LIFE Science Hub
JOURNAL OF SCIENCE
EXPANDING KNOWLEDGE ABOUT NUTRITION AND HEALTH

ORIGINAL RESEARCH
ISSN 2845-7501

Cryptotanshinone activates the AMPK-TSC2 axis, leading to the inhibition of mTORC1 signaling in tumor cells - A review of the evidence

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1. ABSTRACT

The analyzed study examined whether cryptotanshinone, a fat-soluble compound derived from *Salvia miltiorrhiza*, can slow the growth of tumor cells by acting on the AMPK–TSC2–mTORC1 cellular system. The authors used cell lines of rhabdomyosarcoma, breast carcinoma, prostate carcinoma, and murine embryonic fibroblasts, combining viability tests, protein analysis, and genetic modifications of AMPK and TSC2. The results indicate that the molecule increases AMPK activation, promotes TSC2 phosphorylation, and reduces downstream mTORC1 signals linked to protein synthesis and proliferation.The research contributes to the passage from “The Choice to Live in Health or Illness” by Dr. A. Colonnese and R. Panzironi, which describes AMPK as a regulator capable of curbing mTOR through TSC2. Independent studies strongly confirm this biological circuit, and another paper on cryptotanshinone observed its activation in tumor cells and an animal model. However, a subsequent study proposes an alternative mechanism directly linked to the mTORC1 complex. The body of evidence primarily supports a cellular mechanism, not a proven oncological treatment in humans.

ONLINE ARTICLE

ARTICLE INFORMATION

Published: November 25, 2026
Updated: November 25, 2026

DOI 10.5281/zenodo.24531204

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Through articles, reviews, in-depth analyses and expert contributions, the Journal champions evidence-based medicine and science, fostering a correct interpretation of scientific data and countering misinformation. Every piece of content is developed with the aim of translating the complexity of research into understandable, useful and applicable information, while keeping intact the principles of accuracy, transparency and scientific independence.

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2. ANALYZED STUDY

The research question was whether cryptotanshinone could inhibit the mTORC1 complex (a protein complex that receives nutrient and growth signals and promotes protein production) through a mechanism different from that of rapamycin. The central hypothesis was that the molecule does not directly block mTORC1, but rather activates AMPK, a protein that senses conditions of lower energy availability, and that this activation involves TSC2, a protein that acts as a brake on mTORC1.This question is relevant to section 6.3.1 because the publication subject to the Evidence Review precisely documents the AMPK TSC2 reduction of mTORC1 signaling cellular segment. It does not, however, evaluate whether cryptotanshinone prevents or treats tumors in humans, nor does it demonstrate that every activation of AMPK produces the same effect in all tissues or in all neoplasms.

3. MATERIALS AND METHODS OF THE ORIGINAL STUDY

The authors studied human Rh30 rhabdomyosarcoma, DU145 prostate carcinoma, and MCF-7 breast carcinoma cells. They also compared mouse embryonic fibroblasts with functional AMPK and fibroblasts lacking the AMPKa subunit. The cells were exposed to cryptotanshinone at concentrations ranging, depending on the specific experiment, between 0 and 20 micromoles per liter. In some tests, they were first serum-starved and then stimulated with IGF-1, a growth factor that activates signaling toward mTOR.Cell proliferation was evaluated after 48 hours using a colorimetric assay; each test condition was performed in six technical replicates. Proteins and their phosphorylated forms were measured using western blotting (a technique that makes specific proteins in a sample visible). Phosphorylation is a chemical modification that can increase or decrease a protein's activity, depending on the site involved.To verify the role of AMPK, the study used Compound C, a pharmacological inhibitor of AMPK, a dominant-negative form of AMPKa, and short hairpin RNAs, or shRNA, to reduce AMPKa expression. The same silencing approach was applied to TSC2. The authors also performed co-immunoprecipitation, a technique that assesses whether proteins present in the same complex remain associated, and molecular docking, a computer simulation useful for estimating whether a molecule can fit into a region of a protein.Quantitative data were expressed as mean ± standard error and analyzed using analysis of variance and Dunnett's test; the stated significance threshold was $P < 0.05$. The number of independent biological experiments for each western blot is not reported in the available material.

4. RESULTS

In simple terms, cryptotanshinone reduced certain signals through which mTORC1 supports cell growth, but it did not behave like rapamycin. In Rh30 cells expressing a rapamycin-resistant form of mTOR, cryptotanshinone at 10 micromoles per liter continued to reduce the phosphorylation of S6K1 and the expression or signaling of 4E-BP1, proteins downstream of mTORC1 involved in protein synthesis. This suggests that the effect did not depend on the usual rapamycin–FKBP12 binding site.Molecular docking was consistent with this simulated interpretation: the estimated CDOCKER energy for rapamycin was –69.294, compared to –0.443 for cryptotanshinone. In the computer model used by the authors, a lower energy indicates a more stable predicted interaction. This data alone does not prove what happens in cells or in the organism.Proteins in the PTEN–PI3K pathway, another important pathway that can modulate mTOR, did not show evident changes after treatment. The formation of mTORC1 and mTORC2 complexes was also not clearly modified. Conversely, cryptotanshinone increased the phosphorylation of AMPK and ACC, a biochemical target of AMPK, in a concentration- and time-dependent manner. In Rh30 cells, an activation signal was observable at as little as 1 micromole per liter; the increase in phospho-ACC appeared within ten minutes, and that of phospho-AMPK within one hour.When AMPK was inhibited with Compound C or genetically reduced, the effect of cryptotanshinone on mTORC1 signals was attenuated: S6K1 phosphorylation partially recovered, and the inhibition of 4E-BP1 was partially or completely countered, depending on the experimental concentration. The number of surviving cells was also higher with cryptotanshinone plus Compound C

compared to cryptotanshinone alone. Since Compound C can have non-exclusive effects on AMPK, genetic verification is important to support the interpretation.The most direct comparison involved mouse embryonic fibroblasts. Cells with AMPKa had a median inhibitory concentration, or IC50, of 2.45 micromoles per liter; in cells without AMPKa, the IC50 rose to 16.14 micromoles per liter. In practice, in the absence of AMPK, a concentration approximately six times higher was needed to reduce cell viability by 50%, a finding compatible with lower sensitivity to cryptotanshinone.Finally, cryptotanshinone increased the phosphorylation of TSC2. Reducing TSC2 via shRNA made the Rh30 cells only partially less sensitive to the molecule and increased S6K1 and 4E-BP1 signals compared to the control. This result positions TSC2 as a relevant, but not exclusive, component of the observed mechanism.

5. INTERPRETATION

The primary biological significance is that a tumor cell exposed to cryptotanshinone showed an increase in a signal that normally indicates energy conservation. AMPK can be viewed, as a simplification, as a sensor that prompts the cell not to invest resources in growth when the energy balance is unfavorable. In the study, AMPK was associated with the activation of TSC2 and the reduction of signals produced by mTORC1.The pathway effectively documented by the publication is therefore: cryptotanshinone increased AMPK phosphorylation increased TSC2 phosphorylation reduction of downstream mTORC1 markers, with lower proliferation or viability in cell models. Loss-of-function experiments for AMPK and TSC2 make this interpretation more robust than a simple observation of phosphorylated proteins.It remains necessary to distinguish between observation and deduction. The study demonstrates that the effect of cryptotanshinone largely depends on AMPK in the cell lines examined; it does not prove that TSC2 is the sole mediator, because its silencing only partially restored mTORC1 signals. Furthermore, it does not prove that the compound reaches effective and safe concentrations in human tumors, nor that it can be considered an oncological therapy.

6. INDEPENDENT CONFIRMING EVIDENCE

The most general step of the paragraph, namely the brake exerted by AMPK on mTORC1 via TSC2, was independently documented by Inoki and colleagues. In cellular models subjected to reduced energy availability, the authors showed that AMPK phosphorylates TSC2 and increases its activity; this modification was necessary to regulate protein translation and cell size in response to energy deficiency. The study is mechanistic and cellular, but it offers direct confirmation of the AMPK TSC2 mTORC1 control link [1].An independent confirmation closer to the molecule studied comes from Park and colleagues. In HepG2 hepatoma cells and other tumor lines expressing LKB1, a protein upstream of AMPK, cryptotanshinone activated LKB1–AMPK signaling, including TSC2, and suppressed mTORC1. The authors also observed AMPK-dependent cell cycle arrest in the G1 phase and a reduction in HCT116 xenograft growth in mice, accompanied by the activation of AMPK signals. The animal model increases biological relevance, but it is not equivalent to human trials and does not establish clinical efficacy [2].The circuit does not appear to be limited to tumor cells. In vascular smooth muscle cells treated to induce osteoblastic-type differentiation, Zhan and colleagues observed that adiponectin increased AMPK phosphorylation, increased TSC2, and reduced mTOR and S6K1 phosphorylation. AMPK inhibition blocked the effect of adiponectin, while an AMPK activator strengthened it; in parallel, mineralization and osteoblastic differentiation decreased. This confirms the consistency of the AMPK–TSC2–mTOR–S6K1 pathway in a different cellular context, but it does not directly confirm the effect of cryptotanshinone in cancer [3].

7. CONFLICTING EVIDENCE

A subsequent publication obtained a partially different result regarding how cryptotanshinone reduces mTORC1 signaling. In SK-Hep1 and JB6 Cl41 cells, Jeoung and colleagues observed a reduction in S6K1 phosphorylation, clonogenic growth, and IGF-1-induced cell transformation. However, the authors proposed that the molecule prevented the association of S6K1 with the mTOR–Raptor complex, rather than acting primarily on the upstream

regulators of mTORC1; in the same study, no reduction in 4E-BP1 phosphorylation was observed [4].This evidence does not invalidate the observation of the AMPK–TSC2 axis in the analyzed study, but it indicates that the effect of cryptotanshinone may depend on the cell line, the measured target, and the experimental conditions. It is possible that multiple mechanisms coexist, or that some are prevalent only in specific contexts. No sufficiently relevant directly conflicting evidence has been identified regarding the general fact that AMPK can inhibit mTORC1 through TSC2; this does not prove the absence of contradictory results.

8. QUADRO COMPARATIVO E SINTESI DELLE EVIDENZE

The table distinguishes the publication subject to the Evidence Review from the independent research used to confirm or refine the narrative of the paragraph.

Quadro comparativo

Reference	Study type	Population or model	Main result	Direction of evidence	Strength of evidence
Original study	Cellular and mechanistic	Human tumor lines Rh30, DU145, MCF-7, and mouse embryonic fibroblasts with or without AMPKa	Cryptotanshinone increases AMPK and TSC2 signaling, reduces mTORC1 markers, and shows lower efficacy without AMPKa.	direct confirmation	moderate
[1]	Cellular and mechanistic	Cellular models subjected to energy stress	AMPK phosphorylates and activates TSC2; TSC2 mediates the control of translation and cell growth during energy deficiency.	direct confirmation	moderate
[2]	Cellular and animal	Tumor cells, including HepG2, and HCT116 xenografts in mice	Cryptotanshinone activates LKB1–AMPK–TSC2, suppresses mTORC1, arrests the cell cycle, and reduces tumor growth in the mouse model.	direct confirmation	moderate
[3]	Cellular	Vascular smooth muscle cells with induced osteoblastic differentiation	AMPK activation increases TSC2 and reduces mTOR and S6K1 phosphorylation, with lower cellular mineralization.	biologically consistent evidence	moderate

[4]	Cellular	SK-Hep1 and JB6 Cl41 cells	Cryptotanshinone reduces S6K1 phosphorylation and cellular transformation, but an action on the S6K1–mTOR/Raptor interaction is proposed.	discordant evidence	preliminary

9. SINTESI EDITORIALE E CONCLUSIONI

CONCLUSIONS

The book passage is well-supported at a mechanistic level: AMPK can activate TSC2 and contribute to the reduction of mTORC1 activity, limiting cell signaling pathways that favor growth. The analyzed study documents this sequence in tumor cells treated with cryptotanshinone through pharmacological and genetic interventions. Independent evidence confirms both the biological circuit and, in other models, the activation of AMPK by the same molecule. However, validation in humans remains necessary, and the mechanism of cryptotanshinone may vary depending on the cell type and the target measured.

Aggiornato al 25/11/2026. Questo contenuto riflette una sintesi divulgativa delle evidenze scientifiche al momento della pubblicazione e non sostituisce il parere medico.

10. TRANSPARENCY AND SCIENTIFIC RESPONSIBILITY

Artificial Intelligence was used solely as a tool for editorial and documentary support. All content, interpretations, bibliographic citations and conclusions have been verified, reviewed and approved by the author, who assumes full scientific responsibility for them.

The manuscript was also subjected to independent peer review prior to publication.

To ensure the greatest transparency, the complete history of the article, including the dates of submission, revision, acceptance and publication, as well as information on the peer review process, is available on the dedicated Article History page.

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