ID: 03084

Topic: DRUG TOLERANCE

Deciphering the molecular drivers of protein translation in cancer persister cells

Peijin Jiang[1] , Cándida Salvans[1] , Luna Martín[2] , Francesc Canals[2] , Héctor G Palmer[1] , Isabel Puig[1]

1) Stem Cells and Cancer Group, Vall d'Hebron Institute of Oncology (VHIO) 2) Proteomics Group, Vall d'Hebron Institute of Oncology (VHIO)

Introduction: Most colorectal cancer (CRC) patients rapidly develop chemoresistance, leading to disease relapse and remaining a key obstacle to successful cancer therapy. In this context, current standard treatments are increasingly recognized to promote the emergence of resistant cancer cells. Both genetic and non-genetic strategies have been proposed as the culprit of therapy resistance in CRC. Since chemotherapeutic drugs mainly target proliferating cells, the suppression of proliferation, manifesting as *slow-cycliness*, has emerged as a pivotal non-genetic strategy to evading therapy-induced cell death. However, although the slow-cycling resistance mechanism contributes to tumor relapse, strategies to target cancer persistence have not yet been successfully translated into clinical use. To improve patient outcomes, it is imperative to advance the understanding of the molecular mechanisms underlying non-genetic resistance.

To this end, our laboratory has been studying the biology of slow-cycling cancer cells (SCCCs) for over a decade. We recently identified the developmental pluripotent factor DPPA3 as a key regulator of the slow-cycling phenotype in CRC cells, particularly under stress conditions such as hypoxia and chemotherapy. DPPA3-overexpressing cells are prone to adopt a slow-cycling, drug-tolerant state, contributing to cancer persistence. Immunohistochemical and gene-expression analyses revealed that DPPA3 is a predictive biomarker for CRC progression. Therefore, targeting DPPA3-positive cells emerges as a promising therapeutic strategy to overcome CRC persistence.

Objectives: We hypothesize that CRC persistence could be effectively targeted by exploiting vulnerabilities mediated by DPPA3. Our main objective is to decipher the survival pathways regulated by DPPA3 in response to standard-of-care chemotherapy, specifically 5-fluorouracil (5FU), to reveal new therapeutic targets for improving cancer patients' outcomes.

Methods: Previous RNA-seq analysis comparing DPPA3-overexpressing CRC cells to control cells revealed an enrichment of gene programs related to translation and ribosome assembly.

We investigated DPPA3's role in protein translation by assessing the phosphorylation status of 4E-BP1 and EIF2?, key regulators of translational initiation, under naïve conditions and following 5FU treatment by Western Blot. Additionally, we assessed ribosomal RNA (rRNA) levels by immunofluorescence staining and functional protein translation process was evaluated by OPP and puromycin labelling assays.

To further elucidate how DPPA3 regulates translation under both naïve and chemotherapeutic conditions, we performed an interactomic proteomic and phospho-proteomic analyses on SW1222 control and DPPA3-overexpressing cells grown in 3D-cultures and treated with vehicle or 5FU for 3 days. These samples were subsequently analyzed by the VHIO Proteomic Group.

Results:

Western Blot analysis showed that DPPA3 overexpression enhances translation under naïve conditions but leads to a stronger translational blockade in response to 5FU. Moreover, DPPA3 overexpression increased rRNA biogenesis, indicating enhanced ribosome production. Additionally, ribosomal proteins emerged as potential DPPA3 interactors, suggesting that DPPA3 could reprogram tumor cells by reshaping their translatome, thereby contributing to therapy resistance. These findings were functionally validated by OPP and puromycin labelling assays, which quantified total cellular translation.

Conclusions: Overall, our data indicate that in naïve conditions, there is an increase of protein translation level

when DPPA3 is overexpressed but under 5FU treatment, it induces a stronger translation blockage. Contrary to expectations of reduced protein production in slow-cycling cells, all analyses indicated that DPPA3 induces a higher translational activity in these cells. Further studies are needed to decipher the molecular mechanisms behind this phenomenon. Nevertheless, results from the interactomic, proteomic and phospho-proteomic analyses are expected to shed light on how DPPA3 regulates the translation process.