

## **The Nuclear DNA Sensor IFI16 Acts as a Restriction Factor for Human Papillomavirus Replication through Epigenetic Modifications of the Viral Promoters**

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**INTRODUCTION.** Intrinsic immunity is mediated by cellular restriction factors that are constitutively expressed and active even before a pathogen enters the cell.

The host nuclear factor IFI16 acts as sensors of foreign DNA and antiviral restriction factors. It is a multifunctional nuclear protein involved in transcriptional regulation, induction of interferon- $\beta$  (IFN- $\beta$ ), and activation of the inflammasome response. It interacts with the sugar-phosphate backbone of dsDNA and modulates viral and cellular transcription through largely undetermined mechanisms. IFI16 is a restriction factor for human cytomegalovirus (HCMV) and herpes simplex virus (HSV-1).

**METHODS.** HPV18 DNA (religated or minicircle genomes) was introduced into human keratinocytes (NIKS) or U2OS cells. Genome establishment was determined by measurements of DNA replication and viral transcription after IFI16 silencing by small interfering RNA technology or IFI16 overexpression by recombinant AdV infection. The role of IFI16 on HPV18 transcription and epigenetic modification was tested by transient-transfection of HPV18LCR-luciferase reporter constructs and ChIP experiments, respectively.

**RESULTS.** In differentiated IFI16-silenced NIKS-HPV18 cells, viral load values were significantly increased compared with differentiated control cells. Consistent with this, IFI16 overexpression severely impaired HPV18 replication in both NIKS and U2OS cells, thus confirming its antiviral restriction activity. In addition to the inhibition of viral replication, IFI16 was also able to reduce viral transcription, as demonstrated by viral gene expression analysis in U2OS cells carrying episomal HPV18 minicircles and HeLa cells. We also provide evidence that IFI16 promotes the addition of heterochromatin marks and the reduction of euchromatin marks on viral chromatin at both early and late promoters, thus reducing both viral replication and transcription.

**DISCUSSION.** IFI16 restricts chromatinised HPV DNA through epigenetic modifications and executes a broad surveillance role against viral DNA in the nucleus that is not restricted to Herpesviruses.