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Lymphoma overrides a key protein's quadruple locks

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Protein chemists at Johns Hopkins report they are closer to explaining why certain blood cancers are able to crack a molecular security system and run rampant.

In a detailed description of their discoveries in lab-grown human cells published in two companion papers in the *Journal of Biological Chemistry* on April 15, the investigators offer evidence that mutations in cancerous lymphoma cells break through not one but four "locks" on the protein CARD11.

"A lot of the immune system's signaling proteins have a lock built into them to prevent miscommunication with other proteins," says Joel Pomerantz, Ph.D., associate professor of biological chemistry at the Johns Hopkins University School of Medicine and a member of the Johns Hopkins Kimmel Cancer Center. "This is the first one we know of that has four locks."

A few years ago, Pomerantz says, doctors learned that "rogue" or mutated CARD11 proteins are found in about 10 percent of people with the activated B cell-like (ABC) subtype of diffuse large B-cell lymphoma (DLBCL). When his laboratory set out to understand why and how the molecular "lock" that normally keeps CARD11 proteins in check fails, they discovered not one but four separate locks in operation.

Usually, when surveillance cells of the immune system encounter an invading bacterium or virus, they chew it up and display pieces of the

invader on their outer membranes like a "wanted" poster to alert the rest of the immune system, including T and B cells that can launch an attack against the invader. Pomerantz's group previously found that the protein CARD11 is locked in an "off" position in T and B cells until they detect these wanted posters. CARD11 then unlocks so that it can connect with other signaling proteins that command the body's army of T and B cells to multiply to fight the infection.

But sometimes, he says, mutations in the protein effectively unlock CARD11 so that it ramps up the body's white blood cell supply when no infection is present. "Unsurprisingly," he says, "these mutations are sometimes found in patients with lymphoma," particularly in the ABC subtype of DLBCL. "Surprisingly," he adds, "none of the mutations exist in the lock region of CARD11, and we wanted to know why."

In an effort to solve that puzzle, Pomerantz and his team, working with human, lab-grown T cells, first genetically deleted CARD11's "lock," or autoinhibitory domain, so that the protein was always "on" and signaling. Then, to figure out which region of the lock accounted for its function, the team systematically deleted six different segments of it one at a time, rather than deleting the whole thing, expecting most of the deletions to keep CARD11 locked and one or two to unlock it.

Instead, Pomerantz says, none of the deletions unlocked it, suggesting there was more than one lock within the autoinhibitory domain.

To see if that was the case and to find out how many locks there might be, the team deleted the full autoinhibitory domain again, then added back small regions of it, one and two at a time. Doing so, they pieced together the presence of four different biochemical regions that are each capable alone of locking CARD11, which they dubbed "repressive elements."

"Having four redundant repressive elements seems to explain why patients with lymphoma don't have mutations in CARD11's autoinhibitory domain," says Pomerantz. "A mutation in any one of the repressive elements would only unlock one of the four locks, keeping CARD11's signaling under the control of the other three."

So where do the lymphoma-associated mutations occur, and how do they circumvent CARD11's quadruple locks? Clinical data show that the mutations occur in three other regions of CARD11, called the CARD, LATCH and coiled-coil regions. Biochemical tests done by Pomerantz's team on human T cells showed that those three regions clasp the autoinhibitory domain to keep CARD11's activity on lockdown. This makes sense, says Pomerantz, because CARD11 uses those same regions to connect with other signaling proteins to send its message. "When they are interacting with the autoinhibitory domain, they can't be interacting with other proteins," he says.

According to Pomerantz, single mutations in the CARD, LATCH or coiled-coil regions appear to be sufficient to disable the protein's four safety locks and the ultimate goal is to advance the development of therapies that rein in hyperactive CARD11 in patients with lymphoma. "If we can understand how CARD11 is normally kept off, we might be able to mimic that with a drug," he says. "We also hope to shed light on how different mutations in CARD11 affect its function and a patient's prognosis."

More information: 1. Rakhi P. Jattani et al. Cooperative Control of Caspase Recruitment Domain-Containing Protein 11 (CARD11) Signaling by an Unusual Array of Redundant Repressive Elements, *Journal of Biological Chemistry* (2016). DOI: 10.1074/jbc.M115.683714

2. *Journal of Biological Chemistry*, dx.doi.org/10.1074/jbc.M115.717322

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