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STAT3 labels a subpopulation of reactive astrocytes required for brain metastasis

Neibla Priego[1] , Lucía Zhu[1] , Cátia Monteiro[1] , Manon Mulders[1] , David Wasilewski[2] , Wendy Bindeman[1] , Laura Doglio[1] , Liliana Martínez[1] , Elena Martínez-Saez[3] , Santiago Ramón y Cajal[3] , Coral Fustero[4] , Elena Piñeiro[4] , Aurelio Hernández-Laín[5] , Valeria Poli[6] , Javier A. Menéndez[7] , Ricardo Soffietti[8] , Joaquim Bosch-Barrera[7] , Manuel Valiente[1]

1) Brain Metastasis Group, Spanish National Cancer Research Centre (CNIO), Madrid, Spain. 2) Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. 3) Pathology Department, Vall d'Hebron Hospital, Barcelona, Spain. 4) Bioinformatics Unit, Spanish National Cancer Research Centre (CNIO), Madrid, Spain. 5) Neuropathology Unit, Hospital Universitario 12 de Octubre Research Institute, Madrid, Spain. 6) Medical Sciences Department, Division of Pathology, University and City of Health and Science University Hospital of Turin, Turin, Italy. 7) Catalan Institute of Oncology (ICO), Dr. Josep Trueta University Hospital, Girona, Spain. 8) Neuro-Oncology Department, University and City of Health and Science University Hospital of Turin, Turin, Italy.

INTRODUCTION:

The diagnosis of brain metastasis involves high morbidity and mortality and remains as an unmet clinical need in spite of being the most common tumor in the brain. Brain metastasis affects between 10-30% of patients with lung cancer, breast cancer and melanoma. Given the increasing evidences that the microenvironment is crucial to understand the biology of brain colonization by metastatic cells, we were interested in evaluating therapeutic strategies targeting it. Specifically, we focused on a particular glial cell type: the reactive astrocyte (RA). This cell type has a complex behavior associated with brain metastasis limiting the number of metastasis initiating cells during the initial stages of brain colonization and promoting the growth of cancer cells later on.

We hypothesized that during brain colonization cancer cells could alter molecular pathways in the brain microenvironment, including RA. Activation of such signaling pathways could rewire the microenvironment turning it into a pro-metastatic niche. We have found that activation of the STAT3 pathway, by phosphorylation in Tyr705 ($pSTAT3^{+}$), in RA modifies the behavior of this cell type. $pSTAT3^{+}$ RA promote cancer cell survival by blocking antitumor components of the immune system. Consequently, we evaluated $pSTAT3^{+}$ RA as a novel therapeutic target in brain metastasis.

OBJECTIVES:

The main objectives of this study include the analysis of the generality of $pSTAT3^{+}$ RA in different experimental models of brain metastasis as well as in human samples. Once this was confirmed as a common altered molecular pattern of the brain metastasis associated microenvironment, we wanted to test its contribution to the progression of brain metastasis as well as to understand their pro-metastatic function. Our final goal was to develop a proof-of-concept pre-clinical approach to validate $STAT3^{+}$ RA as a therapeutic target that could eventually be translated to patients.

METHODOLOGY:

To achieve these goals, we have used different brain tropic metastatic cell lines from several primary origins (MDA231-BrM, H2030-BrM, 393N1, 482N1, B16/F10-BrM) to perform colonization assays in immunosuppressed and immunocompetent mice models (including the GFAP-Cre/ERT2;$Stat3^{loxP/loxP}$ -cKO-Stat3-). Additionally, we have incorporated PDX models established from human brain metastasis. Primary astrocytes that generate astrospheres with activated STAT3 have been used to mimic the brain metastasis associated RA. Primary T cells

were activated and expanded *in vitro* before being cultured in conditioned media from astrospheres and cancer cells. Brain organotypic cultures of initial and advanced stages of brain metastasis allowed to collect preliminary data before performing brain metastasis assays *in vivo*. Validation in human samples was possible thanks to our collaboration with three different hospitals that provided us with paraffin samples.

RESULTS:

A subpopulation of RA with active STAT3 pathway (phosphorylation in Tyr705) is located in the vicinity of established brain metastases from experimental models and patients independently of the primary tumor source. Targeting STAT3 in RA specifically using genetic and pharmacologic approaches impairs the progression of brain metastasis, even during advanced stages of the disease.

Moreover, a safe and orally bioavailable STAT3 inhibitor reduced brain metastasis in 75% of stage IV lung adenocarcinoma patients with established brain metastasis and, given the mortality associated with metastatic tumors in the brain, increased their survival.

In order to understand the pro-metastatic functions of $pSTAT3^+$ RA, we have established astrosphere cultures as an *in vitro* condition that reproduces key aspects of brain metastasis associated $pSTAT3^+$ RA. This preparation allowed us to demonstrate that $pSTAT3^+$ RA acquire stemness potential linked to the ability of producing secreted and membrane bound molecules with immunosuppressive properties.

In fact, functional studies showed that $pSTAT3^+$ RA decrease the anti-tumor activity of $CD8^+$ T lymphocytes *in vitro* and also promote the expansion of pro-tumor $CD74^+$ microglia/macrophages, all of which allow cancer cells to survive and remain viable.

CONCLUSIONS:

This work emphasizes the potential of novel therapies targeting brain specific survival mechanisms based on the altered molecular patterns induced in the microenvironment. Our study suggests that adaptation of metastatic cells to the brain could also involve an increased dependency on it, which might generate vulnerabilities to be exploited therapeutically. Besides the possibility of becoming a therapeutic target, we conclude that $pSTAT3^+$ RA are key regulators of local immunosuppression.

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