# Intracellular pathways involved in cannabidiol anti-proliferative effect in U87-MG and T98G glioma cell lines

V. Cinquina[1], M. Solinas[1], D. Parolaro[1]

[1] Dept. of Theoretical and Applied Sciences, Biomedical Research Division, Univ. of Insubria, Busto Arsizio (VA), Italy

Several studies have demonstrated that cannabidiol (CBD) possesses anti-proliferative, anti-apoptotic effects and inhibits glioma cell migration and invasion. CBD effect depends on multiple cellular targets that control tumorigenesis through the modulation of different intracellular signaling pathways.
Strong evidence now suggests that Id-1, an inhibitor of basic helix-loop-helix transcriptional factors, is a key regulator of cellular processes related to tumor progression. Higher levels of Id-1 gene expression have been detected in many different types of aggressive tumor cells, when compared to normal cells of the same tissue origin. Interestingly sever studies have suggested that Id proteins are involved in the development of brain tumors. Furthermore, there is a close association between Id-1 expression and angiogenesis: this protein is essential for tumor-associated angiogenesis during cancer progression. It has also been reported that Id-1 is possibly linked to prominent cell-signaling pathways such as phosphatidylinositol-3-kinase (PI3K)/Akt, Mitogen-Activated Protein Kinase (MAPK/ERK), Nuclear Factor kappa B (NFkB), and Forkhead transcriptional factor (FOXO3a) pathways.
Thus, based on these data we decided to investigate the role of Id-1 protein in CBD anti-proliferative effect and its relation with different intracellular pathways, both in U87-MG and in T98G glioma cell lines.

We first evaluated Id-1 expression in U87-MG and T98G human glioma cells in comparison with its control level in astrocytes. As demonstrated by Western Blot analysis, Id-1 level is significantly elevated in glioma cells whereas a very low expression was observed in astrocytes. This data seems to suggest that Id-1 level is involved in tumorigenesis.
Interestingly CBD treatment causes down-regulation of Id-1 level at concentrations causing inhibition of U87-MG and T98G glioma cell viability (MTT test).
As we previously demonstrated that CBD treatment increased ROS production in glioma cells, we investigated the possible correlation between ROS production, Id-1 levels and CBD anti-proliferative effect. According to previous evidences, in U87-MG cells the ROS scavenger alpha-tocopherol (TOC) reversed CBD anti-proliferative effect (MTT test). Interestingly it also counteracted the CBD-induced Id-1 inhibition (Western Blot).
Finally we tried to correlate the CBD-induced inhibition of ERK and Akt phosphorilation with the observed Id-1 inhibition. We found that both ERK inhibitor, U0126, and PI3K inhibitor, LY294002, significantly down-regulated Id-1 expression (Western Blot).
Together these data suggest that CBD anti-proliferative effect in U87-MG and T98G cells is associated with Id-1 inhibition through ERK and Akt pathways and ROS production.
These data add new insight to the cellular mechanisms involved in the cannabidiol-induced inhibition of glioma cell proliferation.