

Human papillomavirus (HPV) causes almost all cancers of the cervix and anus, and a large percentage of cancers of the vagina, vulva, penis, and the back of the throat. The virus is spread by sexual activity, but vaccination can help prevent infection.

Deciphering how HPV gets into cells is a quest for Daniel DiMaio, MD, PhD, Waldemar Von Zedtwitz Professor of Genetics and Professor of Therapeutic Radiology and of Molecular Biophysics and Biochemistry, and Deputy Director of Yale Cancer Center. Several years ago, he and other researchers discovered that HPV follows an unusual path to the cell nucleus. The virus itself is not covered by a membrane, but as it enters cells it is encapsulated in a membrane-bound vesicle, or sac, called the endosome. Dr. DiMaio and his colleagues also showed that for HPV to successfully complete the entry process, a viral protein named L2 must bind to a protein called retromer inside the cell cytoplasm. The retromer then takes the viral cargo into what's called the retrograde pathway, which transports it to the nucleus.

Dr. DiMaio knew what had to happen for viral infection, but he was puzzled about how it occurred. "It wasn't clear how the virus was able to see the retromer and bind to it," explained Dr. DiMaio, "which we knew was required for proper trafficking of the virus."

Now Dr. DiMaio and his colleagues have solved this conundrum. They published their findings in *Cell* last September [2018]. "We found that L2 has a short sequence of only six amino acids that can actually poke through the endosome membrane into the cytoplasm, so it can

bind to retromer," said Dr. DiMaio.

After Dr. DiMaio and his colleagues hypothesized such a mechanism, his lab tested and confirmed it through a novel assay. Most of the experiments in the *Cell* paper were performed by Pengwei Zhang, PhD, a post-doctoral associate in Dr. DiMaio's lab. Other collaborators on this study were Gabriel Monteiro da Silva, MS, Catherine Deatherage, PhD, and Christopher Burd, MS, PhD, Professor of Cell Biology.

In another first, they also discovered that L2 contains a cell-penetrating peptide (CPP). These peptides were discovered in other proteins 30 years ago, but their biological role remained virtually unknown. "This is one of the first times that the normal function of a CPP has been elucidated," said Dr. DiMaio. "People have been studying them for a long time, trying to figure out how they get proteins into cells, but in fact that's not what this one is doing. Rather, it's crossing a membrane that's already inside the cell. It may be a general property of CPPs that they're not used so much to transfer proteins from outside to inside, but rather from one compartment inside a cell to another."

After the probing end of L2 pierces the membrane, it functions as a pipeline into the cell for the HPV particle. The L2 pipeline is the virus's only contact with the cytoplasm. The main body of the virus stays inside the endosome, invisible to the cell.

"Cells have all sorts of mechanisms to halt foreign invaders," said Dr. DiMaio, "and viruses come up with all sorts of strategies to overcome that. HPV's strategy is

to stay inside these vesicles and never expose itself to the cellular immune system during entry."

The ability of cell-penetrating peptides to enter cells and deliver cargo raises the tantalizing possibility of using them to deliver anti-cancer drugs. Dr. DiMaio intends to explore this idea using L2.

In the meantime, these recent discoveries suggest new ways of preventing HPV infection and the cancers it causes. If a targeted drug could stop L2 from binding to retromer, the virus couldn't infect. Blocking protein interactions can be tricky, said Dr. DiMaio, but this one only involves three amino acids on L2. "So, it's at least plausible to find a small molecule to prevent that."

Another possibility would be to stop L2 from protruding through the endosome membrane in the first place. That would quarantine HPV in its vesicle and prevent infection. "Based on our improved understanding of the entry mechanism," Dr. DiMaio said, "we're hopeful that we will be able devise ways to prevent infection."

He emphasizes that the current vaccination for HPV is very good and is likely to remain the mainstay for prevention. Yet not everybody responds to it, it's expensive, and some people refuse it. "Having additional approaches to block infection might be very useful," he explained.

"We think these targeted approaches could be applicable to every single HPV type because all the papillomaviruses have L2 sequences that penetrate membranes and bind to retromer. If you could develop a way to prevent the L2 cell-penetrating peptide from working, that would be a general solution."



Daniel DiMaio, MD, PhD

Unlocking The Mechanisms of HPV Entry