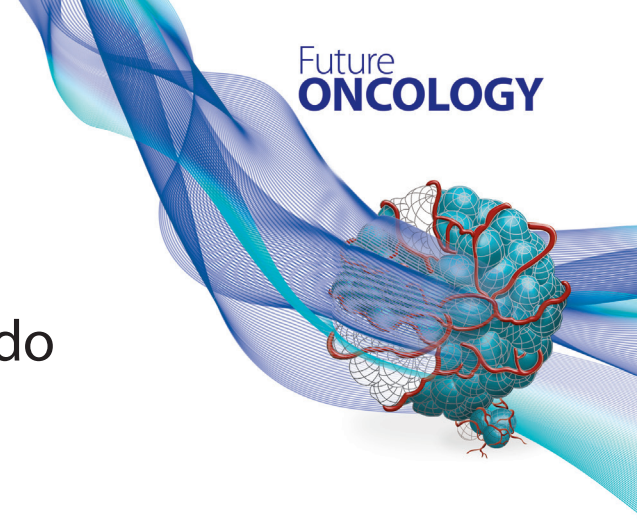
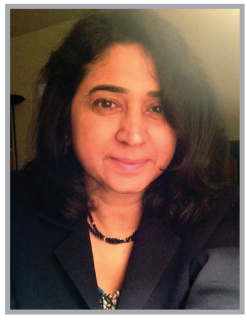
Future ONCOLOGY



EDITORIAL

Applying knowledge of autism to brain cancer management: what do we know?



"Meaningful future therapies are likely to require cocktails of drugs that target the proliferation/invasion of cancer and the development of chemoresistance."

Hari Prasad[1] & Rajini Rao*[,1]

"To raise new questions, new possibilities, to regard old problems from a new angle requires creative imagination and marks real advances in science."
– Albert Einstein (1879–1955)

Autism meets glioblastoma

What do two seemingly unrelated disorders of the brain have in common? Autism encompasses a heterogeneous collection of neurodevelopmental dysfunctions ranging from impaired social behavior, stereotyped interests and language deficit. Diagnosed at increasingly high rates of one in 68 children (one in 42 for boys), autism spectrum disorders are a major impediment to childhood development worldwide. Current treatment options for autism are limited [1]. On the other hand, glioblastoma multiforme (GBM) is the most common and aggressive form of adult primary brain cancer with extremely poor 3-year survival rates of less than 10%. In GBM, clinical trials of drugs inhibiting the EGF receptor (EGFR) kinase, the most prominent oncogenic target, have been disappointing and as a result, patient outcomes have failed to show substantive improvement over median survival times of 12 months [2]. Thus, there is a great, unmet need for effective therapy in both disorders and innovative approaches are urgently needed. In order to identify new drug targets, we need a transdisciplinary approach that breaches the conventional boundaries confining each area of research. There is growing recognition that glioblastoma and many brain disorders share the same fundamental pathophysiological mechanisms at a cellular and molecular level. As a consequence, mutations or gene expression changes in the same gene family can underlie multiple, distinct clinical phenotypes. A compelling example is that of the *SLC9A6* and *SLC9A9* genes encoding endosomal Na^+/H^+ exchangers NHE6 and NHE9, respectively [1]. Genome-wide association studies, gene linkage analysis, homozygosity mapping, transcription profiling and mining patient databases have linked these genes to a collection of neurological disorders, including autism, attention deficit hyperactivity disorder, epilepsy, intellectual disability, Alzheimer's disease and other neurodegenerative disorders, and most recently and unexpectedly brain cancer [1–4].

KEYWORDS
• chemoradioresistance • EGFR
• endosomes • pH regulation
• *SLC9A9* • temozolomide

"There is growing recognition that glioblastoma and many brain disorders share the same fundamental pathophysiological mechanisms at a cellular and molecular level."

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Uncovering the hidden link

A prerequisite of precision medicine involves identifying specific genes and pathways to be targeted in individual patients. Databases of genome and proteome changes in GBM patients can be mined to uncover novel risk factors and potential therapeutic targets. Using this approach, we recently made the unexpected discovery that the autism-associated gene *SLC9A9*, encoding the endosomal Na^+/H^+ exchanger NHE9, was in the top 12% of overexpressed genes in glioblastoma samples, with expression levels approximately sixfold higher relative to control neural stem cells [2]. This subset of GBM overexpressing NHE9, including 36% of mesenchymal tumors, was associated with decreased patient survival and disease-free period after resection. Strikingly, these tumors were essentially nonresponsive to radiochemotherapy. In contrast, patients with unaltered expression of NHE9 showed significantly better response to neo-adjuvant chemotherapy, which included temozolomide (>50% cases) and PCV (procarbazine, CCNU, vincristine), with a median survival of 59 months, compared with 16 months in patients with NHE9 upregulation [2]. Reviewing this striking clinical information, we were compelled to ask: how does NHE9 regulate gliomagenesis?

eNHE: from bloom to brain

According to the pump–leak model, endosomal pH is precisely tuned by a combination of inward proton pumping through the V-ATPase and outward proton leak via endosomal $Na^+(K^+)/H^+$ exchangers (eNHE): both mechanisms that are evolutionarily conserved from yeast to plants, and from simple metazoans like *C. elegans*, to fruit fly and vertebrates including humans [1]. Beginning in the mid-90s, we cloned, localized and recognized eNHE as distinct from the well-studied plasma membrane Na^+/H^+ exchangers and proposed a role for eNHE in vesicle trafficking and lysosomal biogenesis. Given the evolutionary conservation within this family, we were able to harness decades of work on simple model organisms, including bacterial, plant and yeast orthologs, translating them into complex neurological systems where they provide novel insight into human disease [1,5]. For example, in plants, NHE orthologs determine bloom color by altering luminal pH, and confer salt tolerance by sequestering sodium in the vacuolar compartment, while the yeast ortholog Nhx1 regulates vacuolar cargo delivery and intracellular trafficking pathways [1,6]. In humans, there are two eNHE isoforms, NHE6 and NHE9 that have distinct, nonredundant functions. Both are highly expressed in the brain, particularly in hippocampus and cortex, regions associated with communication, memory and cognitive function [1]. We showed that autism-associated mutations in *SLC9A9* led to loss of NHE9 transporter function, resulting in hyperacidification of sorting endosomes and alterations in glutamate clearance by astrocytes that could explain synapse dysfunction and epilepsy in this subset of autism patients [4].

Working from the inside out

An early pathophysiological hallmark of cancer is a perturbation in pH regulation. Alkalization of cytoplasmic pH coupled with extracellular acidification by constitutive activation of plasma membrane, NHE1 is an important and early step in neoplastic transformation [7]. While the role of pH dynamics in the cancer cell and tumor microenvironment has been recognized, the link between endosomal pH regulation and tumorigenesis was unclear. As a starting point, we extended the knowledge gained from our autism research that established a profound pan-specific effect of endosomal NHE9 activity on membrane persistence of multiple cell surface receptors and transporters [4]. Based on these insights, we hypothesized that NHE9 is an upstream activator of oncogenic signaling pathways, and works by maintaining receptor density and persistence at the cell membrane. The prevailing model explaining the role of EGFR in oncogenesis and chemoresistance overwhelmingly focuses on transcript amplification and mutations in the EGFR, with the role of post-translational pathways, including endosomal receptor sorting and degradation, being largely understudied. By functioning as a leak pathway for protons, NHE9 limits luminal acidification to exert post-translational control over EGFR turnover, resulting in persistence of oncogenic signaling pathways that drive tumor growth and migration [2]. We showed that membrane surface expression, turnover, receptor phosphorylation and downstream effectors of EGFR are upregulated by NHE9 overexpression and conversely, attenuated by NHE9 silencing. In a comparative analysis of early passage, brain tumor-initiating cells (BTIC) lines derived from patient tumors, aggressive proliferation and migration correlated with NHE9 levels both *in vitro* and *in vivo*. Using cell migration on nanopatterned surface experiments, we have demonstrated that BTIC with

NHE9 upregulation had enhanced migration speed and directionality, a key property of GBM known to migrate along neurovascular tracks. Furthermore, silencing or inhibition of NHE9 in BTIC drastically attenuated tumorsphere formation and improved the efficacy of EGFR inhibitor, suggesting that targeting endosomal pH might circumvent chemoresistance [2]. Similar 'inside-out' control of signaling is intensively studied for integrin receptors whose function is controlled by the cells that express them [8]. We suggest that the effect of NHE9 extends beyond EGFR to other plasma membrane proteins, including other growth factor and cytokine receptors and neurotransmitter receptors and transporters. Thus, by modulating endosomal pH, NHE9 may be a master regulator of cargo delivery and recycling to mediate inside-out control of both synapse function in autism and oncogenic signaling in brain cancer.

Lessons learned

It is perhaps ironic that although the human brain works with infinite complexity, it fails through basic biological and biochemical mechanisms such as pH dysregulation. The convergence of studies demonstrating dose dependence of NHE9 function in the pathogenesis of brain maladies has been remarkable. It is likely that NHE9 is targeted in multiple disorders including autism and GBM because it impinges on multiple critical pathways. It is, however, important to note that these observations do not directly predicate risk between NHE9-associated cancers and NHE9-associated autism. Rather, we emphasize how perturbations in a common molecular pathway can lead to distinct disease phenotypes. Autism can teach us a lot about brain cancer, and vice versa. Intriguingly, similar to NHE9, studies on several genes involved in the mTOR pathway, including PTEN, TSC1 and TSC2, have also revealed that autism shares common roots with brain cancer [9]. The practical consequence of the rapidly accruing knowledge of shared genetic causes of autism and brain cancer is an expanding effort to identify many more targets for therapeutic manipulation of these disorders. One good example is that of everolimus, an anticancer drug inhibiting mTOR signaling. Early results from clinical trials suggest that everolimus can reduce seizures in tuberous sclerosis and might offer a new treatment approach for comorbidities associated with autism [10].

Conclusion

Most knowledge accrues in pieces, but is assimilated in patterns. Often essential pieces of a pattern might lie beyond the border of any given discipline. Until recent decades, there has been a lot of mechanistic ignorance and therapeutic nihilism about brain cancers, but that is now changing. We now know that the development of novel cancer therapies requires a thorough understanding of cellular and molecular pathways. Systematically integrating what we know about endosomal NHE from decades of work on simple model organisms, including bacterial and yeast orthologs and translating them into complex neurobiological models of autism and cancer identified a novel and 'druggable' mediator of inside-out control of oncogenic signaling that could significantly improve efficacy in the treatment of malignant progression of glioblastoma. Meaningful future therapies are likely to require cocktails of drugs that target the proliferation/invasion of cancer and the development of chemoresistance. Defining the cellular pathways of NHE9-mediated tumorigenesis could pave the way toward new and combinatorial avenues of therapeutic intervention with existing drugs. Furthermore, expression changes and mutations in NHE9 could offer new ways of targeted therapies and stratifying cancer risk and patient prognosis.

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