MicroRNAs dictate the Epstein-Barr virus' elaborate waiting game, cancer formation

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While most commonly associated with mononucleosis, Epstein-Barr virus (EBV) has been linked to many diseases that affect people long after the initial infection takes place, including some forms of cancer. In the current issue of the *Journal of Biological Chemistry*, scientists at The Wistar Institute describe how viral microRNA – small segments of RNA that suppress the effects of gene activity – allows EBV to hide within cells and evade the immune system. The scientists believe their findings may one day enable physicians to flush EBV out of hiding, allowing a healthy immune system to rid the body of the virus.

According to the scientists, EBV uses microRNA encoded among its own genes to create an elaborate timing mechanism that allows it to quietly persist until an opportune moment to reproduce en masse. In particular, a viral microRNA, called BART6, keeps EBV in a latent, or quiet, state by preventing the host cell from creating its own microRNA as part of normal gene regulation.

"Epstein-Barr infection is marked by a period of active infection and replication – the lytic stage – where it causes acute disease, but it can also remain latent, and later emerge as an effective cancer-causing agent," said Kazuko Nishikura, Ph.D., a professor in Wistar's Gene Expression and Regulation program and senior author of the study. "It is a strategy that allows EBV to survive our initial immune response and await conditions, such as weakened immunity, to reemerge."

According to the Centers for Disease Control and Prevention, up to 95

percent of Americans are infected with EBV. While only a small portion of these infections ever lead to cancer, EBV has been associated with diseases that include cancers such as Burkitt lymphoma, Hodgkin's lymphoma, and a form of sinus and throat cancer called nasopharyngeal carcinoma.

"Our findings suggest that EBV and humans have been engaged in a complex microRNA arms race, where EBV evolved microRNA that specifically exploit the human host cell's own microRNA machinery," Nishikura said.

These findings add to the growing body of evidence that suggest microRNA activity has a real and potent effect on health, Nishikura says. MicroRNAs are among a host of objects encoded within our DNA that help regulate how genes are read – or "expressed" – by our cells in the form of proteins.

MicroRNAs suppress gene activity by knocking out messenger RNAs, molecules that serve to convey genetic instructions to our cell's protein-making machinery. In effect, microRNA suppression of messenger RNA is akin to the diner manager who fires a waiter in the middle of a shift – your order may have been placed, but the kitchen will never make that grilled sandwich you want.

Nishikura and her colleagues found that BART6 directly prevents the production of the human protein, called DICER, responsible for creating microRNAs by "dicing" up stretches of RNA encoded in our DNA. In silencing DICER, BART6 also silences an EBV gene, called EBNA2, which creates a protein that can "transform" human cells into a cancerous state in the process of forcing the cell to create new copies of the Epstein-Barr virus.

The result is a complex feedback mechanism that can be tipped into

inciting cancer if the human immune system is weakened by age or HIV/AIDS infection, for example. However, EBV becomes vulnerable to the immune system when infected cells begin producing viral proteins in significant numbers, so BART6 helps EBV maintain a balance between total silence and a degree of activity that would attract the attention of the immune system. BART6 serves as a sort of timer, since EBV relies on its host cell's DICER protein to create viral mircoRNA, including BART6. As BART6 levels drop, DICER and EBNA2 become active, which ultimately leads to BART6 becoming active again. Cancer may occur, for example, when the upswing in EBNA2 coincides with a drop in immune system activity.

"Epstein-Barr virus uses microRNA to achieve a balance between suppressing genes and promoting genes," Nishikura said. "This system has been so precisely tuned through evolution that BART6 only interacts with human DICER messenger RNA, which may explain why EBV doesn't infect other animals."

However, there are two sides to this RNA arms race, since humans evolved a strategy to counteract BART6 through "RNA editing," where members of the ADAR (adenosine deaminase acting on RNA) family of genes actively alter microRNA precursors. Nishikura and her colleagues also found that ADAR1 prevents the cell from making fully matured forms of BART6 and stops the formation of a critical RNA-protein machine consisting of BART6, DICER, and other proteins collectively known as RISC (RNA induced silencing complex). BART6 only works after being integrated into RISC, so RNA editing appears to be an evolutionary adaptation to BART6 activities, Nishikura says.

When the researchers removed BART6 from the feedback cycle, the cells in culture began to produce a number of viral genes, including EBNA2. In humans, Nishikura says, this would expose cells to the immune system, enabling the body to clear itself of EBV in healthy

patients. "Although it may be some time before we can manipulate microRNA as a part of patient care, these findings offer evidence that we may one day use some of the same tools our cells use to regulate gene activity," Nishikura said.

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