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Identification of regulators of intravascular engulfment of circulating tumor cells by patrolling monocytes to combat lung metastases

Patrolling monocytes (PMo) are circulating innate immune cells endowed with the capacity of intravascular surveillance for capture of harmful particles (Moreno-Cañadas et al., Front Immunol 2021). Accordingly, PMo were shown to engulf circulating tumor cells (CTCs) in the lung microvasculature preventing their seeding (Hanna et al., Science 2015). Although boosting this PMo capacity may constitute a new strategy to combat early metastasis, no targets have yet been proposed. Our team recently discovered that the absence of the protease MT4-MMP increased PMo intravascular surveillance (Clemente et al., Nat Commun 2018). These findings led us to implement in vivo and in vitro approaches to decipher the mechanisms underlying CTC capture by PMo in the lung microvasculature. Our recent data indicate that PMo not only uptake tumor cell-derived material but they can also engulf pieces of intact live tumor cells. This process, called trogocytosis, occurs at the immune synapse or during uptake of opsonized tumor cells by neutrophils in immunotherapy, but has not been described in PMo or the bloodstream.

We hypothesize that the identification of enhancers of this ability of PMo to engulf CTCs will help implement more efficient strategies to combat lung metastases by preventing adaptation and resistance of tumor cells at the secondary site.

The JAE Intro fellow will be trained in a variety of techniques to develop the following objectives and working plan:

1. Candidate-based screening of regulators of tumor cell trogocytosis by PMo in vitro. As we established, mouse bone marrow-derived PMo will be co-cultured with murine B16F10 melanoma cells, Lewis lung carcinoma or MMTV-PyM derived breast carcinoma cells (DiL-pre-labeled or biotinylated) on mouse lung endothelial cells, and trogocytosis will be quantified by multi-parametric flow cytometry after 3 hours. The role of the actin

cytoskeleton and PI3K, among other pathways, will be tested by pre-incubating PMo with modulators and of the GTPase RhoG by deriving PMo from bone marrow of deficient mice (in collaboration with B. Alarcón, CBMSO; Martínez-Martín et al., Immunity 2011). In vitro trogocytosis will be evaluated in a complementary way by confocal microscopy of fixed cells and by confocal time-lapse microscopy and 3D reconstruction (Imaris®) of PMo and tumor cells labeled with Qdots.

2. Proof of concept for modulation of tumor cell trogocytosis by PMo in the lung in vivo. The impact of the best in vitro candidates on the trogocytosis of CTCs by PMo in the lung will be analyzed in vivo in wild-type mice (pre-treated with modulators or adoptive transfer with pre-treated DiO-labeled PMo) and in RhoG-null mice. DiL-labeled tumor cells will be injected i.v. and 3 hours later, the lungs will be removed for analysis of tumor cell capture by PMo using flow cytometry, confocal microscopy of fixed lung sections and multiphoton time-lapse microcopy of lung explants.

This project will lay the foundations for a future in-depth characterization of the relevant pathways for the capture and elimination of tumor cells by innate immune cells in the lung, the enhancement of which will help reduce tumor lung metastases and improve patient survival.