EZRIN PROTEIN'S ROLE ON CELL MIGRATION IN GASTRIC CANCER AND IDENTIFICATION OF M6A-MODIFIED SITES

Sara Silva [1*], Patrícia Mesquita [1,2], Bruno Pereira [1,2], Raquel Almeida [1,2,3]

[1] *i3S - Institute for Research and Innovation in Health, University of Porto, 4200-135 Porto, Portugal*
[2] *IPATIMUP - Institute of Molecular Pathology and Immunology of the University of Porto, 4200-465 Porto, Portugal*
[3] *Biology Department, Faculty of Sciences of the University of Porto, Rua Campo Alegre s/n, 4169-007 Porto, Portugal*
** up202104367@up.pt*

Background: Gastric cancer (GC) is the fourth leading cause of cancer-related deaths, often diagnosed in late stages of the disease, limiting treatment options [1-2]. The absence of new therapies and proper diagnosis calls for an exploration of different potential targets. Epitranscriptomics studies RNA chemical modifications such as N6-methyladenosine (m6A), lately studied to understand its effect on cancer progression and potential treatment [3-4]. YTHDF3 is an m6A reader and its role in migration was established by the "Differentiation and Cancer" group at i3S (unpublished data, manuscript submitted). In this study we focused on EZRIN (EZR), which is a target of YTHDF3. **Aims:** To inhibit EZR expression using NSC668384, study the impact on migration/metastization and on Paclitaxel response and identify the m6A methylation sites on the EZR transcript using the SELECT method. **Methods:** We inhibited EZR expression with NSC668384 in two GC cell lines, characterizing the effect on cell viability, cell migration through a migration assay, and performed a combined treatment of NSC668384 with Paclitaxel. We also used the SELECT method to identify m6A-modified sites in the EZR transcript. **Results:** EZR is involved in cell migration and its downregulation sensitizes cells to Paclitaxel treatment. We successfully detected two methylated sites in the EZR transcript. **Conclusions**: The loss of migration capacity upon EZR inhibition aligns with previous studies showing EZR's critical role in cancer cell motility [5-9]. The increased sensitivity of SNU638 cells to Paclitaxel supports findings from other malignancies where EZR inhibition enhances chemotherapy efficacy, indicating a shared mechanism across cancers [10]. Additionally, the presence of m6A modifications in the EZR transcript highlights the need to investigate their functional impact on EZR regulation and therapeutic responses. EZR's inhibition has therapeutical value, and the identification of m6A-sites will allow research on these modifications' role which may result in development of new treatment strategies.

Keywords: Gastric cancer; EZR; epitranscriptomics; m6A; migration.

Referências

[1] López, MJ; Carbajal, J; Alfaro, AL; Saravia, LG; Zanabria, D; Araujo, JM; et al. Characteristics of gastric cancer around the world. *Crit Rev Oncol Hematol* 2023, *181*.

[2] Cavatorta, O; Scida, S; Miraglia, C; Barchi, A; Nouvenne, A; Leandro, G; et al. Epidemiology of gastric cancer and risk factors. *Acta Biomed* 2018, *89*, 82-87.

[3] Zhang, X; Su, H; Chen, H; Li, Q; Liu, X; Zhang, L; et al. RNA modifications in gastrointestinal cancer: current status and future perspectives. *Biomedicines* 2022, *10*, 1-15.

[4] Kadumuri, RV; Janga, SC; Building, WP; Genetics, M; Building, L. Epitranscriptomic code and its alterations in human disease. *Trends Mol Med* 2019, *24*, 886-903.

[5] Bulut, G; Hong, S-H; Chen, K; Beauchamp, EM; Rahim, S; Kosturko, GW; et al. Small molecule inhibitors of ezrin inhibit the invasive phenotype of osteosarcoma cells. *Oncogene* 2012, *31*, 269-281.

[6] Xu, J; Zhang, W. EZR promotes pancreatic cancer proliferation and metastasis by activating FAK/AKT signaling pathway. *Cancer Cell Int* 2021, *21*, 1-15.

[7] Jin, J; Jin, T; Quan, M; Piao, Y; Lin, Z. Ezrin overexpression predicts the poor prognosis of gastric adenocarcinoma. *Diagn Pathol* 2012, *7*, 135.

[8] Ghaffari, A; Hoskin, V; Turashvili, G; Varma, S; Mewburn, J; Mullins, G; et al. Intravital imaging reveals systemic ezrin inhibition impedes cancer cell migration and lymph node metastasis in breast cancer. *Breast Cancer Res* 2019, *21*, 1-11.

[9] Pore, D; Bodo, J; Danda, A; Yan, D; Phillips, JG; Lindner, D; et al. Identification of Ezrin-Radixin-Moesin proteins as novel regulators of pathogenic B-cell receptor signaling and tumor growth in diffuse large B-cell lymphoma. *Leukemia* 2015, *29*, 1857-1867.

[10] Hoskin, V; Ghaffari, A; Laight, BJ; SenGupta, S; Madarnas, Y; Nicol, CJB; et al. Targeting the ezrin adaptor protein sensitizes metastatic breast cancer cells to chemotherapy and reduces neoadjuvant therapy-induced metastasis. *Cancer Res Commun* 2022, *2*, 456-470.