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Nup358 limits connectivity between the endoplasmic reticulum and mitochondria by modulating mTORC2/Akt/GSK3b signaling - A review of the evidence

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1. ABSTRACT

The analyzed study examined how Nup358, a protein primarily known for its role in nuclear pore complexes, contributes to regulating contact sites between the endoplasmic reticulum and mitochondria. The authors primarily used human cell lines, combining genetic silencing, microscopy, biochemical analysis, and experimental rescue of protein function. The reduction of Nup358 increased contacts between the two organelles, enhanced the activation of mTORC2 and AKT, and increased the inhibitory phosphorylation of GSK-3b.In section 4.12, “The Choice to Live in Health or Illness” by Dr. A. Colonnese and R. Panzironi, this research is cited to argue that the mTORC2-AKT-GSK-3b axis also participates in the physical organization of the cell and communication between organelles. Independent studies confirm that mTORC2-AKT acts at endoplasmic reticulum-mitochondria contact sites, that the VAPB-PTPIP51 complex influences calcium transfer, and that GSK-3b can reduce such contacts. The convergence is mechanistic and cellular; however, direct demonstrations in humans and clinical evidence regarding the consequences of this regulation are still lacking.

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2. ANALYZED STUDY

The research question was whether Nup358 regulates contact sites between the endoplasmic reticulum and mitochondria. These contacts are not fusions between organelles: they are zones where the two membranes remain very close and can exchange signals, especially calcium and lipids. The question is relevant because the distance and number of these points influence energy metabolism, the stress response, and certain forms of cell death.The authors' hypothesis was that Nup358 acts as a brake on the mTORC2/AKT/GSK-3b pathway. In simple terms, Nup358 could prevent endoplasmic reticulum-mitochondria contacts from increasing excessively when growth signals, such as those produced by insulin, are received. At the molecular level, the study proposes that Nup358 interacts with the VAPB-PTPIP51 complex, a protein bridge between the endoplasmic reticulum and mitochondria, limiting the activation of mTORC2 and AKT and keeping GSK-3b more active.This is the precise reason why the publication contributes to section 4.12 of "The Choice to Live in Health or Illness" by Dr. A. Colonnese and R. Panzironi: it documents a segment of the chain according to which mTORC2 and AKT, through the inhibition of GSK-3b, can modify intracellular architecture and communication between organelles. However, the study does not demonstrate that this mechanism produces clinical benefits or harm in humans, nor that it alone explains all the metabolic consequences attributed to contacts between organelles.

3. MATERIALS AND METHODS OF THE ORIGINAL STUDY

This is a preclinical experimental study, conducted primarily in human HeLa, HEK293T, U2OS, and Huh7 cells cultured in the laboratory. The authors reduced Nup358 using small interfering RNAs—molecules capable of selectively decreasing the production of a protein—or via CRISPR-Cas9 knockout, a gene-editing technique. They also reintroduced silencing-resistant Nup358 and expressed protein fragments or GSK-3b to verify whether the observed phenomena could be reversed.Contacts between the endoplasmic reticulum and mitochondria were evaluated using several complementary methods: fluorescence microscopy with overlap coefficients, proximity ligation assay or PLA (a method that detects proteins in close proximity within the cell), co-immunoprecipitation (a technique that verifies whether proteins belonging to the same complex are isolated together), cell fractionation, and transmission electron microscopy. For electron microscopy, the authors considered a distance of 30 nanometers or less between membranes as a contact.Signaling was evaluated primarily by measuring the phosphorylation of AKT at serine 473, an indicator of mTORC2 activity, and the phosphorylation of GSK-3b at serine 9—a modification generally associated with its inactivation—using western blot. Insulin and EGF (epidermal growth factor) were used to activate growth signals. Silencing with interfering RNA was performed at 40 nM for 72 hours. Quantitative analyses utilized at least three independent experiments for many biochemical assays; for several microscopy experiments, approximately 58 to 131 cells were analyzed per condition. Exact P-values are not reported in the provided text, which does, however, indicate that the authors used two-tailed t-tests and considered $P \leq 0.05$ to be significant.The study also included an experiment in *Drosophila* fruit flies, in which the reduction of Nup358 in the nervous system was associated with an increase in AKT phosphorylation. However, no functional, behavioral, or clinical outcomes in the animals linked to the endoplasmic reticulum-mitochondria contacts are reported.

4. RESULTS

The first result indicates that a portion of the structures containing Nup358 is localized near the mitochondria. In confocal microscopy, approximately 40% of the Nup358-positive annulate lamellae structures were associated with contact sites between the endoplasmic reticulum and mitochondria; in a quantification of the main figure, the estimate was 42.8 ± 6.6% in 20 cells. Nup358, along with other nucleoporins, was also detected in the cellular fraction enriched in mitochondria-associated membranes.Reducing Nup358 increased the proximity between the endoplasmic reticulum and mitochondria according to four different readings. There was an increase in the overlap of fluorescent markers for the two organelles, an increase in the VAPB-PTPIP51 interaction measured via co-immunoprecipitation and

PLA, and an increase in the portion of the mitochondrial perimeter located within 30 nanometers of the reticulum in electron microscopy. The main result, therefore, is not just a variation in a marker: multiple methods indicate an increase in physical contacts.In parallel, the loss of Nup358 increased AKT phosphorylation at serine 473, which is consistent with increased mTORC2 activity. The same signal was observed both after silencing and in knockout cells. The reintroduction of Nup358 reduced this increase. The reduction of Nup358 in the *Drosophila* brain was also associated with an increase in AKT phosphorylation, suggesting that the regulation may be conserved, although it does not prove that the mechanism is identical in all tissues.Rescue experiments are central to the interpretation. The simultaneous reduction of Rictor, a specific component of mTORC2, reduced the increase in reticulum-mitochondria contacts induced by the loss of Nup358. Conversely, the reduction of Raptor, a component of mTORC1, did not produce the same recovery. This indicates that, in the cell model studied, the increase in contacts depends primarily on mTORC2 and not on mTORC1.The loss of Nup358 also increased the inhibitory phosphorylation of GSK-3b at serine 9. The concomitant reduction of Rictor attenuated this modification. Furthermore, the additional expression of GSK-3b reduced the increase in VAPB-PTPIP51 signals observed in cells lacking Nup358. In practical terms, when Nup358 decreases, the mTORC2-AKT pathway becomes more active, GSK-3b becomes less active, and the contacts between the two organelles increase.Finally, the authors observed that insulin and other growth signals decrease the association between Nup358 and VAPB, while increasing the association of VAPB with mTOR, Rictor, and Sin1, another component of mTORC2. The VAPB-PTPIP51 complex was found to be necessary for the full activation of mTORC2-AKT induced by insulin in the cells used. These data are consistent with a model in which Nup358 and mTORC2 compete or alternate in their interaction with the VAPB-PTPIP51 bridge; however, the directly competitive nature of this relationship has not been definitively proven.

5. INTERPRETATION

The biological significance of this work is that the insulin and growth factor pathway does not act solely on metabolism, growth, and cell survival: it can also modify how different organelles communicate with each other. The endoplasmic reticulum and mitochondria must remain close enough to transfer useful signals, but not necessarily as close as possible. Excessive increase or reduction in these contacts can, in fact, alter calcium transfer, the energy produced by the mitochondria, and the stress response.The segment directly supported by the publication is: decrease in Nup358 increased mTORC2/AKT activity increased inhibitory phosphorylation of GSK-3b increase in endoplasmic reticulum-mitochondria contacts. This finding is reinforced by rescue experiments with Rictor and GSK-3b, which link the alteration of Nup358 to the subsequent steps in the chain. The interaction with VAPB-PTPIP51 also provides possible structural support for the model.The research does not demonstrate that Nup358 controls these processes in an identical manner in human neurons, muscle, liver, or other organs. It does not directly measure, under the main experimental conditions, calcium transfer, mitochondrial respiration, or long-term consequences on cellular health. Nor does it prove that the observed increase in contacts is always favorable: the function of endoplasmic reticulum-mitochondria contacts depends on the context, duration, and intensity of the signal.For the narrative of “The Choice to Live in Health or Illness” by Dr. A. Colonnese and R. Panzironi, the value of the study is therefore mechanistic. It expands the function of the mTORC2-AKT-GSK-3b axis by showing that this sequence can regulate the physical arrangement of cellular structures and, indirectly, processes such as calcium homeostasis and energy metabolism. However, it does not authorize the transformation of this cellular observation into a clinical or therapeutic conclusion.

6. INDEPENDENT CONFIRMATORY EVIDENCE

Initial independent confirmation concerns the link between mTORC2-AKT and endoplasmic reticulum-mitochondria contact sites. Betz and

colleagues studied mammalian cells and mouse embryonic fibroblasts lacking Rictor. They detected mTORC2 in mitochondria-associated membranes following stimulation with growth factors and observed that mTORC2 deficiency impairs the integrity of these contacts. AKT phosphorylated proteins localized at this site, including IP3R, PACS2, and hexokinase 2, which are involved in calcium regulation, contact organization, and glucose metabolism, respectively. The study directly confirms that mTORC2-AKT is part of a signaling platform at the endoplasmic reticulum-mitochondria contact sites, although it utilizes a different set of bridging proteins than VAPB-PTPIP51 and does not evaluate Nup358 [1].A second step in the narrative concerns the function of the VAPB-PTPIP51 bridge. De Vos and colleagues, using human cell models, demonstrated that VAPB, an endoplasmic reticulum protein, interacts with PTPIP51, a protein on the outer mitochondrial membrane. The reduction of either protein decreased and delayed the entry of calcium into the mitochondria following the release of calcium from endoplasmic reticulum stores. This result supports the idea that physical communication between the two organelles can have functional consequences on calcium transfer; however, it does not prove that every variation in the number of contacts necessarily produces the same effect in every cell [2].Stoica and colleagues subsequently linked GSK-3b more directly to the stability of the VAPB-PTPIP51 bridge. In NSC34 and HEK293 neuronal cells, pharmacological inhibition of GSK-3b increased the VAPB-PTPIP51 interaction and endoplasmic reticulum-mitochondria contacts, whereas overexpression of GSK-3b reduced the binding between the two proteins. In a cellular and mouse model of TDP-43 alteration, GSK-3b activation was associated with a reduction in contacts, lower mitochondrial calcium, and higher cytosolic calcium following release from the endoplasmic reticulum. This evidence confirms the direction proposed in the analyzed study: more active GSK-3b tends to reduce the stability of the VAPB-PTPIP51 bridge [3].Further research in the same field, but independent of the publication under review, studied cellular models of FUS, a protein associated with amyotrophic lateral sclerosis and frontotemporal dementia. FUS expression activated GSK-3b and reduced both the VAPB-PTPIP51 interaction and the contacts between the endoplasmic reticulum and mitochondria. The authors also detected a reduction in mitochondrial calcium uptake and ATP production. This work reinforces the biological consistency of the sequence: active GSK-3b reduced endoplasmic reticulum-mitochondria connection alterations in calcium-energy communication. The limitation is that these are experimental models of neurodegenerative disease, not general human physiology [4].

7. CONFLICTING EVIDENCE

No directly conflicting evidence of sufficient relevance has been identified; this does not prove the absence of contradictory results.However, there are elements that call for caution. In the study on mTORC2 in mitochondria-associated membranes, the loss of mTORC2 altered the contacts and was accompanied by an increase in mitochondrial membrane potential, ATP production, and calcium entry into the mitochondria [1]. This shows that the relationship between the number of contacts and mitochondrial function is not linear. Contacts may be more numerous but not necessarily more efficient, or less numerous but associated with compensatory responses.Furthermore, the available studies use different bridging proteins, different cell lines, and heterogeneous experimental conditions. The analyzed work focuses primarily on the VAPB-PTPIP51 and Nup358 complex; the study on mTORC2 also evaluates the IP3R-GRP75-VDAC1 complex. These data are consistent with the idea that multiple contact platforms exist, each regulated in a partially distinct manner. Consequently, it cannot be inferred that the same direction of effect applies to all tissues, all diseases, or every type of endoplasmic reticulum-mitochondria contact.

8. QUADRO COMPARATIVO E SINTESI DELLE EVIDENZE

The table distinguishes the publication under review from the independent evidence used to verify the individual steps of the narrative.

Quadro comparativo

Reference	Study type	Population or model	Main result	Direction of evidence	Strength of evidence
Original study	Cellular and experimental animal	Human HeLa, HEK293T, U2OS, and Huh7 cells; Drosophila brain	Reduction of Nup358 increases mTORC2/AKT, inhibits GSK-3b, and increases endoplasmic reticulum-mitochondria contacts.	biologically consistent evidence	moderate
[1]	Cellular and experimental animal	Mammalian cells and Rictor-deficient mouse embryonic fibroblasts	mTORC2-AKT localized in mitochondria-associated membranes regulates contact integrity, calcium, and mitochondrial metabolism.	direct confirmation	moderate
[2]	Cellular	HEK293 and CV1 cells	VAPB and PTPIP51 form an endoplasmic reticulum-mitochondria bridge; their reduction decreases and delays calcium entry into mitochondria.	indirect confirmation	moderate
[3]	Cellular and experimental animal	NSC34 and HEK293 cells; TDP-43 transgenic mice	GSK-3b activation reduces VAPB-PTPIP51 binding and endoplasmic reticulum-mitochondria contacts; GSK-3b inhibition produces the opposite effect.	direct confirmation	moderate
[4]	Cellular	Cells with ALS/FTD-associated FUS expression	FUS activates GSK-3b and reduces VAPB-PTPIP51, endoplasmic reticulum-mitochondria contacts, mitochondrial calcium entry, and ATP production.	indirect confirmation	moderate

9. SINTESI EDITORIALE E CONCLUSIONI

CONCLUSIONS

The analyzed study convincingly supports, within its experimental model, that Nup358 acts as a negative regulator of the contacts between the endoplasmic reticulum and mitochondria through the mTORC2-AKT-GSK-3b axis. The most relevant point for the reader is that growth factor signaling pathways can influence not only protein activity but also the arrangement and physical communication between organelles.Independent evidence confirms that mTORC2-AKT is active in mitochondria-associated membranes, that VAPB-PTPIP51 contributes to calcium exchange, and that active GSK-3b can reduce the stability of this bridge. The narrative is therefore well-supported at a mechanistic level. However, it requires validation in human tissues, more complex physiological studies, and research to define whether an increase or reduction in these contacts is beneficial or harmful under different biological conditions.

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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