Stimulation of ALK by the growth factor midkine renders glioma cells resistant to autophagy-mediated cell death

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Abbreviations: ALK, anaplastic lymphoma kinase receptor; ER; endoplasmic reticulum; Mdk; midkine; mTORC1, mammalian target of rapamycin complex 1; THC, Δ9-tetrahydrocannabinol; TRB3, tribbles homolog 3

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Δ9-tetrahydrocannabinol (THC), the main active component of marijuana, promotes cancer cell death via autophagy stimulation. We find that activation of the tyrosine kinase receptor ALK by its ligand midkine interferes with the signaling mechanism by which THC promotes autophagy-mediated glioma cell death.

The final outcome of the activation of the autophagy program seems to be highly dependent on the cellular context and the strength and duration of the stress-inducing signals. Thus, besides its role in cellular homeostasis, autophagy can be a form of programmed cell death or play a cytoprotective role, for example in situations of nutrient starvation. Accordingly, autophagy plays a dual role in cancer as this cellular process may help to overcome the stress evoked at the initial steps of tumorigenesis or work as a tumor suppressor mechanism. Moreover, different anticancer treatments activate autophagy in tumor cells, which either enhance cancer cell death or act as a mechanism of resistance to chemotherapy.

Cannabinoids, the active components of marijuana, of which Δ9-tetrahydrocannabinol (THC) is the most important owing to its high abundance and potency, are currently being investigated as potential antitumoral agents. Along these lines, treatment with cannabinoids curbs tumor growth in various animal models of cancer. Our recent findings have revealed that cannabinoids induce autophagy in different types of tumor cells, including glioma/astrocytoma and pancreatic cancer cells, whereas they do not activate this cellular process in nontransformed cells (which are resistant to the cell death-promoting activity of cannabinoids). Of interest, pharmacological or genetic inhibition of autophagy prevents cannabinoid-induced cell death as well as apoptosis, whereas abrogation of apoptosis prevents cell death but not autophagy as induced by these agents. These observations lead us to conclude that induction of autophagy is an essential part of the mechanism by which cannabinoids promote the apoptotic death of cancer cells. These pro-autophagic actions of cannabinoids rely on the stimulation of an ER stress-related signaling route that leads to the upregulation of the transcriptional co-activator p8 and its target the pseudo-kinase tribbles homolog 3 (TRB3). The stimulation of this pathway promotes autophagy via TRB3-mediated inhibition of the Akt-mammalian target of rapamycin complex 1 (mTORC1) axis.

Glioblastoma multiforme (GBM) is the most frequent class of malignant primary brain tumor and one of the most aggressive forms of cancer. These features of GBM are, at least in part, due to the high resistance to standard chemotherapy and radiotherapy exhibited by these tumors. In this study, we investigated the molecular factors responsible for the resistance of glioma cells to THC anticancer action. To this end, we analyzed the gene...
expression profile of a panel of glioma cell lines with different sensitivity to cannabinoid-induced cell death. This experimental approach led us to identify 8 candidate genes whose expression is associated with resistance to THC-treatment in both glioma cell lines and primary cultures of glioma cells.

One of the genes whose high expression is more strongly associated with the resistance to THC-induced cell death is the one encoding the growth factor midkine (Mdk). Mdk is a member of the pleiotrophin family that has been proposed to modulate the proliferation and migration of various types of tumor cells. Likewise, high expression of Mdk is associated with increased malignancy in several types of tumors. In line with these observations, we found a correlation between Mdk expression and lower overall survival of GBM patients. In addition, our data show that Mdk levels are increased in the supernatants of cannabinoid-resistant glioma cells. We therefore analyzed whether this growth factor may play a direct role in the resistance to THC action. In line with this hypothesis, Mdk silencing sensitizes cannabinoid-resistant glioma cells to THC-induced cell death. Moreover, incubation with Mdk-enriched conditioned medium obtained from cannabinoid-resistant cells or with exogenous Mdk renders sensitive cells resistant to THC-induced cell death, and this effect is prevented by incubation with an anti-Mdk antibody. Altogether, these data indicate that Mdk promotes resistance to THC-induced glioma cell death.

The mechanism by which Mdk regulates different biological processes has not been completely clarified yet. Thus, several membrane receptors have been implicated in Mdk actions in different biological systems. We found that the protective effect of Mdk on glioma cells relies on the activation of the tyrosine kinase receptor ALK. Thus, pharmacological or genetic inhibition of ALK sensitizes cannabinoid-resistant cells to THC-induced cell death, and ALK silencing prevents the protective action of Mdk on cannabinoid-sensitive cells.

Next, we asked whether the mechanism by which Mdk, via ALK, promotes resistance to THC could be based on the modulation of the signaling route that regulates autophagy-mediated cell death in response to this agent. In line with this idea, genetic inhibition of Mdk or ALK enhances THC-induced autophagy and apoptosis in glioma cells. Moreover, incubation of sensitive glioma cells with Mdk-enriched conditioned medium or with exogenous Mdk decreases THC-induced autophagy and apoptosis in these cells.

As indicated above, THC promotes autophagy via the upregulation of the ER stress-related proteins p8 and TRB3, and by enhancing the activity of Akt.

Figure 1. Stimulation of the Mdk-ALK axis promotes resistance to THC antitumoral action. Mdk promotes resistance to THC-induced autophagy and apoptosis by interfering with the upregulation of the ER stress-related proteins p8 and TRB3, and by enhancing the activity of Akt.
The in vivo relevance of these findings was confirmed in tumor xenografts generated with the cannabinoid-resistant glioma cell line T98G (these tumors exhibit high levels of Mdk and are resistant to THC anti-tumoral action). Interestingly, in vivo silencing of Mdk or pharmacological inhibition of ALK renders established T98 cell-derived tumors sensitive to THC treatment. Moreover, we found that the THC-induced increase in TRB3 expression, decrease in S6 phosphorylation (a well established readout of mTORC1 activity) and increase in autophagy and apoptosis only take place in these tumors when Mdk has been silenced or ALK has been inhibited. Taken together, these findings support the idea that stimulation of the Mdk-ALK axis promotes resistance to THC-induced autophagy and cell death in vitro and in vivo via modulation of the p8-TRB3 signaling route. In addition, our data suggest that selective targeting of the Mdk-ALK axis could be used to enhance the efficiency of antitumoral therapies based on the stimulation of autophagy-mediated cell death. More specifically, these results could help to set the basis for the potential clinical utilization of THC in combination with inhibitors of the Mdk-ALK axis in gliomas.