GPR12-Mediated Alteration of the mTOR Pathway by Phytocannabinoids

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Introduction

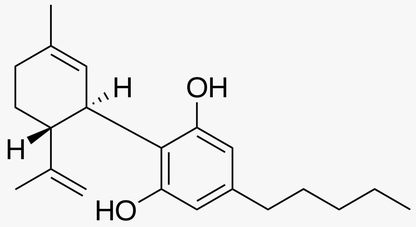
G-protein coupled receptors possess enormous therapeutic potential as pharmacological targets for the treatment of cancer. GPR12 is one such receptor that was first cloned from a mouse cDNA library in 1993, and followed by the cloning of its human counterpart in 1995. Though an endogenous ligand remains unknown, GPR12 can be considered a 'cannabinoid receptor-like orphan receptor' because it shares 35% amino acid sequence identity with established cannabinoid receptors CB1 and CB2. There is promising evidence that the phytocannabinoids cannabidiol (CBD) and cannabidavarin (CBDV) may offer a potential application as antitumor drugs, particularly as ligands of various cannabinoid or cannabinoid-like receptors.

Mechanistic target of rapamycin (mTOR), and its downstream enzymes 4E-BP1 and p70s6k1, are known effectors of cell-signaling pathways commonly altered in several human cancers. The mTOR pathway, as a central regulator for cell metabolism, growth, proliferation and survival, is activated during various processes including tumor formation. Therefore, an mTOR inhibitor may be useful in cancer treatment.

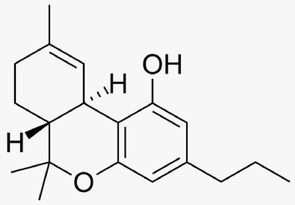
In this study, HEK cells transfected with GPR12 were treated with CBD and CBDV, and the resulting effects on the activation of mTOR, 4E-BP1, and p70s6k1 was tested using western blot analysis.

Phytocannabinoid Structures

Cannabidiol (CBD)



Cannabidavarin (CBDV)

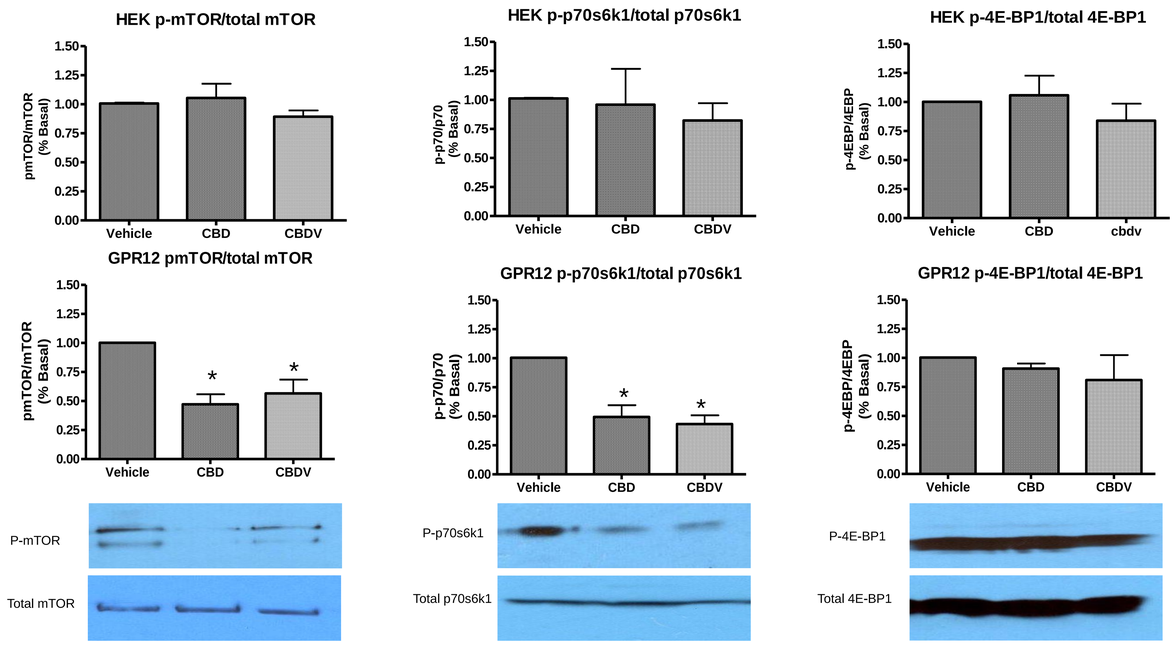


Methods

Membrane samples were prepared from HEK cells according to published procedures. Samples were incubated with 2X Laemmli buffer under reducing conditions at room temperature for 20 minutes and proteins were resolved on a 10% SDS-polyacrylamide gel using a minigel electrophoresis system (Invitrogen, Carlsbad, CA). Protein bands were transferred onto a nitrocellulose membrane. The nitrocellulose membranes were blocked with 5% nonfat dried milk in TBS-T buffer (10 mM Tris-HCl [pH 8.0] 150 mM NaCl, and 0.3% Tween 20) for 1 hour and then incubated overnight at 4°C with primary anti-mTOR, anti-p70s6k1, and anti-4E-BP1 or anti-p-mTOR, anti-p-p70s6k1, and anti p-4E-BP1. Subsequently, the membranes were washed thrice for 5 minutes each time with TBS-T buffer and incubated with horseradish peroxidase (HRP)–conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hour at room temperature. The membranes were then washed three times with TBS-T buffer for 5 minutes each time and the antibody-recognized protein bands were visualized using an enhanced chemiluminescence kit (Fisher Scientific, Waltham, MA).

For Western blot assays, the bands on x-ray films were scanned by the Epson Perfection V39 (Epson, Long Beach, CA) and were semi-quantified with the use of ImageJ program (NIH, Bethesda, MD). One-way ANOVA tests were used to compare the treatment groups. The level of significance for all studies was chosen as $P < 0.05$.

Results



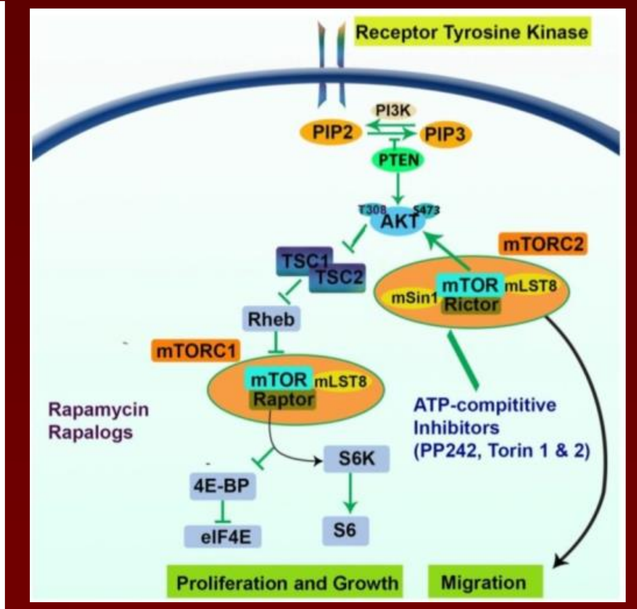
- mTOR was phosphorylated significantly less in response to CBD and CBDV compared to the control (vehicle alone).
- p70-S6K1 was phosphorylated significantly less in response to CBD and CBDV compared to the control.
- 4E-BP1 phosphorylation levels in response to CBD and CBDV were similar to those following application of the control.
- The observed decrease in phosphorylation of p70s6k1 in response to the drugs is consistent with the observed decrease in phosphorylation of mTOR, but the lack of change in phosphorylation of 4E-BP1 is inconsistent with the mTOR result. It is possible that a separate regulation factor of 4E-BP1 is upregulated in response to these two drugs.

Conclusions & Future Directions

The phytocannabinoids CBD and CBDV induce an inhibition of the mTOR pathway through GPR12. This inhibition has therapeutic potential for cancer patients, as it may block the aberrant cell growth that is often associated with upregulation of the mTOR pathway in many human cancers such as prostate, breast, and pancreatic cancer.

Further research is necessary to better understand the effects of these same drugs on the mTOR pathway in cancer cells that express varying levels of GPR12, as well as the effects of mTOR inhibition on the proliferation of cancer cells.

mTOR Pathway



Acknowledgments

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