanoma and triple negative cancer cells, which might be particularly important in the exclusive perivascular growth of these tumour cells. In addition, pericytes secrete insulin-like growth factor 2 (IGF2), which has a very significant pro-proliferative effect on mammary carcinoma, but not on melanoma cells.

List of publications

Full papers directly related to the subject of the thesis

I. **Molnár, K.**, Mészáros, Á., Fazakas C., Kozma, M., Győri, F., Reisz, Z., Tiszlavicz, L., Farkas, A.E., Nyúl-Tóth, Á., Haskó, J., Krizbai, I.A., Wilhelm, I. (2020) Pericyte-secreted IGF2 promotes breast cancer brain metastasis formation. Mol Oncol. 2020 Sep; 14(9): 2040-2057. (IF2019 = 6.574, Journal Ranking: D1)

II. Sereno, M., Haskó, J., **Molnár, K.**, Medina, S.J., Reisz, Z., Malhó, R., Videira, M., Tiszlavicz, L., Booth, S.A., Wilhelm, I., Krizbai, I.A., Brito MA. (2020) Downregulation of circulating miR 802-5p and miR 194-5p and upregulation of brain MEF2C along breast cancer brain metastasization. Mol Oncol. 2020 Mar;14(3):520-538. (IF2019 = 6.574, Journal Ranking: D1)

III. Haskó, J., Fazakas C., **Molnár, K.**, Mészáros, Á., Patai, R., Szabó, G., Erdélyi, F., Nyúl-Tóth, Á., Győri, F., Kozma, M., Farkas, A.E., Krizbai, I.A., Wilhelm, I. (2019) Response of the neurovascular unit to brain metastatic breast cancer cells. Acta Neuropathol Commun. 2019 Aug 19;7(1):133. (IF = 6.270, Journal Ranking: D1)

IV. Herman, H., Fazakas C., Haskó, J., **Molnár, K.**, Mészáros, Á., Nyúl-Tóth, Á., Szabó, G., Erdélyi, F., Ardelean, A., Hermenean, A., Krizbai, I.A., Wilhelm, I. (2019) Paracellular and transcellular migration of metastatic cells through the cerebral endothelium. J Cell Mol Med. 2019 Apr;23(4):2619-2631. (IF = 4.486, Journal Ranking: Q1)

V. Wilhelm, I., Fazakas C., **Molnár, K.**, Végh, A.G., Haskó, J., Krizbai, I.A. (2018) Foe or friend? Janus-faces of the neurovascular unit in the formation of brain metastases. J Cereb Blood Flow Metab. 2018 Apr;38(4):563-587. (IF = 6.040, Journal Ranking: D1)

Full papers not included in the thesis

1. **Molnár, K.**; Lőrinczi, B.; Fazakas, C.; Szatmári, I.; Fülöp, F.; Kmetykó, N.; Berkecz, R.; Ilisz, I.; Krizbai, I.A.; Wilhelm, I.; Vécsei, L. (2021) SZR-104, a Novel Kynurenic Acid Analogue with High Permeability through the Blood–Brain Barrier. Pharmaceutics 2021, 13, 61. (IF2019 = 4.421, Journal Ranking: Q1)
2. Nógrádi, B., Nyúl-Tóth, Á., Kozma, M., **Molnár, K.,** Patai, R., Siklós, L., Wilhelm, I., Krizbai, I.A. (2020) Upregulation of NLRP3 in motoneurons following peripheral nerve injury in mice. Frontiers in Pharmacology. (accepted for publication) (IF2019 = 4.225, Journal Ranking: Q1)
3. Grexa,I., Fekete, T., Molnár, J., **Molnár, K.**, Vizsnyiczai, G., Ormos, P., Kelemen, L. (2020) Single-cell elasticity measurement with an optically actuated microrobot. Micromachines (Basel). 2020 Sep 22;11(9):E882. (IF2019 = 2.523, Journal Ranking: Q2)
4. Mészáros, Á., **Molnár, K.**, Nógrádi, B., Hernádi, Z., Nyúl-Tóth, Á., Wilhelm, I., Krizbai, I.A. (2020) Neurovascular inflammaging in health and disease. Cells. 2020 Jul 4;9(7):1614. (IF2019 = 4.366, Journal Ranking: Q2)
5. Nyúl-Tóth, Á., Kozma, M., Nagyőszi, P., Nagy, K., Fazakas C., Haskó, J., **Molnár, K.**, Farkas, A.E., Végh, A.G., Váró, G., Galajda, P., Wilhelm, I., Krizbai, I.A. (2017) Expression of pattern recognition receptors and activation of the non-canonical inflammasome pathway in brain pericytes. Brain Behav Immun. 2017 Aug;64:220-231. (IF = 6.306, Journal Ranking: D1)
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Manuscripts under revision

1. Figueira, I., Galego, S., Custódio-Santos, T., Vicente, R., **Molnár, K.**, Haskó, J., Malhó, R., Videira, M., Wilhelm, I., Krizbai, I., Brito, M.A. Picturing breast cancer brain metastasis development to unravel molecular players and cellular crosstalk.
2. Fazakas, C., Kozma, M., **Molnár, K.**, Kincses, A., Dér, A., Fejér, A., Horváth, B., Wilhelm, I., Krizbai, IA., Végh, AG. Breast adenocarcinoma-derived exosomes lower first-contact de-adhesion strength of adenocarcinoma cells to brain endothelial layer.
3. Török, N., Maszlag-Török, R., **Molnár, K.**, Szolnoki, Z., Somogyvári, F., Boda, K., Tanaka, M., Klivényi, P., Vécsei, L. Single nucleotide polymorphisms of indoleamine 2,3-dioxygenase 1 influenced the age onset of Parkinson's disease.

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