ImmunoTools *special* Award 2018

Maria Alexandra Brito
Associate Professor

Neuron Glia Biology in Health and Disease Unit, Research Institute for Medicines, Faculty of Pharmacy, University of Lisbon, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal

Blood-brain barrier: role in brain metastasization of breast cancer

Breast cancer (BC) progression towards brain metastasis occurs by a metastatic cascade that includes malignant cells passage across the blood-brain barrier (BBB), known as extravasation. The migration of BC cells (BCCs) across the BBB resembles that of leukocytes in inflammatory conditions, with rolling, adhesion and transendothelial migration/diapedesis involving pairs of ligands/receptors expressed in each cell type *(Custódio-Santos, BBA-Rev Cancer 2017).* However, the metastatic process is poorly characterized pointing to the relevance of profiling the ligands involved in the interaction between BCCs and the brain microvascular endothelial cells (BMEC) that constitute the BBB.

Endothelial cells undergo phenotypic changes characterized by gain of mesenchymal traits and loss of junctional proteins, known as endothelial-mesenchymal transition (EndMT) similarly to the epithelial-mesenchymal transition (EMT) that occurs in BCCs. EndMT is induced by TGF-β family and non-TGF-β cytokines *(Yang, Int J Mol Med 2017; Cho, Front Immunol 2018)*, and was associated with monocyte adhesion and melanoma cells transendothelial migration, and increased endothelial permeability *(Yang, Int J Mol Med 20172017; Krizbai, PLoS ONE 2015).* Interestingly, BMP-7 is considered the most common cytokine promoting an epithelial phenotype in BCCs *(Gonzalez, Sci Signal 2015),* opening new avenues for maintenance of malignant cells and BMEC with an epithelial/endothelial phenotype and to prevent brain metastasis.

It was shown that the neuregulin (NRG)-1 ligand has BBB protective effects in neuroinflammation (Wu, J Neurochem 2016) and ability to hamper macrophages infiltration and inflammatory responses, involving the engagement of ErbB receptor tyrosine kinases through its EGF-like domain *(Liu, J Neuroinflammation 2018).* Moreover, the success of anti-tumor therapies was improved by recombinant NRG-1 and NRG-1-expressing cancer cells *(Le Clorennec, Mol Cancer Ther. 2017).*

However, counteraction of BCCs extravasation into the brain by NRG-1 remains undetermined.

We aim to disclose the extravasation step of the metastatic cascade by profiling the ligands involved and establishing the phenotypic changes of human BMEC (HBMEC). We also aim to devise a novel approach to abrogate the trafficking of BCCs across the BBB towards the brain as a strategy to abrogate brain metastasis.

Monolayers of human BEMC (HBMEC), used as an in vitro BBB model (Palmela, Front Neurosci 2015), will be exposed to a human BC cell line known to metastasize to brain (MDA-MB-231/brain) *(Lorger, Am J Pathol 2010).* It will be performed flow cytometry analyses to assess the markers/ligands CD34, CD54, CD62E, CD66adecb and CD105 in HBMEC, and of CD15, CD44, CD61, CD66adecb, CD29 and CD49d in BCCs. The monolayer will be inspected by confocal microscopy for markers of endothelial integrity and barrier properties (claudin-5 and β-catenin), endothelial-mesenchymal phenotype (claudin-5 and α-SMA), as well as for the trafficking of malignant cells across the endothelium (von Willebrand factor and CD44). To establish the cytokines involved in the expected EndMT, experiments with addition of the recombinant cytokines BMP-2, EGF, IL1β, IL-6, CCL4 and/or TNF-alpha will be performed followed by flow cytometry analysis of ligands and microscopic analysis of barrier, phenotype and transmigration features, as above. A similar set of assays will be finally performed in the presence of NRG-1 and BMP-7 to establish their protective role.

The ImmunoTools flow cytometry antibodies and recombinant cytokines will allow a comprehensive evaluation of the ligands involved in the extravasation of BCCs into the brain and to depict the BBB role in brain metastasization of BC. It will also provide a unique opportunity to disclose the molecular players involved in the BBB alterations that favor the BCCs entrance into the brain. Remarkably, it will allow the testing of promising strategies to prevent brain metastasization of BC.

ImmunoTools *special* AWARD for **Maria Alexandra Brito** includes 25 reagents

FITC - conjugated anti-human CD15, CD34, CD49d, CD61, CD62E, CD105,
Control-IgG1, Control-IgG2a, Control-IgG2b

PE - conjugated anti-human CD29, CD44, CD54, CD66adecb (CEACAM1/3/5/6/8),
Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin-V

recombinant human cytokines: rh BMP-2, rh BMP-7, rh EGF, rh IL-1beta /IL-1F2,
rh IL-6, rh MIP-1b/CCL4, Neuregulin-1b, rh TNF-alpha

DETAILS more AWARDS