# Disclosing molecular alterations in the blood-brain barrier endothelium during interaction with breast cancer cells: a step forward brain metastasis prevention

**AR Garcia[1,2], J Godinho-Pereira[1,2], I Figueira[1,3], KS Kim[4], HM Botelho[5], R Malhó[5], MA Brito[1,2]**

[1]iMed.ULisboa – Instituto de Investigação do Medicamento, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal; [2]Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal; [3]Farm-ID – Associação da Faculdade de Farmácia para a Investigação e Desenvolvimento, Lisboa, Portugal; [4] Division of Infectious Diseases, John Hopkins University School of Medicine, Baltimore, USA; [5]BioISI – Instituto de BioSistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.

arcgarcia@campus.ul.pt

## Background

Brain metastases are amongst the impact factors associated with decreased life expectancy of many breast cancer (BC) patients. The extravasation, a key step for the establishment of metastasis, is thought to involve the disruption of the microvascular endothelium that composes the blood-brain barrier (BBB) by cancer cells. However, little is known about the mechanism involved in BBB transposition by BC cells.

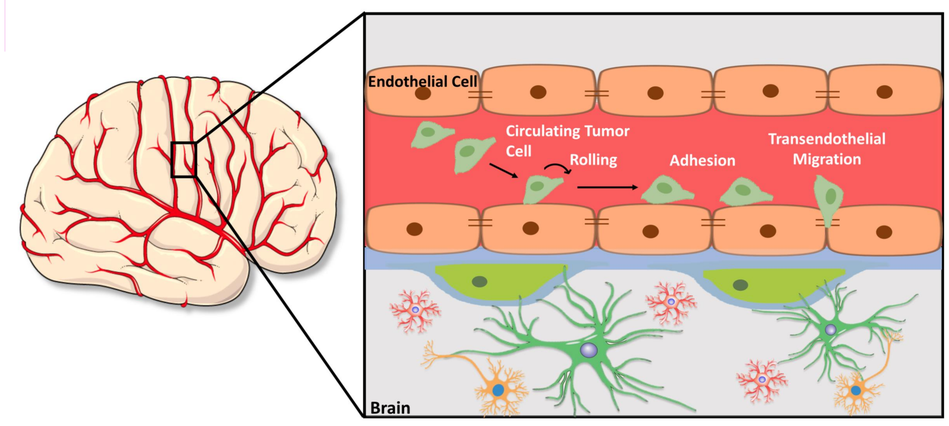
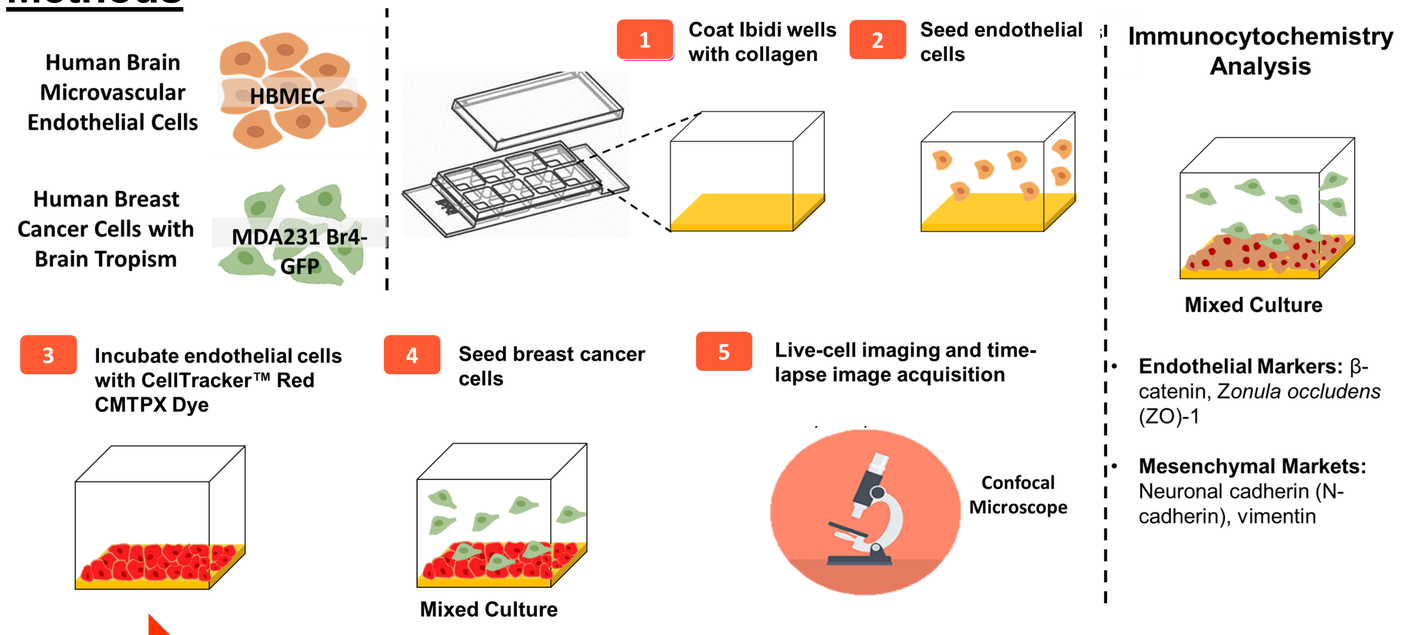



***Fig. 1*** **|** Schematic representation of cancer cells extravasation across blood-brain barrier (BBB), a multi-step process involved in brain metastases development.

## Methods

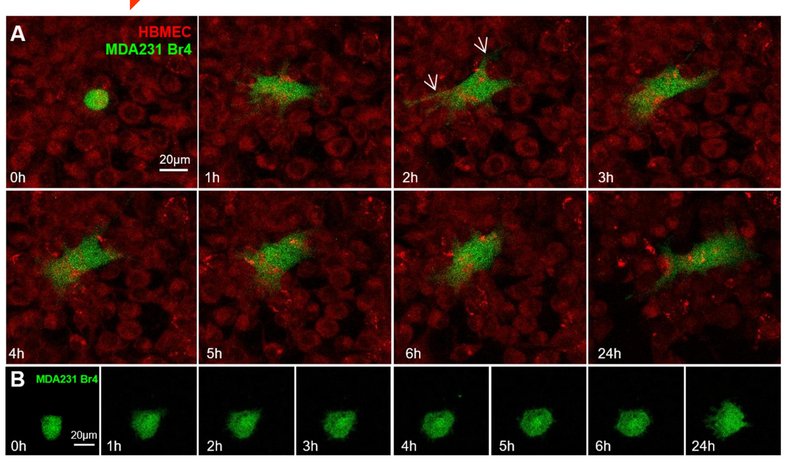



## Aim

**Dissect the interaction between BC cells and BBB endothelial cells**

## Results

### BC cells acquired a highly invasive phenotype during interaction with BBB endotelial cells



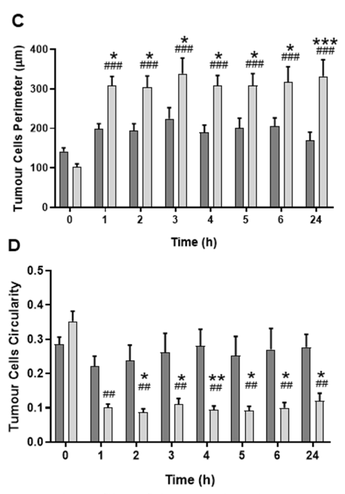




***Fig. 2*** **| MDA231 Br4 cells acquire an invasive phenotype during extravasation.** Temporal profile of interaction between MDA231 Br4 cells (tagged with GFP, green) and HBMEC confluent monolayers (labelled with CellTracker™ Red CMTPX Dye; red) in mixed (**A**) and single cultures of MDA231 Br4 cells (control) (**B**) at 0, 1, 2, 3, 4, 5, 6 e 24 h. Morphological parameters such as tumour cells perimeter (**C**) and circularity (**D**) were analysed at the same timepoints. Arrows indicate the cytoplasmatic cell extensions. Statistical significances are denoted as *P<0.05, **P<0.01 and ***P<0.001 for mixed culture vs control at the same timepoint and #P<0.05, ##P<0.01 and ###P<0.001 for mixed culture of indicated timepoints vs. mixed culture at 0 h.

### BC cells localize at different endothelium levels during the extravasation process

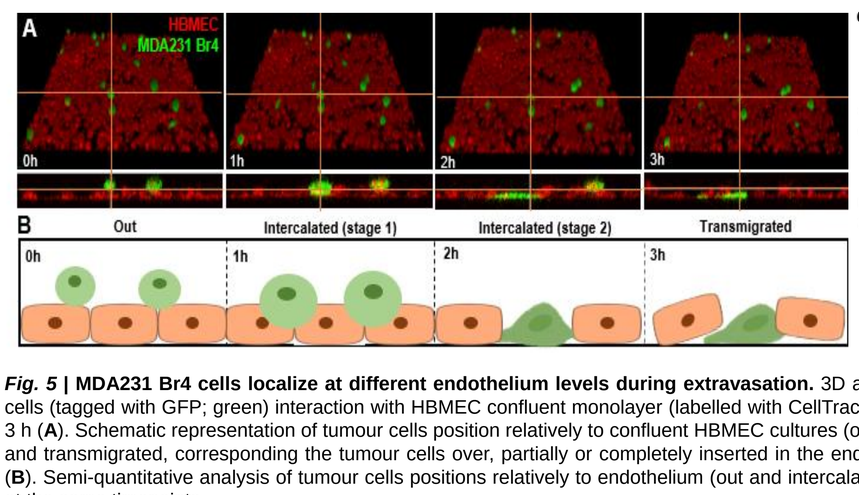
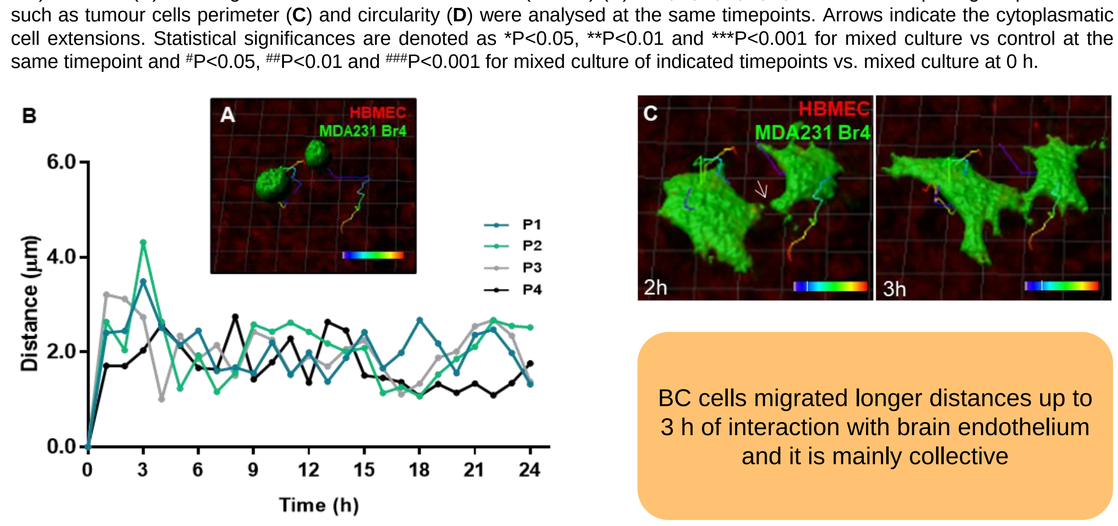
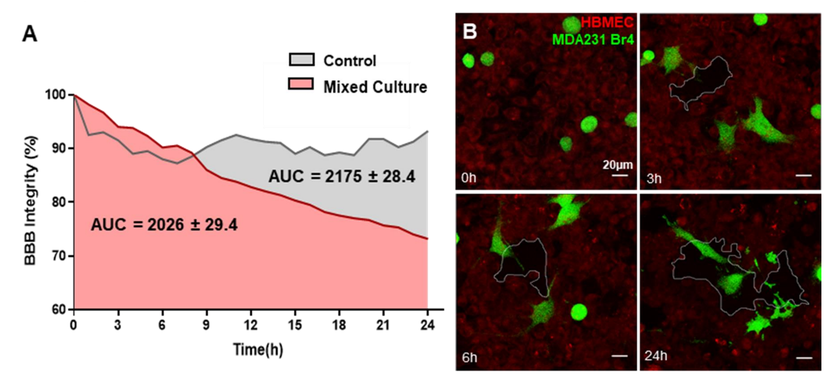



***Fig. 5*** **| MDA231 Br4 cells localize at different endothelium levels during extravasation.** 3D and orthogonal visualization of MDA231 Br4 cells (tagged with GFP; green) interaction with HBMEC confluent monolayer (labelled with CellTrackerTM Red CMTPX Dye; red) at 0, 1, 2 and 3 h (**A**). Schematic representation of tumour cells position relatively to confluent HBMEC cultures (out, intercalated stage 1, intercalated stage 2 and transmigrated, corresponding the tumour cells over, partially or completely inserted in the endothelial monolayer, below the endothelium) (**B**). Semi-quantitative analysis of tumour cells positions relatively to endothelium (out and intercalated stage 1, stage 2 and transmigrated) (**C**) at the same timepoints.




BC cells migrated longer distances up to 3 h of interaction with brain endothelium and it is mainly collective

***Fig. 3*** **| MDA231 Br4 cells acquire a migratory phenotype during extravasation.** 3D representation of the total distance migrated by MDA231 Br4 cells (tagged with GFP; green) along 24 h (**A**). Semi-quantitative analysis of MDA231 Br4 cells migrated distances along 24 h (**B**). Each line in B represents the distances mean of four MDA231 Br4 cells in four different positions on ibidi wells. Representation of tumour cells collective migration at 2 and 3 h of interaction (**C**). Arrows indicates the contact points between tumour cells.




After only 3 h, the endothelial monolayer already presented some gaps, whose size increases over time.

**About 27% of monolayer is disrupted**

***Fig. 4*** **| HBMEC and MDA231 Br4 cells interaction decrease the blood-brain barrier (BBB) integrity.** The effect of malignant-endothelial cells interaction on BBB integrity was evaluated in mixed and endothelial cultures (control) by live-cell imaging microscopy and area under curve was determined along 24 h (**A**). Tumor cells transmigration promoted an impairment of BBB along time, confirmed by the increase of monolayer holes size upon the interaction with MDA231 Br4 cells (circled with a white line) (**B**).

### BBB endothelial cells undergone a Endothelial-Mesenchymal Transition that favors the paracellular transmigration

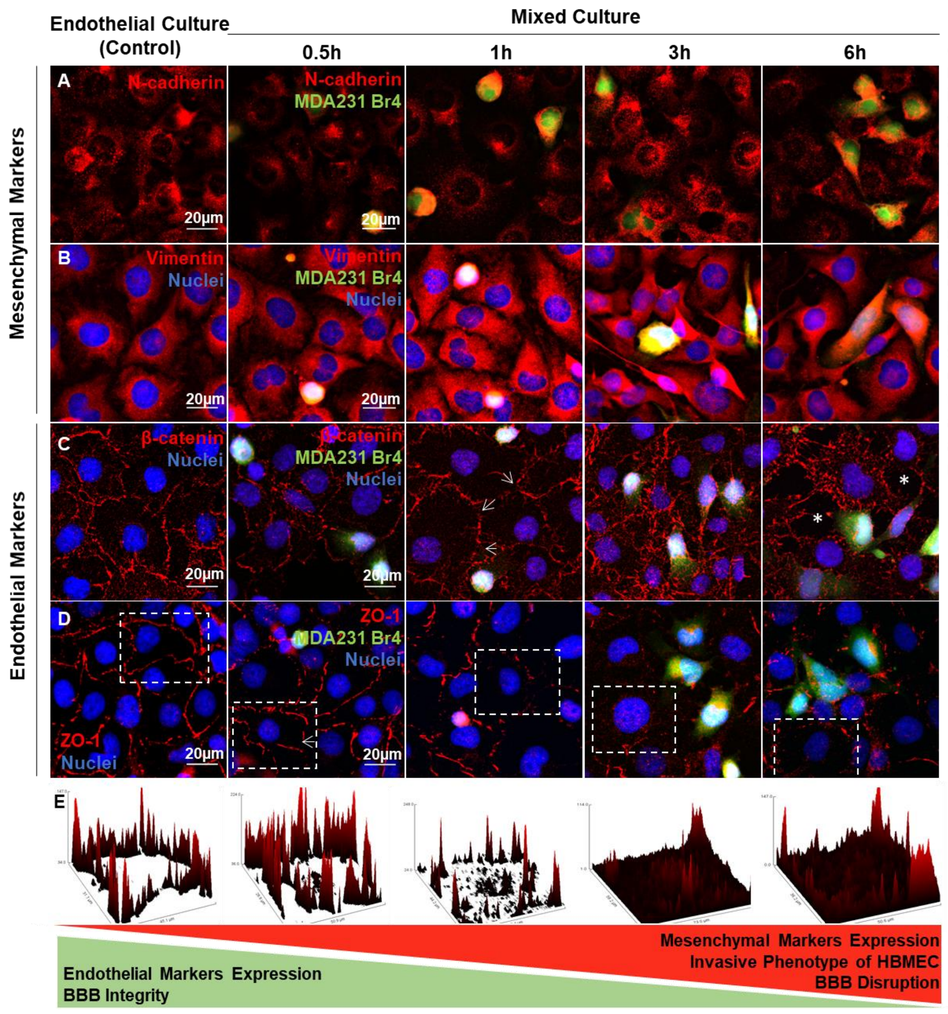



***Fig. 6*** **| HBMECs acquired mesenchymal features during interaction with MDA231 Br4 cells.** Fluorescence intensity analysis of N-cadherin (**A**), vimentin (**B**), β-catenin (**C**) and zonula occludens (ZO)-1 **(D)** in endothelial and mixed cultures at 0.5, 1, 3 e 6 h of interaction. The ZO-1 labelling localization was evaluated by the plot profile of a representative cell (represented inside the corresponded dashed square) in endothelial and mixed cultures (**E**) at the same timepoints. Asterisks and white arrows indicate endothelial holes and gaps localization, respectively.

## Conclusion

These findings indicate that along extravasation BCCs acquire invasive properties, while the endothelium undergoes profound phenotypic changes, which altogether impact on BBB integrity and culminate in BCCs transmigration.

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA EDUCAÇÃO E CIÊNCIA

PTDC/MED-ONC/29402/2017 and 2020.07115.BD scholarship; UIDB/04138/2020 and UIDP/04138/2020; PPBI-POCI-01-0145-FEDER-022122; LPCC/Pfizer 2019 scholarship.