

# Evolutionary arms race

A 400 million-year-old battle between HIV and ancient genes, *HERC5* and *HERC6*

Two human genes, *HERC5* and *HERC6* have protective effects against HIV and other viruses. Dr Stephen D. Barr from Western University, Canada, traces the evolution of these genes throughout human history and investigates their protective effects. His work has shown that these genes are involved in an evolutionary arms race, with host defence mechanisms and the virus ever trying to outwit one another. Increased understanding of the viral mechanisms involved may present novel therapeutic targets for antiviral treatments.

The immune system is part of the host's defence mechanism against potentially dangerous pathogens, such as bacteria and viruses. However, many viruses have evolved ways in which they can avoid elimination by the cells of the immune system. These adaptations include avoiding detection or hiding from immune cells, interfering with host processes or preventing recruitment of immune cells to the site of infection.

HIV is one such virus which evades the immune response by preventing antibodies from binding to itself and by destroying immune cells, amongst other strategies. Understanding more about the mechanisms which viruses use to avoid antiviral activity is vital for the future development of antiviral drugs.

## THE HERC GENE FAMILY AND HIV

Several proteins induced by signalling molecules, called interferons, have previously been identified as exhibiting antiviral activity towards HIV. These proteins include those encoded by a family of genes called *HERC* genes. There are six *HERC* members that are classified into 'large *HERC*' proteins (*HERC1* and *HERC2*) and 'small *HERC*' proteins (*HERC3*, *HERC4*, *HERC5* and *HERC6*). The small *HERC* proteins are highly similar proteins of which only *HERC5* and *HERC6* are interferon-induced in humans. Although the functions of the small *HERC* family members are not fully understood, they do have antiviral effects.

Dr Stephen D. Barr and his team at Western University, Canada, previously discovered that the human protein called *HERC5* potently inhibited the replication of human immunodeficiency virus (HIV), identifying it as a new

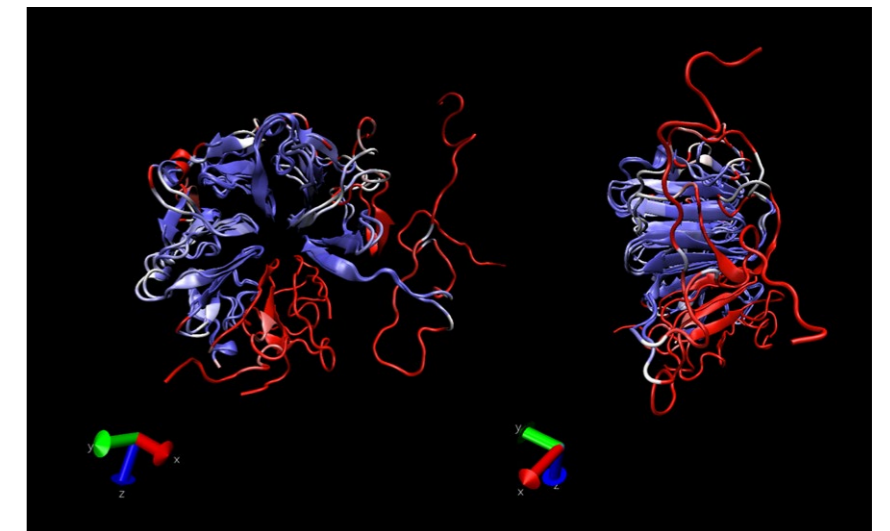
candidate for HIV therapy. In contrast, whilst *HERC6* also inhibited HIV replication, it did so to a much lesser extent than *HERC5*.

Based on this work, the Barr lab were also interested in the origin of the *HERC5* gene and how its antiviral activity has evolved in vertebrates.

In order to do this, they searched genome databases to find the earliest emergence of the small *HERC* gene members and tracked their evolution through vertebrates over time. More recent versions, or distantly related versions, of genes can be recognised by matching similarities in their genetic sequences to their ancestral genes. In this way a family tree of genes can be made, which identifies the original genes and follows the changes and divergence of these genes over time and between species.

Dr Barr's team showed that the oldest member of the small *HERC* gene family is *HERC4*. *HERC4* is present in one of the few lineages of jawless fish that survive today, sea lampreys, which originated almost 600 million years ago. *HERC4* has since duplicated itself three times at different evolutionary timepoints in the tree of life. The last expansion of the *HERC* family happened after the divergence of the ray-finned fish, such as eels, salmon and sturgeon, around 400 million years ago and this probably resulted in a duplication of the *HERC6* gene which gave rise to *HERC5*. The earliest vertebrate identified to possess *HERC5* was an ancient marine organism called the coelacanth, a type of fish that emerged over 400 million years ago.

To better understand how *HERC5* and the other *HERC* proteins evolved in vertebrates, Dr Barr's team compared



Three dimensional models of evolutionarily similar *HERC5* proteins.

dozens of evolutionarily divergent *HERC* proteins, looking for similarities and differences that might give clues about how the antiviral function of *HERC5* emerged. One example of a program which they used for this complex analysis is the Structural Alignment of Multiple Proteins (STAMP), which aligns proteins based on their three-dimensional structures. The STAMP analysis showed that a particular region of the *HERC5* protein that is key for antiviral activity, the RCC1-like domain, was highly similar in other *HERC* proteins and in *HERC* proteins from evolutionarily distant species, such as coelacanth and human. This highlighted the potential that *HERC5* in other vertebrates, and perhaps other *HERC* proteins, may possess antiviral activity and that this activity has an ancient origin.

## AN EVOLUTIONARY ARMS RACE

In order to avoid detection and destruction by the host immune defences, viruses continually evolve mechanisms to escape detection. To combat this, host defences must also evolve to keep pace with the viral adaptations. This evolutionary arms race, sometimes called the Red Queen hypothesis, is the driving force behind the battle to simply survive in a constantly evolving environment. This dynamic coevolution process is caused by a positive feedback loop; gain of fitness, or the ability to survive, in one organism

must be met by an increase in fitness by the competing organism.

*HERC* genes originated in marine species; however, not all vertebrates possess *HERC* genes. This suggests that different forms of the genes emerged

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at different points during evolution, when they would confer a survival advantage to the host. Two of the more recent *HERC* genes, *HERC5* and *HERC6*, were observed to have been involved in an evolutionary arms race with viruses for hundreds of millions of years. Because of this battle, these genes have developed sophisticated ways to put up a shield in cells to block viruses.

Using sequence analysis, the Barr lab showed that positive selection pressure is causing both *HERC5* and *HERC6* to evolve. For example, they have previously demonstrated that part of the RCC1-like domain in *HERC5* has been evolving for over 100 million years in this way, providing further evidence that these genes are inextricably linked with viral evolution. Interestingly, *HERC3* and *HERC4*, which also possess the RCC1-like domain, do not seem to be evolving under selective pressure despite being much older than *HERC5* and *HERC6*.

## ANTIVIRAL EFFECTS AGAINST OTHER VIRUSES

In order to assess whether the antiviral effects of the genes were effective against related viruses, Dr Barr and his team tested their antiviral activity towards a non-human virus, the monkey version of HIV called simian immunodeficiency virus (SIV). SIV is thought to be at least 32,000 years older than HIV, so it could be hypothesised that primate immune responses may

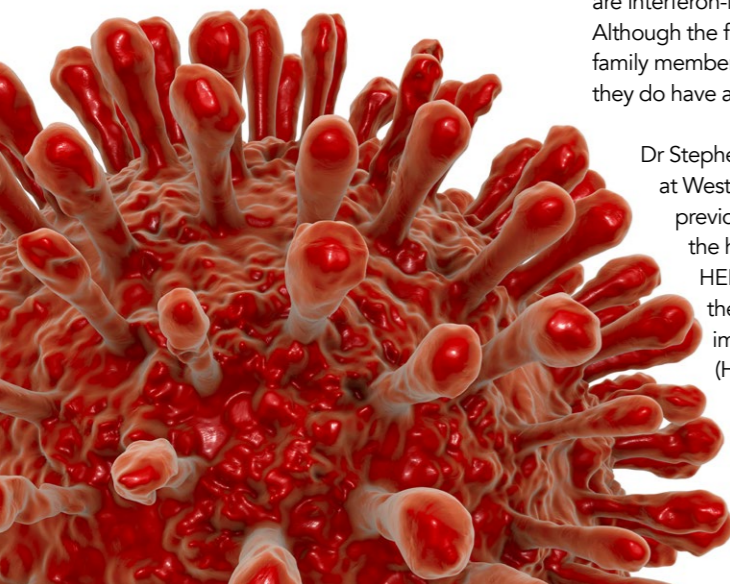
be more evolved to target SIV compared to HIV. They tested *HERC5* and *HERC6* as these exhibited both the strongest and the weakest antiviral effects in human cells.

Human cells expressing increasing concentrations of coelacanth *HERC5*, human *HERC5* or human *HERC6* were infected with either SIV or HIV. As the team predicted, human *HERC5* showed the greatest protective effect against



A coelacanth, the earliest vertebrate identified to possess *HERC5*.

The HIV virus. Some human genes have protective effects against the virus.





Salmon (large image), sea lamprey (top inset) and sturgeon (bottom inset) all carry versions of the *HERC* genes.

HIV, with coelacanth *HERC5* and human *HERC6* showing little inhibition of HIV. Surprisingly, all *HERCs* potentially inhibited SIV virus replication. This suggests that the protective responses of *HERC5* and *HERC6* exhibit species- and virus-specific activity.

Given the knowledge that the RCC1-like domain is important in the immune response against viral infection, Dr Barr asked why *HERC5* and *HERC6* varied in their antiviral activities, with particular focus on this domain. Indeed, his team showed that if part of the RCC1-like domain from *HERC5* was transferred into *HERC6*, then HIV particle production was inhibited to a level similar to *HERC5*. Dr Barr mentions that, "additional structure-function studies are needed to understand exactly how

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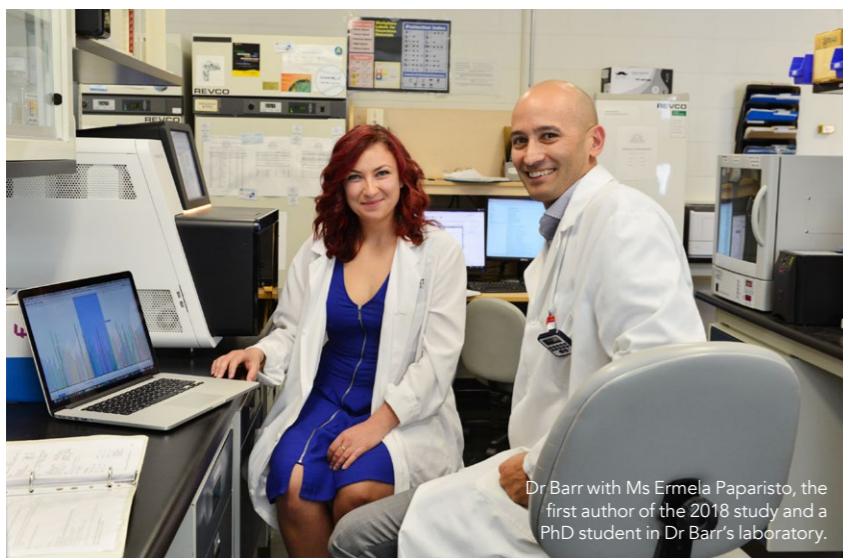
this part of the RCC1-like domain exerts its antiviral activity."

Since viruses such as HIV have been in battle for so long, they have had time to learn ways to get around the host defence shields and become smarter. Consequently, this new level of sophistication can allow these viruses to jump the species barrier

to establish new infections in humans, a process called zoonosis. An interesting comparison would be to infect monkey cells with the viruses to see whether the observations from human cells are also seen in another species.

### DEVELOPMENT OF NOVEL THERAPIES

Dr Barr's work investigating the arms race between genes and viruses has provided new insights into how both the immune system and viruses have evolved. The work by the Barr lab has highlighted that the *HERC* family are likely to have an important role in intrinsic immunity. Dr Barr's ultimate goal is to discover more about the mechanisms which viruses use to inactivate *HERCs* and other similar antiviral proteins. In doing so, he provides a window of opportunity by which this knowledge can be exploited for the development of novel antiviral drugs. If these mechanisms can be specifically targeted, antiviral genes such as *HERCs* will remain active during infection and will increase viral clearance and therefore improve the outcome for the patient.



Dr Barr with Ms Ermela Paparisto, the first author of the 2018 study and a PhD student in Dr Barr's laboratory.

Photo Credit: Crystal MacKay, Schulich School of Medicine & Dentistry, Western University



# Behind the Research

## Dr Stephen Barr

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### Research Objectives

Dr Stephen Barr's work aims to illuminate the evolution of the HIV virus and the family of *HERC* genes that inhibit HIV.

### Detail

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### Bio

Dr Barr obtained his PhD in Molecular Biology from the University of Calgary under the supervision of Dr Lashitew Gedamu, studying a family of antioxidant proteins called Peroxiredoxins and how they protected cells from infection by Leishmania. He then studied various aspects of HIV biology as a Postdoctoral Fellow with Dr Rick Bushman at the University of Pennsylvania. He continued his Fellowship with Dr James Smiley at the University of Alberta where he studied the host interferon response towards HIV. In 2008, Dr Barr became an independent investigator where his laboratory continues to study the host interferon response towards viruses such as HIV and Ebola virus.

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- Natural Sciences and Engineering Research Council of Canada (NSERC)
- Canadian Institutes of Health Research (CIHR)
- CWRU/UH Center for AIDS Research (CFAR)
- National Institute of Allergy and Infectious Diseases (NIAID)

### Collaborators

- Dr Eric Arts
- Dr Yong Gao



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### Personal Response

#### How do you plan to further develop your understanding of the antiviral effects of the *HERC* proteins?

“ We are currently comparing the antiviral activities of evolutionarily diverse *HERC* proteins towards HIV and other viruses such as Ebola virus to better understand how broad the antiviral activity of *HERCs* are, and to discover how viruses evade these *HERC* proteins for survival. We also hope to test these effects in animal models that are more biologically relevant to the human immune system. ”