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Autophagic Punctum

TRB3 links ER stress to autophagy in cannabinoid anti-tumoral action

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Abbreviations: Atg, autophagy-related; eIF2α, eukaryotic translation initiation factor 2α; ER, endoplasmic reticulum; mTORC1, mammalian target of rapamycin complex 1; THC, Δ^9-tetrahydrocannabinol; TRB3, tribbles homologue 3

Key words: cancer, cannabinoids, autophagy, ER stress, TRB3

Δ^9-tetrahydrocannabinol (THC), the main active component of marijuana, is being investigated as a potential anti-tumoral agent. We find that THC stimulates an endoplasmic reticulum (ER) stress-related signaling pathway, which activates autophagy via inhibition of the Akt/mTORC1 axis. We also show that autophagy is upstream of apoptosis in cannabinoid-induced cancer cell death and that activation of this pathway is necessary for the anti-tumoral action of cannabinoids in vivo.

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. On one hand, this cellular process may help to overcome the stress evoked at the initial steps of tumorigenesis, and on the other, autophagy has been proposed to work as a tumor suppressor. 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 Δ^9-tetrahydrocannabinol (THC) is the most important owing to its high abundance and potency, are currently being investigated as potential anti-tumoral agents. Along these lines, treatment with cannabinoids curbs tumor growth in various animal models of cancer. These anti-tumoral actions of cannabinoids are based on the ability of these agents to inhibit tumor angiogenesis and activate apoptosis of cancer cells.

Our recent findings have unraveled 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 part of the mechanism by which cannabinoids promote the apoptotic death of cancer cells. The in vivo relevance of these findings is demonstrated by the observation that cannabinoid treatment reduces tumor growth and activates autophagy and apoptosis in subcutaneous tumor xenografts derived from human U87MG astrocytoma cells and transformed mouse embryonic fibroblasts (MEFs). Furthermore, autophagy-deficient tumors (generated by subcutaneous injection of transformed Atg5$^{-/-}$ MEFs) are resistant to THC anti-tumoral action, strongly supporting the idea that autophagy is essential for the antineoplastic activity of cannabinoids. In addition, analysis of samples obtained from two glioblastoma multiforme patients indicates that THC administration might also trigger autophagy-mediated cell death in human tumors.

We also investigated the molecular mechanisms responsible for the activation of autophagy upon THC administration. Our results indicate that cannabinoids stimulate this process via an endoplasmic reticulum (ER) stress-related signaling pathway (Fig. 1). ER stress is an adaptive response that becomes activated in the cell when the ER is altered. We found that THC triggers

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an early accumulation of de novo-synthesized ceramide (an event that takes place in the ER), which leads in turn to ER dilation and increased eukaryotic initiation translation factor 2α (eIF2α) phosphorylation, two hallmarks of the ER stress response. Activation of this ER stress response induces the upregulation of several genes, including the stress-regulated protein p8 (also named candidate of metastasis 1, Com-1) and its downstream target, the pseudokinase Tribbles homologue 3 (TRB3), that we had previously implicated in cannabinoid anti-tumoral action. Genetic inhibition of p8 and TRB3 prevents cannabinoid-induced autophagy as well as cell death, demonstrating that these proteins play a major role in connecting ER stress and autophagy in the context of cannabinoid action.

Our investigations show that a key step in the induction of autophagy by this ER stress-related signaling pathway relies on the inhibition of the Akt/mammalian target of rapamycin complex 1 (mTORC1) axis by TRB3. It is widely accepted that one of the crucial events for the initiation of autophagy in many cellular settings is the inhibition of mTORC1, which plays a central role in the control of protein synthesis, cell growth and cell proliferation through the regulation of several downstream targets. In addition, mTORC1 is proposed to regulate autophagy by repressing the activity of Atg1. As a result of its central position in the control of cellular homeostasis, mTORC1 integrates signals from different inputs. One of the most important upstream regulators of mTORC1 is the pro-survival kinase Akt, which phosphorylates and inactivates TSC2 (an inhibitor of the mTORC1 activator Rheb) as well as PRAS40. Thus, Akt activation stimulates mTORC1 and inhibits autophagy.

In our study we found that cannabinoid treatment, via the above-described ER stress-related signaling route, increases TRB3 levels and promotes the inhibitory interaction of this protein with Akt, leading in turn to mTORC1 inhibition. In agreement with these observations, treatment of mice with THC decreases mTORC1 activity, stimulates autophagy and apoptosis and reduces tumor growth in xenografts generated with $p8^{+/+}$ cells but not in those generated with $p8^{-/-}$ cells (in which TRB3 is not upregulated in response to THC), further confirming that the p8/TRB3 pathway plays an essential role in the activation of autophagy and cell death by cannabinoids also in vivo.

As discussed above, research by different laboratories shows that autophagy is involved in both promotion and inhibition of tumor growth. However, the molecular determinants of this dual role of autophagy in cancer remain obscure. The identification by our laboratories of a new ER stress-related pathway that activates autophagy and promotes the apoptotic death of tumor cells may help to clarify the molecular events that lead to activation of autophagy-mediated cell death by anticancer drugs. The crucial role of p8 and TRB3 in cannabinoid anti-tumoral action points to these two proteins as important regulators of the autophagy process in the context of tumor cell death. Nevertheless, further research should still clarify whether the extent of ceramide accumulation, the simultaneous activation of other ER stress-regulated signaling pathways, the time frame and degree of mTORC1 inhibition, and the participation of specific autophagy genes are also key elements for the activation of the autophagy-mediated cell death pathway in tumor cells. Characterization of the molecular mechanisms that differentially regulate autophagy in situations in which this cellular process is activated to promote cell death will be crucial to design new therapeutic strategies based on the modulation of autophagy in cancer cells.

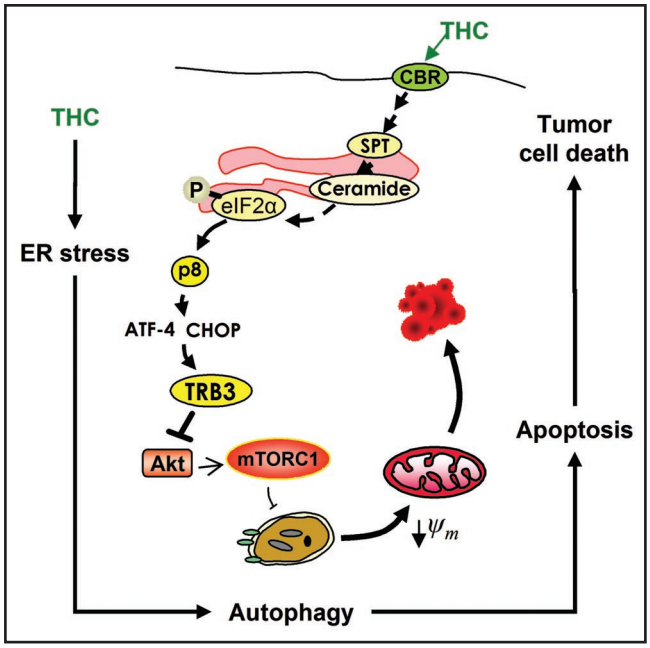

Figure 1. Proposed mechanism of cannabinoid anti-tumoral action. This panel is modified from a schematic shown in Figure 7C of the original article published at J Clin Invest 2009; 119:1359–72.