

The Epstein-Barr virus, (EBV) is one of the most common viruses in humans but also one of the most mysterious. In many people it remains latent for many years, but in others it causes several forms of cancer – Hodgkin lymphoma, Burkitt lymphoma, nasopharyngeal carcinoma, and B-cell lymphoma, which occurs in immunodeficient people such as those with AIDS. For cancer scientists, the question is ‘What causes the virus to wake up and trigger disease?’

I. George Miller, Jr., MD, John F. Enders Professor of Pediatrics and Professor of Epidemiology and of Molecular Biophysics and Biochemistry, has been working on EBV since 1967. It has given up its secrets slowly.

In 1985, he and his colleague, Jill Countryman, discovered a viral protein that acts as a master switch between the latent and replicating stages of EBV. They named this gene ZEBRA (Z EB Replication Activator). Dr. Miller has been studying it ever since. His most recent finding is one of his most exciting.

ZEBRA belongs to a family of related cellular proteins called AP-1 (activator protein). Both ZEBRA and AP-1 are transcription factors – they recognize specific sequences in DNA and bind to them. “We noticed that there are five amino acids in AP-1 that contact DNA specifically,” Dr. Miller said. “Four of them were in exactly the same position as on ZEBRA. But the fifth one in ZEBRA was a serine and in AP-1 it was an alanine.”

Dr. Miller’s lab made a mutant ZEBRA that replaced that serine amino acid with the alanine from AP-1. Dr. Miller and his colleague, Amy Francis, described the surprising consequence of that tiny change in a paper published in 1997: “It basically inactivated the ability of the ZEBRA protein to drive the lytic cycle of EBV,” he said. That is, it blocked the protein’s ability to push the virus into the replication that leads to cancer.

Next, in a paper published last May in *Proceedings of the National Academy of Sciences*, Miller and his colleague, Kuan-Ping Yu, did the opposite experiment; they created a mutated AP-1 by substituting the variant amino acid from ZEBRA – the serine – for the alanine on AP-1. With that change, AP-1 suddenly was able to drive EBV into replication. And like ZEBRA proteins, these mutated AP-1 proteins preferentially bound to methylated DNA, a feature associated with cancer.

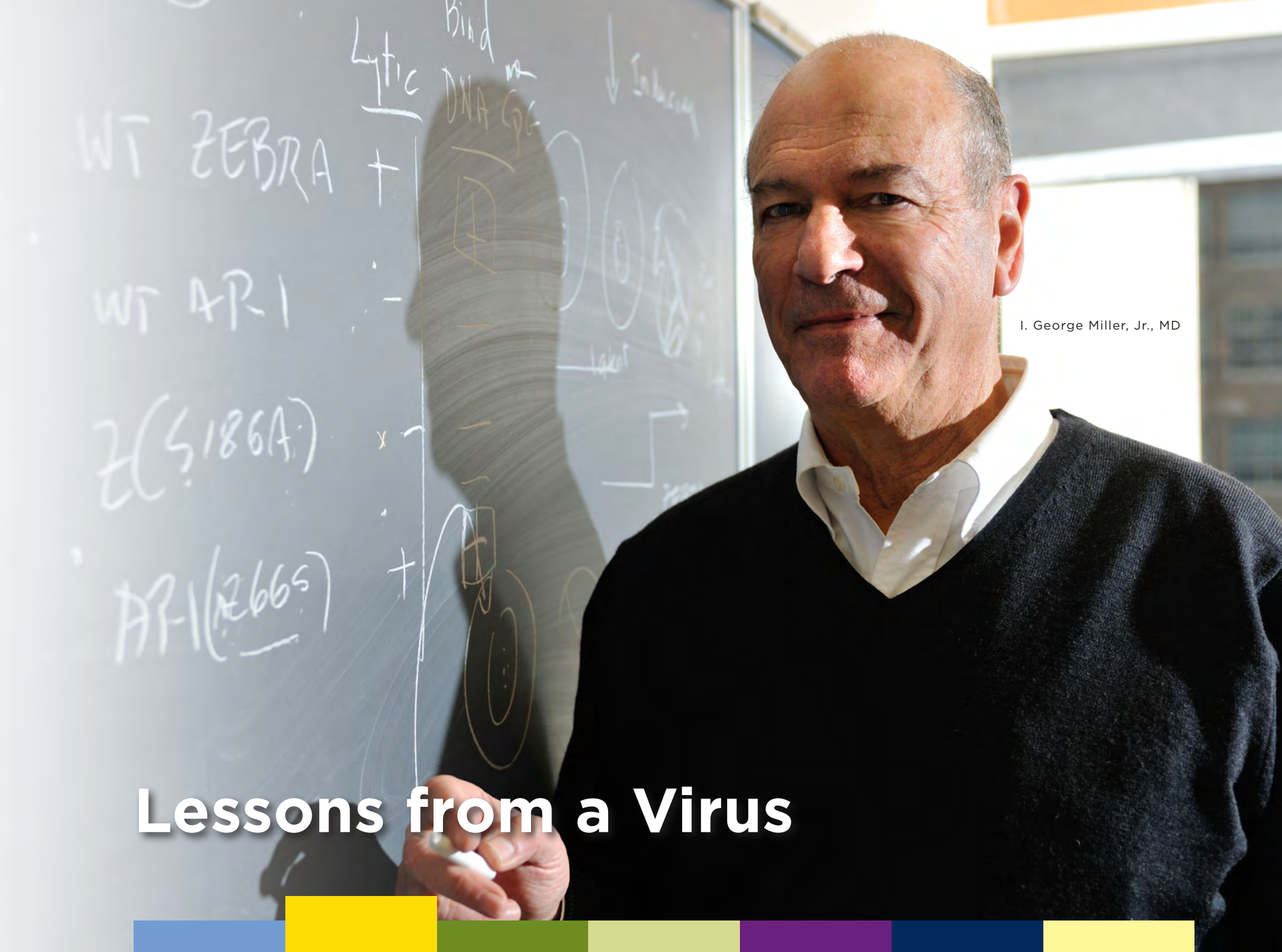
“That’s important,” Dr. Miller said. “It overturns the dogma, which you’ll find in all the textbooks, that DNA methylation is inhibitory to gene expression. As a general tool to explore the role of promoter methylation in cancer biology, I think this is going to be very powerful.”

This finding also makes Dr. Miller wonder whether mutations in other cellular proteins, similar to the ones he made, could cause a virus to move from latency to replication. If so, molecular profiling could reveal those mutations in a patient and provide advance warning about the risk factors before the virus activated into replication and cancer.

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Dr. Miller and his collaborators are now trying to understand which genes are regulated by methylation. That knowledge could provide clues for designing drugs to inhibit the binding of transcription factors to methylated DNA. “We’re looking for ways to wake up silenced genes with these cellular proteins that now bind to methylated DNA,” he said. “We might be able to program cells to go back into normal cells, to activate or repress gene expression. Ultimately we’ll be able to alter the way methylation regulates the gene expression of the cell.” But first, he added, there’s a lot of basic science to do.

Dr. Miller expects EBV and ZEBRA to keep surprising him, like the way they overturned the received wisdom that DNA methylation represses gene expression. “The thing I like is that it’s a long story,” he said. “I think there’s going to turn out to be cellular genes where methylation helps gene expression. The virus keeps teaching us about what’s going on in the cell.”



I. George Miller, Jr., MD

Lessons from a Virus