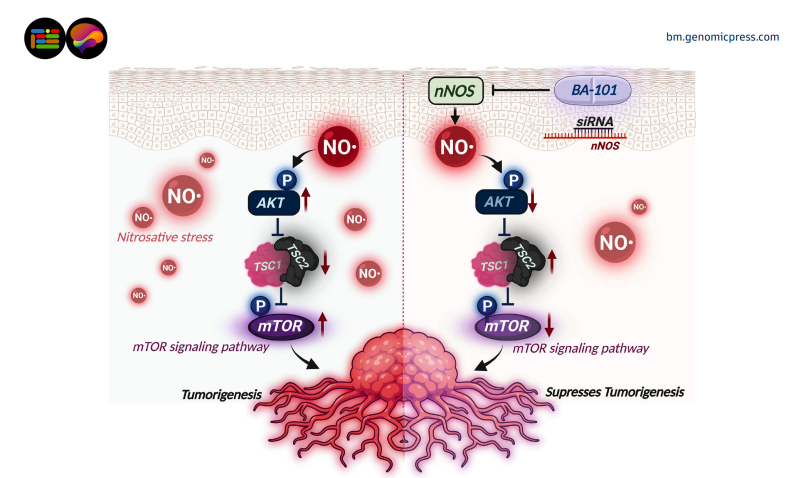
New research identifies enzyme driving neuroblastoma and shows inhibitor collapsed tumor growth in mice

Neuronal nitric oxide synthase sustains neuroblastoma through the mTOR pathway, blocking it shrank tumors dramatically in a mouse model.



Schematic model illustrating the NO-mTOR signaling axis in neuroblastoma. Under basal/pathological conditions (left panel), and nNOS inhibition (right panel).

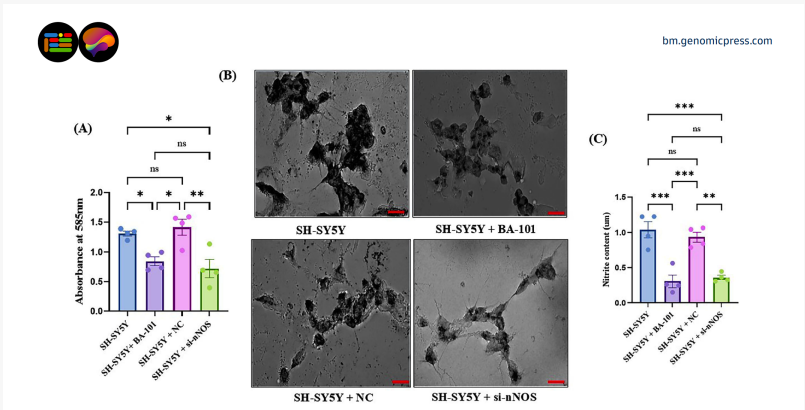
JERUSALEM, ISRAEL, April 7, 2026 /EINPresswire.com/ -- The tumor begins before birth. Somewhere in the developing fetus, neural crest cells that should have matured into adrenal tissue or sympathetic ganglia take a wrong turn, and a child is born harboring a malignancy that may not declare itself for months. Neuroblastoma accounts for roughly 28 percent of all cancers diagnosed in infants across Europe and the United States. In its gentlest form it regresses on its own, a fire that puts itself out. In its cruelest, it metastasizes with a velocity that makes oncologists reach for words they would rather not use in front of parents. High-risk neuroblastoma carries a five-year survival rate of approximately 40 percent. That number has barely moved in a generation.

Now, a study published in the peer-reviewed journal Brain Medicine (Genomic Press) offers something that has been in short supply: a mechanistic explanation for how this cancer sustains itself, and a way to cut the wire.

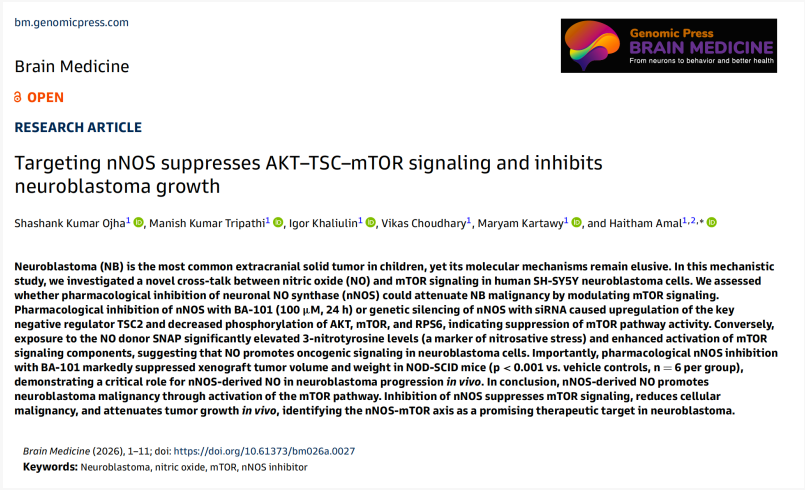
Nitric oxide is among the most ancient signaling molecules in biology. It dilates blood vessels. It carries messages between neurons. At physiological concentrations it is indispensable, a quiet civil servant. But at elevated concentrations it becomes reactive, generating nitrogen species that chemically modify proteins through a process called S-nitrosylation -- a chemical tagging of proteins that has been implicated in every stage of cancer progression.

The relationship between nitric oxide and tumors is not simple. Very high concentrations can

damage DNA and trigger apoptosis, the process by which cells self-destruct. Lower, sustained levels appear to do the opposite, promoting survival and metastasis. Prof. Haitham Amal and colleagues at the Hebrew University of Jerusalem had previously demonstrated that nitric oxide drives glioblastoma progression. The question that remained was whether the same enzyme, neuronal nitric oxide synthase (nNOS), was performing a similar service for neuroblastoma, and if so, through which downstream pathway.



Effects of the BA-101 treatment and nNOS silencing on the nNOS activity and NO levels in SH-SY5Y cells.



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RESEARCH ARTICLE

Targeting nNOS suppresses AKT–TSC–mTOR signaling and inhibits neuroblastoma growth

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Neuroblastoma (NB) is the most common extracranial solid tumor in children, yet its molecular mechanisms remain elusive. In this mechanistic study, we investigated a novel cross-talk between nitric oxide (NO) and mTOR signaling in human SH-SY5Y neuroblastoma cells. We assessed whether pharmacological inhibition of neuronal NO synthase (nNOS) could attenuate NB malignancy by modulating mTOR signaling. Pharmacological inhibition of nNOS with BA-101 (100 μM, 24 h) or genetic silencing of nNOS with siRNA caused upregulation of the key negative regulator TSC2 and decreased phosphorylation of AKT, mTOR, and RPS6, indicating suppression of mTOR pathway activity. Conversely, exposure to the NO donor SNAP significantly elevated 3-nitrotyrosine levels (a marker of nitrosative stress) and enhanced activation of mTOR signaling components, suggesting that NO promotes oncogenic signaling in neuroblastoma cells. Importantly, pharmacological nNOS inhibition with BA-101 markedly suppressed xenograft tumor volume and weight in NOD-SCID mice ($p < 0.001$ vs. vehicle controls, $n = 6$ per group), demonstrating a critical role for nNOS-derived NO in neuroblastoma progression *in vivo*. In conclusion, nNOS-derived NO promotes neuroblastoma malignancy through activation of the mTOR pathway. Inhibition of nNOS suppresses mTOR signaling, reduces cellular malignancy, and attenuates tumor growth *in vivo*, identifying the nNOS-mTOR axis as a promising therapeutic target in neuroblastoma.

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Keywords: Neuroblastoma, nitric oxide, mTOR, nNOS inhibitor

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The answer turned out to be mTOR, a master regulator of cell growth and metabolism.

The team attacked nNOS from two directions. They treated human SH-SY5Y neuroblastoma cells with BA-101, a selective pharmacological inhibitor, at 100 micromolar for 24 hours. Separately, they silenced the nNOS gene with small interfering RNA (siRNA), a molecular tool that switches off a specific gene. The logic was deliberate: if a drug and a genetic tool produce the same result, you are looking at biology, not pharmacological noise.

They produced the same result.

BA-101 reduced NADPH-diaphorase activity, the standard readout of NOS function, by 35 to 40 percent. Genetic silencing cut it by 45 to 50 percent. Nitrite levels, a stable proxy for nitric oxide production, fell 65 to 70 percent with BA-101 and 55 to 60 percent with siRNA. Colony formation, the most direct measure of proliferative capacity, dropped significantly after both BA-101 treatment ($p < 0.001$) and nNOS silencing ($p < 0.01$). The cells were losing their ability to multiply.

What followed downstream was systematic. Protein tyrosine nitration, measured by 3-nitrotyrosine immunoreactivity, fell sharply after BA-101 treatment ($p < 0.01$) and nNOS silencing ($p < 0.001$). The chemical signature of nitrosative stress was fading.

Then the dominoes. AKT phosphorylation decreased ($p < 0.01$ with BA-101; $p < 0.05$ with siRNA), while total AKT remained unchanged. Phosphorylation of mTOR itself declined under both

> The consistency of the neuroblastoma results across every assay points to nNOS as something more than a contributor. It appears to be a central driver."
>
> *Professor Haitham Amal, Hebrew University of Jerusalem*

conditions ($p < 0.01$ each). The downstream mTORC1 substrate ribosomal protein S6 followed ($p < 0.05$ with BA-101; $p < 0.01$ with siRNA). And here was the most telling detail: TSC2, a master negative regulator of mTOR signaling, rose significantly under both treatments ($p < 0.05$). Removing the nitric oxide signal had allowed the cell's own braking system to reengage, like releasing a foot from an accelerator that someone had wired to the floor.

Synaptophysin, a neuroendocrine tumor marker used to gauge the malignant identity of neuroblastoma cells, decreased significantly with BA-101 ($p < 0.01$) and nNOS knockdown ($p < 0.05$). The tumor cells were not merely growing more slowly. They were becoming, at a molecular level, less recognizably cancerous.

Good science asks the question in reverse. If blocking nitric oxide suppresses mTOR signaling, then flooding the cell with nitric oxide should amplify it. The researchers exposed SH-SY5Y cells to SNAP, a nitric oxide donor, at 200 micromolar for 24 hours. Every needle swung the other way. 3-nitrotyrosine rose ($p < 0.05$). TSC2 fell ($p < 0.01$). Phosphorylation of AKT, mTOR, and RPS6 all increased ($p < 0.05$ for each). The converse experiment produced the converse result, which is the kind of symmetry that separates a finding from a fluke.

Cell culture can tell you a great deal. It cannot tell you whether a tumor inside a living body will respond the same way. The team established xenograft neuroblastoma by injecting SH-SY5Y cells subcutaneously into the flanks of six-week-old NOD-SCID mice, waited for palpable tumors, then administered BA-101 intraperitoneally at 80 mg/kg/day for 22 days. Control animals received vehicle alone. Six mice per group.

The control tumors grew to approximately 1.5 cm in their largest dimension. The treated tumors did not. Final tumor volume and weight were dramatically reduced in the BA-101 group ($p < 0.001$ for both). Body weight did not differ significantly between groups, suggesting that the compound was tolerated without gross systemic toxicity. The in vivo data did what in vivo data must do: they confirmed that the mechanism observed in the dish operates in a whole organism.

"What convinced me was the concordance between the pharmacological and genetic approaches," said Dr. Shashank Kumar Ojha, first author of the study and a researcher at the Institute for Drug Research, Hebrew University of Jerusalem. "When BA-101 and siRNA independently produce the same pattern of effects across NADPH-diaphorase activity, nitrosative stress markers, mTOR pathway phosphorylation, and clonogenic growth, you can be confident the biology is real. That reproducibility is what gives you a therapeutic hypothesis worth testing further."

The authors are forthright about limitations. The in vitro work relies on a single cell line, SH-SY5Y, which cannot capture the full genetic heterogeneity of neuroblastoma or the complexity of the tumor microenvironment. The chemical identity of BA-101 is currently undisclosed pending patent issuance, which means independent replication by other laboratories must wait. Whether nitrosative stress directly underlies its functional impairment, or whether an intermediary mechanism is involved, remains an open question that the authors explicitly flag for future investigation.

These are honest caveats, and they matter. But they do not diminish the central finding. The nNOS-mTOR axis is real, it is druggable, and in mice it responds to intervention with a force that demands further study.

mTOR inhibitors such as rapalogs and catalytic mTOR inhibitors have shown limited efficacy as monotherapies in neuroblastoma, undermined by feedback activation and resistance mechanisms. The present study suggests a different geometry of attack: rather than targeting mTOR at the lock, intervene upstream at the hand that turns the key. By reducing nitric oxide-dependent mTOR activation, nNOS inhibition may sidestep the compensatory pathways that have frustrated direct mTOR blockade.

There is a long distance between a mouse flank and a child's bedside. The researchers know this better than anyone. But a door that was previously invisible has now been located, measured, and shown to open. What walks through it next will depend on the studies that follow.

The peer-reviewed research article in Brain Medicine titled "Targeting nNOS suppresses AKT-TSC-mTOR signaling and inhibits neuroblastoma growth," is freely available via Open Access, starting on 7 April 2026 in Brain Medicine at the following hyperlink:
https://doi.org/10.61373/bm026a.0027

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