

Viewpoint

Is Overexpression of the Plasma Membrane Transporter (*SLC29A4*) a New Option to Stratify Patients with High-Risk Neuroblastoma for Treatment with ^{131}I -mIBG?

Neuroblastoma (NB) is a heterogeneous disease with solid tumors in infants and young children originating from neural crest tissue and frequently manifests in the adrenal glands of the thoracic, abdominal, or cervical paraspinal ganglia (Rivera et al., 2023). Patients are stratified in low-, intermediate-, and high-risk categories based on age, stage of disease, and tumor biology. More than 50% of patients are metastatic and, unfortunately, with current treatment options, long-term survival is <40% in the high-risk patient group with metastatic disease (Olecki and Grant, 2019).

A broadly used method to diagnose NB is by injecting ^{123}I -labeled meta-iodobenzylguanidine (^{123}I -mIBG) followed by imaging. An analog of ^{123}I -mIBG, ^{131}I -mIBG is being explored as one of the approaches to treat relapsed or refractory high-risk NB (Olecki and Grant, 2019). mIBG is a stable analog of norepinephrine that is transported into tumor cells by the norepinephrine transporter (NET; gene name *SLC6A2*) (Parisi et al., 2016), and it has been shown that mIBG is retained intracellularly in several NB-derived cell lines by an unknown mechanism (Iavarone et al., 1993). In an interesting paper in this issue of the *Journal of Pharmacology and Experimental Therapeutics*, Vieira et al. (2023) investigate the potential role of monoamine and organic cation transporters beyond NET in the transport of ^{131}I -mIBG in patients with high-risk neuroblastoma. They also explored to what extent the expression of these transporters correlated with the expression of the MYC oncogene family member *MYCN*. The latter is an important question because *MYCN* amplification is a driver in neuroblastoma, and amplification is correlating with a poor prognosis in 50% of advanced-stage and high-risk NB (Otte et al., 2021).

In 154 samples from a publicly available database [Therapeutically Applicable Research to Generate Effective Treatments (TARGET)] with genomic and clinical data from NB tumor samples from patients, Vieira et al. (2023) found expression of *SLC6A2*, confirming previous findings. In addition, increased expression was detected of the plasma membrane monoamine transporter (PMAT; *SLC29A4*) and the vesicular membrane monoamine transporters 1 and 2 (*VMAT1/2*; *SLC18A1/2*) but not of other organic cation transporters. The highest expression was found for *SLC29A4*, and, intriguingly, there was a correlation between high expression of this transporter and lack of amplification of *MYCN*. Data were confirmed in nine independently obtained NB samples from the Seattle Children's Hospital. The findings were consistent with previous observations showing a decreased abundance of NET and *VMAT1/2* in tumors in which *MYCN* was amplified (Temple et al., 2016). The authors subsequently noticed that high expression of *SLC29A4* and *SLC18A2*, but not of *SLC6A2* and *SLC18A1*, correlated with a more favorable overall survival. Additionally, it was found that in *MYCN* nonamplified tumors, high expression of *SLC29A4* and *SLC18A1/2* correlated with better survival. Surprisingly, although PMAT is normally localized in the plasma membrane, immuno-fluorescence colocalization studies conducted in tumor samples and cell lines derived from NB patients suggested localization of PMAT primarily in the mitochondria, in addition to the endoplasmic reticulum and in intracellular storage vesicles. Although immunofluorescence is a suitable method to determine localization of proteins, higher resolution techniques such as electron microscopy will be needed for more unequivocal proof that PMAT is in the outer membrane of mitochondria.

The authors also investigated whether mIBG was a substrate of PMAT in HEK293 cells overexpressing a *SLC29A4* cDNA. Much higher concentration-dependent uptake of mIBG was found relative to control cells, and this transport was inhibited by the inhibitor decynium-22 (D22). To investigate whether PMAT does contribute to the accumulation of mIBG in mitochondria, uptake was measured in mitochondria isolated

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from two NB-derived patient cell lines. Indeed, accumulation was observed that was inhibited partially by D22. Based on these data, the authors concluded that PMAT is involved in the accumulation of mIBG in mitochondria in NB tumors (Fig. 1). Because D22 is not specific for PMAT—it also inhibits NET—knockout or knockdown of this transporter would be needed to prove its role more definitively. Attempts were made to address this point, but these were unsuccessful thus far.

In drug development, PMAT is not routinely considered as a transporter that must be taken into account in the characterization of mechanisms relevant for the disposition of drugs (Zamek-Gliszczyński et al., 2018), but based on the findings by Vieira et al. (2023), it can be important. PMAT is detected in various human tissues, including multiple regions in the brain, such as the cerebral cortex, hippocampus, substantia nigra, medulla, oblongata, cerebellum, and choroid plexus (Wang, 2016). In recombinant polarized cell monolayers and in epithelial cells of, for instance, the choroid plexus, PMAT has been detected in the apical plasma membrane. Typical substrates for PMAT are small and polar organic cationic drugs such as metformin and atenolol, and a range of biogenic amines like, for instance, serotonin and dopamine. Potent inhibitors of PMAT include D22 and quinidine. PMAT-mediated transport of organic cations is Na^+ and Cl^- independent, but it is sensitive to membrane potential (Itagaki et al., 2012), suggesting that the physiologic inside-negative membrane potential is a driving force for transport. Because of the favorable membrane potential, PMAT could function in the outer membrane of the mitochondria (Zorova et al., 2018).

Currently, it is not understood why PMAT would route to the mitochondria in NB cells, whereas it is localized in the plasma membrane in normal cells. The equilibrative nucleoside transporter 3 (ENT3; *SLC29A3*) is localized in mitochondria (Liu et al., 2015), so there is precedence for *SLC29* family members to be localized in intracellular organelles. As also discussed by the authors, it is not clear why there would be a change in membrane routing of PMAT in NB because drugs to treat the disease—besides mIBG—are not substrates of this transporter.

It is remarkable that the expression of PMAT is highest in high-risk NB patients without amplification of *MYCN*. This suggests that *MYCN* could be involved in the suppression of *SLC29A4* expression (Fig. 1), but functional promoter activity analysis would be needed to substantiate this hypothesis.

From a clinical perspective, an important implication of the work by Vieira et al. (2023) is that PMAT could be used as a diagnostic marker for high-risk patients without *MYCN* amplification. This is an interesting possibility because *MYCN* amplification is only found in less than half of patients with advanced or high-risk NB (Otte et al., 2021). As also acknowledged by the authors, independent confirmation of the correlation between survival and high level of expression of *SLC29A4* will be needed before further consideration as a potential novel prognostic marker.

Treatment options for high-risk patients with NB are currently chemotherapy, surgery, radiation, autologous hematopoietic stem cell rescue, and 13-cis-retinoic acid (Parket al., 2010). Additional treatment options

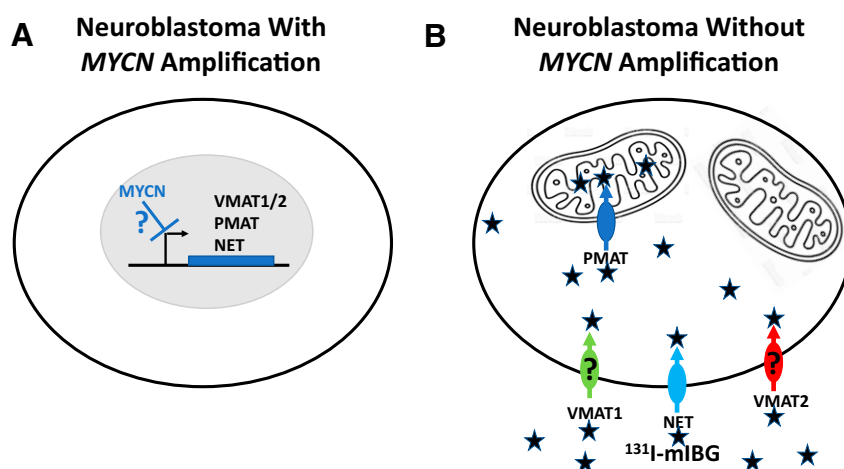


Fig. 1. Potential transcriptional regulation and transport activity of PMAT (*SLC29A4*) and related transporters in neuroblastoma. (A) Potential role of *MYCN* in the (partial) suppression of *SLC6A2* (NET), *SLC29A4* (PMAT), and *SLC18A1* and 2 (VMAT1 and 2) expression in neuroblastoma with *MYCN* amplification. Promoter analysis will be needed to confirm a direct role of *MYCN* in the transcriptional regulation of these transporter genes. (B) Involvement of NET, VMAT1, and 2 and PMAT in the transport of mIBG across the plasma and mitochondrial membrane in neuroblastomas without *MYCN* amplification. Mitochondria with PMAT in the membrane will accumulate ^{131}I -mIBG preferentially. Question marks indicate that transport of mIBG has not been demonstrated directly. The localization of VMAT in the plasma membrane in neuroblastoma has not been confirmed. Stars represent ^{131}I -mIBG molecules.

are needed to increase the overall survival of patients, and clinical trials to test ^{131}I -mIBG as a treatment option have been ongoing in high-risk patients since the 1990s. The aim of these trials is to develop the most optimal dosing and combination regimen (Olecki and Grant, 2019; Rivera et al., 2023). The findings by Vieira et al. (2023) raise the question of whether the success rate of ^{131}I -mIBG therapy can be increased by stratifying patients for the expression of *SLC29A4*. Correlating overall survival and tumor regression with expression of *SLC29A4* in patients treated with ^{131}I -mIBG therapy will be highly informative.

In summary, the paper by Vieira et al. (2023) provides novel insights into the transport activity of PMAT and opens the possibility for novel diagnostic approaches to predict the prognosis in patients with NB.

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