

## WAITING FOR GADD45B

**Neuronal Activity-Induced Gadd45b Promotes Epigenetic DNA Demethylation and Adult Neurogenesis.** Ma DK, Jang MH, Guo JU, Kitabatake Y, Chang ML, Pow-Anpongkul N, Flavell RA, Lu B, Ming GL, Song H. *Science* 2009 Feb 20;323(5917):1074–1077. The mammalian brain exhibits diverse types of neural plasticity, including activity-dependent neurogenesis in the adult hippocampus. How transient activation of mature neurons leads to long-lasting modulation of adult neurogenesis is unknown. Here we identify Gadd45b as a neural activity-induced immediate early gene in mature hippocampal neurons. Mice with Gadd45b deletion exhibit specific deficits in neural activity-induced proliferation of neural progenitors and dendritic growth of newborn neurons in the adult hippocampus. Mechanistically, Gadd45b is required for activity-induced DNA demethylation of specific promoters and expression of corresponding genes critical for adult neurogenesis, including brain-derived neurotrophic factor and fibroblast growth factor. Thus, Gadd45b links neuronal circuit activity to epigenetic DNA modification and expression of secreted factors in mature neurons for extrinsic modulation of neurogenesis in the adult brain.

## COMMENTARY

The mammalian hippocampal dentate gyrus contains neural stem-like cells that generate neurons throughout life (1,2). Adult hippocampal neurogenesis is implicated in certain learning and memory tasks, patient response to antidepressants, as well as anxiety-related behaviors, although its precise role in hippocampal function remains uncertain (3,4). Both molecular and environmental stimuli, including growth factors, neurotransmitters, enriched environment, exercise, and brain injuries influence adult neurogenesis (4,5). Among brain insults, seizures are especially potent stimulators of neuronal birth (6) and accelerate the differentiation of adult-born neurons (7).

One missing link is how neuronal activity, either physiological or pathological, stimulates neural progenitor proliferation and neurogenesis. Work by Ma et al. now sheds light on this link (see also Basic Review by Naegel in this issue). The investigators found that single electroconvulsive seizures (described by the authors as “electroconvulsive treatment”) in adult mice increase dentate gyrus Gadd45b expression, and this expression stimulates dentate cell proliferation. Gadd45b seems to act via transient epigenetic modifications of mature neurons, leading to persistent changes in neurogenesis. So, what is Gadd45b and how did the investigators reach these conclusions?

Gadd45b (Growth Arrest and DNA-Damage-inducible protein 45 β), originally known as MyD118, is one isoform of the highly homologous Gadd45 family of proteins that consist of Gadd45a, b, and g. They are 18kd proteins found in

the nucleus, known to act as stress-response genes, and classically are induced by genotoxic agents but also by other forms of stress, terminal cell differentiation, and cytokines (8). They function in DNA repair, apoptosis, cell survival, growth arrest, and probably DNA demethylation. In terms of the latter function, Gadd45a alone is implicated in epigenetic gene activation by DNA demethylation (9).

What is the function of DNA methylation/demethylation? DNA methylation at cytosine residues (by DNA methyltransferases yielding 5-methylcytosine) occurs at CpG dinucleotides, which are found in high-density areas or so-called islands located within proximal promoters. While demethylation typically activates gene expression, DNA methylation is one of the various epigenetic mechanisms for silencing gene expression. Promoter methylation silences genes by blocking binding of transcriptional activators or by recruiting repressors. DNA methylation status is heritable and methylation changes, which typically are of a global and relatively static nature, regulate early developmental processes when widespread gene activation or silencing is required (10). Altered DNA methylation also occurs in pathological processes, such as silencing of tumor repressor genes in cancer cells. Only recently has DNA methylation/demethylation been recognized to occur dynamically to regulate gene expression (11). Little is known, however, about this function in adult organisms or even postmitotic cells, such as neurons.

Ma and colleagues have begun to fill in details of this knowledge gap in gene function. To understand how neuronal activity increases adult hippocampal neurogenesis, they used electroconvulsive seizures to stimulate neurogenesis, and their screen for candidate epigenetic regulators, induced in the dentate after

electroconvulsive seizures, yielded Gadd45b. Using in situ hybridization, reverse transcriptase polymerase chain reaction, and Western blot, the investigators showed that mRNA and protein expression of Gadd45b, but not the closely related Gadd45a or g, transiently increased in the dentate within hours following the electroconvulsive seizures. This finding is consistent with an immediate early gene function for Gadd45b. Interestingly, the authors found that a spatial learning task similarly increased expression specifically of Gadd45b, suggesting that rather than sensing stress, Gadd45b was serving as a neuronal activity sensor. Another finding was that Gadd45b expression was predominantly induced in mature dentate granule cells and not in newborn granule neurons or their progenitors, an important result for understanding how Gadd45b might affect adult granule cell neurogenesis. The authors also performed pharmacologic experiments to show that seizure-induced Gadd45b up-regulation required NMDA-receptor activation, calcium influx, and calcium/calmodulin-dependent protein kinase signaling—pathways also involved in activation of established immediate early genes.

To test whether Gadd45b induction is necessary for seizure-induced hippocampal cell proliferation, the authors labeled dividing cells with bromodeoxyuridine. They delivered a single electroconvulsive seizure, either to Gadd45b null mice or after knockdown of Gadd45b, using a lentivirus-delivered short hairpin RNA, and then quantified proliferating cells 3 days later. Both treatments suppressed, but did not entirely abolish, electroconvulsive seizure-induced increases in bromodeoxyuridine labeling. A similar effect was found using exercise to stimulate cell proliferation instead of electroconvulsive seizures. Because seizure activity accelerates dendritic development of adult-born neurons in mice (7), the authors examined how the absence of Gadd45b influenced electroconvulsive seizure-induced changes in dendritic complexity of immature (adult-born) granule neurons. They found that electroconvulsive seizures increased dendritic growth of adult-born neurons as expected, an effect that was partially attenuated in Gadd45b null mice.

The next critical question for these investigators concerned the mechanism that underlies these effects. Focusing on a potential role for Gadd45b in DNA demethylation, the authors first examined global methylation status and found that electroconvulsive seizures did not produce widespread changes in DNA methylation. They then assayed for demethylation of candidate growth factor genes that may influence neurogenesis. This work involved two techniques. One is methylated DNA immunoprecipitation, in which an antibody specific for methylated DNA (anti-5-methylcytosine) essentially pulls down pieces of digested DNA, which are then amplified, using polymerase chain reaction with primers for specific gene promoter regions. The other technique, bisulfite sequencing, involves treating DNA with bisulfite to convert unmethylated cytosines to uracils, while methylated cytosines are left unmodified. Sequencing of DNA

after this treatment is a sensitive method to determine methylation status of specific promoters.

Using these techniques, the authors found that electroconvulsive seizures induced selective demethylation of a subset of candidate genes that are likely to influence adult neurogenesis. Both the brain-specific promoter B region of fibroblast growth factor-1 (FGF-1B) and the regulatory region IX of the brain-derived neurotrophic factor (BDNF) promoter showed significant demethylation after electroconvulsive seizures. The time course of BDNF and FGF-1B demethylation paralleled induction of Gadd45b after electroconvulsive seizures, both being transient and reversible. Gadd45b null mice showed no decreased methylation of these regulatory regions after electroconvulsive seizures, despite having baseline methylation levels equivalent to wild-type mice. In contrast, overexpressing Gadd45b increased gene demethylation. The authors then showed that Gadd45b binds to the FGF-1B and BDNF IX regulatory regions, and importantly, that electroconvulsive seizure-induced gene expression from these regions as well as total expression of *Bdnf* and *Fgf-1B* was suppressed in Gadd45b null mice.

Together, these data suggest a model in which seizure or physiological activity stimulates neurogenesis in a paracrine manner, via transient actions of Gadd45b on mature neurons. Short-lived Gadd45b expression leads to reversible epigenetic modifications involving demethylation of specific gene regulatory regions, both transient events resulting in more long-lasting changes in cell proliferation. Thus, activation of gene expression by epigenetic modification is a feasible means to translate physiological activity into structural brain alterations, as may occur with learning and memory, or to translate pathological activation into aberrant plasticity, such as after seizures.

Further work is needed to clarify the link between transient epigenetic modifications and increased neurogenesis after seizures or physiological activity. One potential aspect involves the fact that the authors assayed cell proliferation with bromodeoxyuridine labeling (and at only one time point after electroconvulsive seizures) but did not look at neurogenesis, per se. Thus, it remains unclear how temporal changes in neurogenesis correlate with alterations in Gadd45b, BDNF, and FGF-1B expression. In addition, loss of Gadd45b only partially reduced the increased cell proliferation and dendritic growth seen after seizures, so this mechanism likely comprises a single pathway in a complex set of events that translate activity into accelerated neuronal birth and development in the adult. Lastly, no causative relationship was established between altered BDNF and FGF-1B expression and increased neurogenesis after electroconvulsive seizures. Nonetheless, this large and elegant body of work shows that neuronal activity in general, and seizures in particular, lead to dynamic epigenetic alterations of gene expression, and it provides a compelling mechanism for how transient brain activation may alter gene expression. This

knowledge will no doubt lead to new insights into brain function and potential targets for simulating the effects of physiological activity or attenuating maladaptive gene expression that may be seen with pathological activity, such as seizures.

by Jack M. Parent, MD

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