Study demonstrates effectiveness of new approach to protect humans from infectious disease

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There are two common approaches to protecting humans from infectious disease: Targeting pathogens and parasites with medicines like antibiotics, or dealing with the conditions that allow transmission. A paper published today in the journal Nature *Scientific Reports* demonstrates the effectiveness of a third strategy: Adjusting the landscape of the human body to remove the mechanism that allows pathogens to cause disease. The discovery is the result of serendipity and collaboration between high-level scientists in different fields.

"It was pure luck that I ended up on this paper," says Dan Theodorescu, MD, PhD, director of the University of Colorado Cancer Center. "Bill Petri and I had been social friends for years - Christmas parties, that kind of thing. When I was at Virginia it happened that we were on a recruitment committee together and the candidate was late, so we started talking."

His conversation with William A. Petri, Jr., MD, PhD, chief of the Division of Infectious Diseases & International Health at the University of Virginia led to the idea of applying an innovative cancer science technique to the study of infectious disease. With first author Chelsea Marie, PhD, postdoctoral researcher in the Petri Laboratory at Virginia, the group decided to silence genes in human cells to discover if the loss of any single gene would confer immunity to the parasite *E. histolytica*, which infects 50 million people and causes 40,000-110,000 deaths via severe diarrhea worldwide.

"Chelsea is a fearless experimenter. She took a library of cells that Dan had developed in his work with bladder cancer and then sequentially killed them with *E. histolytica* parasites," Petri says.

Specifically, the group used the technique called RNAi to create a library of bladder cancer cells with thousands of independent, silenced genes. Then they challenged these cultures with the parasite *E. histolytica*.

"We do this all the time in cancer research," Theodorescu says. "Commonly, we're looking for genes that, when silenced, will make cells more susceptible to chemotherapy."

In this case the analogue of chemotherapy was the infectious, dangerous pathogen.

"This amoeba is a cluster bomb - a voracious killer. In the back of my mind I was thinking the parasite was going to decimate the host cells no matter what we did with their genetics," Marie says.

For the vast majority of cells in this genome-wide screen, Chelsea Marie was correct; *E. histolytica* decimated many thousands of these independent cell cultures. However, a small number of cells seemed to resist the parasite. Was this the random chance of lucky survival or had silenced genes somehow offered immunity to these cells? To find out, Marie discarded the killed cells and retested the cells that had survived; again she infected these survivor cells with *E. histolytica*.

"It wasn't a fluke," says Marie. "We did this over nine generations of cells, each time selecting the cells that survived and then re-applying the parasite. Over these generations of selection, we saw the cultures becoming more and more enriched for cells lacking specific genes."

Using next generation sequencing, Marie identified the genes that conferred resistance and found that many were involved in managing the flow of potassium into and out of human cells. Specifically, the identified genes KCNA3, KCNB2, KCNIP4, KCNJ3, and SLC24A3 are involved in what is called potassium transport. A follow-up experiment showed that new intestinal cells treated with *E. histolytica* showed potassium efflux - the flow of potassium from inside a cell out through the cell wall - directly before cell death.

"We started to see a pretty clear line of reasoning," says Theodorescu. "The parasite was causing potassium efflux right before cell death and cells that happened to be unable to transport potassium didn't die."

To ensure that lack of potassium transport was, in fact, causing resistance to the parasite, the group reversed the direction of their experiments. Marie started with new cells and used drugs to block their ability to transport potassium. Blocking potassium efflux created cells that were resistant to *E. histolytica*.

"There is a clear need for new drugs targeting *E. histolytica*," Petri says. "Right now there is a single antibiotic that works against this parasite. We know that eventually the parasite will develop resistance to the antibiotic and at that point there's no plan B. This could be the plan B - targeting the human genes that enable the parasite to cause disease."

Marie is pushing forward. She recently learned from a mentor at John's Hopkins how to isolate stem cells from human tissue to grow what she calls "mini guts" to test therapeutics that may be useful in human patients. And technological advances make this study's general technique more efficient, allowing the use of what are called CRISPR libraries instead of RNAi screens.

"This is a major finding with translational implications for this infection that causes so many deaths worldwide, but also proof that this cancer-science approach can be used to explore genetic mechanisms of resistance in the field of infectious disease," Theodorescu says.

The field of infectious disease has been focused on the infection, targeting pathogens and their transmission. This study shows that in addition to characteristics of the parasite, mortality due to disease can be prevented by manipulating characteristics of the host.

Source:
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