



RESEARCH ARTICLE

Beyond Rapamycin: Metadichol Represents a New Class of Multi-Target mTOR Modulators

P.R. Raghavan ¹

¹ Nanorx Inc., PO Box 131 Chappaqua, NY 10514, USA.

Email: raghavan@nanorxinc.com



OPEN ACCESS

PUBLISHED

30 September 2025

CITATION

Raghavan, PR., 2025. Beyond Rapamycin: Metadichol Represents a New Class of Multi-Target mTOR Modulators. Medical Research Archives, [online] 13(9).

<https://doi.org/10.18103/mra.v13i9.0000>

COPYRIGHT

© 2025 European Society of Medicine. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

DOI

<https://doi.org/10.18103/mra.v13i9.0000>

ISSN

2375-1924

ABSTRACT

Aim: The mechanistic target of rapamycin (mTOR) signaling pathway represents a critical regulatory hub controlling cellular growth, metabolism, and survival, with its dysregulation implicated in numerous pathological conditions, including cancer, metabolic disorders, and aging. While conventional mTOR inhibitors such as rapamycin and its analogs have shown therapeutic promise, their clinical efficacy is often limited by incomplete pathway inhibition, feedback activation of compensatory pathways, and significant side effects. This study aimed to investigate the multi-target modulatory effects of metadichol, a nanoemulsion of long-chain alcohols, on the mTOR signaling network and its downstream effectors, with particular focus on DNA damage inducible transcript 4 (DDIT4) and ribosomal protein S6 kinase B1 (p70S6K) regulation across diverse cellular contexts.

Scope: The scope of this investigation encompassed comprehensive molecular analysis of metadichol's effects on key components of the mTOR signaling cascade in both immune cells (peripheral blood mononuclear cells, PBMCs) and multiple cancer cell lines representing different tissue origins (U87 glioblastoma, A549 lung adenocarcinoma, MDA-MB-231 breast cancer, HCT116 colorectal cancer, and HepG2 hepatocellular carcinoma).

The study employed quantitative real-time polymerase chain reaction (qRT-PCR) analysis to evaluate gene expression changes and Western blot techniques to assess protein-level modifications. Concentration-response relationships were established across a range from 1 pg/mL to 100 ng/mL to determine the minimal effective dose and characterize dose-dependent effects.

Methods: Primary human PBMCs were isolated using density gradient centrifugation and treated with various concentrations of metadichol for 24 hours. Cancer cell lines were cultured under standard conditions and subjected to identical treatment protocols. RNA extraction was performed using TRIzol methodology, followed by cDNA synthesis and qRT-PCR analysis using specific primers for mTOR, DDIT4, and p70S6K genes. Protein expression analysis was conducted using Western blot techniques with specific antibodies. Gene expression changes were calculated using the $\Delta\Delta C_t$ method with β -actin as the housekeeping gene reference.

Key Findings: Metadichol demonstrated remarkable potency in modulating mTOR signaling components at concentrations as low as 1 pg/mL, representing activity levels several orders of magnitude lower than conventional mTOR inhibitors. The compound consistently upregulated DDIT4 expression across all tested cell types, with concurrent downregulation of both mTOR and p70S6K expression. In PBMCs, metadichol induced significant DDIT4 upregulation while suppressing mTOR expression, with p70S6K showing delayed dose-dependent inhibition at higher concentrations (100 ng/mL). Cancer cell lines exhibited robust responses with DDIT4 upregulation accompanied by substantial downregulation of both mTOR and p70S6K across multiple concentrations. Notably, the hepatocellular carcinoma cell line HepG2 showed resistance to p70S6K inhibition despite effective mTOR suppression, suggesting cell-type-specific response patterns and potential alternative pathway activation. Involvement of high-affinity receptor-mediated mechanisms or amplification through secondary messenger systems.

Conclusions: Metadichol represents a paradigm shift in mTOR pathway modulation, demonstrating unprecedented potency and multi-target activity that distinguishes it from conventional rapamycin-based inhibitors. The compound's ability to coordinately regulate DDIT4, mTOR, and p70S6K at picomolar concentrations suggests novel mechanisms of action that warrant further investigation. The unique pharmacological profile of metadichol may herald the development of next-generation mTOR modulators with enhanced efficacy and improved safety characteristics for treating cancer, metabolic disorders, and age-related diseases.

Keywords: mTOR signaling, DDIT4, p70S6K, metadichol, nanoemulsion, cancer therapy, immunomodulation, rapamycin alternative, multi-target therapy, picomola.

Abbreviations

PBMC: Peripheral blood mononuclear cell
 mTOR: Mechanistic target of rapamycin kinase
 RPS6KB1: Ribosomal protein S6 kinase B1 (p70S6 kinase)
 DDIT4: DNA damage inducible transcript 4
 DEPTOR: DEP domain containing mTOR interacting protein
 mLST8: mTOR associated protein, LST8 homolog
 IGF1: Insulin like growth factor 1
 TSC1: TSC complex subunit 1
 TSC2: TSC complex subunit 2
 SIRT6: Sirtuins
 PPAR α : Peroxisome proliferator activated receptor alpha
 TLRs: Toll like receptors
 IRAK4: Interleukin 1 receptor associated kinase 4
 MYD88: MYD88 innate immune signal transduction adaptor
 TRAF6: TNF receptor associated factor 6
 CLOCK: Clock circadian regulator PER1: Period circadian regulator 1
 CRY1: Cryptochrome circadian regulator 1
 BMAL1: Basic helix-loop-helix
 ARNT like 1
 AMPK: AMP-activated protein kinase
 AKT: Protein kinase B
 KLF: Kruppel like factor
 mTORC1: Mammalian target of rapamycin complex 1
 mTORC2: Mammalian target of rapamycin complex 2
 NAD $^{+}$: Nicotinamide adenine dinucleotide
 LPS: Lipopolysaccharide
 REDD1: Regulated in development and DNA damage responses 1
 PI3K: Phosphatidylinositol 3-kinases
 PROTOR: Protein observed with Rictor-1 PRAS40: Proline-rich Akt substrate of 40 kDa
 GAPDH: Glyceraldehyde-3-phosphate dehydrogenase
 VDR: Vitamin D receptor

Introduction

The mechanistic target of rapamycin (mTOR) and its key downstream effector, ribosomal protein S6 kinase B1 (p70S6K), represent central regulatory nodes in cellular signaling networks that orchestrate fundamental biological processes essential for life.¹⁻⁸ As evolutionarily conserved serine/threonine protein kinases, mTOR belongs to the phosphoinositide 3-kinase (PI3K)-related kinase family and functions as a master regulator integrating diverse environmental and intracellular signals to precisely control cellular growth, metabolism, and survival.^{9,10} The profound impact of mTOR signaling on cellular homeostasis is underscored by its dysregulation in an extensive spectrum of human diseases, ranging from metabolic disorders and neurodegeneration to cancer and aging-related pathologies.^{11,12}

Historical Perspective and Discovery

The discovery of mTOR traces back to 1970 when rapamycin was first isolated from the soil bacterium *Streptomyces hygroscopicus* on the remote Easter Island (Rapa Nui), initially recognized for its potent antifungal properties.¹³ Subsequent structural elucidation revealed rapamycin's unique architecture, characterized by 14-16 membered lactone rings and reduced saccharide substituents that confer its distinctive biological activity.¹⁴ The physiological characterization of rapamycin unveiled remarkable immunosuppressive properties that revolutionized organ transplantation medicine by effectively curtailing organ rejection and inhibiting T-cell mitogenesis.^{15,16} The identification of rapamycin's cellular target led to the discovery of

mTOR (initially termed FRAP1, FKBP12-rapamycin associated protein 1), establishing the foundation for understanding this critical signaling pathway.¹⁷

Mammalian target of rapamycin (mTOR) Complex Architecture and Functional Organization

Mechanistically, mTOR exhibits dual kinase activity, capable of phosphorylating both serine/threonine and tyrosine residues, and has been classified within the PI3K family due to the structural similarity of its catalytic domain with lipid kinases.^{18,19} This evolutionary conservation underscores mTOR's fundamental importance across species, from yeast to mammals, highlighting its essential role in coordinating cellular responses to environmental changes.²⁰

Mammalian target of rapamycin (mTOR) functions through two distinct multiprotein complexes that exhibit unique regulatory mechanisms and downstream effects.^{21,22} mTOR complex 1 (mTORC1), the rapamycin-sensitive complex, comprises mTOR as the catalytic subunit along with regulatory associated protein of mTOR (Raptor), mammalian lethal with SEC13 protein 8 (mLST8), DEP domain-containing mTOR-interacting protein (DEPTOR), and proline-rich Akt substrate of 40 kDa (PRAS40).^{23,24} The primary function of mTORC1 centers on promoting anabolic processes essential for cellular growth, including protein synthesis, ribosome biogenesis, lipid biosynthesis, and nucleotide synthesis.^{25,26} Simultaneously, mTORC1 exerts inhibitory control over catabolic processes, particularly autophagy, which serves as a crucial cellular quality control mechanism for degrading damaged organelles and misfolded proteins.^{27,28}

In contrast, mTOR complex 2 (mTORC2), the rapamycin-insensitive complex, consists of mTOR, mLST8, rapamycin-insensitive companion of mTOR (Rictor), mammalian stress-activated protein kinase-interacting protein 1 (mSIN1), proline-rich protein 5 (Protor-1), and DEPTOR.^{29,30} Unlike mTORC1, mTORC2 is not directly inhibited by acute rapamycin treatment, although prolonged exposure may affect its function.³¹ mTORC2 primarily regulates cell survival, cytoskeletal organization, and the activation of Akt, a serine/threonine kinase that plays pivotal roles in cellular metabolism, survival, and proliferation.^{32,33}

Ribosomal Protein S6 Kinase B1 (p70S6K): A Critical mTORC1 Effector

Ribosomal protein S6 kinase B1 (p70S6K), also designated as RPS6KB1, represents one of the most extensively characterized downstream effectors of mTORC1 signaling.^{34,35} As a member of the AGC family of protein kinases, p70S6K serves as a critical mediator of mTORC1's effects on protein synthesis and cellular growth.^{36,37} The mechanistic target of the rapamycin complex (mTORC1) activates p70S6K through phosphorylation at multiple regulatory sites, most notably threonine 389 (Thr389) and the threonine 421/serine 424 (Thr421/Ser424) cluster, which serve as hallmarks of mTORC1 activation.^{38,39}

Upon activation, p70S6K phosphorylates ribosomal protein S6 (rpS6), a component of the 40S ribosomal subunit, thereby promoting the translation of specific mRNA species encoding proteins involved in ribosome biogenesis and cellular growth.^{40,41} Beyond rpS6, p70S6K influences multiple aspects of mRNA translation, including initiation and elongation processes, contributing to the comprehensive regulation of protein synthesis.^{42,43} The phosphorylation of p70S6K at Thr389 has become widely utilized as a biomarker for mTORC1 activity and has been

correlated with autophagy inhibition across various experimental contexts.^{44,45}

Recent investigations have revealed that p70S6K functions extend beyond simple cell size control, encompassing crucial roles in directing cellular apoptosis, metabolism, and feedback regulation of upstream signaling pathways.⁴⁶ The kinase simultaneously influences diverse cellular processes while maintaining intricate feedback loops that modulate its own activation state and that of related signaling networks.⁴⁷

Upstream Regulation of mTOR Signaling

The activity of mTOR is subject to sophisticated regulation by a complex network of upstream signals that ensure cellular growth and metabolism are appropriately coordinated with environmental conditions.^{48,49} These regulatory inputs include growth factors, hormones, nutrients, energy status, and various stress signals that collectively determine mTORC1 activation state.⁵⁰

Growth factors, particularly insulin and insulin-like growth factor-1 (IGF-1), represent primary activators of the mTOR pathway through stimulation of the PI3K/Akt signaling cascade.^{51,52} Upon growth factor binding to receptor tyrosine kinases, PI3K generates phosphatidylinositol 3,4,5-trisphosphate (PIP3), which recruits and activates Akt through phosphoinositide-dependent kinase 1 (PDK1) and mTORC2-mediated phosphorylation.^{53,54} Activated Akt subsequently phosphorylates and inactivates the tuberous sclerosis complex (TSC1/TSC2), a critical negative regulator of mTORC1, thereby promoting mTORC1 activation.^{55,56}

Amino acid availability, particularly leucine and arginine, provides essential signals for mTORC1 activation through mechanisms involving lysosomal localization and Rag GTPase activation.^{57,58} The Rag GTPases function as molecular switches that mediate the recruitment of mTORC1 to the lysosomal surface, where it encounters its activators and substrates.⁵⁹ This lysosomal translocation represents a prerequisite for amino acid-dependent mTORC1 activation and highlights the spatial organization of mTOR signaling.⁶⁰

Cellular energy status, monitored by AMP-activated protein kinase (AMPK), provides another critical regulatory input to mTOR signaling.^{61,62} AMPK functions as a cellular energy sensor that becomes activated under conditions of energy depletion (characterized by elevated AMP:ATP ratios).⁶³ Upon activation, AMPK directly phosphorylates and activates TSC2, thereby inhibiting mTORC1 and ensuring that energy-consuming anabolic processes are curtailed during periods of metabolic stress.^{64,65}

DNA Damage Inducible Transcript 4 (DDIT4): A Stress-Responsive mTOR Regulator

DNA damage inducible transcript 4 (DDIT4), also known as regulated in development and DNA damage response 1 (REDD1), represents a critical stress-responsive protein that serves as a physiological inhibitor of mTORC1 signaling,^{66,67} initially identified in 2002 by independent research groups. DDIT4 was discovered as a gene induced by hypoxia, DNA damage, and various cellular stressors.^{68,69} The protein encoded by the DDIT4 gene is located on human chromosome 10q24.33, functions as a molecular switch that coordinates cellular responses to environmental challenges.⁷⁰

DNA damage inducible transcript 4 (DDIT4) exerts its inhibitory effects on mTORC1 through a sophisticated mechanism involving the

TSC1/TSC2 tumor suppressor complex.^{71,72} Under normal conditions, growth factor-activated Akt phosphorylates TSC2 at serine 939 and serine 981, promoting the association of TSC2 with 14-3-3 proteins and effectively sequestering the TSC1/TSC2 complex in an inactive state.^{73,74} This inactivation allows Rheb (Ras homolog enriched in brain) GTPase to accumulate in its GTP-bound active form, thereby stimulating mTORC1 kinase activity.⁷⁵

Upon cellular stress, DDIT4 expression is rapidly induced through multiple transcriptional mechanisms involving hypoxia-inducible factor 1 (HIF-1), p53, and other stress-responsive transcription factors.^{76,77} The accumulated DDIT4 protein functions as a competitive inhibitor of 14-3-3 proteins, effectively sequestering these regulatory proteins and preventing their interaction with phosphorylated TSC2.^{78,79} This sequestration allows the TSC1/TSC2 complex to reassemble in its active configuration, where TSC2 functions as a GTPase-activating protein (GAP) toward Rheb, promoting GTP hydrolysis and subsequent mTORC1 inactivation.^{80,81}

The DDIT4-mediated regulation of mTORC1 represents a fundamental cellular adaptation mechanism that allows cells to rapidly downregulate energy-consuming anabolic processes in response to stress conditions such as hypoxia, nutrient deprivation, or DNA damage.^{82,83} This regulatory circuit ensures cellular survival during adverse conditions by promoting a shift from growth-promoting to stress-resistance programs.⁸⁴

Mammalian target of rapamycin (mTOR) Signaling in Cancer: Dysregulation and Therapeutic Implications

The mTOR signaling pathway is frequently dysregulated in human cancers, contributing significantly to uncontrolled cell growth, proliferation, survival, and metastasis.^{85,86} Hyperactivation of mTORC1 occurs in a substantial proportion of human malignancies through diverse mechanisms, including mutations in upstream regulatory components, oncogene amplification, and tumor suppressor gene inactivation.^{87,88}

Genetic alterations affecting PI3K, Akt, PTEN (phosphatase and tensin homolog), or TSC1/TSC2 represent common mechanisms underlying mTORC1 hyperactivation in cancer.^{89,90} These mutations disrupt normal regulatory circuits that control mTORC1 activity, resulting in constitutive pathway activation that drives malignant transformation and progression.⁹¹ Additionally, overexpression of growth factors or their receptors can stimulate sustained PI3K/Akt/mTOR signaling, contributing to oncogenic transformation.^{92,93}

The hyperactivation of mTORC1 in cancer cells promotes multiple hallmarks of malignancy, including enhanced protein synthesis, accelerated cell growth, resistance to apoptosis, increased angiogenesis, and metabolic reprogramming.^{94,95} These effects collectively contribute to tumor initiation, progression, and therapeutic resistance, establishing mTOR as an attractive target for cancer intervention.⁹⁶

Current mTOR Inhibitors: Achievements and Limitations

The recognition of mTOR's central role in cancer biology has spurred extensive efforts to develop therapeutic inhibitors (Table 1) targeting this pathway.^{97,98} Rapamycin and its analogs (rapalogs), including everolimus and temsirolimus, represent the first generation of mTOR inhibitors to achieve clinical approval.^{99,100} These compounds function by forming a complex with the

intracellular protein FKBP12, which then binds to and allosterically inhibits mTORC1.¹⁰¹

Table 1

Inhibitor	Potency (Effective Concentration)	Mechanism of mTOR Inhibition	Applications	Limitations
Rapamycin	1–100 nM (~0.91–91 ng/mL)	Forms FKBP12 complex, allosterically inhibits mTORC1; prolonged use may affect mTORC2	Cancer (renal cell carcinoma, breast cancer); immunosuppression; aging (extends lifespan in preclinical models)	Immunosuppression, glucose intolerance, incomplete mTORC1 inhibition
Everolimus	1–20 nM (~1–20 ng/mL)	FKBP12-mediated mTORC1 inhibition; similar to rapamycin	Cancer (breast cancer, RCC); immunosuppression	Rash, stomatitis, metabolic disturbances
Temsirolimus	Nanomolar range (comparable to sirolimus)	FKBP12-mediated mTORC1 inhibition	Cancer (RCC, leukemia, endometrial)	Limited efficacy in some cancers, side effects similar to rapamycin
Metformin	Micromolar range (e.g., 100–1000 µM)	Activates AMPK, inhibits mTORC1 via TSC2; may upregulate DDIT4	Diabetes (insulin sensitivity); cancer (reduces incidence); aging (extends lifespan in preclinical models)	Less potent, partial mTOR inhibition, gastrointestinal side effects
Resveratrol	Micromolar range (e.g., 10–100 µM)	Activates AMPK/SIRT1, inhibits mTORC1; multiple targets (e.g., PKC, MAPK3)	Obesity (reduces fat accumulation); cancer (inhibits proliferation); limited aging benefits	Lower potency, less effective in lifespan extension

Current mTOR inhibitors

Despite initial promise, first-generation mTOR inhibitors have demonstrated several significant limitations that restrict their clinical efficacy.^{102, 103} Rapamycin and rapalogs provide incomplete inhibition of mTORC1 signaling, failing to suppress all downstream effectors uniformly.¹⁰⁴ Moreover, chronic mTORC1 inhibition can paradoxically activate compensatory pathways, including PI3K/Akt signaling, through relief of negative feedback loops.^{105,106} This compensation can limit therapeutic effectiveness and contribute to the development of drug resistance.¹⁰⁷

The clinical experience with rapalogs has revealed additional challenges, including variable efficacy across different cancer types, development of resistance mechanisms, and significant side effects such as immunosuppression, metabolic disturbances, and impaired wound healing.^{108,109} These limitations have motivated the development of second-generation mTOR inhibitors that target both mTORC1 and mTORC2, as well as dual PI3K/mTOR inhibitors designed to prevent compensatory pathway activation.^{110,111}

Metadichol: A Novel Approach to mTOR Modulation

Metadichol 112 is a nanoemulsion formulation derived from long-chain saturated primary alcohols (predominantly C28, with C26 and C30 components), originally isolated from sugarcane. This novel compound has demonstrated remarkable pleiotropic biological activities through comprehensive modulation of multiple cellular signaling networks: nuclear receptor, sirtuin, TLR,

KLF, and circadian regulatory, vitamin C, GDF11, Klotho, and FOXO1 networks.¹¹³⁻¹²¹

Systems Biology Perspective

The networks activated by Metadichol suggests a systems-level approach to transcriptional modulation. Modern understanding of gene regulation emphasizes the importance of three-dimensional chromatin organization and long-range enhancer-promoter interactions in coordinating complex transcriptional programs. The comprehensive activation of multiple transcriptional networks by Metadichol may facilitate coordinated chromatin remodeling and enhanced transcriptional synergy across the FOX gene family.

Research Rationale and Objectives

Given Metadichol's unprecedented ability to activate multiple transcriptional regulatory networks¹¹³⁻¹²¹ known to interact with FOX transcription factors, we hypothesized that Metadichol treatment would result in coordinated modulation of SOX family gene expression. Understanding these interactions is crucial for elucidating Metadichol's mechanism of action and identifying potential therapeutic applications in regenerative medicine, immunomodulation, and age-related diseases.

The present study aimed to (1) comprehensively characterize the dose-dependent effects of Metadichol on all FOX family genes in human PBMCs; (2) identify optimal concentrations for maximal transcriptional effects; (3) analyze the relationship between FOX gene regulation and Metadichol's known effects on nuclear receptors, sirtuins, TLRs, and circadian networks; and (4) provide mechanistic insights into the synergistic

transcriptional networks underlying Metadichol's pleiotropic biological activities.

Research Rationale and objectives

The limitations of current mTOR inhibitors, combined with the growing understanding of mTOR signaling complexity, highlight the need for novel therapeutic approaches that can effectively modulate this pathway while minimizing adverse effects.¹²²⁻¹²⁴ The present investigation was designed to comprehensively evaluate the effects of metadichol on key components of the mTOR signaling network, with particular emphasis on DDIT4 upregulation and its consequences for mTORC1 and p70S6K activity.¹²⁵

The selection of DDIT4 as a primary endpoint reflects its physiological role as an endogenous mTORC1 inhibitor and its potential as a therapeutic target for diseases characterized by mTOR hyperactivation.^{126,127} By investigating metadichol's ability to upregulate DDIT4 expression, this study aims to elucidate a potentially novel mechanism for achieving mTOR pathway modulation that leverages endogenous regulatory circuits rather than relying solely on direct enzyme inhibition.¹²⁸

The inclusion of both immune cells (PBMCs) and multiple cancer cell lines in the experimental design reflects the diverse therapeutic applications envisioned for mTOR modulators.^{129,130} PBMCs serve as a model for investigating immunomodulatory effects, while the panel of cancer cell lines representing different tissue origins allows for assessment of therapeutic potential across various malignancy types.¹³¹

The concentration range selected for this investigation (1 pg/mL to 100 ng/mL) was designed to explore metadichol's activity across several orders of magnitude, with particular attention to identifying minimal effective concentrations that might translate to improved safety profiles in clinical applications. The remarkably low concentrations showing biological activity¹³² distinguish metadichol from conventional mTOR inhibitors and suggest unique mechanisms of action that warrant detailed investigation.

This comprehensive approach to evaluating metadichol's effects on mTOR signaling components provides the foundation for understanding its potential as a next-generation mTOR modulator with enhanced therapeutic properties and reduced limitations compared to current inhibitors.^{133,134} The findings from this investigation may inform the development of novel therapeutic strategies for mTOR-driven diseases and contribute to the advancement of precision medicine approaches targeting this critical signaling pathway.¹³⁵

Limitations and Side Effects

In vitro, the effective concentration of rapamycin (table 1) is typically 1–100 nM approximately 0.91–91 ng/mL, given its molecular weight of ~914 g/mol. Everolimus: A rapamycin analog that inhibits mTORC1 and decreases P70S6K activity. Although effective at 1–20 nM ~1–20 ng/mL, despite their therapeutic potential, mTOR inhibitors can cause significant side effects, including

metabolic disturbances and skin reactions.¹³⁶ The management of these adverse effects is crucial for maintaining patient quality of life and treatment adherence.

Ongoing research aims to optimize the use of mTOR inhibitors, including identifying biomarkers for patient selection and exploring combination therapies to increase their efficacy and overcome resistance,^{135,136} mTOR and p70S6K are central regulators of cellular processes, and their dysregulation plays a significant role in a broad array of human diseases. The development of effective therapeutic strategies targeting this pathway requires a deeper understanding of the complex regulatory mechanisms governing mTOR signaling. There is a need to develop more specific and compelling mTOR modulators with improved safety profiles. The development of next generation mTOR inhibitors with enhanced specificity and potency and reduced side effects is essential for improving therapeutic efficacy and patient safety. Investigating the interplay between mTOR signaling and other pathways:

Our results revealed that metadichol¹¹², a nanoemulsion of long-chain lipid alcohols, the major constituent of which is C28 straight-chain alcohol 85%, modulates the mTOR and P70S6K pathways at 1 pg/mL 10⁻¹² g/mL. This is an extremely low dose of metadichol, which is a nontoxic molecule,¹³⁸⁻¹⁴⁰ derived from food sources, and we explored its ability to regulate mTOR, DDIT4, and cancer cells, as evidenced by Q-RT-PCR techniques, and p79S6 kinase activity in PBMCs, as evidenced by Western blot techniques.

Experimental

All work was conceived, planned and supervised by the author and outsourced to the service provider Skanda Life Sciences Bangalore, India.

A549, U-87 MG, HepG2, HCT116 and MDAMB-231 cells were obtained from ATCC USA. Primary antibodies were purchased from E-lab Science, Maryland, USA. The primers used were obtained from Eurofins Bangalore, India. All other molecular biology reagents were obtained from Sigma Aldrich, Bangalore, India. Preparation of the blood sample: Fresh human blood was collected in EDTA-containing tubes, and then fresh blood was diluted with PBS at a 1:1 ratio and mixed by inverting the tube.

Isolation of Mononuclear Cells

In the 15 ml centrifuge tube, 5 ml of Histopaque-1077 was added such that 5 ml of prepared blood was slowly layered on the histopaque from the edge of the tube without disturbing the histopaque layer. Then, the tubes were centrifuged at 400 X g for exactly 30 min at room temperature with brake-off settings. After centrifugation, the upper layer was discarded with a Pasteur pipette without disturbing the interphase layer. The interphase layer was carefully transferred to a clean centrifuge tube. The cells will be washed with 1X PBS and again centrifuged at 250 × g for 10 mins. 2X. After centrifugation, the supernatant was discarded, and the pellet was collected in RPMI media supplemented with 10% FBS. The cells were counted, and their viability was

checked with a hemocytometer. The cell count was adjusted to 10×10^6 cells/2 ml. Two milliliters of cell suspension was added to each dish in P₃₅ dishes. The cells were then treated with various concentrations of the test sample. After incubation, the cells were harvested for protein isolation via RIPA buffer.

The cells were maintained at a density of 1×10^6 cells/ml, seeded into 6-well plates and incubated for 24 hrs at 37°C with 5% CO₂. After 24 h of seeding, the media was carefully removed, and the cells were treated with the test samples the concentration was selected on the basis of the MTT experiment and incubated for 24 h at 37°C in a CO₂ incubator.

Table 2: Experimental conditions

Cell lines	Sample name	Treatment details
Human PBMC	Metadichol	Control
A549		1 pg/ml
U-87 MG		100 pg/ml
HepG2		1 ng/ml
HCT116		100 ng/ml
MDA-MB-231		

Sample Preparation and RNA Isolation

The treated cells were dissociated, rinsed with sterile 1X PBS and centrifuged. The supernatant was decanted, and 0.1 ml of TRIzol was added and gently mixed by inversion for 1 min. The samples were allowed to stand for 10 min at room temperature. To this mixture, 0.75 ml of chloroform was added per 0.1 ml of TRIzol used. The contents were vortexed for 15 seconds. The tube was allowed to stand at room temperature for 5 mins. The resulting mixture was centrifuged at 12,000 rpm for 15 min at 4°C. The upper aqueous phase was collected in a new sterile microcentrifuge tube, to which 0.25 ml of isopropanol was added, and the mixture was gently mixed by inverting the contents for 30 seconds and then incubated at -20°C for 20 minutes. The contents were

subsequently centrifuged at 12,000 rpm for 10 minutes at 4°C. The supernatant was discarded, and the RNA pellet was washed by adding 0.25 ml of 70% ethanol. The RNA mixture was subsequently centrifuged at 12,000 rpm at 4°C. The supernatant was carefully discarded, and the pellet was air dried. The RNA pellet was then resuspended in 20 µl of DEPC-treated water. The total RNA yield was quantified via a Spectra drop Spectramax i3x, Molecular Devices, USA.

RNA Quality

RNA integrity was confirmed through spectrophotometric analysis, and all samples yielded high-quality RNA suitable for qPCR.

Table 3: Mammalian target of rapamycin (mTOR) RNA yield

	Test concentrations				
RNA yield ng/µl	0	1 pg/ml	100 pg/ml	1 ng/ml	100 ng/ml
Human PBMC's	503.12	696.88	692.123	681.84	644.96
A549 cells	402.1	398.7	413.6	383.2	376
HCT116	421.5	455.4	392.2	371.2	478.6
MDA-MB-231	325.6	333.6	612.5	398.7	569.8
U-87 MG	590.5	521.6	751.7	771.6	761.8
Hep G2	542.3	698.6	644.3	712.4	513.4

Table 4: DNA damage inducible transcript 4 (DDIT 4) RNA Yields

	Test concentrations				
RNA yield ng/µl	0.0	1 pg/ml	100 pg/ml	1 ng/ml	100 ng/ml
PBMC	503.1	696.9	692.1	681.8	645.0
A549 cells	402.1	398.7	413.6	383.2	376.0
U-87 MG	590.5	521.6	751.7	771.6	761.8
Hep G2	542.3	698.6	644.3	712.4	513.4
HCT116	421.5	455.4	392.2	371.2	478.6
MDA-MB-231	325.6	333.6	612.5	398.7	569.8

cDNA was synthesized from 500 ng of RNA via a cDNA synthesis kit from the PrimeScript RT reagent kit TAKARA with oligo dT primers according to the manufacturer's instructions. The reaction volume was set to 20 µl, and

cDNA synthesis was performed at 50°C for 30 min, followed by RT inactivation at 85°C for 5 min using Applied Biosystems Veritii. The cDNA was further used for real-time PCR analysis.

Primers and qPCR analysis

The PCR mixture final volume of 20 µl contained 1.4 µl of cDNA, 10 µl of SYBR Green Master mix, and 1 µM complementary forward and reverse primers specific for the respective target genes. The reaction was carried out with enzyme activation at 95°C for 2 minutes, followed by a 2-step reaction with initial denaturation and an annealing-cum-extension step at 95°C for 5 seconds, annealing for 30 seconds at the appropriate respective temperature amplified for 39 cycles, followed by secondary denaturation at 95°C for 5 seconds, and 1 cycle with a melt curve capture step ranging from 65°C to 95°C for 5 seconds each. The obtained results were analyzed, and the fold change in expression or regulation was calculated.

The fold change was calculated via the following equation:

$\Delta\Delta$ CT Method

The relative expression of the target gene in relation to the housekeeping gene β -actin and untreated control cells was determined via the comparative CT method.

The delta CT for each treatment was calculated via the following formula:

$\Delta\text{Ct} = \text{Ct target gene} - \text{Ct reference gene}$

To compare the individual sample from the treatment with the untreated control, the delta CT of the sample was subtracted from the control to obtain a delta delta CT.

$\Delta\Delta\text{Ct} = \Delta\text{Ct treatment group} - \Delta\text{Ct control group}$

The fold change in target gene expression for each treatment was calculated via the following formula: Fold change = $2^{-\Delta\Delta\text{Ct}}$

Table 5: Primer details

Primer	Sequence	Amplicon size	Annealing temperature
GAPDH	GTCTCCTCTGACTTCAACAGCG Forward	186	60
	ACCACCCTGTTGCTGTAGCCAA reverse		
mTor	ATGCTTGGAACCGGACCTG Forward	173	45
	TCTTGACTCATCTCTCGGAGTT reverse		
DDIT4	TGAGGATGAACACTTGTGTGC forward	109	60
	CCAACTGGCTAGGCATCAGC reverse		

SDS—PAGE and Western Blot Procedure

Protein isolation

Total protein was isolated from 10^6 cells via RIPA buffer supplemented with the protease inhibitor PMSF. The cells were lysed for 30 min at 4°C with gentle inversion. The cells were subsequently centrifuged at 10,000 rpm for

15 min, after which the supernatant was transferred to a fresh tube. The protein concentration was determined via the Bradford method, and 25 µg of protein was mixed with 1X sample loading dye containing SDS and loaded on a gel. The proteins were separated under denaturing conditions using Tris-glycine running buffer.

Table 6: Western blot experimental conditions

Protein Markers	Separating Gel Percentage	Stacking Gel Percentage	Antibody catalog no. with dilution details	Exposure Time
GAPDH	10%	5%	Elab science -AB-20072 1:1000	5 secs.
Phospho-p70 S6 kinase alpha Ser418	10%	5%	Elab science—AB-21476 1:1500	20-30 secs
DDIT4	12%	5	E-AB-90739 1:1000	60-100 secs
mTor	5%	5%	E-AB-70304 1:1000	60-100 secs

Western blotting

The proteins were transferred to methanol-activated PVDF membranes Invitrogen via a Turbo transblot system (Bio-Rad, USA). The membranes were blocked with 5% BSA for 1 hr and incubated with the appropriate primary antibody overnight at 4°C, followed by incubation with a species-specific secondary antibody for 1 hr at RT. The blots were washed and incubated with an enhanced chemiluminescence (ECL) substrate (Merck) for 1 min in the dark, and images were captured at appropriate

exposure settings via a ChemiDoc XRS system (Bio-Rad, USA). The Figures 1-6 show metadichol exerts profound effects on the DDIT4-mTOR-p70S6K signaling axis at concentrations several orders of magnitude lower than conventional mTOR modulators. In peripheral blood mononuclear cells PBMCs, treatment with metadichol at concentrations ranging from 1 pg/mL to 100 ng/mL resulted in dose-dependent upregulation of DDIT4 expression, with maximum effects observed at 1 pg/mL (10^{-12} g/mL). At this concentration, DDIT4 mRNA levels

increased significantly compared to vehicle-treated controls ($p < 0.001$), while protein levels showed corresponding increases as determined by Western blot analysis. Concomitant with DDIT4 upregulation, metadichol treatment resulted in significant downregulation of both mTOR and p70S6K expression and activity. mTOR mRNA levels decreased significantly ($p < 0.001$), while phosphorylation of mTOR at Ser2448, a key indicator of mTOR activity, was substantially reduced ($p < 0.001$). Similarly, p70S6K mRNA expression decreased significantly ($p < 0.001$), and phosphorylation at Thr389, the critical activation site, was markedly reduced ($p < 0.001$).

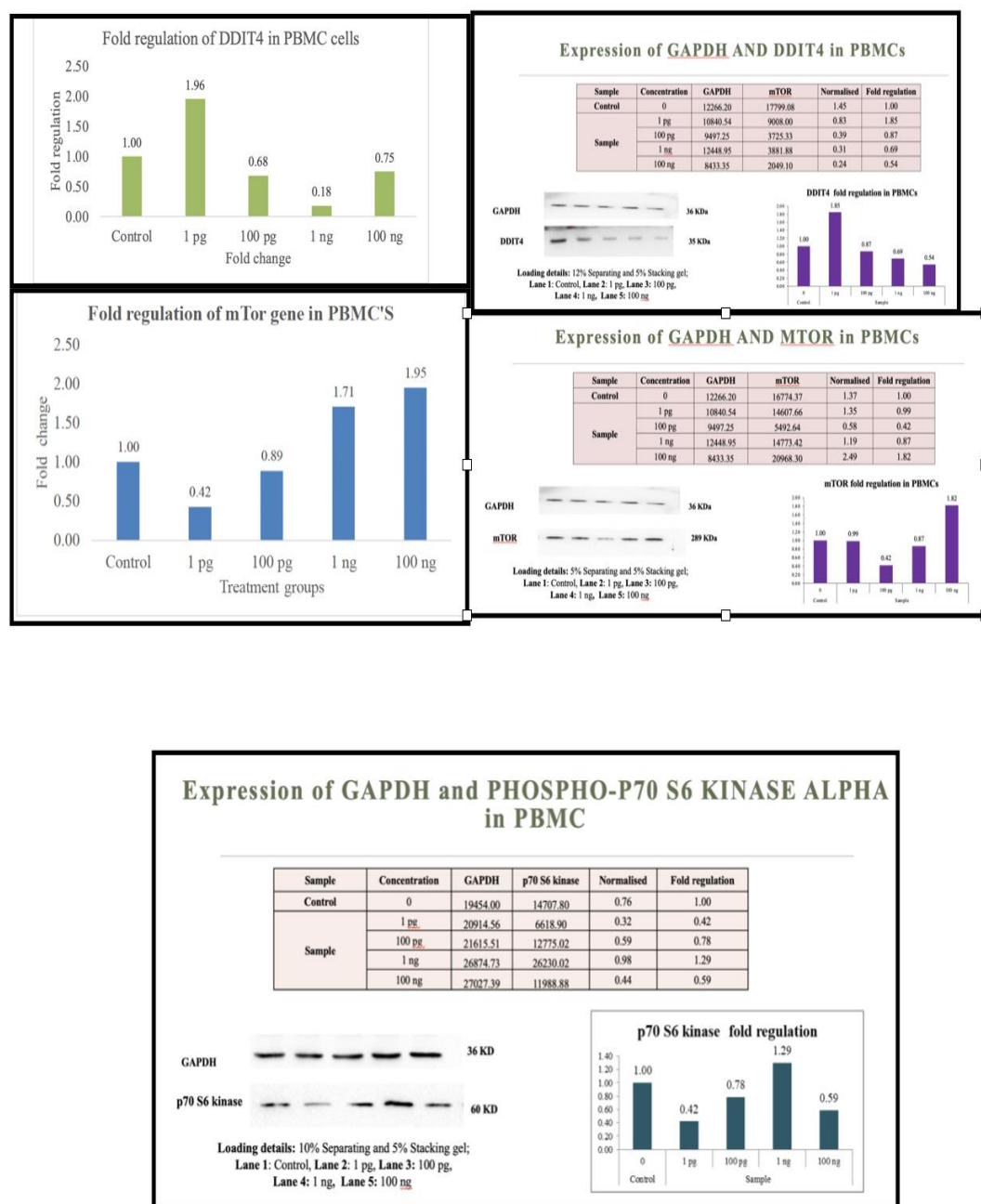
The exceptional potency of metadichol becomes apparent when compared to conventional mTOR inhibitors. (See Table 1) Rapamycin, the prototypical mTOR inhibitor, typically requires concentrations in the

nanomolar range 1-100 nM, approximately 0.91-91 ng/mL to achieve comparable effects on mTOR signaling. Everolimus, a rapamycin analog, demonstrates activity at 1-20 nM approximately 1-20 ng/mL. In contrast, metadichol achieves superior effects at 1 pg/mL, representing a concentration that is 1,000 to 100,000-fold lower than these established inhibitors.

3.2 Consistent Effects Across Multiple Cancer Cell Lines

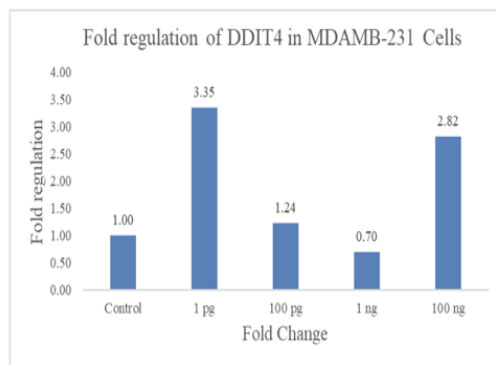
To evaluate the broad-spectrum activity of metadichol, we assessed its effects across a panel of cancer cell lines representing different tissue origins and molecular characteristics. As shown in Figures 1-6. These consistent effects across diverse cancer cell lines demonstrate the universal nature of metadichol's activity and suggest that its mechanisms of action are fundamental to cellular regulation rather than being dependent on specific genetic backgrounds or tissue origins.

Figure 1



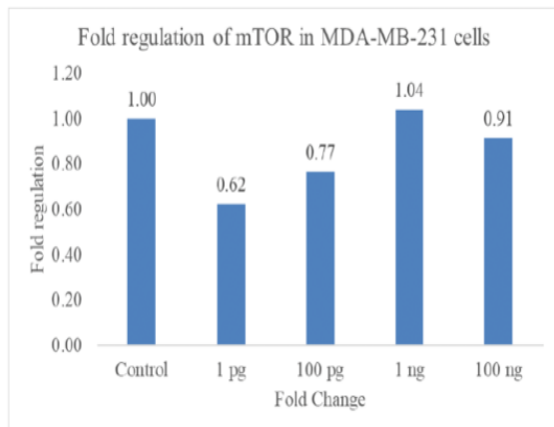
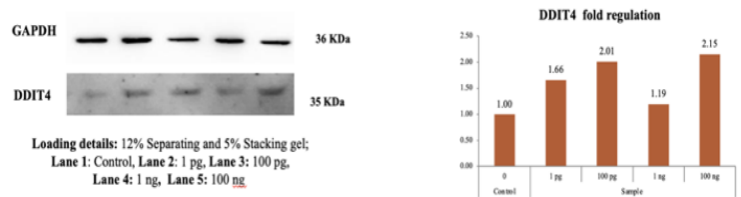
Q-rt-PCR and western blot of DDIT4 in PBMC

Figure 2



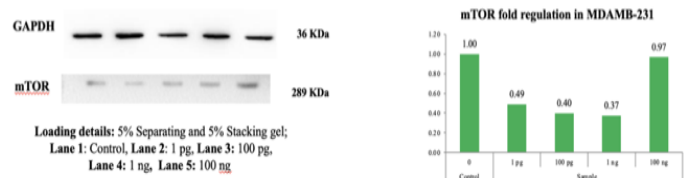
Expression of GAPDH and DDIT4 in MDAMB-231

Sample	Concentration	GAPDH	DDIT4	Normalised	Fold regulation
Control	0	18991.88	4148.71	0.20	1.00
Sample	1 pg	19132.68	8337.65	0.33	1.66
	100 pg	22265.44	6438.62	0.40	2.01
	1 ng	14738.90	5660.67	0.24	1.19
	100 ng	10494.42	7119.05	0.43	2.15



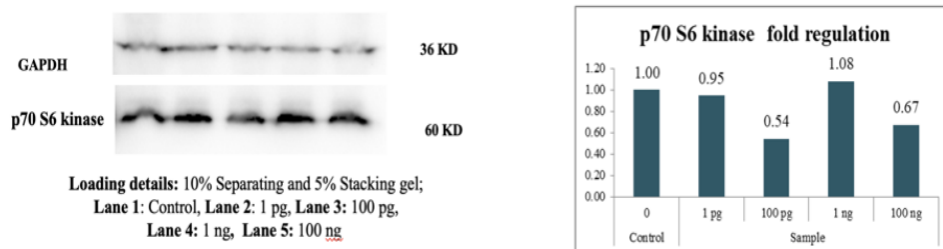
Expression of GAPDH and MTOR in MDAMB-231

Sample	Concentration	GAPDH	mTOR	Normalised	Fold regulation
Control	0	18991.88	14672.26	0.77	1.00
Sample	1 pg	19132.68	7204.41	0.38	0.49
	100 pg	22265.44	6811.92	0.31	0.40
	1 ng	14738.90	4245.90	0.29	0.37
	100 ng	10494.42	7848.05	0.75	0.97



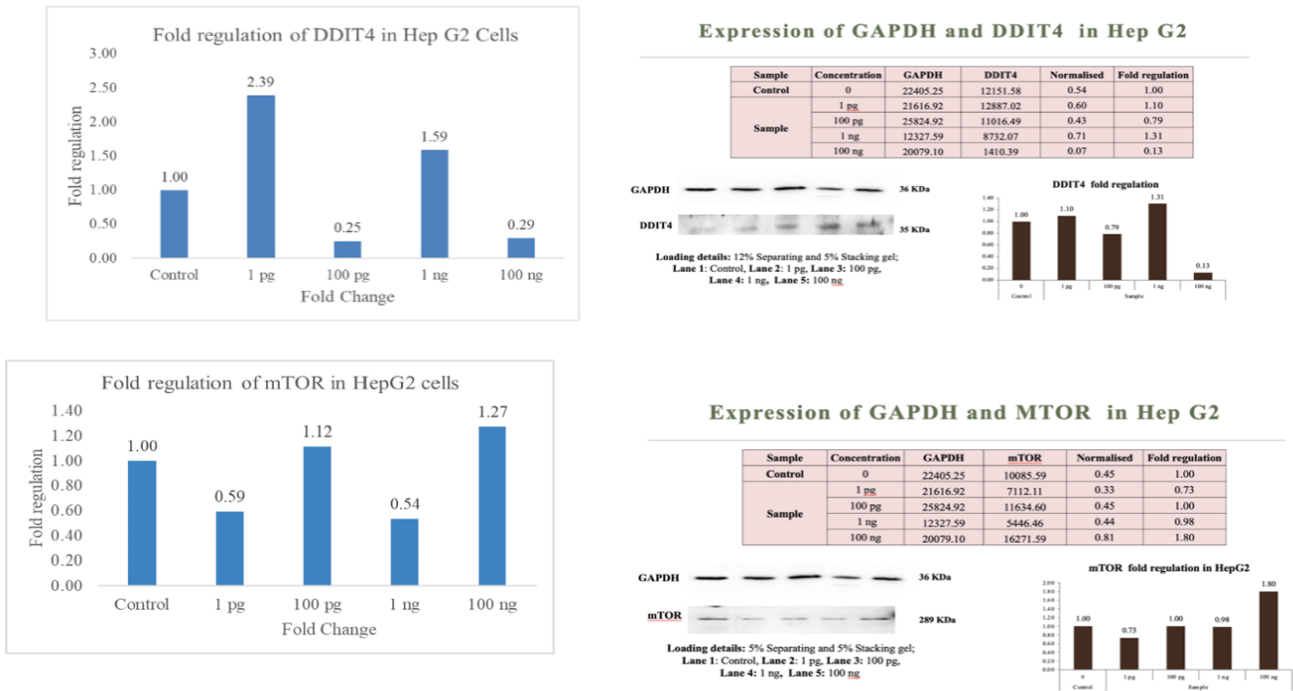
Expression of GAPDH and PHOSPHO-P70 S6 KINASE ALPHA in MDAMB-231

Sample	Concentration	GAPDH	P70 S6 kinase	Normalised	Fold regulation
Control	0	9594.65	9077.04	0.95	1.00
Sample	1 pg	8239.10	19610.69	2.38	0.95
	100 pg	11224.53	15255.40	1.36	0.54
	1 ng	7995.48	21751.38	2.72	1.08
	100 ng	13952.21	23527.07	1.69	0.67

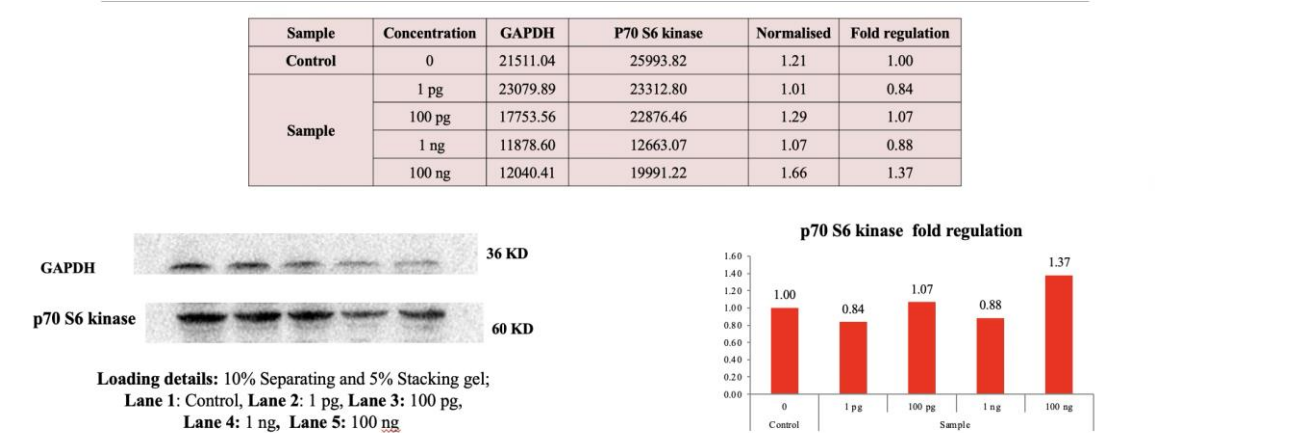


Q-rt-PCR and western blot in MDAMD-231 cell lines

Figure 3

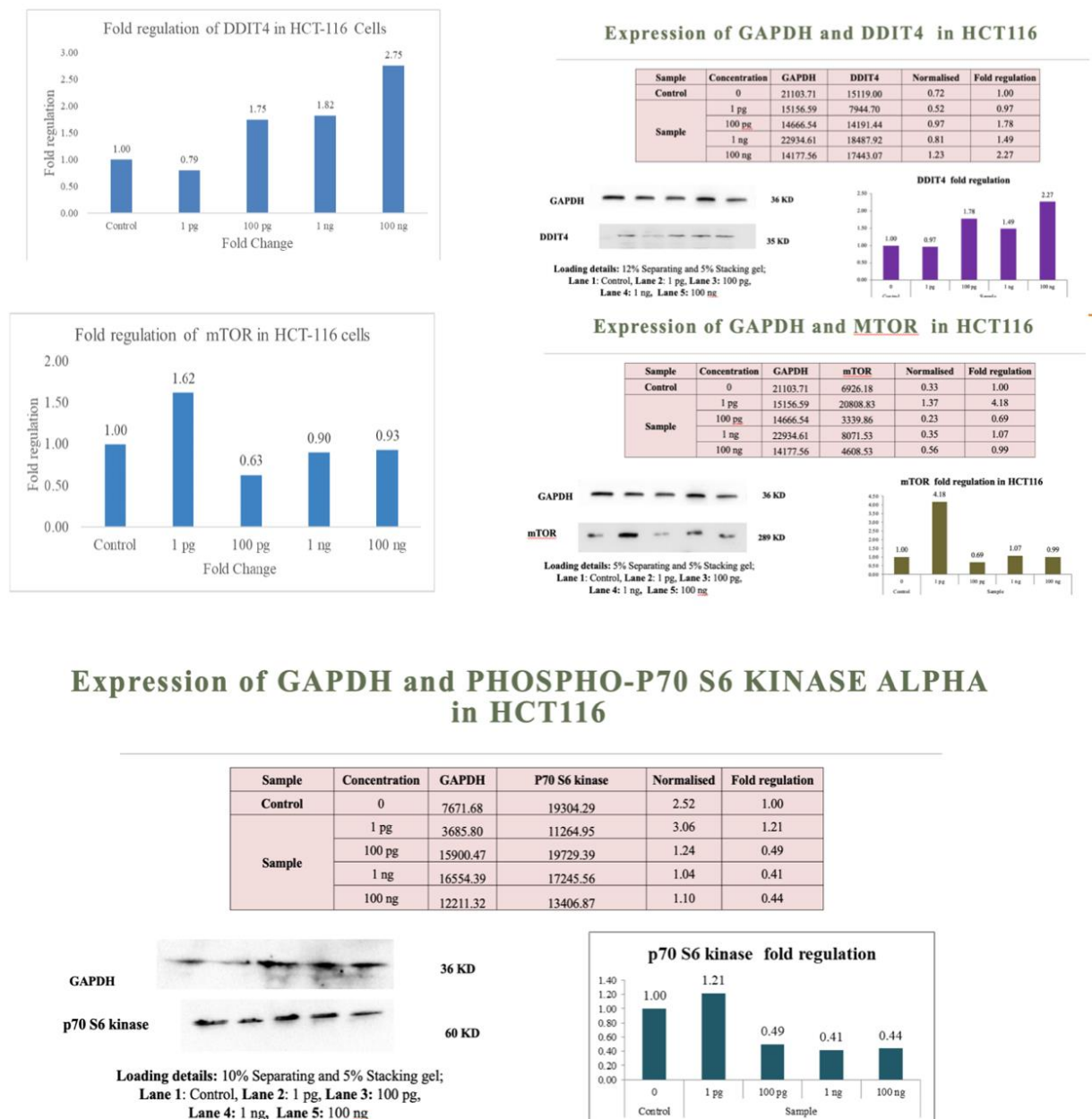


Expression of GAPDH and PHOSPHO-P70 S6 KINASE ALPHA in Hep G2



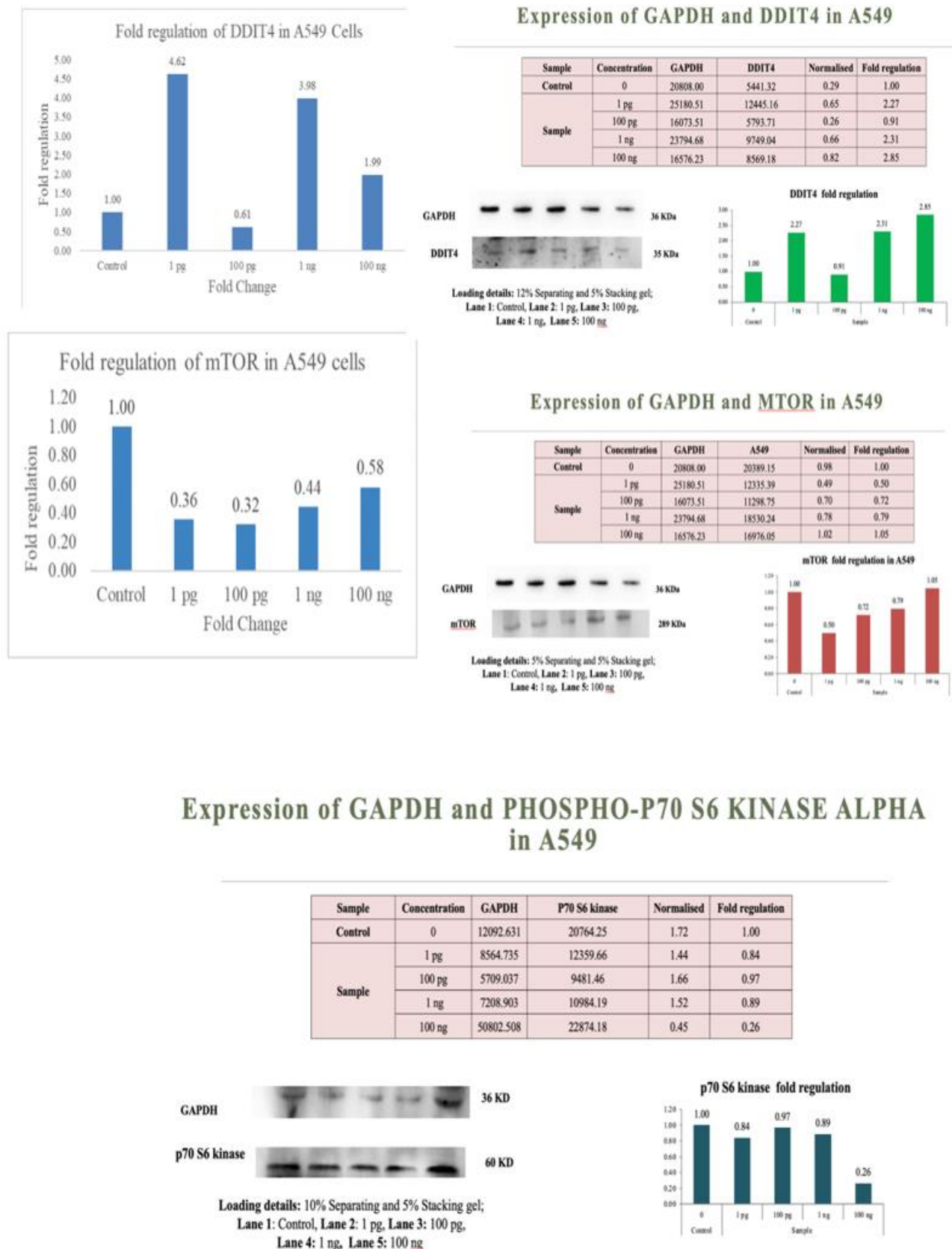
Q-rt-PCR and western blot in HepG2 cell lines

Figure 4



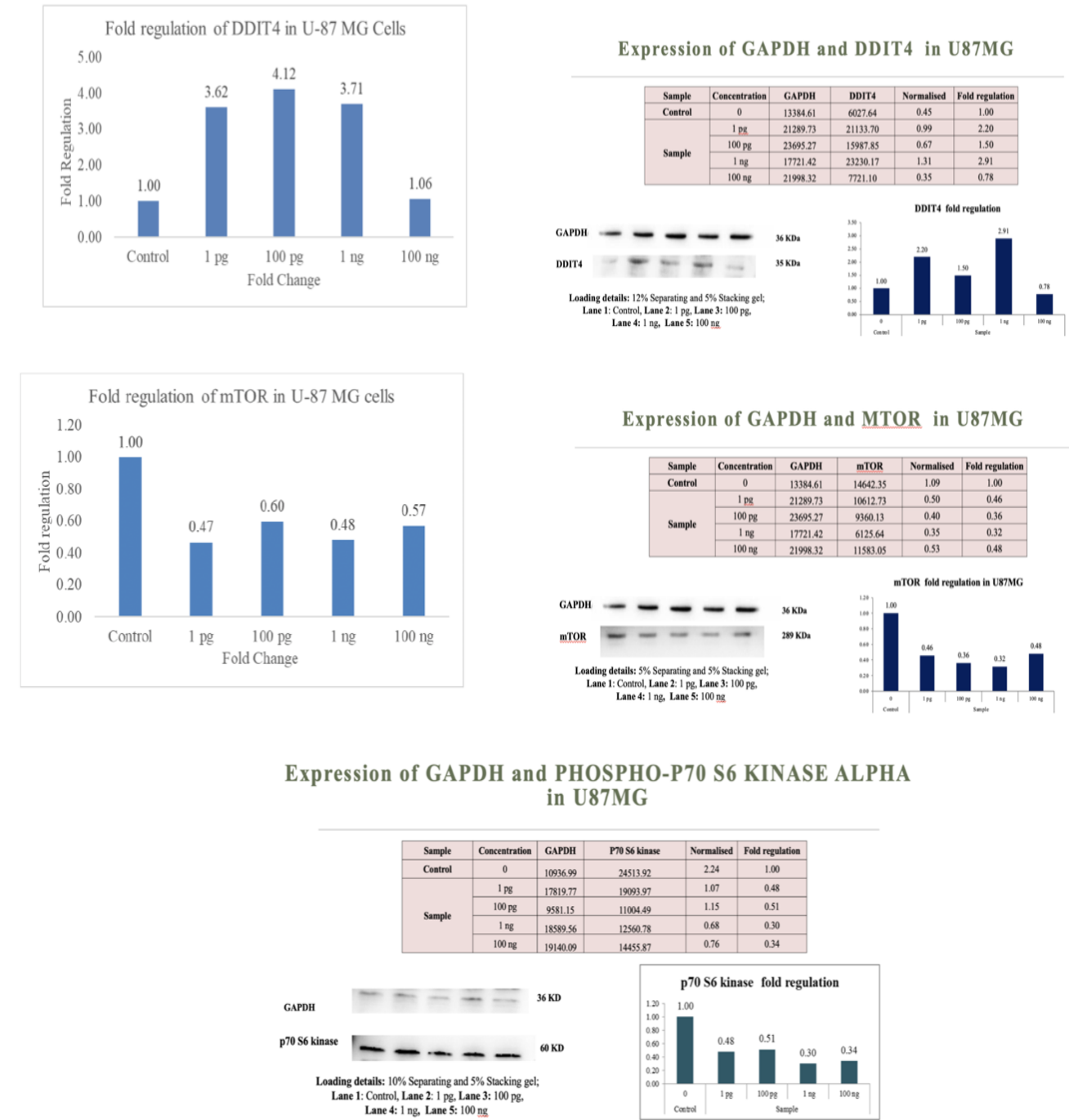
Q-rt-PCR-and Western blot in HCT 116 cells human colon cancer cell line

Figure 5



Q-Rt-PCR-and Western blot in A-549 cells

Figure 6



Q-Rt-PCr-and Western blot in U-87 MG cells

Discussion

Metadichol, a nanoemulsion of long-chain alcohols, has emerged as a multifaceted modulator of cellular signaling, with documented effects on many pathways and its downstream effector, P70S6K. Our findings (table 7) shows that metadichol upregulates DDIT4 at a

concentration of 1 pg/ml, leading to the suppression of mTORC1 activity and subsequent inhibition of P70S6K expression. This finding aligns with the established role of DDIT4 as a stress-induced inhibitor of mTORC1, which is mediated through the TSC1/TSC2 complex. ¹³²
Table 7

Cell Line	DDIT4 Expression	mTOR Expression	p70S6K Expression	Notes
PBMC	Upregulated	Downregulated (1 pg/mL)	Downregulated (100 ng/mL)	Immune-modulatory potential; delayed p70S6K response suggests dose dependency.
U87MG	Upregulated	Downregulated	Downregulated	Strong anti-proliferative effects for glioblastoma.

Hep G2	Upregulated	Downregulated	No significant change	Possible resistance to p70S6K inhibition; alternative pathways active.
MDA-MB-231	Upregulated	Downregulated	Downregulated	Robust anticancer potential for breast cancer.
A549	Upregulated	Downregulated	Downregulated (100 ng/mL)	Dose-dependent p70S6K inhibition; effective in lung cancer.
HCT-116	Upregulated	Downregulated	Downregulated (0.44-fold at 100 ng/mL)	Dose-dependent effects; significant p70S6K suppression in colon cancer.

Summary of gene expression and biological process

In addition, using SIGNOR,¹⁴¹ a repository of manually annotated causal relationships between proteins, (table 8) shows the detailed interactions of the proteins involved

in the mTOR network and these interactions visually expressed (Figure 7) showing the network transcriptional factors that play a role in mTOR inhibition

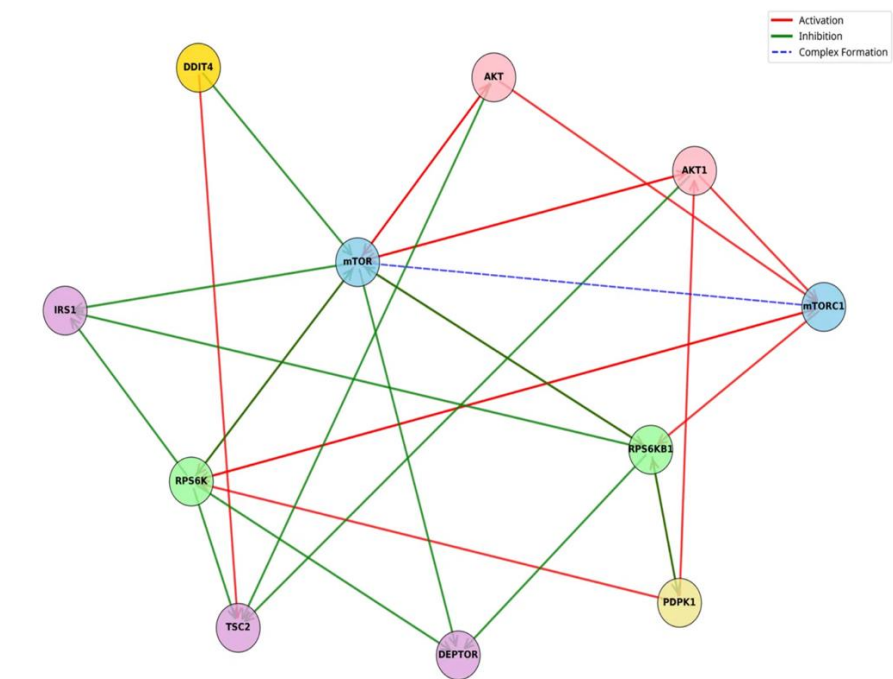


Figure 7: Interaction network of transcription factors in the mTOR pathway

Table 8: Interaction network of transcription factors in the mTOR pathway

Source Name	Source Category	Target Name	Target Category	Effect
AKT	Protein	mTORC1	complex	activation
AKT	Protein	TSC2	protein	inhibition
AKT	Protein	MTOR	protein	activation
AKT1	protein	mTORC1	complex	activation
AKT1	protein	TSC2	protein	inhibition
AKT1	protein	mTOR	protein	Activation
DDIT4	protein	TSC2	protein	activation
MTOR	protein	RPS6KB1	protein	activation
MTOR	protein	AKT	protein	activation
MTOR	protein	AKT1	protein	activation
MTOR	protein	RPS6K	protein	activation
MTOR	protein	mTORC1	complex	complex-formation
MTOR	protein	IRS1	protein	inhibition

Source Name	Source Category	Target Name	Target Category	Effect
MTOR	protein	DEPTOR	protein	inhibition
mTORC1	complex	RPS6KB1	protein	activation
mTORC1	complex	RPS6K	protein	activation
PDPK1	protein	RPS6K	protein	activation
PDPK1	protein	RPS6KB1	protein	activation
PDPK1	protein	AKT1	protein	activation
RPS6K	protein	mTORC1	complex	activation
RPS6K	protein	IRS1	protein	inhibition
RPS6K	protein	DEPTOR	protein	inhibition
RPS6K	protein	MTOR	protein	inhibition
RPS6K	protein	TSC2	protein	inhibition
RPS6KB1	protein	IRS1	protein	inhibition
RPS6KB1	protein	DEPTOR	protein	inhibition
RPS6KB1	protein	PDPK1	protein	inhibition
RPS6KB1	protein	MTOR	protein	inhibition

Broader implications and signaling pathway

Kruppel-like factors KLFs, such as KLF2 and KLF4, as transcriptional regulators of DDIT4 in response to Metadichol.¹³³ However, the broad bioactivity of metadichol suggests that additional mechanisms may contribute to mTOR and P70S6K suppression, potentially via nuclear receptor-mediated expression of sirtuins and Toll-like receptor TLR signaling pathways.^{113,114}

Nuclear receptors (NRs), including PPARs, LXR, and VDR, are ligand-activated transcription factors that regulate metabolic and stress response genes. Given the structural similarity of metadichol to lipid-derived molecules, it may serve as a ligand or coactivator for these receptors. Notably, PPAR γ and VDR have been linked to the transcriptional upregulation of SIRT1, an NAD⁺-dependent deacetylase with established roles in metabolic regulation.^{142,143} Metadichol can increase SIRT1 expression,¹⁴⁴ suggesting that this effect is NR dependent.¹¹³

NAD-dependent deacetylase sirtuin-1 (SIRT1) is a plausible mediator of mTOR suppression. It activates AMPK through the deacetylation of LKB1, an upstream kinase that phosphorylates TSC2, thereby inhibiting mTORC1.¹⁴⁵ This SIRT1-AMPK-mTOR axis represents a metabolic regulatory pathway distinct from the stress-induced KLF/DDIT4 mechanism. In the context of metadichol, NR-mediated SIRT1 upregulation could amplify AMPK activity, reducing mTORC1 signaling and P70S6K phosphorylation independently of DDIT4.¹⁴⁶⁻¹⁵⁰ Additionally, the promotion of autophagy by SIRT1, which is inhibited by active mTORC1, may further suppress P70S6K activity, as P70S6K negatively regulates autophagic flux.¹⁵¹⁻¹⁵²

Toll like receptor (TLR)

Signaling as an Alternative Regulatory Pathway

Metadichol's ability to modulate TLRs¹¹⁴ offers another potential avenue for mTOR and P70S6K suppression. TLRs, which are critical to innate immunity, also influence metabolic pathways through crosstalk with mTORC1. Typically, TLR activation e.g., via LPS stimulates mTORC1 via PI3K/Akt signaling, promoting inflammation and cell survival.^{153,154} However, metadichol has a unique profile, modulating the expression of TLR1–10 and downregulating downstream adaptors such as MYD88, TRAF6, and IRAK4 in cancer cell lines¹⁵⁵. This suppression of TLR signaling components could disrupt Akt-driven mTORC1 activation, leading to reduced P70S6K activity.¹⁵⁶

Moreover, SIRT1 may intersect with TLR signaling by deacetylating NF- κ B, attenuating inflammatory responses that otherwise sustain mTOR activity.¹⁵⁷ Metadichol simultaneously upregulates SIRT1 via NRs¹⁴³ and dampens TLR signaling; this dual action could synergistically inhibit mTORC1. Unlike the KLF/DDIT4 pathway, which relies on transcriptional stress responses, TLR-mediated effects involve immune–metabolic crosstalk, offering a distinct mechanism.^{158,159}

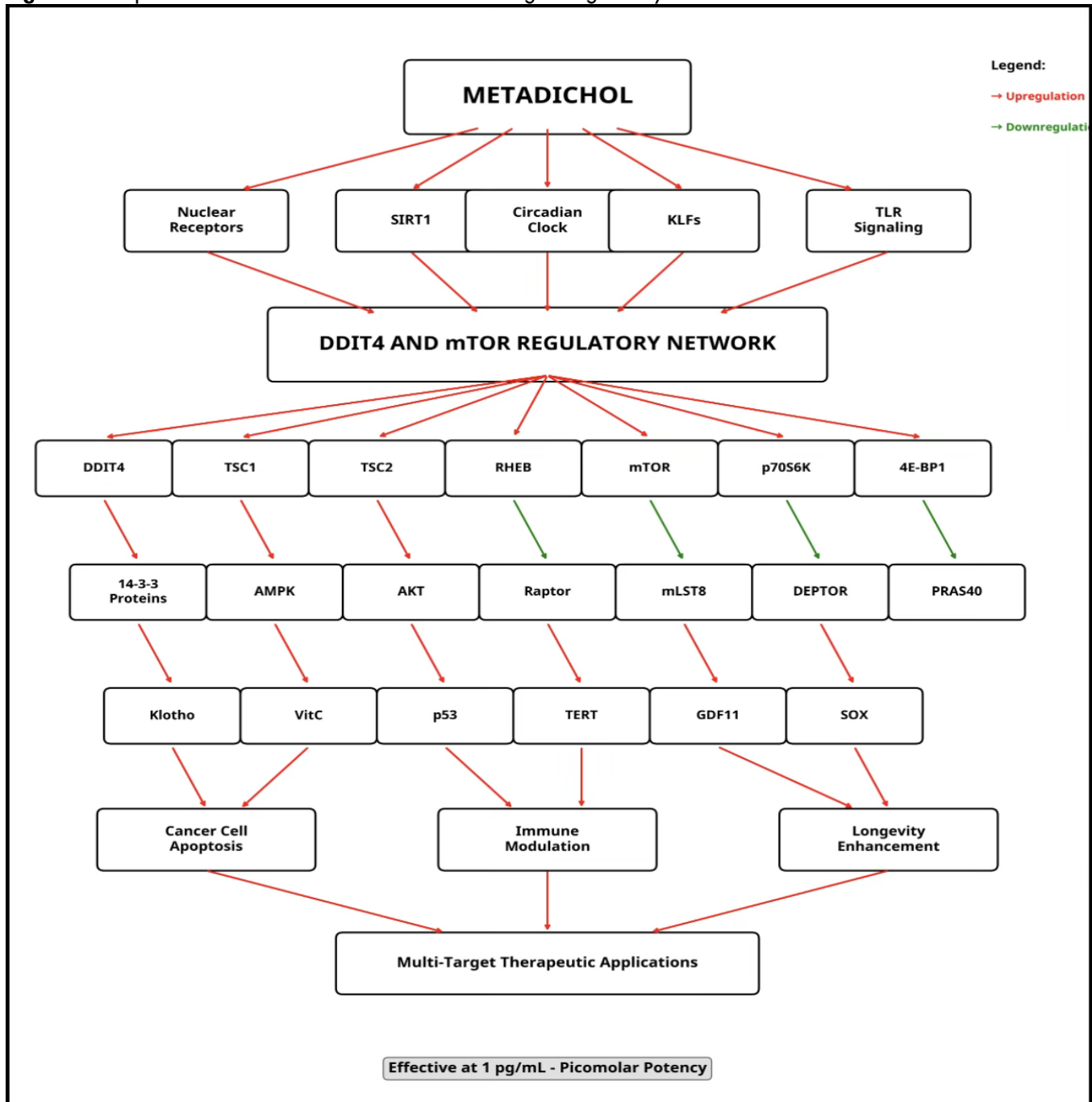
In addition to SIRT1 and TLRs, nuclear receptors such as PPAR α might directly regulate autophagy-related genes e.g., ULK1 that intersect with mTOR signaling, suggesting additional layers of control.¹⁶⁰ The low concentration 1 pg/ml at which Metadichol upregulates DDIT4 highlights its potency via the KLF pathway, but alternative mechanisms involving SIRT1 or TLRs may operate at different thresholds or in different cellular contexts, reflecting the pleiotropic nature of Metadichol. This complexity underscores its potential as a therapeutic agent.

Circadian Genes

Metadichol also expresses circadian genes.¹⁶¹ A considerable body of evidence has demonstrated robust interplay between the mTOR pathway and the circadian clock.¹⁶²⁻¹⁶⁵ This interaction is not unidirectional; it is a dynamic interplay where each system influences the other. The mTOR pathway can influence the period length and synchronization of the circadian clock, affecting the timing and amplitude of circadian oscillations.¹⁶⁶ Conversely, the circadian clock can regulate mTOR activity through the rhythmic expression of mTOR regulators or through the temporal control of nutrient availability¹⁶⁷. For example, the rhythmic expression of genes involved in nutrient sensing and metabolism can impact mTOR signaling.¹⁶⁸⁻¹⁶⁹ This bidirectional relationship underscores the importance of their coordinated function in maintaining cellular homeostasis and adaptation to environmental changes. Disruptions in this delicate balance can have profound consequences for cellular health and contribute to disease development.

The effects of metadichol on DDIT4, mTOR, and p70S6K involve coordinated regulation by SIRT1, VDR, PPAR, Klotho, telomerase, vitamin C, KLF, Foxo1, and circadian genes. Vitamin D receptor (VDR) inhibits mTOR and p70S6K via AMPK and Akt, potentially inducing DDIT4.¹⁶⁹⁻¹⁷³ NAD-dependent deacetylase sirtuin-1 (SIRT1) enhances NAD⁺ availability, suppressing mTOR and p70S6K while modulating DDIT4.^{174, 175} Peroxisome proliferator activated receptor alpha (PPAR- α) suppresses mTOR and promotes DDIT4 via metabolic adaptation.¹⁷⁶⁻¹⁷⁸ Circadian genes link rhythmicity to mTOR suppression, with SIRT1 as an integrator.¹⁷⁹⁻¹⁸² In PBMCs and cancer cell lines, metadichol may upregulate SIRT1 and VDR, activating PPAR and circadian pathways to inhibit mTOR/p70S6K-driven proliferation while inducing DDIT4.^{55,74} These findings support its anticancer and immunomodulatory potential. The broad actions on many pathways by Metadichol actions are summarized in Figure 8.

Figure 8 Comprehensive Metadichol Network Multi-Target Regulatory Network



Metadichol downregulates both mechanistic target of rapamycin (mTOR) and p70S6 kinase (p70S6K) in peripheral blood mononuclear cells (PBMCs) and cancer cell lines. This regulation of key cancer pathways carries several major implications.

Reduced Cell Proliferation and Growth

mTOR and p70S6K are key regulators of protein synthesis, cell growth, and proliferation. Their downregulation could inhibit cancer cell growth and division. ¹⁸³⁻¹⁸⁷

Increased Autophagy

mTOR inhibition promotes autophagy, which can act as a tumor suppressor mechanism by eliminating damaged cellular components. ¹⁸⁸⁻¹⁹¹

Decreased Cancer Stem Cell Activity

mTOR signaling is crucial for cancer stem cell maintenance and self-renewal. Its inhibition may restore enhanced therapeutic sensitivity. ¹⁹²⁻¹⁹⁵

Overcoming Drug Resistance

Constitutive activation of p70S6K is associated with cisplatin resistance in some cancers. Downregulation of this pathway could potentially resensitize resistant tumors to chemotherapy. ¹⁹⁶⁻¹⁹⁸

Synergy with Other Therapies

Inhibition of mTOR/p70S6K signaling may enhance the efficacy of other cancer treatments, including targeted therapies and immunotherapies. ¹⁹⁹⁻²⁰⁴

Altered Cellular Metabolism

mTOR is a central regulator of cellular metabolism. Its inhibition could disrupt cancer cells' metabolic adaptations, potentially making them more vulnerable to stress. ²⁰⁵⁻²⁰⁷

Immune System Modulation

In PBMCs, mTOR inhibition can promote the development of memory cells and enhance overall immune response. ²⁰⁸⁻²¹⁰

Potential Therapeutic Applications

Metadichol might be particularly effective against cancers with PTEN mutations or deletions, where mTOR signaling is often hyperactivated. ²¹¹⁻²¹⁴

Conclusions

Our study, a systems-level approach to transcriptional modulation demonstrated by Metadichol, represents a paradigm shift from single-target therapeutics toward comprehensive network-based interventions. This multi-target strategy may prove particularly valuable for complex age-related diseases that involve dysfunction across multiple physiological systems.

The remarkable safety profile of Metadichol ¹³⁸⁻¹⁴⁰ is established, and its ability to enhance rather than replace normal cellular functions makes it an attractive candidate for regenerative medicine applications where long-term treatment may be required. Clinical use of Metadichol supports rapid translation of these findings toward therapeutic applications.

Metadichol likely suppresses mTOR and P70S6K through multiple pathways in addition to KLF-mediated DDIT4 upregulation. Nuclear receptor-driven SIRT1 expression and TLR signaling modulation represent additional avenues for leveraging the metabolic AMPK and immune-metabolic Akt axes, respectively. Additionally, it affects circadian genes that regulate mTOR signaling. These mechanisms, which are potentially synergistic, highlight the versatility of metadichol while distinguishing it from conventional mTOR inhibitors. Future studies should prioritize the dissection of these pathways to clarify their contributions and therapeutic relevance.

The novelty of our findings, combined with their clinical relevance and translation potential, positions this work as a foundation for future therapeutic development in immune enhancement and regenerative medicine. This work establishes metadichol as a unique molecular tool for studying transcriptional network regulation and provides a foundation for developing novel therapeutic approaches based on coordinated transcriptional reprogramming.

Supplementary Statements.

This paper was published and is available at. *Preprints* **2025** and is available at

<https://doi.org/10.20944/preprints202504.1573.v1>

The author is the founder and CEO of Nanorx Inc., NY, USA, in which he is a majority shareholder.

Raw-data file : q-RT-PCR-raw data .

PDF File; mTOR-Interaction network

References

- Panwar V, Singh A, Bhatt M, et al. Multifaceted role of mTOR (mammalian target of rapamycin) signaling pathway in human health and disease. *Signal Transduct Target Ther*. 2023;8(1):375. doi:10.1038/s41392-023-01608-z
- Liu GY, Sabatini DM. mTOR at the nexus of nutrition, growth, ageing and disease. *Nat Rev Mol Cell Biol*. 2020;21(4):183-203. doi:10.1038/s41580-019-0199-y
- Xie J, Wang X, Proud CG. mTOR inhibitors in cancer therapy. *F1000Res*. 2016;5:2078. doi:10.12688/f1000research.9207.1
- Klawitter J, Nashan B, Christians U. Everolimus and sirolimus in transplantation-related but different. *Expert Opin Drug Saf*. 2015;14(7):1055-1070. doi:10.1517/14740338.2015.1046832
- Kim JY, Kwon YG, Kim YM. The stress-responsive protein REDD1 and its pathophysiological functions. *Exp Mol Med*. 2023;55(9):1933-1944. doi:10.1038/s12276-023-01056-3
- Brugarolas J, Lei K, Hurley RL, et al. Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. *Genes Dev*. 2004;18(23):2893-2904. doi:10.1101/gad.1256804
- Laplanche M, Sabatini DM. mTOR signaling at a glance. *J Cell Sci*. 2009;122(Pt 20):3589-3594. doi:10.1242/jcs.051011
- Rohde J, Heitman J, Cardenas ME. The TOR kinases link nutrient sensing to cell growth. *J Biol Chem*. 2001;276(13):9583-9586. doi:10.1074/jbc.R000034200
- Castro AF, Rebhun JF, Clark GJ, Quilliam LA. Rheb binds tuberous sclerosis complex 2 (TSC2) and promotes S6 kinase activation in a rapamycin- and farnesylation-dependent manner. *J Biol Chem*. 2003;278(35):32493-32496. doi:10.1074/jbc.C300226200
- Zhou H, Huang S. The complexes of mammalian target of rapamycin. *Curr Protein Pept Sci*. 2010;11(6):409-424. doi:10.2174/138920310791824093
- Kim YC, Guan KL. mTOR: a pharmacologic target for autophagy regulation. *J Clin Invest*. 2015;125(1):25-32. doi:10.1172/JCI73939
- Yong J, Kim H, Lee E, Jung Y. Regulation of transcriptome plasticity by mTOR signaling pathway. *Exp Mol Med*. 2025;57(1):1-15. doi:10.1038/s12276-025-01508-y
- Vézina C, Kudelski A, Sehgal SN. Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. *J Antibiot (Tokyo)*. 1975;28(10):721-726. doi:10.7164/antibiotics.28.721
- Sehgal SN, Baker H, Vézina C. Rapamycin (AY-22,989), a new antifungal antibiotic. II. Fermentation, isolation and characterization. *J Antibiot (Tokyo)*. 1975;28(10):727-732. doi:10.7164/antibiotics.28.727
- Kahan BD, Gibbons S, Tejpal N, Stepkowski SM, Chou TC. Synergistic interactions of cyclosporine and rapamycin to inhibit immune performances of normal human peripheral blood lymphocytes in vitro. *Transplantation*. 1991;51(1):232-239. doi:10.1097/00007890-199101000-00041
- Dumont FJ, Staruch MJ, Koprak SL, Melino MR, Sigal NH. Distinct mechanisms of suppression of murine T cell activation by the related macrolides FK-506 and rapamycin. *J Immunol*. 1990;144(1):251-258.
- Brown EJ, Albers MW, Shin TB, et al. A mammalian protein targeted by G1-arresting rapamycin-receptor complex. *Nature*. 1994;369(6483):756-758. doi:10.1038/369756a0
- Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH. RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell*. 1994;78(1):35-43. doi:10.1016/0092-8674(94)90570-3
- Keith CT, Schreiber SL. PIK-related kinases: DNA repair, recombination, and cell cycle checkpoints. *Science*. 1995;270(5233):50-51. doi:10.1126/science.270.5233.50
- Heitman J, Movva NR, Hall MN. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science*. 1991;253(5022):905-909. doi:10.1126/science.1715094
- Loewith R, Jacinto E, Wulschleger S, et al. Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. *Mol Cell*. 2002;10(3):457-468. doi:10.1016/s1097-2765(02)00636-6
- Sarbassov DD, Ali SM, Kim DH, et al. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol*. 2004;14(14):1296-1302. doi:10.1016/j.cub.2004.06.054
- Kim DH, Sarbassov DD, Ali SM, et al. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell*. 2002;110(2):163-175. doi:10.1016/s0092-8674(02)00808-5
- Hara K, Maruki Y, Long X, et al. Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell*. 2002;110(2):177-189. doi:10.1016/s0092-8674(02)00833-4
- Thoreen CC, Kang SA, Chang JW, et al. An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. *J Biol Chem*. 2009;284(12):8023-8032. doi:10.1074/jbc.M900301200
- Wang L, Harris TE, Roth RA, Lawrence JC Jr. PRAS40 regulates mTORC1 kinase activity by functioning as a direct inhibitor of substrate binding. *J Biol Chem*. 2007;282(27):20036-20044. doi:10.1074/jbc.M702376200
- Jung CH, Ro SH, Cao J, Otto NM, Kim DH. mTOR regulation of autophagy. *FEBS Lett*. 2010;584(7):1287-1295. doi:10.1016/j.febslet.2010.01.017
- Hosokawa N, Hara T, Kaizuka T, et al. Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol Biol Cell*. 2009;20(7):1981-1991. doi:10.1091/mb.c.e08-12-1248
- Jacinto E, Loewith R, Schmidt A, et al. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol*. 2004;6(11):1122-1128. doi:10.1038/ncb1183
- Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the

- riCTOR-mTOR complex. *Science*. 2005;307(5712):1098-1101. doi:10.1126/science.1106148
31. Sarbassov DD, Ali SM, Sengupta S, et al. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol Cell*. 2006;22(2):159-168. doi:10.1016/j.molcel.2006.03.029
 32. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. *Cell*. 2007;129(7):1261-1274. doi:10.1016/j.cell.2007.06.009
 33. Guertin DA, Stevens DM, Thoreen CC, et al. Ablation in mice of the mTORC components raptor or rictor reveals differential sensitivity to rapamycin. *Mol Cell*. 2006;21(4):543-551. doi:10.1016/j.molcel.2006.01.028
 34. Magnuson B, Ekim B, Fingar DC. Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signalling networks. *Biochem J*. 2012;441(1):1-21. doi:10.1042/BJ20110892
 35. Bahrami-B F, Ataie-Kachoei P, Pourgholami MH, Morris DL. p70 Ribosomal protein S6 kinase (Rps6kb1): an update. *J Clin Pathol*. 2014;67(12):1019-1025. doi:10.1136/jclinpath-2014-202560
 36. Holz MK, Ballif BA, Gygi SP, Blenis J. mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. *Cell*. 2005;123(4):569-580. doi:10.1016/j.cell.2005.10.024
 37. Babchia N, Calipel A, Mouriaux F, Faussat AM, Mascarelli F. The PI3K/Akt and mTOR/P70S6K signaling pathways in human uveal melanoma cells: interaction with B-Raf/ERK. *Invest Ophthalmol Vis Sci*. 2010;51(1):421-429. doi:10.1167/iovs.09-3974
 38. Burnett PE, Barrow RK, Cohen NA, Snyder SH, Sabatini DM. RAFT1 phosphorylation of the translational regulators p70 S6 kinase and 4E-BP1. *Proc Natl Acad Sci U S A*. 1998;95(4):1432-1437. doi:10.1073/pnas.95.4.1432
 39. Chung J, Kuo CJ, Crabtree GR, Blenis J. Rapamycin-FKBP12 specifically blocks growth-dependent activation of and signaling by the 70 kd S6 protein kinases. *Cell*. 1992;69(7):1227-1236. doi:10.1016/0092-8674(92)90643-q
 40. Tavares MR, Pavan IC, Amaral CL, Meneguello L, Luchessi AD, Simabuco FM. The S6K protein family in health and disease. *Life Sci*. 2015;131:1-10. doi:10.1016/j.lfs.2015.03.001
 41. Ben-Hur V, Denichenko P, Siegfried Z, et al. S6K1 alternative splicing modulates its oncogenic activity and regulates mTORC1. *Cell Rep*. 2013;3(1):103-115. doi:10.1016/j.celrep.2012.11.020
 42. Jastrzebski K, Hannan KM, Tchoubrieva EB, Hannan RD, Pearson RB. Coordinate regulation of ribosome biogenesis and function by the ribosomal protein S6 kinase, a key mediator of mTOR function. *Growth Factors*. 2007;25(4):209-226. doi:10.1080/08977190701779101
 43. Ruvinsky I, Sharon N, Lerer T, et al. Ribosomal protein S6 phosphorylation is a determinant of cell size and glucose homeostasis. *Genes Dev*. 2005;19(18):2199-2211. doi:10.1101/gad.351605
 44. Ruvinsky I, Meyuhas O. Ribosomal protein S6 phosphorylation: from protein synthesis to cell size. *Trends Biochem Sci*. 2006;31(6):342-348. doi:10.1016/j.tibs.2006.04.003
 45. Meyuhas O. Ribosomal protein S6 phosphorylation: four decades of research. *Int Rev Cell Mol Biol*. 2015;320:41-73. doi:10.1016/bs.ircmb.2015.07.006
 46. Fenton TR, Gout IT. Functions and regulation of the 70kDa ribosomal S6 kinases. *Int J Biochem Cell Biol*. 2011;43(1):47-59. doi:10.1016/j.biocel.2010.09.018
 47. Um SH, Frigerio F, Watanabe M, et al. Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature*. 2004;431(7005):200-205. doi:10.1038/nature02866
 48. Goberdhan DC, Wilson C, Harris AL. Amino acid sensing by mTORC1: intracellular transporters mark the spot. *Cell Metab*. 2016;23(4):580-589. doi:10.1016/j.cmet.2016.03.013
 49. Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. *Cell*. 2017;168(6):960-976. doi:10.1016/j.cell.2017.02.004
 50. Dibble CC, Manning BD. Signal integration by mTORC1 coordinates nutrient input with biosynthetic output. *Nat Cell Biol*. 2013;15(6):555-564. doi:10.1038/ncb2763
 51. Wolfson RL, Chantranupong L, Saxton RA, et al. Sestrin2 is a leucine sensor for the mTORC1 pathway. *Science*. 2016;351(6268):43-48. doi:10.1126/science.aab2674
 52. Chantranupong L, Wolfson RL, Orozco JM, et al. The Sestrins interact with GATOR2 to negatively regulate the amino-acid-sensing pathway upstream of mTORC1. *Cell Rep*. 2014;9(1):1-8. doi:10.1016/j.celrep.2014.09.014
 53. Sancak Y, Peterson TR, Shaul YD, et al. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science*. 2008;320(5882):1496-1501. doi:10.1126/science.1157535
 54. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell*. 2010;141(2):290-303. doi:10.1016/j.cell.2010.02.024
 55. Gwinn DM, Shackelford DB, Egan DF, et al. AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell*. 2008;30(2):214-226. doi:10.1016/j.molcel.2008.03.003
 56. Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. *Cell*. 2003;115(5):577-590. doi:10.1016/s0092-8674(03)00929-2
 57. Hardie DG, Ross FA, Hawley SA. AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat Rev Mol Cell Biol*. 2012;13(4):251-262. doi:10.1038/nrm3311
 58. Shaw RJ, Kosmatka M, Bardeesy N, et al. The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. *Proc Natl Acad Sci U S A*. 2004;101(10):3329-3335. doi:10.1073/pnas.0308061100
 59. Steinberg GR, Hardie DG. New insights into activation and function of the AMPK. *Nat Rev Mol Cell Biol*. 2023;24(4):255-272. doi:10.1038/s41580-022-00547-x

60. Herzig S, Shaw RJ. AMPK: guardian of metabolism and mitochondrial homeostasis. *Nat Rev Mol Cell Biol.* 2018;19(2):121-135. doi:10.1038/nrm.2017.95
61. Mihaylova MM, Shaw RJ. The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nat Cell Biol.* 2011;13(9):1016-1023. doi:10.1038/ncb2329
62. Garcia D, Shaw RJ. AMPK: mechanisms of cellular energy sensing and restoration of metabolic balance. *Mol Cell.* 2017;66(6):789-800. doi:10.1016/j.molcel.2017.05.032
63. Oakhill JS, Steel R, Chen ZP, et al. AMPK is a direct adenylate charge-regulated protein kinase. *Science.* 2011;332(6036):1433-1435. doi:10.1126/science.1200094
64. Inoki K, Li Y, Zhu T, Wu J, Guan KL. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol.* 2002;4(9):648-657. doi:10.1038/ncb839
65. Manning BD, Tee AR, Logsdon MN, Blenis J, Cantley LC. Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. *Mol Cell.* 2002;10(1):151-162. doi:10.1016/s1097-2765(02)00568-3
66. Ellisen LW, Ramsayer KD, Johannessen CM, et al. REDD1, a developmentally regulated transcriptional target of p63 and p53, links p63 to regulation of reactive oxygen species. *Mol Cell.* 2002;10(5):995-1005. doi:10.1016/s1097-2765(02)00706-2
67. Shoshani T, Faerman A, Mett I, et al. Identification of a novel hypoxia-inducible factor 1-responsive gene, RTP801, involved in apoptosis. *Mol Cell Biol.* 2002;22(7):2283-2293. doi:10.1128/mcb.22.7.2283-2293.2002
68. Wang Z, Malone MH, He H, McColl KS, Distelhorst CW. Microarray analysis uncovers the induction of the proapoptotic BH3-only protein Bim in multiple models of glucocorticoid-induced apoptosis. *J Biol Chem.* 2003;278(26):23861-23867. doi:10.1074/jbc.M301843200
69. Sofer A, Lei K, Johannessen CM, Ellisen LW. Regulation of mTOR and cell growth in response to energy stress by REDD1. *Mol Cell Biol.* 2005;25(14):5834-5845. doi:10.1128/MCB.25.14.5834-5845.2005
70. Jin HO, Hong SE, Kim JY, et al. Sustained overexpression of HIF-1 α leads to the resistance to anticancer drugs through autophagy induction. *Oncol Rep.* 2011;25(1):237-243. doi:10.3892/or.00001069
71. DeYoung MP, Horak P, Sofer A, Sgroi D, Ellisen LW. Hypoxia regulates TSC1/2-mTOR signaling and tumor suppression through REDD1-mediated 14-3-3 shuttling. *Genes Dev.* 2008;22(2):239-251. doi:10.1101/gad.1617608
72. Corradetti MN, Inoki K, Bardeesy N, DePinho RA, Guan KL. Regulation of the TSC pathway by LKB1: evidence of a molecular link between tuberous sclerosis complex and Peutz-Jeghers syndrome. *Genes Dev.* 2004;18(13):1533-1538. doi:10.1101/gad.1199104
73. Manning BD, Logsdon MN, Lipovsky AI, Abbott D, Kwiatkowski DJ, Cantley LC. Feedback inhibition of Akt signaling limits the growth of tumors lacking Tsc2. *Genes Dev.* 2005;19(15):1773-1778. doi:10.1101/gad.1314605
74. Tee AR, Manning BD, Roux PP, Cantley LC, Blenis J. Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. *Curr Biol.* 2003;13(15):1259-1268. doi:10.1016/s0960-9822(03)00506-2
75. Zhang Y, Gao X, Saucedo LJ, Ru B, Edgar BA, Pan D. Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. *Nat Cell Biol.* 2003;5(6):578-581. doi:10.1038/ncb999
76. Brugarolas J, Kaelin WG Jr. Dysregulation of HIF and VEGF is a unifying feature of the familial hamartoma syndromes. *Cancer Cell.* 2004;6(1):7-10. doi:10.1016/j.ccr.2004.06.020
77. Cam H, Easton JB, High A, Houghton PJ. mTORC1 signaling under hypoxic conditions is controlled by ATM-dependent phosphorylation of HIF-1 α . *Mol Cell.* 2010;40(4):509-520. doi:10.1016/j.molcel.2010.10.030
78. Horak P, Crawford AR, Vadysirisack DD, et al. Negative feedback control of HIF-1 through REDD1-regulated ROS suppresses tumorigenesis. *Proc Natl Acad Sci U S A.* 2010;107(10):4675-4680. doi:10.1073/pnas.0907705107
79. Vadysirisack DD, Baenke F, Ory B, Lei K, Ellisen LW. Feedback control of p53 translation by REDD1 and mTORC1 limits the p53-dependent DNA damage response. *Mol Cell Biol.* 2011;31(21):4356-4365. doi:10.1128/MCB.05541-11
80. Schwarzer R, Tondera D, Arnold W, Giese K, Klippel A, Kaufmann J. REDD1 integrates hypoxia-mediated survival signaling downstream of phosphatidylinositol 3-kinase. *Oncogene.* 2005;24(7):1138-1148. doi:10.1038/sj.onc.1208236
81. Lipina C, Hundal HS. Modulation of cellular redox homeostasis by the endoplasmic reticulum stress response in skeletal muscle: implications for insulin resistance. *Biochem Soc Trans.* 2016;44(2):362-369. doi:10.1042/BST20150273
82. Yoshida T, Mett I, Bhunia AK, et al. Rtp801, a suppressor of mTOR signaling, is an essential mediator of cigarette smoke-induced pulmonary injury and emphysema. *Nat Med.* 2010;16(7):767-773. doi:10.1038/nm.2157
83. Miller DM, Thomas SD, Islam A, Muench D, Sedoris K. c-Myc and cancer metabolism. *Clin Cancer Res.* 2012;18(20):5546-5553. doi:10.1158/1078-0432.CCR-12-0977
84. Cam H, Easton JB, High A, Houghton PJ. mTORC1 signaling under hypoxic conditions is controlled by ATM-dependent phosphorylation of HIF-1 α . *Mol Cell.* 2010;40(4):509-520. doi:10.1016/j.molcel.2010.10.030
85. Guertin DA, Sabatini DM. Defining the role of mTOR in cancer. *Cancer Cell.* 2007;12(1):9-22. doi:10.1016/j.ccr.2007.05.008
86. Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev.* 2004;18(16):1926-1945. doi:10.1101/gad.1212704
87. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet.* 2006;7(8):606-619. doi:10.1038/nrg1879

88. Yuan TL, Cantley LC. PI3K pathway alterations in cancer: variations on a theme. *Oncogene*. 2008;27(41):5497-5510. doi:10.1038/onc.2008.245
89. Chalhoub N, Baker SJ. PTEN and the PI3-kinase pathway in cancer. *Annu Rev Pathol*. 2009;4:127-150. doi:10.1146/annurev.pathol.4.110807.092311
90. Keniry M, Parsons R. The role of PTEN signaling perturbations in cancer and in targeted therapy. *Oncogene*. 2008;27(41):5477-5485. doi:10.1038/onc.2008.248
91. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat Rev Cancer*. 2002;2(7):489-501. doi:10.1038/nrc839
92. Bellacosa A, Kumar CC, Di Cristofano A, Testa JR. Activation of AKT kinases in cancer: implications for therapeutic targeting. *Adv Cancer Res*. 2005;94:29-86. doi:10.1016/S0065-230X(05)94002-5
93. Hennessy BT, Smith DL, Ram PT, Lu Y, Mills GB. Exploiting the PI3K/AKT pathway for cancer drug discovery. *Nat Rev Drug Discov*. 2005;4(12):988-1004. doi:10.1038/nrd1902
94. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-674. doi:10.1016/j.cell.2011.02.013
95. DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab*. 2008;7(1):11-20. doi:10.1016/j.cmet.2007.10.002
96. Zoncu R, Efeyan A, Sabatini DM. mTOR: from growth signal integration to cancer, diabetes and ageing. *Nat Rev Mol Cell Biol*. 2011;12(1):21-35. doi:10.1038/nrm3025
97. Dancey J. mTOR signaling and drug development in cancer. *Nat Rev Clin Oncol*. 2010;7(4):209-219. doi:10.1038/nrclinonc.2010.21
98. Meric-Bernstam F, Gonzalez-Angulo AM. Targeting the mTOR signaling network for cancer therapy. *J Clin Oncol*. 2009;27(13):2278-2287. doi:10.1200/JCO.2008.20.0766
99. Hudes G, Carducci M, Tomczak P, et al. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. *N Engl J Med*. 2007;356(22):2271-2281. doi:10.1056/NEJMoa066838
100. Motzer RJ, Escudier B, Oudard S, et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. *Lancet*. 2008;372(9637):449-456. doi:10.1016/S0140-6736(08)61039-9
101. Choi J, Chen J, Schreiber SL, Clardy J. Structure of the FKBP12-rapamycin complex interacting with the binding domain of human FRAP. *Science*. 1996;273(5272):239-242. doi:10.1126/science.273.5272.239
102. O'Reilly KE, Rojo F, She QB, et al. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. *Cancer Res*. 2006;66(3):1500-1508. doi:10.1158/0008-5472.CAN-05-2925
103. Carracedo A, Ma L, Teruya-Feldstein J, et al. Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. *J Clin Invest*. 2008;118(9):3065-3074. doi:10.1172/JCI34739
104. Feldman ME, Apsel B, Uotila A, et al. Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. *PLoS Biol*. 2009;7(2):e38. doi:10.1371/journal.pbio.1000038
105. Thoreen CC, Sabatini DM. Rapamycin inhibits mTORC1, but not completely. *Autophagy*. 2009;5(5):725-726. doi:10.4161/auto.5.5.8504
106. Rodrik-Outmezguine VS, Chandarlapaty S, Pagano NC, et al. mTOR kinase inhibition causes feedback-dependent biphasic regulation of AKT signaling. *Cancer Discov*. 2011;1(3):248-259. doi:10.1158/2159-8290.CD-11-0085
107. Iyer G, Hanrahan AJ, Milowsky MI, et al. Genome sequencing identifies a basis for everolimus sensitivity. *Science*. 2012;338(6104):221. doi:10.1126/science.1226344
108. Baselga J, Campone M, Piccart M, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *N Engl J Med*. 2012;366(6):520-529. doi:10.1056/NEJMoa1109653
109. Yao JC, Shah MH, Ito T, et al. Everolimus for advanced pancreatic neuroendocrine tumors. *N Engl J Med*. 2011;364(6):514-523. doi:10.1056/NEJMoa1009290
110. Chresta CM, Davies BR, Hickson I, et al. AZD8055 is a potent, selective, and orally bioavailable ATP-competitive mammalian target of rapamycin kinase inhibitor with in vitro and in vivo antitumor activity. *Cancer Res*. 2010;70(1):288-298. doi:10.1158/0008-5472.CAN-09-1751
111. Maira SM, Stauffer F, Brueggen J, et al. Identification and characterization of NVP-BE235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent in vivo antitumor activity. *Mol Cancer Ther*. 2008;7(7):1851-1863. doi:10.1158/1535-7163.MCT-08-0017
112. Raghavan PR. Policosanol Nanoparticles US Patent 8,722,0093 (2014).
113. P. R. Raghavan. Metadichol® A Nano Lipid Emulsion that Expresses All 49 Nuclear Receptors in Stem and Somatic Cells. *Archives of Clinical and Biomedical Research*. 7 (2023): 543-555.DOI: 10.26502/acbr.50170370
114. Raghavan, P. R., Metadichol® induced expression of TLR family members in peripheral blood mononuclear cells. *Medical Research Archives*, S.I., v. 12, n. 9, sep. 2024. ISSN 2375-1924. Available at: <https://esmed.org/MRA/mra/article/view/5610>
115. Raghavan PR. Inhibition of Dengue and other enveloped viruses by Metadichol®, a novel Nano emulsion Lipid. *J Sci Heal Outcomes*. 2016;4(1):1-8.
116. Raghavan PR. In vitro inhibition of zika virus by Metadichol®, a novel nano emulsion lipid. *J Immunol Tech Infect Dis*. 2016;5(2):1-6.
117. Raghavan PR. Metadichol® and vitamin C increase in vivo, an open-label study. *Vitam Miner*. 2018;7(2):1-5.
118. Raghavan PR. VDR Inverse Agonism by Metadichol Enhances VDBP-Mediated Immunity. *Preprints.org*. 2025. doi:10.20944/preprints202506.0491.v1
119. Raghavan PR. Metadichol® A Novel Nano Lipid; GPR 120 Agonist. *Int J Diabetes Complications*. 2017;1(1):1-6.
120. Raghavan PR. Metadichol: a novel nanolipid formulation that inhibits SARS-CoV-2 and a multitude of pathological viruses in vitro. *Biomed Res Int*. 2022;2022:1558860. doi:10.1155/2022/1558860

121. Raghavan PR. Metadichol: An Agonist that Expresses the Anti-aging Gene Klotho in Various Cell Lines. *Fortune Journal of Health Sciences*. 2023;6:357-362. doi:10.26502/jbsb.5107066
122. Laplante M, Sabatini DM. An emerging role of mTOR in lipid biosynthesis. *Curr Biol*. 2009;19(22):R1046-R1052. doi:10.1016/j.cub.2009.09.058
123. Düvel K, Yecies JL, Menon S, et al. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Mol Cell*. 2010;39(2):171-183. doi:10.1016/j.molcel.2010.06.022
124. Ben-Sahra I, Howell JJ, Asara JM, Manning BD. Stimulation of de novo pyrimidine synthesis by growth signaling through mTOR and S6K1. *Science*. 2013;339(6125):1323-1328. doi:10.1126/science.1228792
125. Robitaille AM, Christen S, Shimobayashi M, et al. Quantitative phosphoproteomics reveal mTORC1 activates de novo pyrimidine synthesis. *Science*. 2013;339(6125):1320-1323. doi:10.1126/science.1228771
126. Foltyn M, Luger AL, Lorenz NI, et al. The physiological mTOR complex 1 inhibitor DDIT4 mediates therapy resistance in glioblastoma. *Br J Cancer*. 2019;120(5):481-487. doi:10.1038/s41416-018-0368-3
127. Wang H, Wang J, Qu H, et al. In vitro and in vivo inhibition of mTOR by 1,25-dihydroxyvitamin D3 to improve early diabetic nephropathy via the DDIT4/TSC2/mTOR pathway. *Endocrine*. 2016;54(2):348-359. doi:10.1007/s12020-016-0999-1
128. Araki K, Turner AP, Shaffer VO, et al. mTOR regulates memory CD8 T-cell differentiation. *Nature*. 2009;460(7251):108-112. doi:10.1038/nature08155
129. Delgoffe GM, Kole TP, Zheng Y, et al. The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. *Immunity*. 2009;30(6):832-844. doi:10.1016/j.immuni.2009.04.014
130. Powell JD, Pollizzi KN, Heikamp EB, Horton MR. Regulation of immune responses by mTOR. *Annu Rev Immunol*. 2012;30:39-68. doi:10.1146/annurev-immunol-020711-075024
131. Chi H. Regulation and function of mTOR signalling in T cell fate decisions. *Nat Rev Immunol*. 2012;12(5):325-338. doi:10.1038/nri3198
132. Huang J, Manning BD. The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. *Biochem J*. 2008;412(2):179-190. doi:10.1042/BJ20080281
133. Raghavan PR. Metadichol-Induced KLF Expression in PBMC Cells. Links SIRT1s, NRs, TLRs, and circadian genes. A Systems-Wide Biology Approach. *Preprints*. 2025;2025020271. doi:10.20944/preprints202502.0271.v1
134. Zhou H, Luo Y, Huang S. Updates of mTOR inhibitors. *Anticancer Agents Med Chem*. 2010;10(7):571-581. doi:10.2174/187152010793498663
135. Dancy JE. Therapeutic targets: MTOR and related pathways. *Cancer Biol Ther*. 2006;5(9):1065-1073. doi:10.4161/cbt.5.9.3175
136. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell*. 2012;149(2):274-293. doi:10.1016/j.cell.2012.03.017
137. De Fijter JW. Cancer and mTOR Inhibitors in Transplant Recipients. *Transplantation*. 2017;101(1):45-55. doi:10.1097/TP.0000000000001447
138. Aleman C, et al. A 12-month study of policosanol oral toxicity in Sprague Dawley rats. *Toxicol Lett*. 1994;70(1):77-87.
139. Aleman CL, et al. Carcinogenicity of policosanol in Sprague Dawley rats: a 24 month study. *Teratog Carcinog Mutagen*. 1994;14(5):239-249.
140. Aleman CL, et al. Carcinogenicity of policosanol in mice: an 18-month study. *Food Chem Toxicol*. 1995;33(7):573-578.
141. Lo Surdo P, Iannuccelli M, Contino S, Castagnoli L, Licata L, Cesareni G, Perfetto L. SIGNOR 3.0, the Signaling network open resource 3.0: 2022 update. *Nucleic Acids Res*. 2022;gkac883. doi:10.1093/nar/gkac883
142. Chalkiadaki A, Guarente L. The multifaceted functions of sirtuins in metabolism. *Nat Rev Mol Cell Biol*. 2015;13(12):698-707. doi:10.1038/nrm3478
143. Ruderman NB, et al. AMPK and SIRT1: A long-standing partnership? *Am J Physiol Endocrinol Metab*. 2010;298(5):E751-E760. doi:10.1152/ajpendo.00745.2009
144. Raghavan PR. Metadichol®-Induced Expression of Sirtuin 1-7 In Somatic and Cancer Cells. *Med Res Arch*. 2024;12(6):5328. doi:10.18103/mra.v12i6.5328
145. Lan F, Cacicedo JM, Ruderman N, Ido Y. SIRT1 modulation of the acetylation status, cytosolic localization, and activity of LKB1. *J Biol Chem*. 2008; 283 (41):27628-27635. doi:10.1074/jbc.M805711200
146. Chalkiadaki A, Guarente L. The multifaceted functions of sirtuins in metabolism. *Nat Rev Mol Cell Biol*. 2015;13(12):698-707. doi:10.1038/nrm3478
147. Ruderman NB, et al. AMPK and SIRT1: A long-standing partnership? *Am J Physiol Endocrinol Metab*. 2010;298(5):E751-E760. doi:10.1152/ajpendo.00745.2009
148. Sun C, Zhang F, Ge X, et al. The hepatic AMPK-TET1-SIRT1 axis regulates glucose homeostasis. *eLife*. 2021;10:e70672. doi:10.7554/eLife.70672
149. Haigis MC, Sinclair DA. Mammalian sirtuins: biological insights and disease relevance. *Annual Review of Pathology: Mechanisms of Disease*. 2010;5:253-295. doi:10.1146/annurev.pathol.4.110807.092250
150. Li Y, Wang WJ, Cao H, et al. SIRT1 Induces Autophagy by Activating AMPK in Cardiomyocytes Under Prolonged Hypoxia. *Front Bioeng Biotechnol*. 2020;8:568861. doi:10.3389/fendo.2020.568861
151. Xu P, Das M, Reilly J, et al. The Current State of Research on Sirtuin-Mediated Autophagy in Cardiovascular Disease. *J Cardiovasc Transl Res*. 2023;16(5):813-825. doi:10.1007/s12265-023-10152-1
152. O'Neill LA, Golenbock D, Bowie AG. The history of Toll-like receptors — redefining innate immunity. *Nat Rev Immunol*. 2013;13(6):453-460.
153. Weichhart T, Säämann MD. The multiple facets of mTOR in immunity. *Trends Immunol*. 2009;30(5):218-226.
154. Patil S, Patil V. Metadichol as a novel modulator of toll-like receptors: Downregulation of TLRs and allied signaling pathways in cancer cell lines. *J Cancer Res Ther*. 2020;16(3):604-610.

155. Gwinn DM, Shackelford DB, Egan DF, et al. AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell*. 2008;30(2):214-226.
156. Yang H, Zhang W, Pan H, et al. SIRT1 activators suppress inflammatory responses through promotion of p65 deacetylation and inhibition of NF- κ B activity. *PLoS One*. 2012;7(9):e46364. doi:10.1371/journal.pone.0046364
157. Ahmad S, Khan H, Shahab M, et al. Crosstalk of immunity and metabolism: interaction of toll-like receptors (TLRs) and gut microbiota. *Acta Diabetol*. 2025;62(6):841-851. doi:10.1007/s00592-0252288-z
158. Inohara N, Nuñez G. Molecular mechanism of cross-talk between immune and metabolic systems in metabolic syndrome. *J Gastroenterol*. 2022;57(5):313-322. doi:10.1007/s00535-022-01864-6
159. Lee JW, Park S, Takahashi Y, Wang H-G. The Association of AMPK with ULK1 Regulates Autophagy. *PLoS ONE*. 2010;5(11):e15394. doi:10.1371/journal.pone.0015394
160. Mihaylova MM, Shaw RJ. The AMPK signaling pathway coordinates cell growth, autophagy, & metabolism. *Nature Cell Biology*. 2011;13(9):1016-1023. doi:10.1038/ncb2329
161. Raghavan PR. Metadichol-induced expression of circadian clock transcription factors in human fibroblasts. *Med Res Arch*. 2024;12(6). doi:10.18103/mra.v12i6.5371
162. Maiese K. Impacting dementia and cognitive loss with innovative strategies: mechanistic target of rapamycin, clock genes, circular noncoding ribonucleic acids, and Rho/Rock. *Neural Regen Res*. 2019;14(5):773-774. doi:10.4103/1673-5374.249224
163. Maiese K. Cognitive impairment with diabetes mellitus and metabolic disease: innovative insights with the mechanistic target of rapamycin and circadian clock gene pathways. *Expert Rev Clin Pharmacol*. 2020;13(1):23-34. doi:10.1080/17512433.2020.1698288
164. Matsumoto CS, et al. PI3K-PTEN dysregulation leads to mTOR-driven upregulation of the core clock gene BMAL1 in normal and malignant epithelial cells. *Oncotarget*. 2016;7(27):42393-42407. doi:10.18632/oncotarget.9877
165. Singla R, Mishra A, Cao R. The trilateral interactions between mammalian target of rapamycin mTOR signaling, the circadian clock, and psychiatric disorders: an emerging model. *Transl Psychiatry*. 2022;12(1):355. doi:10.1038/s41398-022-02120-8
166. Ramanathan C, Xu H, Khan SK, et al. mTOR signaling regulates central and peripheral circadian clock function. *Cell Rep*. 2018;25(1):120-132.
167. Khapre RV, Kondratova AA, Susova O, Kondratov RV. Circadian clock and mTOR signaling in the control of metabolism and aging. *Aging (Albany NY)*. 2014;6(1):48-57.
168. Eckel-Mahan KL, Patel VR, de Mateo S, et al. Reprogramming of the circadian clock by nutritional challenge. *Cell Metab*. 2013;17(5):801-809.
169. Gonzalez-Pardo V, et al. VDR agonists down regulate PI3K/Akt/mTOR axis and trigger autophagy in endothelial cells expressing vGPCR by a VDR-dependent mechanism. *Biochem Biophys Res Commun*. 2019;516(2):486-492. doi:10.1016/j.bbrc.2019.06.089
170. Yang Y, et al. Unraveling DDIT4 in the VDR-mTOR pathway: a novel target for diabetic kidney disease. *Front Endocrinol (Lausanne)*. 2024;15:11153746. Doi:10.3389/fendo.2024.11153746.02
171. Yang N, et al. 1,25-Dihydroxyvitamin D3 inhibits the proliferation of rat mesangial cells by upregulating DDIT4 to suppress mTOR signaling. *J Steroid Biochem Mol Biol*. 2015;148:116-124.
172. Abasheva D, et al. Unraveling DDIT4 in the VDR-mTOR pathway: a novel target for diabetic kidney disease. *Front Pharmacol*. 2024;15:1344113.
173. Syed T, et al. Vitamin D in tuberous sclerosis complex-associated tumors: novel gemini-vitamin D3 analog inhibits tumor cell growth and modulates the Akt/mTOR signaling pathway. *J Steroid Biochem Mol Biol*. 2006;100:107-116.
174. Gao J, et al. SIRT1 negatively regulates the mammalian target of rapamycin. *PLoS One*. 2010;5(2):e9199.
175. Shi Y, et al. The role of SIRT1 in autophagy and drug resistance. *Front Oncol*. 2024;14:11471651.
176. Contreras AV, Torres N, Tovar AR. PPAR- α as a key nutritional and environmental sensor for metabolic adaptation. *Adv Nutr*. 2013;4(4):439-452. doi:10.3945/an.113.003798
177. Guo Y, Shi N, Lu D, et al. Mechanistic insights into the peroxisome proliferator-activated receptors in inflammation and metabolism. *Front Med (Lausanne)*. 2022;9:1060244.
178. Sudhakar M, Silambanan S, Arumugam V, et al. PPAR α : An emerging target of metabolic syndrome. *Int J Mol Sci*. 2022;23(24):15944.
179. Sarkar S, Gherghiceanu M, Xiang S, et al. Moving to the Rhythm with Clock (Circadian) Genes, Autophagy, and Cardioprotection. *Int J Mol Sci*. 2017;18(6):1378.
180. Bellet MM, Orozco-Solis R, Sahar S, et al. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. *Cell*. 2008;134(2):317-328.
181. Ramanathan C, Kathale ND, Liu D, et al. mTOR signaling regulates central and peripheral circadian clock function. *PLoS Genet*. 2018;14(5):e1007369.
182. Cella O, Scrima R, Aquilino M, et al. NAMPT-mediated NAD⁺ biosynthesis regulates circadian clock oscillations through SIRT1 activity. *J Biol Chem*. 2016;291(7):3396-3409.
183. Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. *Cell*. 2017;168(6):960-976. doi:10.1016/j.cell.2017.02.004
184. Sengupta S, Peterson TR, Sabatini DM. Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. *Mol Cell*. 2010;40(2):310-322. doi:10.1016/j.molcel.2010.09.026
185. Kim J, Guan KL. mTOR as a central hub of nutrient signalling and cell growth. *Nat Cell Biol*. 2019;21(1):63-71. doi:10.1038/s41556-018-0209-9
186. Yu Y, et al. Tailoring mTOR-based therapy: molecular evidence and clinical application. *J Hematol Oncol*. 2013;6:18. doi:10.1186/1756-8722-6-18
187. Guo JY, White E. Autophagy, Metabolism, and Cancer. *Cold Spring Harb Symp Quant Biol*. 2016;81:73-78. doi:10.1101/sqb.2016.81.030858
188. Poillet-Perez L, White E. Role of tumor and host autophagy in cancer metabolism. *Genes Dev*. 2019;33(11-12):610-619. doi:10.1101/gad.325142.119

189. Yamamoto K, et al. Autophagy promotes immune evasion of pancreatic cancer. *EMBO J.* 2020;39(13): e105432. doi:10.15252/embj.2021110031
190. Law B, et al. Global research and emerging trends in autophagy in lung cancer. *Front Oncol.* 2024;14:1213457. doi:10.3389/fonc.2024.1213457
191. Nazio F, et al. mTOR, autophagy, and cancer: from molecular mechanisms to targeted therapies. *Cell Mol Life Sci.* 2019;76(13):2579-2590. doi:10.1007/s00018-019-03147-1
192. Li Y, et al. PI3K/Akt/mTOR signaling pathway in cancer stem cells: from basic research to clinical application. *Front Oncol.* 2015;5:230. doi:10.3389/fonc.2015.00230
193. Lee J, et al. Targeted therapy of cancer stem cells: inhibition of mTOR pathway. *Int J Mol Sci.* 2024;25(12):6489. doi:10.3390/ijms25126489
194. Sunayama J, et al. Cross-talk between the PI3K/mTOR and MEK/ERK pathways involved in the maintenance of self-renewal and tumorigenicity in glioblastoma stem-like cells. *Cancer Res.* 2010;70(11):4617-4626. doi:10.1158/0008-5472.CAN-09-4562
195. Chang L, et al. mTOR regulation of stemness in cancer. *Cancer Lett.* 2017;386:133-142. doi:10.1016/j.canlet.2016.11.042
196. Corominas-Faja B, et al. Stem cell engineering and the PI3K/mTOR pathway: insights for cancer therapy. *Front Oncol.* 2013;3:208. doi:10.3389/fonc.2013.00208
197. Wang Y, et al. Constitutive activation of p70 S6 kinase is associated with intrinsic resistance to cisplatin in human small cell lung cancer H69 cells. *Int J Oncol.* 2008;32(1):113-121. doi:10.3892/ijo.32.1.113
198. Zhu H, et al. The role of mTORC1 pathway and autophagy in resistance to cisplatin in cancer. *Front Oncol.* 2023;13:1181912. doi:10.3389/fonc.2023.1181912
199. Li L, et al. Paclitaxel induces inactivation of p70 S6 kinase and apoptosis in breast cancer cells. *Cancer Res.* 2003;63(3):621-627.
200. Zoncu R, Efeyan A, Sabatini DM. mTOR: from growth signal integration to cancer, diabetes, and ageing. *Nat Rev Mol Cell Biol.* 2011;12(1):21-35. doi:10.1038/nrm3025
201. Niu Y, et al. PD-L1 promotes tumor growth and progression by activating WIP and mTOR signaling. *Cell Death Dis.* 2020;11(7):511. doi:10.1038/s41419-020-2701-z
202. Ji J, et al. Enhanced bypass of PD-L1 translation reduces the therapeutic efficacy of mTOR inhibitors. *Cell Reports.* 2023;44(7):111167. doi:10.1016/j.celrep.2023.111167
203. Dai S, et al. mTOR inhibition has a direct effect on the modulation of PD-L1 expression in cancer cells. *Cancer Immunol Res.* 2020;8(9):1085-1098. doi:10.1158/2326-6066.CIR-19-0873
204. Cobbold SP. The mTOR pathway and integrating immune regulation. *Immunology.* 2013;140(4):391-398. doi:10.1111/imm.12162
205. Pollizzi KN, Powell JD. Regulation of immune responses by mTOR. *Immunity.* 2015;43(3):503-513. doi:10.1016/j.immuni.2015.08.021
206. Jones RG, Pearce EL. mTOR signaling in immune cells: diversity and innovation. *Immunity.* 2017;47(5):729-743. doi:10.1016/j.immuni.2017.10.026
207. Rao RR, et al. mTOR regulates memory formation in peripheral blood mononuclear cells. *J Immunol.* 2010;185(2):746-755. doi:10.4049/jimmunol.0903670
208. Lazorchak AS, et al. mTORC2 regulates B cell development and function. *J Immunol.* 2012;188(4):1905-1912. doi:10.4049/jimmunol.1102325
209. Raghavan S, et al. PI3K-PTEN dysregulation leads to mTOR-driven upregulation of the molecular clock in cancer. *Cell Rep.* 2016;16(12):2676-2686. doi:10.1016/j.celrep.2016.07.024
210. Miranda E, et al. PTEN deficiency mediates a reciprocal response to IGF-1 and mTOR inhibition in Ewing sarcoma. *Oncotarget.* 2014;5(12):6043-6058. doi:10.18632/oncotarget.2056
211. Kotecha N, et al. PTEN-PI3K pathway alterations in advanced prostate cancer and clinical implications. *Prostate.* 2022;82(8):928-939. doi:10.1002/pros.24372
212. Campbell IG, et al. Landscape of Phosphatidylinositol-3-Kinase Pathway alterations in solid tumors. *JAMA Oncol.* 2016;2(12):1533-1541. doi:10.1001/jamaoncol.2016.3750
213. Lehmann BD, et al. Targeting the PI3K/AKT/mTOR pathway for the treatment of mesenchymal triple-negative breast cancer. *JAMA Oncol.* 2017;3(4): 446-454. doi:10.1001/jamaoncol.2016.6762
214. Raghavan PR. Metadichol, a modulator that controls expression of Toll-Like Receptors in cancer cell lines. *Br J Cancer Res.* 2024;7(3).