Meganuclease-mediated Inhibition of HSV1 Infection in Cultured Cells

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Herpes simplex virus type 1 (HSV1) is a major health problem. As for most viral diseases, current antiviral treatments are based on the inhibition of viral replication once it has already started. As a consequence, they impair neither the viral cycle at its early stages nor the latent form of the virus, and thus cannot be considered as real preventive treatments. Latent HSV1 virus could be addressed by rare cutting endonucleases, such as meganucleases. With the aim of a proof of concept study, we generated several meganucleases recognizing HSV1 sequences, and assessed their antiviral activity in cultured cells. We demonstrate that expression of these proteins in African green monkey kidney fibroblast (COS-7) and BSR cells inhibits infection by HSV1, at low and moderate multiplicities of infection (MOIs), inducing a significant reduction of the viral load. Furthermore, the remaining viral genomes display a high rate of mutation (up to 16%) at the meganuclease cleavage site, consistent with a mechanism of action based on the cleavage of the viral genome. This specific mechanism of action qualifies meganucleases as an alternative class of antiviral agent, with the potential to address replicative as well as latent DNA viral forms.

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INTRODUCTION

Herpes simplex virus type 1 (HSV1) is a major health problem with substantial impact on quality of life and disability-related costs.[1] As with two other human *α-herpesvirinae* (namely HSV type 2 and varicella-zoster virus), HSV1 is able to become latent in neurons before inducing recurrent infections in peripheral tissues. The primary infection, asymptomatic in >90 % of cases,[2] takes place in the oral mucosae, as a consequence of contact with infected particles in saliva. After replication in epithelial tissues, viruses propagate in neurons before becoming latent. Following various triggering factors, HSV1 may reactivate and thus reinvade the peripheral tissues connected to the reactivated neuron. Since the principal location of latent HSV1 is the trigeminal ganglion (TG), responsible for sensory innervation of the face, most recurrences are located in the eyes or the lips. The seroprevalence of HSV1 in the general population ranges from 24.5% to 67%, with 15 to 45% of positive subjects experiencing recurrent herpes labialis.[1] The eye, and particularly the cornea, is the second most frequent location of HSV1 infection. The prevalence of herpes simplex keratitis is 149/100,000,[3] with >30 new events per 100,000 inhabitants annually.[4] As a consequence, HSV1 is a leading cause of blindness throughout the world. In addition, despite the use of topical or oral antiviral agents, herpes simplex keratitis remains the most frequent cause of infectious corneal opacities in the most developed areas of the world,[5] accounting for about 10% of patients undergoing corneal transplantation.[6] Moreover, the natural risk of HSV1 reactivation in recently grafted cornea is about 25% in the first year following surgery.[7] Today, HSV1 remains a major cause of corneal graft failure, accounting for about 22% of all cases of regrafting.[8]

To date, commercially available anti-HSV1 agents are able to inhibit viral replication through an inhibition of the DNA-polymerase,[9] meaning that any reduction in the bioavailability of the drug and/or the sensitivity of the virus may result in treatment failure. Moreover, current treatments do not reduce the load of the DNA matrix, and thus are unable to reduce the risk of further viral reactivation. Ideally, an ultimate weapon against HSV1 infection should be durably present in the cells, avoid questions of sensitivity and, if possible, reduce the load of viral genomes. Very specific endonucleases such as zinc finger nucleases[10,11] or meganucleases[12,13] could be used to address these goals, especially if they are delivered by a gene transfer process.

With the aim of a proof of concept study, we assessed anti-HSV1 activity in cells transfected with meganuclease-encoding plasmids. Meganucleases are endonucleases that recognize large (>12 bp) DNA sequences. In nature, they induce the spreading of mobile genetic elements by a process called homing,[14] and are therefore also called homing endonucleases. Homing endonucleases have also been used to stimulate targeted recombination

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