LOCALIZATION OF THE MACROPHAGE COLONY STIMULATING FACTOR RECEPTOR IN NEOPLASTIC CELLS

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INTRODUCTION

Macrophage colony stimulating factor (CSF1) is a cytokine that is known to act in the proliferation, differentiation and survival of M2 macrophages, involved in elimination of debris, angiogenesis, tissue remodeling, and wound healing. CSF1 also regulates macrophage and osteoclast differentiation, trophoblast implantation, and mammary gland development (where its receptor is expressed at puberty, pregnancy, and breastfeeding). In addition, the expression of CSF1 as well as its receptor has been observed in several types of cancer as prostate carcinoma, breast tumors, ovary, uterus, Hodgkin lymphoma, and acute myeloid leukemia. It has been reported in the literature that CSF1 makes the immune system permissive to tumors and can act on tumor cells themselves, stimulating their growth and migration (figure 1). Although classically described as a membrane protein, the CSF1 receptor (CSF1R) has already been detected in the nucleus of healthy monocytes as well as breast tumor cells (figure 1). CSF1R nuclear localization has been associated with the transcriptional activity of genes related to cell proliferation in tumor cells such as: CCND1, c-JUN and c-myc, which codes for a central protein in lymphomagenesis. Leukemias and lymphomas are hematological malignancies. In this context, this project aims to investigate the subcellular localization of CSF1R in two cell lines derived cells diffuse large B-cell lymphomas (LDGCB) upon exposure or not to its ligand and compare its location in neoplastic cells from different types of tumors such as of chronic myeloid leukemia (CML), acute myeloid leukemia (AML) and breast cancer (BC).

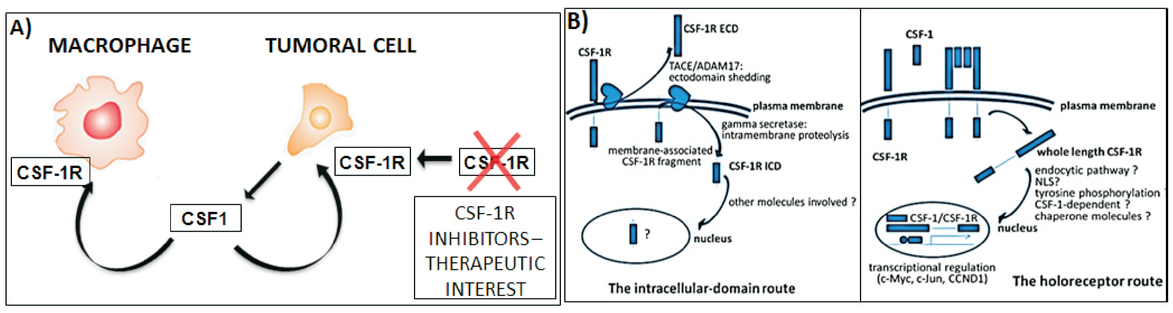

Figure 1: CSF1R in cancer. **A)** CSF1 may contribute to tumor progression by signaling either through CSF1R of macrophages in the tumor microenvironment (TAM) or through CSF1R of tumor cells. Upon CSF1R activation macrophages become permissive to tumor cells and produce angiogenic, metastatic, and immunosupressor factors and tumor cells proliferate, migrate, invade the tissue, and become more agressive. **B)** Schematic representation of subcellular localization of CSF1R (Adapted from Rovida & Sbarba, 2014).

MATERIAL AND METHODS

Human DLBCL-derived cell lines SUDHL4 and Toledo, CML-derived cell lines K562, Lucena and K-IM, AML-derived cell lines HL60, U937 and Kasumi were studied. Immunofluorescence technique (IF) is used to morphologically evaluate the cellular location of CSF1R. Immunostaining with the anti-CSF1R monoclonal antibody (#3152 Cell Signaling) was followed by incubation with Alexa 488-labeled anti-Rabbit secondary antibody as well as with DAPI for visualization of cells nuclei under the confocal microscope. The location of CSF1R will still be compared with that of molecules such as α-tubulin and β-actin, by confocal microscopy, to investigate cell membrane localization. All cell lines will be exposed to 100 ng/ml of recombinant CSF1 to evaluate a possible modulation of the subcellular localization of CSF1R upon activation.

RESULTS AND DISCUSSION

So far, in all cell lines tested the presence of CSF1R was confirmed through the IF assay, corroborating the literature. Regarding the LDGCB-derived cell lines, in the SUDHL4 cell line, it was possible to detect the localization of CSF1R in the cytoplasm and in the nucleus, distributed in nuclear foci. For Toledo cells, still more experiments are needed to better visualize and confirm CSF1R localization (figure 2).

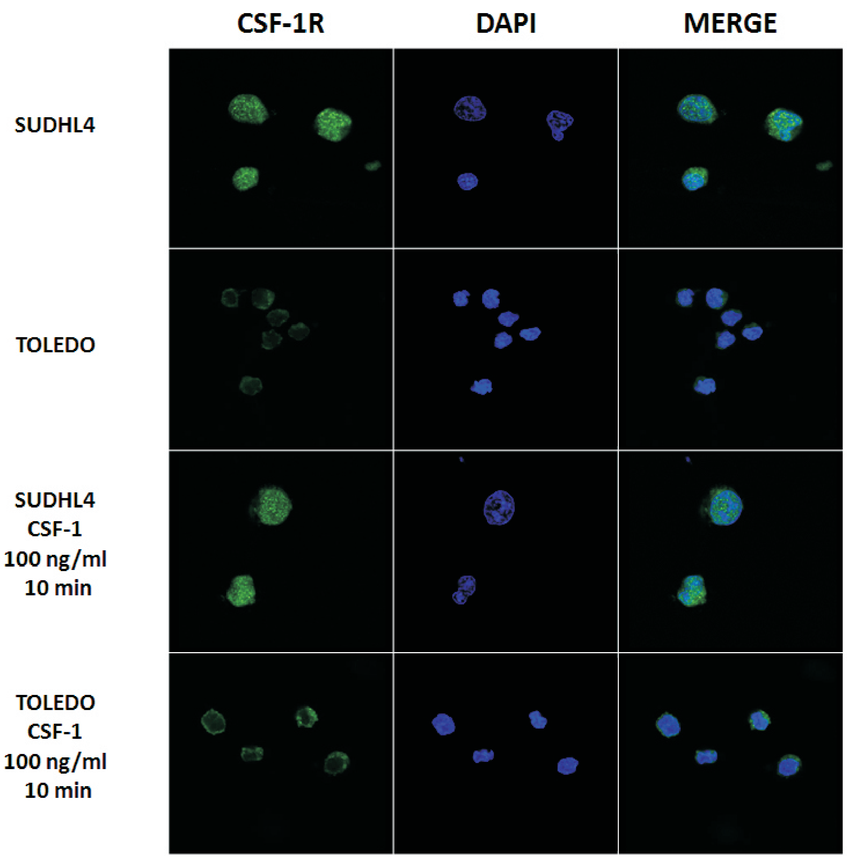

Figure 2: CSF1R localization in SUDHL4 and Toledo LDGCB derived cells. Upper pictures: steady state localization. Lower pictures: localization upon CSF1 exposure. Green represents the detection of the anti-CSF1R monoclonal antibody (# 3152 Cell Signaling) by the anti-Rabbit secondary antibody conjugated to the Alexa fluorochrome 488. Blue represents DAPI staining. Representative image of three experiments.

In cells derived from CML, K562 and Lucena, CSF1R localization appears to be cytoplasmic. In the Imatinib resistant cell line, also derived from CML, namely K-IM, CSF1R localization appears to be perimembranar (figure 3).

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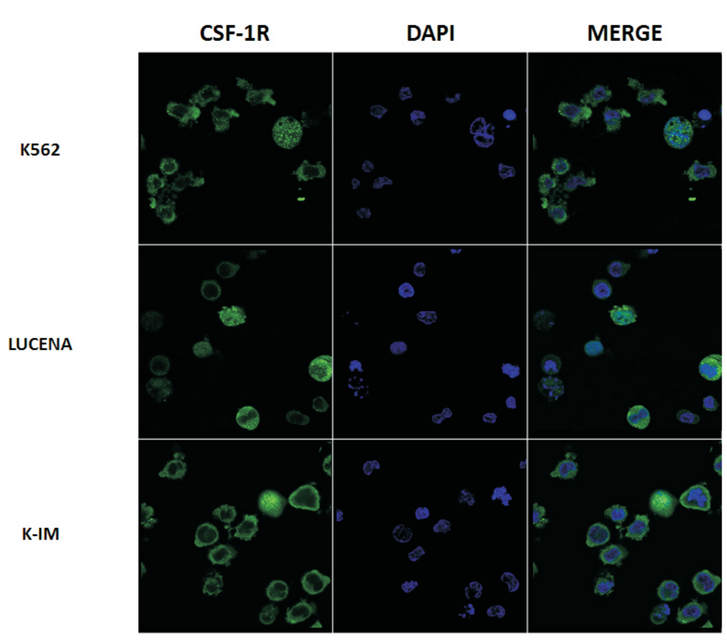

Figure 3: CSF1R localization in CML derived cells. K562 is the parental cell line. Lucena is K562-derived, vincristine resistant. K-IM is K562-derived, imatinib resistant. Green represents the detection of the anti-CSF1R monoclonal antibody (# 3152 Cell Signaling) by the anti-Rabbit secondary antibody conjugated to the Alexa fluorochrome 488. Blue represents DAPI. staining. Representative image of two experiments.

As for AML-derived cell lines, experiments were performed with the HL60 cell line, and the localization of CSF1R appears to be cytoplasmic and nuclear, requiring further experiments to confirm this result (figure 4).

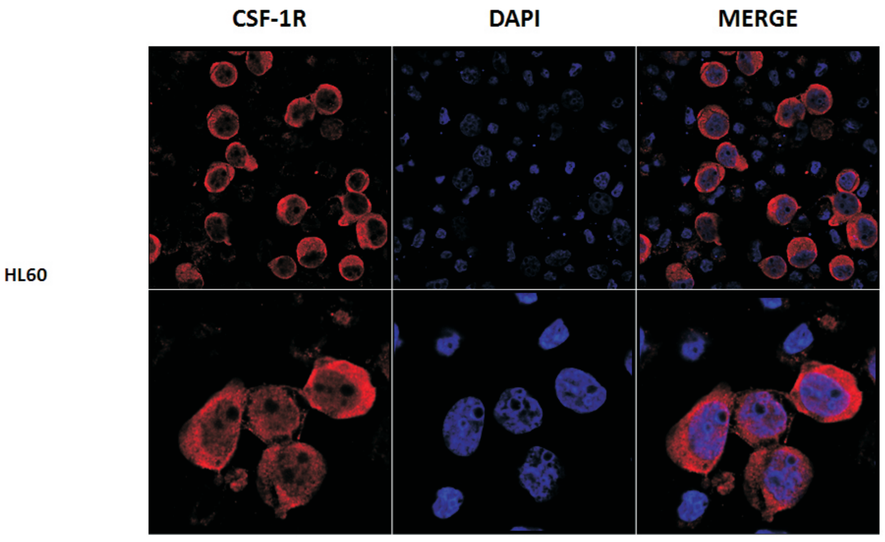

Figure 4: CSF1R localization in HL60 AML derived cells. Red represents the detection of the anti-CSF1R monoclonal antibody by the anti-Rabbit secondary antibody conjugated to the Alexa fluorochrome 594. Blue represents DAPI staining. Representative image of two experiments.

Additionally, further experiments will be carried out by treating cells with recombinant CSF1 to evaluate a possible modulation of the subcellular localization of CSF1R upon activation. The localization of the CSF1R receptor will also be investigated comparing CSF1R localization with that of α-tubulin and β-actin. The respective antibodies were recently titrated: for α-tubulin the most appropriate dilution was 1:1300 and for β-actin the most appropriate dilution was 1:500 (figure 5).

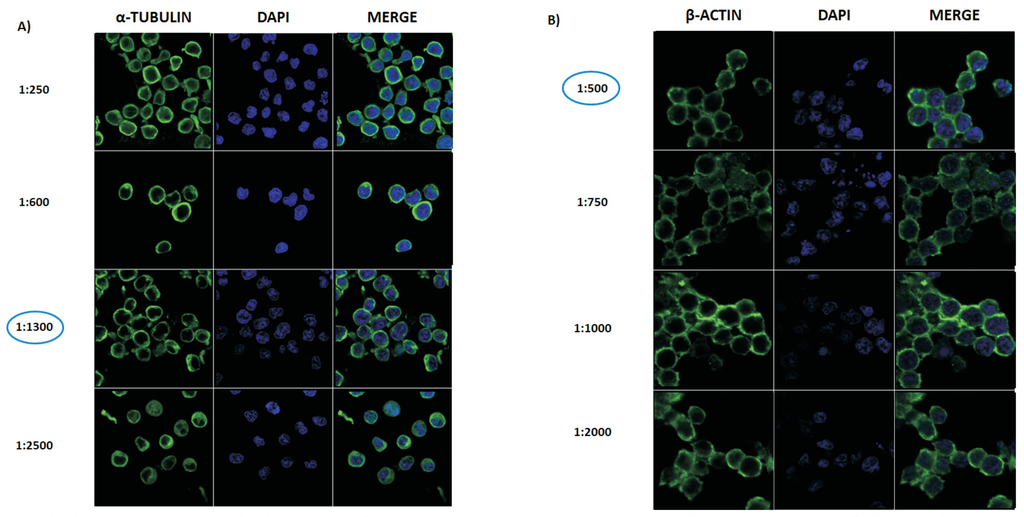

Figure 5: Standardization of subcellular localization markers. **A)** α-tubulin wil be emplyoed as a cytoplasmic localization marker. **B)** β-actin wil be employed as a perimembranar localization marker. Image of one experiment. Green represents the detection of anti- α-tubulin or anti- β-actin the by the anti-Rabbit secondary antibody conjugated to the Alexa fluorochrome 488. Blue represents DAPI staining. Result of one experiment

CONCLUSION AND PERSPECTIVES

These early results lead us to hypothesize the possible relationship between CSF1R localization and the treatment resistance phenotype, and also to emphasize the importance of CSFR inhibitors as of therapeutic interest. To confirm the extranuclear localization of CSF1R (either at the membrane or at the cytoplasm), the co-localization experiments will be carried out. Moreover, to evaluate a possible modulation of the subcellular localization of CSF1R upon activation all cell lines will be exposed to recombinant CSF1R and CSF1R subcellular localization will be evaluated. To further confirm CSF1R subcellular localization, cell fractioning assays will also be crucial.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA