Gene targeting discovery opens door for vaccines and drugs

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In a genetic leap that could help fast track vaccine and drug development to prevent or tame serious global diseases, DMS researchers have discovered how to destroy a key DNA pathway in a wily and widespread human parasite. The feat surmounts a major hurdle for targeting genes in *Toxoplasma gondii*, an infection model whose close relatives are responsible for diseases that include malaria and severe diarrhea.

"This opens a wide window on a complex parasite family and can help accelerate the development of safe and effective genetically modified vaccines and drug therapies," says team leader David Bzik, PHD, professor of microbiology and immunology. The work is reported in the April issue of *Eukaryotic Cell* with Barbara Fox, senior research associate of microbiology and immunology who is the lead author and innovator of the study.

Parasites steal shamelessly from their hosts, co-opting resources to survive and infect. T gondii, however is a clever contrarian: it invites destruction and goes underground.

"Most parasites, along with bacteria and viruses, are shape shifters, so the immune system can't catch up with them; but *T. gondii* actually wants to be destroyed," says Bzik. "It has a unique strategy to elicit an immune response that stops the actively growing parasite and something in that response drives it to a latent stage which is necessary for its transmission."

The food borne parasite, often transmitted from cats, can be serious, even fatal for immune deficient people or newborns of mothers infected in pregnancy. While the *T. gondii* infection is harmless in most people, the parasite does takes up permanent residence inside its host. Its virulent cousins include Plasmodium, which causes lethal malaria and Cryptosporidium, a common source of waterborne diarrhea that can be severe or intractable in children or those with HIV.

"There is an amazing immune response hard-wired into this parasite to deliver life-long immunity to *T. gondii*," Fox says. "So our work has been recently focused at creating safe, attenuated (weakened), and genetically defined *T. gondii* strains that also piggyback antigens to deliver sorely needed vaccines for malaria, cryptosporidiosis, tuberculosis, HIV/AIDS, or even cancer. This finding overcomes the bottleneck for quickly developing multiple manipulated and completely safe strains where each genetic manipulation is precisely defined and irreversible."

T. gondii is easy to grow in the lab and has other amenable attributes that have made it a leading model for understanding intracellular pathogens. It belongs to the Apicomplexan family of protozoa, along with its other medically important relatives. Family members share numerous genes, but many are unique to Apicomplexa, making it difficult to predict or determine gene functions.

Employing a cut and paste genetic engineering technique, scientists can knock out or replace a gene to determine or change its functions. Most model organisms rejoin the manipulated pieces at the location of their proper and predictable sequence.

The dominant pathway in *T. gondii*, however, is random insertion. The parasite uses a pathway of nonhomologous end joining (NHEJ), which is also used to repair DNA in broken chromosomes, and arbitrarily reinserts targeting DNA segments at incorrect locations. That makes

isolating strains with defined and targeted gene knockouts a difficult, time consuming and painstaking adventure.

Using a strategy Fox devised, the DMS team disrupted and killed a parasite gene called KU80 that is involved in the NHEJ DNA repair pathway. Their success effectively turned the parasite into a dependable genetic workhorse for all the diverse organisms in the Apicomplexa phylum. It permits a direct approach to determine gene function by examining mutants lacking a specific gene.

"The KU80 knockout strain holds much genetic magic," says Bzik. "Remarkably, it exhibits 100 percent homologous recombination and gene targeting efficiency compared to the parent strain. This also provides the first biological proof of a functional NHEJ DNA repair pathway in a protozoan."

The work makes *T. gondii* an effective model for understanding a globally significant parasite family and holds promise for speeding up new therapies. "To create safe, genetically modified products or vaccines to put into people, we need to be able to efficiently and reliably target strains for genetic manipulation," Fox explains.

"Fundamentally, all possible growth and virulence factors as well as the potential for transmission must be first genetically deleted; then key protective antigens or genes from other sources must be introduced in a precisely defined way. We needed to be able to do this efficiently, reliably and, cleanly. Now we can."

Source: Dartmouth Medical School (news : web)