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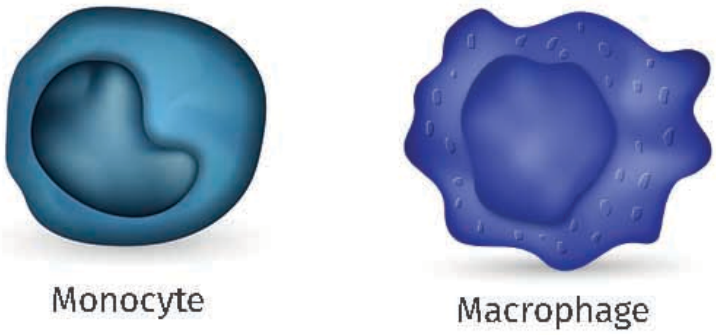
LJI scientists flip molecular switches to distinguish closely related immune cell populations
Investigators test-drive enhancer targeting as next-gen gene targeting strategy

LA JOLLA, CA—The cornerstone of genetics is the loss-of-function experiment. In short, this means that to figure out what exactly *gene X* is doing in a tissue of interest—be it developing brain cells or a pancreatic tumor—you somehow cut out, switch off or otherwise destroy *gene X* in that tissue and then watch what happens. That genetic litmus test has been applied since before people even knew the chemical DNA is what makes up genes. What has changed radically are the tools used by biologists to inactivate a gene.

Until now, scientists wishing to delete a gene in a model organism like a mouse did it by clipping out stretches of DNA encoding entire genes or very big chunks of them from the animal's genome. This type of gene "knockout" is what La Jolla Institute for Allergy and Immunology (LJI) investigator Catherine C. Hedrick, Ph.D., used in 2011, when her lab discovered that mice without the gene *Nr4a1* lack an anti-inflammatory subtype of white blood cells, nicknamed 'patrolling monocytes'.



Selectively inactivating a molecular switch that turns on the gene Nr4a1 in monocytes but not macrophages leads to the loss of patrolling monocytes, which play an important role in suppressing lung metastasis in lung cancer.

Now, the Hedrick group's latest study reports a next-generation molecular manipulation aimed at inactivating *Nr4a1* in a more precise manner. That study, published in the November 15, 2016, edition of *Immunity*, reports the loss of the same patrolling monocyte population following inactivation of a molecular switch that turns on *Nr4a1.* "This new work is exciting,

because it shows that we can directly target genes within a specific cell type, which is important for targeted therapies," says Hedrick, a Professor in the Division of Inflammation Biology.

The Hedrick laboratory's previous demonstration that patrolling monocytes disappear following global *Nr4a1* loss proved that the gene is necessary for development of that cell type. Later, her group reported that cancer cells injected into mice lacking *Nr4a1* (therefore lacking patrolling monocytes) underwent unchecked metastasis, supporting the idea that patrolling monocytes play anti-cancer roles. But an important experimental question lingered: could the cancer metastasis seen in *Nr4a1* knockout mice have anything to do with potential loss of *Nr4a1* in a closely related group of cells called macrophages, which use *Nr4a1* to control inflammation*?*

The new paper answers this question by silencing *Nr4a1* only in patrolling monocytes. The Hedrick group accomplished this by applying good old-fashioned biochemistry to isolate stretches of DNA that flank the gene and define the on-switch for monocytes. Scientists call tissue-specific gene regulatory elements like this "enhancers." They then showed that when activated, that DNA region, which they called "enhancer #2" (E2), was capable of switching on *Nr4a1* expression only in patrolling monocytes, and not in related cells like macrophages.

The group proved the specificity of the enhancer by engineering mice whose genomes lacked *only* the E2 enhancer—*not* the gene itself—and indeed observed a lack of patrolling monocytes. "Until now, we did not have a way to delete a gene only in monocytes without also deleting it in macrophages," says Graham Thomas, Ph.D., a postdoc in the Hedrick lab and the study's first author. "Targeting the enhancer allows us to study particular cell types in a highly specific way," says Thomas. "Also, eliminating enhancers teaches us what turns these genes on in the first place. That knowledge is essential if we are going to design rational targets to go after these cells."

To confirm that macrophages throw an entirely different molecular switch to turn on *Nr4a1*, the group exposed mice missing the monocyte E2 switch to a noxious toxin found in bacterial membranes, as a way of seeing whether macrophages can still mount normal inflammatory responses. Indeed, the macrophage response was entirely normal in E2 mutants, unlike the global *Nr4a1* "knockout", showing that macrophages do not use the genetic E2 switch.

Finally, to make sure that E2 enhancer loss mimicked deletion of the entire gene in monocytes the group revisited a tumor model previously used to test *Nr4a1*'s anti-cancer effect. To do so, they injected melanoma cells into the bloodstream of normal or E2 mutant mice and monitored lung metastasis. Remarkably, outcomes following loss of the switch mirrored what the group had previously observed when they physically removed the gene itself: the lungs of mutant mice contained many more melanoma cells than did lungs of normal mice. This confirmed that the gene regulatory switch is highly specific to one cell type, monocytes and that tumor cell invasion in the absence of this population had nothing to do with deregulated macrophage activity.

Hedrick also thinks the new findings provide new understanding of just how important DNA enhancer regions can be. "Being able to selectively target specific cell types opens up a new world for understanding how to design therapies to treat disease," she says.

Also contributing from LJI were Richard Hanna, Ph.D., Amy Blatchley, Debbie Yoakum, Sara McArdle, Ph.D., and Zbigniew Mikulski, Ph.D. Other contributors include Neelakantan T. Vasudevan, PhD., and Mukesh Jain, M.D., from Case Western Reserve University in Cleveland; Casey Romanoski, PhD., from the University of Arizona; and Kevin Ross, Bruce Hamilton, Ph.D., and Chris Glass, PhD, all from UCSD.

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About La Jolla Institute
La Jolla Institute for Allergy and Immunology is dedicated to understanding the intricacies and power of the immune system so that we may apply that knowledge to promote human health and prevent a wide range of diseases. Since its founding in 1988 as an independent, nonprofit research organization, the Institute has made numerous advances leading towards its goal: life without disease®.

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