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Targeting ganglioneuromas with mTOR inhibitors

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ABSTRACT

We recently identified activated protein kinase B (PKB/AKT) as a tumorigenic driver in childhood ganglioneuroma. Inhibition of the mechanistic target of rapamycin (mTOR), a serine/threonine kinase downstream of AKT, effectively reduced the tumor burden in zebrafish with ganglioneuroma. We propose a clinical trial of mTOR inhibitors as a means to shrink large ganglioneuromas prior to surgical resection.

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Peripheral sympathetic nervous system (PSNS) tumors are the most common solid tumors of childhood, other than brain tumors. They originate from the sympathoadrenal lineage of neural crest cells and are classified into three categories, each with several subtypes: neuroblastoma (undifferentiated, poorly differentiated, and differentiating), ganglioneuroma (maturing and mature) and ganglioneuroblastoma (intermixed and nodular).[1] Neuroblastoma, the most malignant of the three, consists of immature neuroblasts, while ganglioneuroma is comprised of differentiated ganglion cells and mature stroma. Ganglioneuroblastoma possesses an intermediate phenotype characterized by immature neuroblasts similar to those of neuroblastoma, and more mature tissues resembling those of ganglioneuroma.[2] Surgery is the only effective therapy for ganglioneuroma and well-differentiated ganglioneuroblastoma, although these tumors are frequently large and intimately associated with vasculature and neuronal bundles. For these reasons, surgical resection can be difficult and is associated with significant post-operative morbidities, including bowel dysfunction, chronic neuropathy, and vascular insufficiency, among other complications.[3,4] Thus, children with either ganglioneuroma or ganglioneuroblastoma would likely fare significantly better if their tumors could be reduced in size and extent before surgery. Unfortunately, agents capable of reducing mature ganglioneuroma cells have not been identified.[3]

In recent work, we identified activated protein kinase B (PKB/AKT) as a tumorigenic driver in ganglioneuroma, and proposed that inhibitors of the mechanistic target of rapamycin (mTOR), including sirolimus and everolimus, might be ideal drugs for shrinking large ganglioneuromas before resection in order to reduce surgical morbidity.[5] In our study, we first found that AKT and its downstream effectors, mTOR and ribosomal protein S6, are frequently phosphorylated and activated in human differentiated ganglioneuromas and less activated in poorly differentiated neuroblastomas. These findings implicate the AKT-mTOR-S6 pathway as an important signal transduction pathway in the pathogenesis of ganglioneuroma. To test whether activated AKT is a *bona fide* tumorigenic driver of ganglioneuroma or merely a consequence of aberrant growth, we generated a zebrafish model by coexpressing mCherry and a constitutively active, myristoylated murine Akt2 (myr-Akt2) in the PSNS under control of the zebrafish *dopamine-β-hydroxylase* (*dβh*) gene promoter.[6] Overexpression of myr-Akt2 led to the development of ganglioneuromas in the interrenal gland (IRG), the zebrafish counterpart of the human adrenal gland, in each of two independent zebrafish lines. These tumors closely resembled human ganglioneuromas, both histologically and transcriptionally. More interestingly, no neuroblastoma is detected in these fish, despite the fact that the same promoter-driven *MYCN* oncogene expression is sufficient to induce neuroblastoma.[6] With this fish model, we further tested the efficacy of several mTOR kinase inhibitors on zebrafish ganglioneuromas. The results identified sirolimus and everolimus as effective at inducing apoptosis of ganglioneuroma cells and reducing the tumor burden in zebrafish. Sirolimus and everolimus are two clinically available mTOR kinase inhibitors that have been approved by the Food and Drug Administration for use in pediatric patients. On balance, our results suggest that sirolimus and other rapalogs would also be effective for the presurgical treatment of children with ganglioneuroma, allowing these tumors to be removed more safely at the time of surgery. These drugs should also be tested as a means to reduce the size and extent of ganglioneuromas that are deemed surgically unresectable at diagnosis.

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In contrast to neuroblastoma, very little is known about the molecular pathogenesis of mature ganglioneuroma. Thus, our discovery of a driver for ganglioneuroma assumes increased importance while raising a pivotal question: *how is AKT activated in human primary ganglioneuroma?* The upstream signals that drive AKT activation are often initiated by receptor tyrosine kinases or G-protein–coupled receptors, leading to membrane recruitment and activation of one or more isoforms of phosphoinositide 3-kinase (PI3K), which in turn recruit and activate phosphoinositide-dependent protein kinase 1 (PDK1) and the mechanistic target of rapamycin complex 2 (mTORC2), resulting in AKT activation mediated by phosphorylation on Thr308 and Ser473 (Figure 1).[7,8] In patients with ganglioneuroma, mutations or epigenetic alterations affecting the expression levels of proteins in the PI3K-AKT-mTOR pathway may be responsible for tumor initiation and progression. The AKT family of serine/threonine protein kinases consists of three members: AKT1, AKT2 and AKT3. Previous studies have indicated that each AKT isoform has unique substrates and play distinct roles in regulating tumor progression and cell migration.[7,9,10] In our work, ganglioneuromas were induced by myr-Akt2.[5] Further study is required to clarify upstream mechanisms that aberrantly activate AKT in ganglioneuroma, and whether AKT1 or AKT3 is also activated and plays similar roles as AKT2 during ganglioneuroma pathogenesis. AKT proteins have over 100 substrates, and control key multifunctional downstream effectors and signaling nodes including glycogen synthase kinase 3 (GSK3), Forkhead Box O1 (FOXO1) and the mechanistic target of rapamycin complex 1 (mTORC1) (Figure 1).[7] Further, although mTOR and ribosomal protein S6 are more frequently activated in human primary ganglioneuromas than in poorly differentiated human neuroblastomas,[5] it is still unclear if other AKT effectors are involved in the pathogenesis of ganglioneuroma. Systematic evaluation of the activation of AKT family members and downstream effectors will provide a comprehensive landscape of the roles of AKT network elements in ganglioneuroma.

Taken together, these studies with primary patient samples and a new zebrafish model have advanced our understanding of ganglioneuroma biology by implicating activated AKT as a previously unappreciated driver of this disease, and calling attention to the potential clinical impact of mTOR-targeting drugs on the clinical management of ganglioneuroma patients.[5] However, many questions remain to be answered: (i) What phenotype emerges if we overexpress distinct activated *AKT* genes in the PSNS of mice? Will they develop ganglioneuroma? Will the ganglioneuromas be similarly sensitive to mTOR inhibitors? (ii) Will clinical trials of rapalogs such as sirolimus or everolimus reduce tumor burden in children with ganglioneuroma? (iii) What are the upstream activators and other signaling pathways involved in the pathogenesis of childhood ganglioneuroma? By taking advantage of reliable

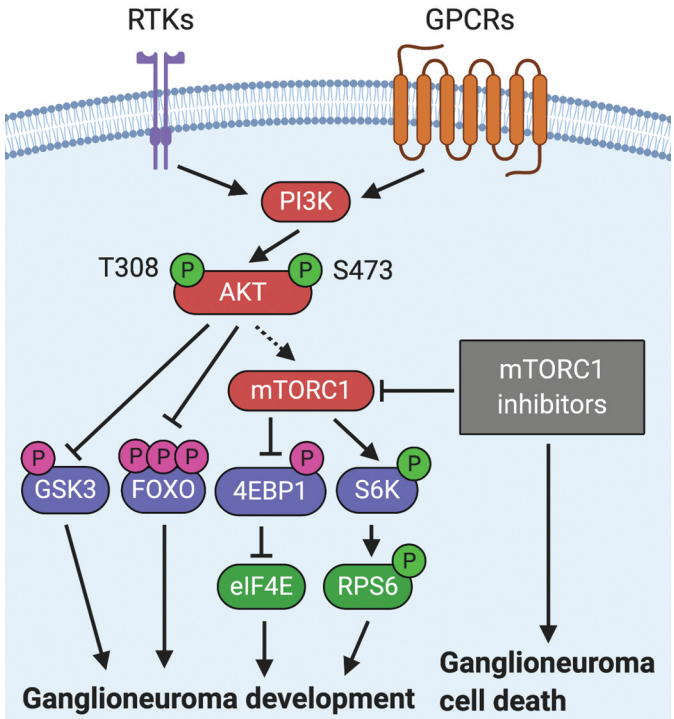

Figure 1. Targeting protein kinase B (PKB/AKT)-driven ganglioneuromas with mechanistic target of rapamycin (mTOR) inhibitors. The activation of receptor tyrosine kinases (RTKs) or G-protein–coupled receptors (GPCRs) leads to membrane recruitment and activation of phosphoinositide 3-kinase (PI3K), which in turn results in AKT activation (phosphorylation on T308 and S473). AKT proteins control key multifunctional downstream effectors and signaling nodes including glycogen synthase kinase 3 (GSK3, phosphorylation on S21 for GSK3α and S9 for GSK3β), Forkhead Box O (FOXO, phosphorylation on T24, S256 and S319 for FOXO1) and mechanistic target of rapamycin complex 1 (mTORC1). mTOR and ribosomal protein S6 are more frequently activated in human primary ganglioneuromas than in poorly differentiated human neuroblastomas, implicating the AKT-mTOR-S6 pathway as an important element in the pathogenesis of ganglioneuroma. Inhibition of the downstream AKT target mTOR in zebrafish with ganglioneuroma effectively induces cell death and reduces the tumor burden. P indicates phosphorylation, with green and magenta indicating activation and inhibition, respectively. This figure is created with BioRender.com.

animal disease models, clinical trials and exome and genome-wide high-throughput sequencing, these questions will be answered in the foreseeable future. The resultant insights will help to further define the etiology and molecular basis for childhood ganglioneuroma and expand the available drugs beyond sirolimus and everolimus as candidates for clinical trials to improve the therapy for this disease.

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Disclosure of potential conflicts of interest

The authors declare no potential conflicts of interest.

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References

1. Shimada H, Ambros IM. Pathology of peripheral neuroblastic tumors. In: Cheung NV, Cohn SL, editors. Neuroblastoma. Berlin (Heidelberg): Springer-Verlag; 2005. p. 87–95. doi:10.1007/3-540-26616-X_8.
2. Lonergan GJ, Schwab CM, Suarez ES, Carlson CL. Neuroblastoma, ganglioneuroblastoma, and ganglioneuroma: radiologic-pathologic correlation. Radiographics. 2002;22(4):911–934. doi:10.1148/radiographics.22.4.g02jl15911.
3. Decarolis B, Simon T, Krug B, Leuschner I, Vokuhl C, Kaatsch P, von Schweinitz D, Klingebiel T, Mueller I, Schweigerer L, et al. Treatment and outcome of ganglioneuroma and ganglioneuroblastoma intermixed. BMC Cancer. 2016;16:542. doi:10.1186/s12885-016-2513-9.
4. Retrosi G, Bishay M, Kiely EM, Sebire NJ, Anderson J, Elliott M, Drake DP, Coppi P, Eaton S, Pierro A. Morbidity after ganglioneuroma excision: is surgery necessary? Eur J Pediatr Surg. 2011;21(1):33–37. doi:10.1055/s-0030-1263195.
5. Tao T, Shi H, Wang M, Perez-Atayde AR, London WB, Gutierrez A, Lemos B, Durbin AD, Look AT. Ganglioneuromas are driven by activated AKT and can be therapeutically targeted with mTOR inhibitors. J Exp Med. 2020;217(10):e20191871. doi:10.1084/jem.20191871.
6. Zhu S, Lee JS, Guo F, Shin J, Perez-Atayde AR, Kutok JL, Rodig SJ, Neuberg DS, Helman D, Feng H, et al. Activated ALK collaborates with MYCN in neuroblastoma pathogenesis. Cancer Cell. 2012;21(3):362–373. doi:10.1016/j.ccr.2012.02.010.
7. Manning BD, Toker A. AKT/PKB signaling: navigating the network. Cell. 2017;169(3):381–405. doi:10.1016/j.cell.2017.04.001.
8. 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.
9. Dillon RL, Marcotte R, Hennessy BT, Woodgett JR, Mills GB, Muller WJ. Akt1 and akt2 play distinct roles in the initiation and metastatic phases of mammary tumor progression. Cancer Res. 2009;69(12):5057–5064. doi:10.1158/0008-5472.CAN-08-4287.
10. Irie HY, Pearline RV, Grueneberg D, Hsia M, Ravichandran P, Kothari N, Natesan S, Brugge JS. Distinct roles of Akt1 and Akt2 in regulating cell migration and epithelial-mesenchymal transition. J Cell Biol. 2005;171(6):1023–1034. doi:10.1083/jcb.200505087.