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The TSC1-2 tumor suppressor controls insulin-PI3K signaling through the regulation of IRS proteins - Evidence review

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1. ABSTRACT

The analyzed study examined how the loss of the TSC1-TSC2 complex, a physiological regulator of cell growth, modifies the response to insulin and IGF-1 in murine embryonic fibroblasts. The authors observed that the absence of TSC2 keeps the mTOR-S6K pathway excessively active and reduces both the quantity and functional capacity of IRS proteins, which are essential for transferring the signal from the insulin receptor to PI3K.This evidence contributes to the narrative of "The Choice to Live in Health or Illness" by Dr. A. Colonnese and R. Panzironi: a physiological feedback mediated by S6K1 can become unfavorable when it remains persistently active. Independent studies confirm the link in cells, animal models, and healthy volunteers experimentally exposed to high concentrations of amino acids. However, genetic research in mice indicates that the single IRS-1 Ser302 site is not enough to explain insulin resistance on its own. The general model is therefore well-supported at a mechanistic level, but not every molecular detail is universally valid in all tissues.

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

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2. ANALYZED STUDY

The research question was whether the TSC1-TSC2 complex participates only in the control of cell growth or if it is also necessary for insulin and insulin-like growth factor 1 (IGF-1) to correctly activate PI3K, a protein that transmits metabolic and survival signals within the cell. The hypothesis was that the loss of TSC2 would stably increase S6K activity and, through this increase, make IRS-1 and IRS-2 proteins—the adapters that connect the insulin receptor to PI3K—less effective.The study is relevant to sections 4.8 and 14.10 of "The Choice to Live in Health or Illness" by Dr. A. Colonnese and R. Panzironi because it documents the cellular segment of the chain: loss of TSC1-TSC2 control excess mTOR-S6K signal reduced IRS function lower activation of PI3K and AKT. It does not evaluate people with type 2 diabetes and does not directly measure the insulin sensitivity of the entire organism. Instead, it demonstrates, in a murine cell model, that the persistent hyperactivation of this pathway can hinder the initial transmission of the insulin signal.

3. MATERIALS AND METHODS OF THE ORIGINAL STUDY

The authors compared mouse embryonic fibroblasts (MEFs, cells obtained from murine embryos) with functional TSC2 and MEFs lacking TSC2. They also reintroduced a normal copy of the gene or a pathological variant into the TSC2-deficient cells, creating very closely matched genetic comparisons. The cells were serum-starved and then exposed for 10 minutes to insulin, IGF-1, or epidermal growth factor (EGF).The response of the insulin pathway was evaluated by measuring the activity of PI3K, the signaling lipid PIP3, and the phosphorylation of AKT/PKB and GSK3. Phosphorylation is a chemical modification that can switch a protein's function on or off. To study IRS-1 and IRS-2, microarrays, Northern blots, quantitative PCR, Western blots, immunoprecipitation, and protein-protein binding assays were used. The authors inhibited mTOR with 20 nM rapamycin, silenced S6K1 and S6K2 using small interfering RNAs, and artificially increased the expression of IRS-1 or IRS-2.To verify functional effects, chemotaxis assays—that is, cell migration directed by a signal—and survival assays after serum deprivation were conducted. Many measurements were repeated in triplicate; some experiments report three independent experiments. The total number of biologically independent cell lines and the overall statistical plan are not systematically reported in the available material.

4. RESULTS

The main finding was that cells lacking TSC2 responded poorly to insulin and IGF-1, while maintaining a marked response to EGF. In other words, the defect was not a generic inability of the cell to receive growth signals: it selectively concerned the pathway that normally passes through IRS and PI3K. After stimulation with IGF-1, TSC2-deficient MEFs did not increase PI3K activity or PIP3, whereas PIP3 production induced by EGF remained similar to that of control cells.Cells without TSC2 showed a reduction in IRS-1 messenger RNA and protein, with no detected decrease in IRS-2, the insulin receptor, or the IGF-1 receptor. Rapamycin for approximately 24 hours restored IRS-1 messenger RNA abundance; the addition of actinomycin D, which blocks gene transcription, nullified this recovery. Silencing of S6K1 or S6K2 increased IRS-1 RNA again, suggesting that both kinases contribute to the repression of its production.A more rapid effect concerned the function of the protein already present. In cells lacking TSC2, IRS-1 and IRS-2 showed a profile consistent with increased phosphorylation. After one hour of rapamycin, before the amount of IRS-1 increased significantly, tyrosine phosphorylation of IRS-1 and its binding to the p85 subunit of PI3K improved. In parallel, AKT only partially recovered its activation; after 24 hours of rapamycin, the recovery was complete in the experimental model.Biochemical experiments indicated that S6K can phosphorylate IRS-1 at the murine Ser302 residue. Phosphorylation of this site was increased in cells lacking TSC2, decreased with rapamycin, and was reduced primarily after S6K1 silencing. In vitro, phosphorylation of the IRS-1 domain that interacts with the insulin receptor reduced binding to the activated receptor. This demonstrates a possible direct mechanism for the reduction of IRS-1 function, but an in vitro assay alone does not prove that the same single site is decisive in all tissues of an organism.Finally, TSC2-negative cells showed reduced migration toward IGF-1 and derived

little benefit from IGF-1 during serum deprivation. Prolonged pretreatment with rapamycin restored IGF-1-induced migration. These data link reduced PI3K-AKT activation to measurable cellular consequences, without directly demonstrating metabolic disease in humans.

5. INTERPRETATION

In simple terms, the study describes an automatic brake on insulin signaling. When mTOR-S6K remains highly active, IRS-1 becomes less available and less capable of binding to the insulin receptor. Consequently, PI3K produces less PIP3, and AKT is activated less effectively. The receptor may therefore be present and bind insulin, but the message is transmitted with lower efficiency.The directly documented segment is: hyperactivation induced by the loss of TSC2 increased S6K activity reduced IRS-1 transcription and inhibitory phosphorylation of IRS-1/IRS-2 reduced IRS-PI3K-AKT signaling. This corresponds to the section of the book that attributes to S6K1 a negative control that is both acute, through phosphorylation, and more prolonged, through a decrease in IRS availability.However, the significance for insulin resistance must be qualified. TSC2 was genetically deleted in murine embryonic fibroblasts, an experimental condition more extreme than common chronic nutrient exposure in humans. Furthermore, rapamycin is useful for investigating the pathway, but it does not prove that chronic pharmacological inhibition of mTORC1 necessarily improves human metabolism: mTOR is also involved in other processes, and effects can vary between tissues and the duration of treatment.

6. INDEPENDENT CONFIRMING EVIDENCE

Confirmation in humans comes from a crossover study conducted on seven healthy men, who were randomly subjected to an infusion of amino acids or saline solution during an insulin clamp. The increase in amino acids amplified the insulin-induced muscle activation of S6K1 from approximately 1.9 to approximately 3.7 times compared to baseline. In the same context, inhibitory phosphorylations of IRS-1 increased, and PI3K activity associated with IRS-1 was reduced. Whole-body glucose disappearance was approximately 33% lower during the amino acid infusion, 50 ± 4 versus 75 ± 6 micromoles per kilogram per minute, indicating a reduced insulin response. This data confirms the nutrient S6K1 IRS-1/PI3K link in humans, but the small sample size and acute exposure are not equivalent to chronic type 2 diabetes [1].A second independent cellular study specifically verified the quantitative part of the model. In mammalian cells lacking TSC1 or TSC2, or with overexpressed Rheb, continuous activation of the Rheb-mTOR-S6K axis reduced IRS-1 and IRS-2. For this reason, insulin and IGF-1 were no longer able to activate AKT, while a signal independent of IRS proteins, activated by PDGF, remained effective. This reinforces the interpretation that the defect is located primarily between the receptor and PI3K, rather than in every component of the cell. This remains a mechanistic confirmation obtained in cell cultures, not a clinical demonstration [2].An animal model added pathophysiological coherence. Mice lacking S6K1 were protected against age-related and high-fat diet-induced obesity and maintained greater insulin sensitivity compared to controls. In normal mice subjected to a high-fat diet, and in two genetic models of obesity, S6K1 activity and IRS-1 phosphorylation at sites Ser307 and Ser636/639 increased; these increases did not appear in mice lacking S6K1. The work therefore supports the idea that S6K1 causally contributes to the negative feedback on IRS-1 in mice, but the global metabolic modifications of the knockout animals prevent attributing every effect to a single cellular mechanism [3].

7. CONFLICTING EVIDENCE

An important piece of contextual evidence concerns the Ser302 residue of IRS-1, identified in the original study as a possible target of S6K1. In mice genetically modified so that IRS-1 could not be phosphorylated at Ser302, glucose homeostasis and muscle insulin signaling were similar to those of the controls. Furthermore, the acute deletion of Tsc1 in the liver moderately reduced the IRS-1/PI3K association and AKT phosphorylation, but did not cause glucose intolerance or hyperinsulinemia. The authors therefore concluded that, in the murine liver, mTORC1-S6K can influence AKT even through mechanisms that are largely independent of IRS-1 Ser302 [4].This evidence does not refute the general mTOR-S6K-IRS circuit observed in the original study. However, it indicates that Ser302 is not necessarily the

sole, or dominant, determinant of physiological insulin sensitivity. The feedback may depend on the combination of multiple phosphorylation sites, the total amount of IRS-1 and IRS-2, the tissue examined, the duration of mTORC1 activation, and other signaling pathways. Therefore, no directly conflicting evidence sufficiently relevant to the general direction of the negative feedback has been identified; instead, there is a significant discrepancy regarding the universal centrality of the single Ser302 site.

8. QUADRO COMPARATIVO E SINTESI DELLE EVIDENZE

The table distinguishes the cellular study that is the subject of the Evidence Review from independent publications that confirm or define the same narrative.

Quadro comparativo

Reference	Study type	Population or model	Main result	Direction of evidence	Strength of evidence
Original study	Cellular and molecular	Mouse embryonic fibroblasts with or without TSC2	Loss of TSC2 increases mTOR-S6K signaling, reduces IRS-1, and impairs insulin-stimulated PI3K and AKT activation.	direct confirmation	moderate
[1]	Human metabolic crossover intervention	Seven healthy men undergoing amino acid or saline infusion	Amino acids increase S6K1 and inhibitory phosphorylations of IRS-1, reduce PI3K, and decrease glucose disappearance by 33%.	direct confirmation	moderate
[2]	Cellular and molecular	Mammalian cells lacking TSC1 or TSC2 or with overexpressed Rheb	Constitutive Rheb-mTOR-S6K activation reduces IRS-1 and IRS-2 and blocks AKT activation by insulin and IGF-1.	direct confirmation	moderate
[3]	Genetic animal	S6K1 knockout mice and mouse models of obesity	Absence of S6K1 preserves insulin sensitivity and prevents the increase of certain inhibitory IRS-1 phosphorylations.	indirect confirmation	moderate
[4]	Genetic animal and primary cells	IRS-1 Ser302A knock-in mice, mouse liver and hepatocytes	Blocking Ser302 does not alter physiological insulin sensitivity; hepatic effects of Tsc1 do not primarily depend on this site.	discordant evidence	moderate

9. SINTESI EDITORIALE E CONCLUSIONI

CONCLUSIONS

The study analyzed provides a well-structured cellular demonstration that mTOR-S6K hyperactivation can weaken insulin signaling by acting on IRS proteins. The mechanism includes a rapid effect on IRS-1 function and a slower effect on its availability.

Independent evidence confirms the same direction of the phenomenon in cellular and animal models, as well as in a small metabolic experiment in humans. The link between persistent S6K1 activation, reduced IRS-PI3K function, and lower insulin response is therefore well-supported. However, it remains necessary to distinguish the general circuit from individual phosphorylation sites and potential differences between the liver, muscle, adipose tissue, and clinical contexts.

Aggiornato al 24/11/2026. Questo contenuto riflette una sintesi divulgativa delle evidenze scientifiche al momento della pubblicazione e non sostituisce il parere medico.

10. TRANSPARENCY AND SCIENTIFIC RESPONSIBILITY

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

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

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

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