

■ VIROLOGY

Human Alpha-defensins Block Papillomavirus Infection

Buck CB, Day PM, Thompson CD, Lubkowski J, Lu W, Lowy DR, and Schiller JT. Human alpha-defensins block papillomavirus infection. *Proc Natl Acad Sci U S A* 103: 1516–21, 2006.

Sexually transmitted human papillomaviruses (HPVs) are the primary cause of cervical cancer. HPVs have also been implicated in a substantial fraction of other genital cancers, as well as some head, neck, and anal cancers. Although only a minority of infected individuals develop cancer, HPV infection is very common, and cervical cancer kills several hundred thousand women worldwide each year.

Papillomaviruses replicate exclusively in stratified squamous epithelial tissues such as the skin or the genital mucosa. Because the viral life cycle is closely linked to cellular differentiation in these tissues, papillomaviruses cannot be cultured using conventional monolayer cell lines. Recently, we in CCR's Laboratory of Cellular Oncology have developed systems to efficiently mass-produce papillomavirus-based gene delivery vectors, known as papillomavirus pseudoviruses. HPV pseudoviruses, which are capable of efficiently delivering reporter plasmids to a wide variety of cell lines, have rapidly become a useful tool for studying the initial infectious entry phase of the HPV life cycle. The availability of reporter pseudoviruses has made it possible to perform targeted screens to identify compounds that might inhibit the infectious entry of papillomaviruses.

We used HPV pseudoviruses to perform targeted screens of commercially available candidate inhibitor compounds. Initial screens focused on peptide effectors of innate immunity that have previously been found in the human female genital tract. By far, the most promising compounds identified were a group of human innate immune effector peptides known as α -defensins. Defensins fold into a disulfide-stabilized β -sheet structure that allows them to kill various types of bacteria and viruses. Initially, it was believed that defensins, which have a high cationic charge, acted primarily by disrupting the relatively anionic lipid membranes of various bacteria and lipid-enveloped viruses. More recently, it has been appreciated that another important action of these molecules is to antagonize viruses, such as HIV-1, in ways that may involve signaling or other modifications of host cells. Other recent reports have shown that defensins can block the uptake of bacterial toxins, such as *Anthrax* lethal toxin and *Pseudomonas* exotoxin. Furthermore, some defensins trigger cellular signaling through toll-like receptors, which are normally involved in detecting common molecular patterns displayed by microbial pathogens.

Using pseudoviruses transducing a green fluorescence protein (GFP) gene as a marker of infection, we showed that α -defensin types 1, 2, 3, and 5 are potent inhibitors of the infectious entry of HPVs into cultured cells (Figure 1). In contrast, β -defensin types 1 and 2 displayed much less or no inhibitory effect against HPV infection. Microscopy studies revealed that α -defensins block HPV escape from endocytic vesicles but do not interfere with virion binding, internalization, or virion uncoating. Because HPVs have a naked (non-enveloped) surface, the result adds to the growing realization that the antimicrobial power of defensins extends beyond their ability to chemically disrupt microbial membranes.

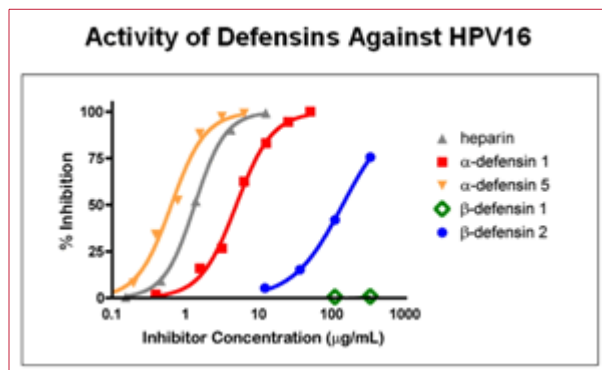


Figure 1. Inhibition of HPV16-GFP (green fluorescent protein–labeled human papillomavirus 16) pseudovirus infection in HeLa cells by human defensins. Pseudovirus and inhibitors were added to subconfluent cells in 96 well plates and GFP-positive cells were counted by a fluorescence-activated cell sorter (FACS) 3 days post infection. Heparin is a highly sulfated form of heparan sulfate previously described as a potent inhibitor of papillomavirus infection.

Recent reports have shown that some α -defensins may be present in the female genital tract at concentrations that inhibit HPV infection *in vitro*. Although the levels of α -defensins 1 through 3 increase under conditions of inflammation, α -defensin 5 appears to be produced constitutively at levels near the *in vitro* inhibitor concentrations. The average level of various defensins probably differs between individuals, perhaps reflecting the fact that humans are genetically polymorphic at the level of defensin gene copy number. In one intriguing study, women who appear to exhibit natural resistance to infection with HIV-1 were shown to have consistently higher levels of α -defensins 1 through 3 in cervical biopsy samples. It will be important to determine whether higher α -defensin 5 levels correlate with women's relative resistance to HPV infection.

Recent meta-analyses have concluded that condoms, as routinely used, have limited effectiveness in preventing the transmission of HPVs. Thus, there is significant interest in the development of compounds that might be used as topical microbicides to block the transmission of HPVs and other sexually transmitted infections. The fact that α -defensins exhibit potent, broad-spectrum antimicrobial activity *in vitro* raises the possibility that they might function to block HPV transmission if applied as topical microbicides. Furthermore, the fact that α -defensins are ordinarily present in the female genital tract suggests that they would be safe to use for routine topical application.

John T. Schiller, PhD

Senior Investigator

Laboratory of Cellular Oncology

NCI-Bethesda, Bldg. 37/Rm. 4112B

Tel: 301-496-6539

Fax: 301-480-5322

schillej@dc37a.nci.nih.gov

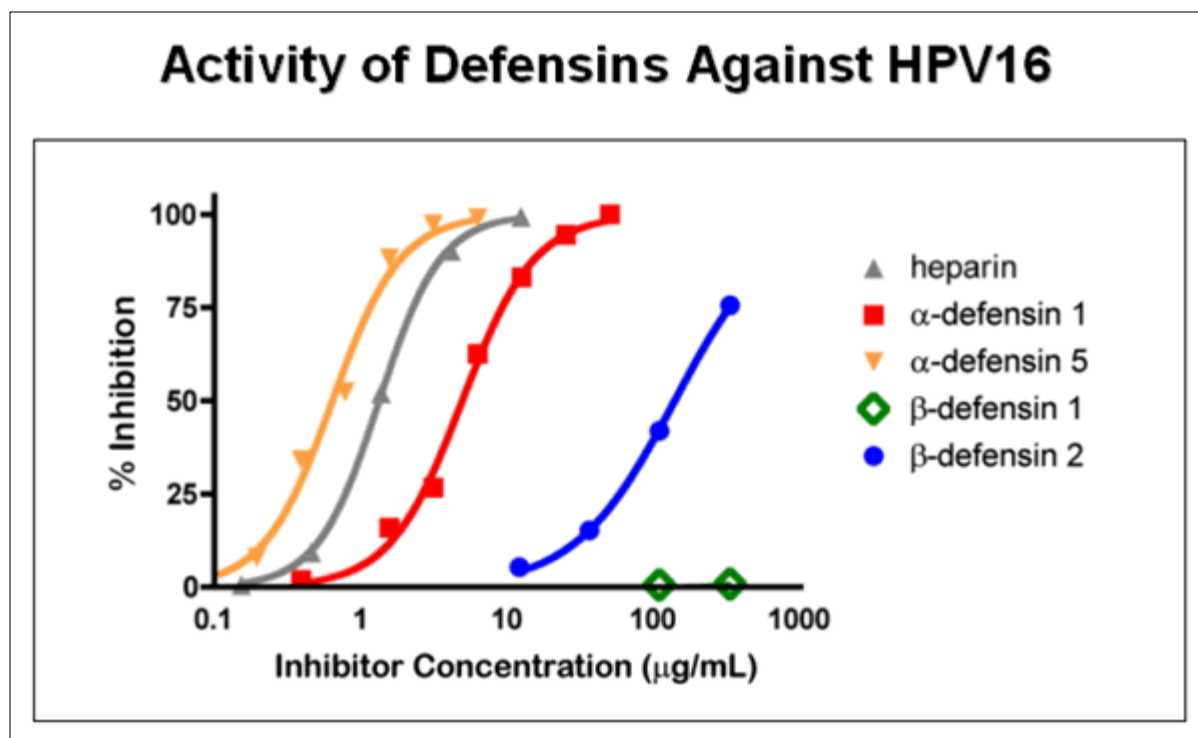


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