Symposium date: 8th September 2008

Nucleic Acids Symposium Series No. 52 **185–186**
doi:10.1093/nass/nrn094

Inhibition of human papillomavirus replication by using artificial zinc-finger nucleases

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ABSTRACT

Recently, we have designed artificial zinc-finger proteins (AZPs) that prevent a viral replication protein, E2, of human papillomavirus type 18 (HPV-18) from binding to its replication origin and demonstrated that the gene-delivered AZPs inhibited HPV-18 DNA replication in mammalian cells. In the present study, we examined a new approach to inhibition of DNA virus replication by using an AZP-nuclease fusion. In transient replication assays for HPV-18, the gene-delivered AZP-nuclease fusion reduced the viral DNA replication rate significantly. Moreover, it was demonstrated by ligation-mediated PCR that viral DNA regions close to the AZP-binding site were cleaved in the cells by the AZP-nuclease. Thus, our results demonstrate that AZP-nucleases have potentials to inhibit replication of any DNA viruses whose replication mechanisms remain unsolved.

INTRODUCTION

Previously, we demonstrated that inhibition of the binding of a viral replication protein (Rep) to its replication origin by using artificial zinc-finger proteins (AZPs, reference 1) is an effective approach to prevent DNA virus infection in both plants and mammalian cells.[2,3] Because this approach targets binding of the Rep to its replication origin, emergence of no or few resistant viruses is expected (see references 2 and 3 for the detailed discussion). For beet severe curly top virus (BSCTV) as a plant DNA virus, we introduced the gene of an AZP designed for inhibition of BSCTV replication into plant germ lines by a floral dip method so that the gene was expressed in all tissues, and demonstrated that the transgenic Arabidopsis plants were resistant to BSCTV infection.[2] For human DNA viruses, we first applied the AZP technology to human papillomaviruses (HPVs).[3] HPVs are double-stranded DNA viruses that induce benign proliferative squamous epithelial and fibroepithelial lesions (warts and papillomas) in their natural hosts, and more than 100 HPV types have been identified (reviewed in reference 4). A subgroup of HPVs classified as "high-risk" viruses, including HPV types 16, 18, 31, 35, 39, 45, 51, 52, 58, and 59, has been found to be associated with the development of cervical cancer.[5,6] Each year, about 500,000 such infections of the uterine cervix undergo malignant conversion, making cervical cancer the second most common malignancy in women worldwide.[7] About 90% of such tumors contain high-risk HPVs, with HPV-16 and -18 being the most prevalent. We demonstrated in transient replication assays with expression vectors encoding AZPs designed for HPV-18 that AZPs could inhibit HPV-18 DNA replication in mammalian cells as well.[3] Furthermore, we have recently reported that the cell-permeable AZPs inhibited HPV-18 DNA replication when added to the culture medium.[8] The cell-permeable AZPs were generated by fusing cell-penetrating peptides to AZPs as previously described.[9]

Our current approach with AZPs alone are effective against DNA viruses such as HPVs and herpes simplex viruses,[10] whose Rep's binding site(s) in their replication origins have been identified. In the present study, we explore a new approach to inhibition of DNA viruses by using AZP-nuclease fusions so that DNA viruses whose replication mechanisms remain unsolved can be targeted. In this study, we targeted HPV-18 to demonstrate the concept.

RESULTS AND DISCUSSION

A six-finger AZP was designed and constructed using our nondegenerate recognition code table[1] to recognize a 19-bp DNA in the HPV-18 replication origin. In gel-shift assays, it was demonstrated that the purified AZP strongly bound to its target site. Then the AZP was fused to a nuclease via a flexible peptide linker.

First, we examined whether the AZP-nuclease fusion purified from E. coli cleaved its target site in vitro. After incubation of the AZP-nuclease with a DNA plasmid containing its 19-bp DNA target, the resulting DNA samples were separated on a agarose gel. We confirmed in the assays that the AZP-nuclease completely digested the target plasmid DNA at 37°C. Under the same conditions, both the AZP alone and nuclease alone used as controls did not cleave the target DNA.

Next, we examined whether the AZP-nuclease inhibited HPV-18 DNA replication in mammalian cells. HPV DNA replication can be transiently reconstituted in mammalian cells by using mammalian expression plasmids for viral E1 and E2 proteins and a plasmid containing an HPV replication origin (HPV ori plasmid).[11] In the transient replication assays with a HPV-18 ori plasmid, the gene-delivered AZP-nuclease fusion inhibited the DNA

replication more efficiently than a control, the AZP alone. In the transient replication assays, the same amounts of plasmids used for the assays were introduced into 293H cells, as judged from Southern blot analysis with input DNA samples.

Finally, we investigated whether AZP-nucleases cleaved the target site on the HPV DNA in mammalian cells. Ligation-mediated PCR (LM-PCR)[12] was performed to detect the cleavage. Namely, DNA samples used for the Southern blot analysis were ligated with a adapter and then amplified by using a HPV-genome-specific primer. In the assays, a DNA band corresponding to the cleavage product was detected in the presence of the AZP-nuclease. In contrast, such a DNA band was not detected in the presence of controls, including AZP alone.

CONCLUSION

In this study, we demonstrated that the AZP-nuclease fusion was effective for inhibition of HPV-18 DNA replication. We confirmed that viral DNA regions close to the AZP-binding site were cleaved in the cells, suggesting that inhibition of HPV replication was due to cleavage of the viral genome by the AZP-nuclease. We hope that the new AZP technology will contribute to reduction of infection events due to any DNA viruses whose genomes were cloned but whose replication mechanisms remain unclear.

ACKNOWLEDGMENTS

We thank Tadayuki Imanaka and Haruyuki Atomi for the use of their DNA sequencer. This work was supported in part by the Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science to T.S.

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