Abstract

Phytocannabinoids, the major secondary metabolites of cannabis plants, exert a wide range of biological activities. The present work was focused on investigating the mechanism of action of cannabidiolic acid (CBDA) in U87MG glioblastoma cell line, exploiting the efficacy of chemical-proteomics based approaches in identifying target proteins of uncharacterized drugs. DARTS experiments showed Eukaryotic Initiation Translation Factor 2A (EIF2A) as a putative target of CBDA. This interaction was further validated by western blot and CETSA, thus showing a thermal stabilization of Eukaryotic Translation Complex conferred by CBDA. Moreover, Limited Proteolysis showed that the EIF2A C-terminal portion 460-480 could play a critical role in the molecular recognition of CBDA by the protein. This result was also confirmed by Molecular Dynamics (MD) calculations, which revealed that CBDA interacts with a stretch of residues in the 460-480 portion and in the adjacent C-terminal helix, acting as a bridge between these regions. Hence, since EIF2A is the initiator factor of translation process, the impact of CBDA-EIF2A interaction on proteins synthesis was investigated by p-SILAC and enrichment via click-chemistry. Comparing CBDA and EIF2A-silencing treatments, a similar remodeling of nascent proteome was detected in the two conditions in terms of protein expression reduction and biological effect. Particularly, CBDA appeared to induce an UPR response, triggering as a balancing effect between the ER-stress response and the attempt to restore cellular homeostasis. Moreover, EIF2A revealed to interact not only with eukaryotic translation proteins but also with the proteins involved in triggering of UPR response and the CBDA-induced reorganization of eukaryotic translation machinery. Interestingly, these proteins seem to be involved in several pathways already highlighted by nascent proteome investigation.

In order to evaluate the protein-CBDA interaction in a cell model closer to the tumor in vivo, a 3D cell culture was set up using a classic-sandwich model. Based on the observation that in 2D- and 3D- cell model U87MG cells grow differently since in 3D they show a natural shape and more cellular interactions, a global proteome comparative analysis was firstly carried out. Interestingly, the obtained results highlighted a higher-amount of proteins involved in invasion cellular processes and cell-ECM interaction expressed by 3D-U87MG, compared to 2D-cultured cells. These findings prompted us to further study the effects of CBDA in 2D and 3D cellular models. In 3D cultured cells, CBDA showed a different cytotoxicity depending on the concentration of FBS in the upper and lower- gels and in the culture medium as well. DARTS assay performed in this cell system also suggested a direct

correlation between the percentage of FBS used in cell culture conditions and the ability of CBDA to interact with EIF2A, thus confirming the critical role played by the molecule-FBS interaction on its availability. Furthermore, comparing the results of DARTS assays performed on 2D and 3D, a difference of the EIF2A interactome with respect to the entire translational complex was revealed in the two conditions. In contrast to 2D-cellular model, EIF2A was highly resistant to proteolysis in untreated 3D cultured cells, but was more digested after CBDA treatment. This result suggested that EIF2A in 3D-U87MG could be likely associated with the other protein partners more strongly than in the 2D model. In closing of this study, it is possible to state that the use of a multi-proteomic approach allowed us to highlight the potential impact of CBDA on the eukaryotic translation machinery, also suggesting the importance of investigating the interactome differences that exist between innovative three-dimensional and conventional cellular models.