Knockouts in human cells point to pathogenic targets

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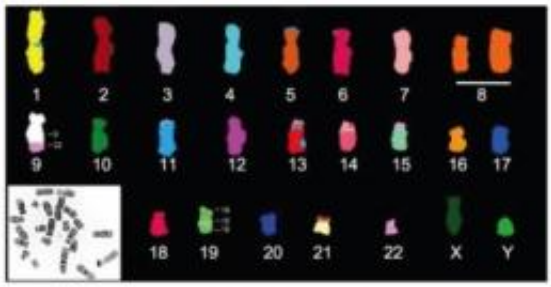

In the rare human cell line used for this genetic screen, the cells have only one copy of each chromosome, except for chromosome 8, which has two copies. Because this cell line has only one copy of almost all of the chromosomes, it is ideal for efficiently making knockout human cells. Photo - Courtesy of the Whitehead Institute

(PhysOrg.com) -- Whitehead researchers have developed a new approach for genetics in human cells and used this technique to identify specific genes and proteins required for pathogens.

With the ability to generate knockout cells for most human genes, the authors were able to find genes used by pathogens to enter and kill human cells. The identification of such factors could aid the future development of new therapeutics to combat infectious disease.

Whitehead researchers have developed a new type of genetic screen for human cells to pinpoint specific genes and proteins used by pathogens,

according to their paper in *Science*.

In most human cell cultures genes are present in two copies: one inherited from the father and one from the mother. Gene inactivation by mutation is therefore inefficient because when one copy is inactivated, the second copy usually remains active and takes over.

In yeast, researchers have it easier: they use yeast cells in which all genes are present in only one copy (haploid yeast).

Now Carette and co-workers have used a similar approach and used a human cell line, in which nearly all human chromosomes are present in a single copy.

In this rare cell line, Carette and co-workers generated mutations in almost all human genes and used this collection to screen for the host genes used by pathogens. By exposing those cells to influenza or to various bacterial toxins, the authors isolated mutants that were resistant to them. Carette then identified the mutated genes in the surviving cells, which code for a transporter molecule and an enzyme that the influenza virus hijacks to take over cells.

Working with Carla Guimaraes from Whitehead Member Hidde Ploegh's lab, Carette subjected knockout cells to several bacterial toxins to identify resistant cells and therefore the genes responsible.

The experiments identified a previously uncharacterized gene as essential for intoxication by diphtheria toxin and exotoxin A toxicity, and a cell surface protein needed for cytolethal distending toxin toxicity.

"We were surprised by the clarity of the results," says Jan Carette, a postdoctoral researcher in the Brummelkamp lab and first author on the Science article. "They allowed us to identify new genes and proteins

involved in infectious processes that have been studied for decades, like diphtheria and the flu. In addition we found the first human genes essential for host-pathogen interactions where few details are known, as is the case for cytolethal distending toxin secreted by certain strains of E. coli. This could be important for rapidly responding to newly emerging pathogens or to study pathogen biology that has been difficult to study experimentally."

Brummelkamp sees the work as only the beginning.

"Having knockout cells for almost all human genes in our freezer opens up a wealth of biological questions that we can look at," he says. "In addition to many aspects of cell biology that can be studied, knockout screens could also be used to unravel molecular networks that are exploited by a battery of different viruses and bacteria."

Provided by Massachusetts Institute of Technology (news : web)