COMMENTARY

Excessive activation of tissue plasminogen activator makes a mouse nervous

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In an experimental tour de force, using a series of cell and molecular biological approaches, Li et al. (1) identify the misexpression of the tissue plasminogen activator (tPA) protein as the causal basis for the cerebellar phenotype of the *nervous* (allele symbol, *nr*) mutant mouse. The original full report of the *nervous* mutant showed a dramatic loss of Purkinje cells in the lateral cerebellum and a significant, but attenuated, loss in the midline cerebellum (2). The loss of Purkinje cells was precipitous from postnatal day 23–50 and the Purkinje cells that survived remained into adulthood (3).

Early work from the laboratory of Nick Seeds identified early postnatal expression of tPA in granule cells and a role for tPA in cerebellar development that focused on granule cell migration using the tPA KO mouse (4). The ontogeny of later expression of tPA in cerebellar Purkinje cells was unexplored, and any possible connection with the *nr* mouse was not made, although the induction of tPA was seen in Purkinje cells after motor learning (5). The mutant gene had been mapped to mouse chromosome 8 (6), but no genomic data had pinpointed the affected gene. The story largely stopped there until the Sidman laboratory reentered the exploration of the *nervous* mutant and found that a gene product, tPA, whose gene was located in this region, was expressed at 10-fold higher amounts in the mutant cerebellum (7). The current Li et al. paper provides significant information that points more definitively at tPA as the causal molecule for the *nervous* phenotype.

Pathways from tPA Overexpression to Purkinje Cell Death

From one perspective, cell biologists and biochemists will find in this article three diverse pathways linked together by tPA, all involved in the development and survival of Purkinje cells. The work of Li et al. and previous efforts from this group provide convincing evidence that the *nervous* allele acts as a gain-of-function mutation of the *Plat* (plasminogen activator, tissue) gene. However, lacking the genomic data to validate this point, the authors apply an impressive, multipronged approach to reach this conclusion. They use single cell transcript profiling, quantitative ultrastructural analysis of synapses and mitochondria, cerebellar organotypic slice and dissociated Purkinje cell cultures, double mutant *nr*:tPA-null mice, injection of tPA into WT neonatal cerebellar cortex, and lentiviral vector-based shRNA knockdown toward the analysis of enzymatic dynamics, dendritic tree quantification, parallel fiber synaptic contacts with Purkinje cells, and mitochondrial anatomy and function to demonstrate that three molecular pathways downstream of tPA/plasmin-based proteolysis affect postnatal Purkinje cell development and survival. Likely the most incapacitating is the decreased membrane potential and enlargement of mitochondria and loss of integrity of the plasma membrane. Both of these events are linked to the production of excess kringle 5, a product of plasminogen proteolysis, that binds to voltage-dependent anion channel 1 (VDAC1) and diminishes VDAC1 function and/or localization. A second pathway that is impacted by the *nervous* mutation is tPA inhibition of dendritic growth through the activation of protein kinase Cγ and microtubule-associated protein 2 phosphorylation. A final pathway that results in a deficit of parallel fiber–Purkinje cell synapses specifically involves a blockade of brain-derived neurotrophic factor (BDNF) and signaling through its tyrosine kinase receptor B.

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What emerges are unappreciated views that the *nervous* mutant now reveals, such as a dissociation between dendritogenesis and synaptogenesis as seen by finding that BDNF can correct the deficits in synaptogenesis but not in dendritogenesis and the primal role of VDAC1's interaction with kringle 5, a proteolytic peptide of plasminogen, in the *nr* phenotype of Purkinje cell death.

Neurological Mutant Mice and the Genetics of Nervous System Development and Function

From a second perspective, this work on the *nervous* mutant mouse serves as a reminder of how the availability of spontaneous and induced mutations in the mouse helped catapult this species into its preeminent role as a preclinical model. The *nervous* mutant mouse arose in a colony of mice at the Jackson Laboratories and was reported in one of the early compendiums of mutations in the mouse (8). As with many mutations found at the Jackson Laboratories, *nervous* fostered a cottage industry in its use that involved multiple avenues of research that included neuron–glia interactions (9) and the stabilization of synaptic contacts (10). These mutant mouse strains were the byproducts of a fruitful collaboration between the Jackson Laboratories and Richard Sidman's research group at Boston Children's Hospital. This highly productive relationship between the Jackson Laboratories and Richard Sidman is epitomized in their classic *Catalog of the Neurological Mutants of the Mouse* (11). From this catalog came some of the classic mutations that were to lay the groundwork for mammalian neurogenetics and provide the genetic keys for some of the molecular events in neurodevelopment such as migration and synaptogenesis.

Only a few of the mice noted in the catalog have evaded genetic discovery, and they likely speak to the complexities of the genome. A recent example of the finding of the Danforth's short-tail mutation (*Sd*), which had

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evaded discovery of the mutant gene for many years, illustrates where the *nervous* mutation may lie. Three reports converged on the identification of a retrotransposon insertion over 12 kb upstream to the start site of the *Ptf1a* gene as the causal basis of the *Sd* mutation (12). The insertional mutation caused ectopic expression of the *Ptf1a* gene, which gave rise to the phenotype of the mutant. The question posed by Hamilton may be apropos to the *nervous* mutant. "The discovery of the *Sd* mutation after so many decades might also prompt us to ask how often regulatory mutations might account for the remaining classical alleles that have been refractory to intragenic-centered analysis and exome sequencing." The current data on the *nervous* mutant are consistent with an upstream, homozygous perturbation of the *Plat* gene that would lead to the unbridled expression of tPA. Many questions exist that can only be answered with the finding of the genetic lesion responsible for *nervous*.

In the meantime, however, this report by Li et al. (1) shines light on an old mutation that could yield new truths about nervous system development and function. For example, there is an interesting convergence between the studies from the Seeds' laboratory that have shown a role for tPA and learning and memory and a study from the Thompson laboratory that has demonstrated a pivotal role of the cerebellum in eyeblink conditioning (13). Might the *nervous* mutant provide an experimental model to provide a systems approach to the role of Purkinje cells in a learned response? Another interesting point of convergence is a second identified substrate of tPA activity: hepatocyte growth factor/scatter factor (HGF/SF) (14). The receptor for this substrate is Met (previously known as the c-met proto-oncogene), which has been shown to be important for cerebellar development (15) and a gene that has been associated with autism (16). Might the *nervous* mutant provide a further link between cerebellar pathogenesis and the etiology of autism spectrum disorder (17)?

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Goldowitz