

Researchers solve questions about phenobarbital action in the liver

By Heather Franco

A new [study](http://www.ncbi.nlm.nih.gov/pubmed/23652203) (<http://www.ncbi.nlm.nih.gov/pubmed/23652203>) led by Masahiko Negishi, Ph.D., head of the NIEHS [Pharmacogenetics Group](#), was recognized by the Faculty of 1000 (F1000) for its contribution to the fields of phenobarbital and epidermal growth factor research. The paper appeared in the May 7 issue of the journal *Science Signaling*.

The study by first author Shingo Mutoh, Ph.D., a postdoctoral fellow with the group, and colleagues, addressed an issue that has plagued the research community of drug metabolism and disposition for decades, by determining the mechanism by which phenobarbital activates the constitutive active androstane receptor (CAR). They found that phenobarbital directly binds to the epidermal growth factor receptor (EGFR), blocking a signaling cascade that normally prevents the dephosphorylation and activation of CAR.

This article is the first from the Negishi lab to be recognized by [F1000](#), (<http://f1000.com/prime>) which consists of more than 10,000 faculty and associate faculty members who highlight recent scientific publications of importance to their fields of research. As Linda Pike, Ph.D., notes in her [recommendation](#) (<http://f1000.com/prime/718008469>) of the paper, “This manuscript is interesting from two perspectives.” In addition to resolving the mechanism by which phenobarbital activates CAR, the study provides new evidence for a drug interacting with EGFR to inhibit its action (see text box).

Identifying a novel mechanism of phenobarbital action

Phenobarbital is a barbiturate used to treat epilepsy. As Negishi explained, “In 1963, phenobarbital was found to increase the expression of drug metabolizing enzymes in the liver and, because of this, patients developed drug resistance.” He remembers this discovery well, since it occurred while he was in graduate school. Since then, the search for the phenobarbital receptor has been underway. More than fifteen years ago, Negishi’s lab was the first to identify the nuclear receptor CAR as the mediator of phenobarbital action, but it turned out to not be the receptor for phenobarbital. He has sought to identify the phenobarbital receptor and to understand its mechanism of action ever since.

Continuing his pioneering work, Negishi’s lab determined that phenobarbital promotes the dephosphorylation and nuclear translocation of CAR to activate gene transcription. Mutoh joined the Negishi lab in 2006, after earning his Ph.D. from the Tokyo University of Agriculture, and began to work towards understanding this mechanism further.

Using primary mouse hepatocytes, Mutoh observed that the EGFR ligand epidermal growth factor (EGF) prevented phenobarbital-induced CAR activation. He further demonstrated that EGF treatment prevented the association of the scaffold protein RACK1 and the protein phosphatase PP2Ac with CAR, thereby blocking its dephosphorylation and activation. Since RACK1 is a common effector in a number of signaling cascades, these findings implicated RACK1 as a hub for multiple inputs that regulate CAR actions, as well as diverse phenobarbital actions.

Closure after decades of uncertainty

These data suggested to Mutoh that phenobarbital may interact directly with EGFR, and he took two approaches to address this hypothesis. Initially, Mack Sobhany, a biologist with the group and an expert in isothermal titration calorimetry, utilized this technique to demonstrate direct binding of phenobarbital to EGFR. Simultaneously, Negishi reached out to his longtime collaborators, NIEHS lead researchers Lee Pedersen, Ph.D. and Lalith Perera, Ph.D., of the Computational Chemistry/Molecular Modeling Support Group, to model the potential binding of phenobarbital to EGFR. Their model predicts that phenobarbital binds to EGFR, overlapping with the EGF binding site, suggesting that phenobarbital may interfere with EGF binding to EGFR.

As Negishi explained, “Each result confirmed the other.” These results not only resolved the half-century-old question of what is

Findings could have broad implications for human health

The identification of a novel mechanism that explains how a drug activates a nuclear receptor opens up new avenues for discovery.

First, a number of environmental agents perform activation functions similar to those of phenobarbital. However, their mechanisms of action are unclear. It is possible that these chemicals act in a similar manner to phenobarbital, by directly binding to a kinase receptor at the cellular membrane to regulate a signaling cascade that activates a nuclear receptor.

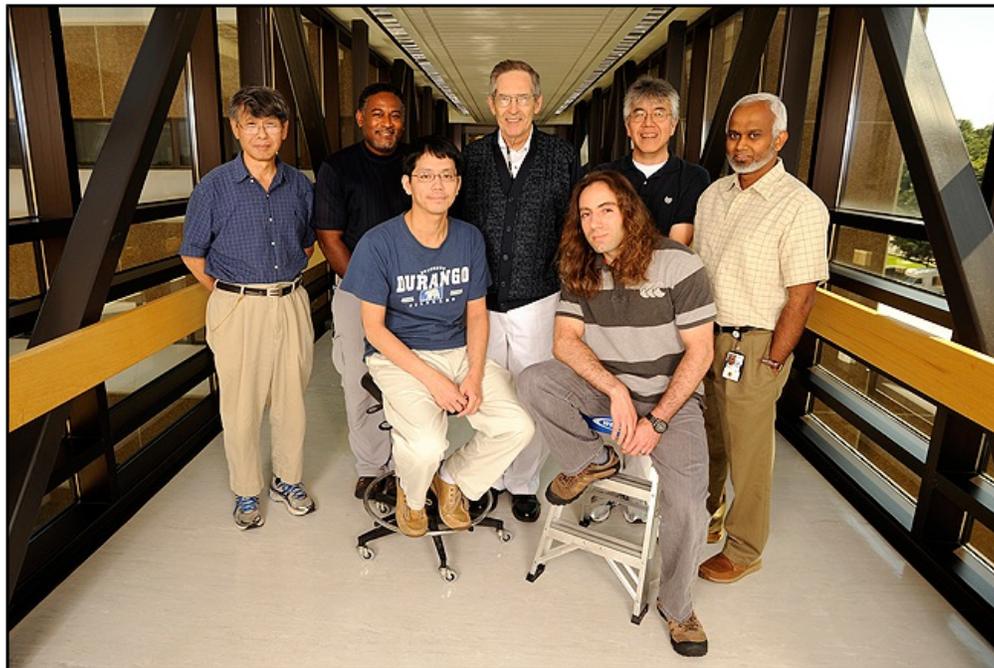
Second, as Mutoh explained, “This mechanism may help in drug discovery, as companies that screen chemicals for their induction of drug-metabolizing enzymes can now also use EGFR activity as an additional readout of their efficacy.”

Lastly, Negishi said, “These results greatly expand the field of nuclear receptor research, by demonstrating their regulation by membrane receptors.” Thus, the results of this study could have broad implications for a number of human health issues associated with aberrant growth factor and nuclear receptor signaling.

the phenobarbital receptor, by identifying EGFR, but also addressed the mechanism by which phenobarbital regulates drug metabolism in the liver.

Citation: Mutoh S, Sobhany M, Moore R, Perera L, Pedersen L, Sueyoshi T, Negishi M. (<http://www.ncbi.nlm.nih.gov/pubmed/23652203>) 2013. Phenobarbital indirectly activates the constitutive active androstane receptor (CAR) by inhibition of epidermal growth factor receptor signaling. Sci Signal 6 (274):ra31.

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The study grew out of collaboration between researchers in the Laboratory of Reproductive and Developmental Biology, and the Laboratory of Structural Biology. Shown, from left, are Negishi, Rick Moore, Mutoh, Pedersen, Sobhany, Tatsuya Sueyoshi, Ph.D., and Perera. (Photo courtesy of Steve McCaw)

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