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CRAFTing a New Approach to Antiepileptic Drug Discovery

Srivastava PK, van Eyll J, Godard P, Mazzuferi M, Delahaye-Duriez A, Steenwinckel JV, et al. A systems-level framework for drug discovery identifies Csf1R as an anti-epileptic drug target. *Nat Commun*. 2018;9(1):3561. doi:10.1038/s41467-018-06008-4.

The identification of drug targets is highly challenging, particularly for diseases of the brain. To address this problem, we developed and experimentally validated a general computational framework for drug target discovery that combines gene regulatory information with causal reasoning ("Causal Reasoning Analytical Framework for Target discovery"-CRAFT). Using a systems genetics approach and starting from gene expression data from the target tissue, CRAFT provides a predictive framework for identifying cell membrane receptors with a direction-specified influence over disease-related gene expression profiles. As proof of concept, we applied CRAFT to epilepsy and predicted the tyrosine kinase receptor Csf1R as a potential therapeutic target. The predicted effect of Csf1R blockade in attenuating epilepsy seizures was validated in 3 preclinical models of epilepsy. These results highlight CRAFT as a systems-level framework for target discovery and suggest Csf1R blockade as a novel therapeutic strategy in epilepsy. The CRAFT is applicable to disease settings other than epilepsy.

Commentary

Drug discovery is a challenging enterprise filled with stories of dramatic cures and heartbreaking clinical trial failures. The classical approach to drug development begins with "target identification," whereby a particular molecule (most often a protein) is thought to have clinical significance. Identification could occur serendipitously but is typically the result of hypothesis-driven basic science research in model systems, such as mice. Ultimately, a clinically oriented team becomes convinced that the target has potential and sets in motion an array of biochemists and pharmacologists in order to characterize the target and develop screening tools to try to identify small molecules that will bind to and disrupt the target's function.[1] The story for monoclonal antibodies and newly developed gene therapies is similar, but promising leads may fail at any stage in the process. Even more concerning, these therapies may make it to clinical trials but ultimately lack the benefit that was anticipated, suggesting that the original target was not after all, an effective target.

In response to the difficulties in drug development, a growing number of investigators are seeking to develop a new paradigm for identifying therapeutic targets. Rather than conceiving of therapies that work by disrupting a single protein target, the goal is to match changes in gene transcription caused by therapies to disease states where transcription is perturbed.[2] In the case of disorders such as epilepsy, this would represent a change from saying "What drugs stop seizures?" to asking "What drugs will produce a change in transcription so that the epileptic brain is more similar to the nonepileptic brain?"

Although many groups have attempted this approach for different disorders, Srivastava et al have made an exceptional amount of progress in applying this method to the problem of epilepsy. To begin, they performed RNA sequencing on the hippocampi of 100 mice treated with pilocarpine (a model for temporal lobe epilepsy [TLE]) and 100 control animals. They then used computer algorithms in order to find groups of genes that changed in the same direction in the different animals. This approach generated "modules" that behaved similarly (so that if a given mouse expressed 50% more of gene "X," the other genes in the module were also increased in their expression). Overall, they found 28 modules representing clusters of genes related to distinct functions such as "inflammation" and "synaptic transmission." Afterward, they correlated the change in each module's expression with the seizure frequency of the mice (prior to their sacrifice for RNA sequencing) and found that increasing activity in the inflammation module was positively correlated with seizure frequency. To validate their approach, they performed a similar analysis on human neurosurgical specimens. They observed that the same pattern of gene modules emerged in the human samples, suggesting that the research team had indeed identified genetic signatures of the epileptic brain.

The next step in their study justifies the method's title (CRAFT: "Causal Reasoning Analytical Framework for Target

Discovery") and is what separates their approach from previous efforts. Their goal was to identify a surface receptor whose activity would change the transcription levels of the entire inflammation genetic module from the "epileptic" version back to the "healthy" version. To do this, they built a computer model of how different membrane receptors influence changes in gene transcription using published databases of membrane receptor function. Put another way, if one considers the set of genes that is altered in TLE as a set of dominoes that has toppled, the authors asked which one was pushed first? This insight led them to identify the gene *Csf1R*, which encodes the macrophage-colony stimulating factor (M-CSF) receptor expressed by microglia. In their computational model, reducing the activity of the M-CSF receptor changed the expression of a wide range of genes and ultimately pushed the network back to the expression level of genes seen in the healthy samples. After an exhaustive modeling study, they had finally arrived at the testable hypothesis that the microglial M-CSF receptor was overactivated in TLE and that this was producing a wide range of downstream transcriptional changes to promote seizures.

To test their hypothesis, they used an inhibitor of the M-CSF receptor (PLX3397) in their original mouse model of TLE. Fascinatingly, treating the TLE mice with PLX3397 both rescued the expression of the genes altered in TLE and limited seizure frequency. To confirm that this wasn't an idiosyncratic result, they then validated their results in another mouse model of TLE (intrahippocampal kainate) and obtained similar seizure-reducing results. These results raise the possibility of a novel disease-modifying treatment for TLE as well as a new paradigm for finding additional antiepileptic drugs.

The role of inflammation in TLE has been increasingly recognized and represents a possible unifying mechanism for the diversity of neurologic insults that can produce TLE.[3] Inflammatory mechanisms have been described as attractive therapeutic targets because they may be disease-modifying without the side effects that characterize antiepileptic drugs targeting ion channel function.[3] However, enthusiasm for directly targeting inflammatory mechanisms should also be tempered by the many unknowns of microglial function in the brain. Beyond their role in inflammation, microglia play a crucial role in remodeling synaptic function, and dysregulation of microglial function has been implicated in a range of neurologic and psychiatric diseases.[4-6] While Srivastava et al have raised the possibility that limiting microglial activity may abrogate TLE, there is the distinct possibility of unforeseen cognitive consequences that may not be readily apparent in animal models.[4]

A more general challenge for employing CRAFT for target identification is in distinguishing the mechanism of disease from the body's response to it. In the case of TLE, limiting seizures is an end in and of itself that may curb disease progression.[7] It is much less obvious that this paradigm can distinguish between homeostatic and pathologic changes in transcription in other diseases. Perhaps a more challenging limitation for CRAFT is its reliance on models of how surface receptor activity translates into changes in transcription. Many neurological diseases feature mutations in proteins as a key pathological mechanism that could invalidate models for predicting transcriptional responses to receptor activation. Trinucleotide repeat disorders such as spinocerebellar ataxia type I often feature "gain-of-function" mutant proteins that affect a wide variety of cellular processes and whose transcriptional networks may be harder to model.[8] As a result, while CRAFT represents an exciting new opportunity for epilepsy drug discovery, there is more work to be done in order to generalize the method across neurological disorders.

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References

1. Lyman KA, Han Y, Chetkovich DM. Animal models suggest the TRIP8b-HCN interaction is a therapeutic target for major depressive disorder. *Expert Opin Ther Targets*. 2017;21(3):235-237.
2. Lamb J, Crawford ED, Peck D, et al. The connectivity map: using gene-expression signatures to connect small molecules, genes, and disease. *Science*. 2006;313(5795):1929-1935.
3. Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. *Nat Rev Neurol*. 2011;7(1):31-40.
4. Monji A, Kato T, Kanba S. Cytokines and schizophrenia: microglia hypothesis of schizophrenia. *Psychiatry Clin Neurosci*. 2009; 63(3):257-265.
5. Blank T, Prinz M. Microglia as modulators of cognition and neuropsychiatric disorders. *Glia*. 2013;61(1):62-70.
6. Wu Y, Dissing-Olesen L, MacVicar BA, Stevens B. Microglia: dynamic mediators of synapse development and plasticity. *Trends Immunol*. 2015;36(10):605-613.
7. Reynolds EH. Early treatment and prognosis of epilepsy. *Epilepsia*. 1987;28(2):97-106.
8. Orr H, Zoghbi H. Trinucleotide repeat disorders. *Annu Rev Neurosci*. 2007;30:575-621.