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Middle East respiratory syndrome coronavirus infection is inhibited by griffithsin

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ABSTRACT

Highly pathogenic human coronaviruses associated with a severe respiratory syndrome, including Middle East respiratory syndrome coronavirus (MERS-CoV), have recently emerged. The MERS-CoV epidemic started in 2012 and is still ongoing, with a mortality rate of approximately 35%. No vaccine is available against MERS-CoV and therapeutic options for MERS-CoV infections are limited to palliative and supportive care. A search for specific antiviral treatments is urgently needed. Coronaviruses are enveloped viruses, with the spike proteins present on their surface responsible for virus entry into the target cell. Lectins are attractive anti-coronavirus candidates because of the highly glycosylated nature of the spike protein. We tested the antiviral effect of griffithsin (GRFT), a lectin isolated from the red marine alga *Griffithsia* sp. against MERS-CoV infection. Our results demonstrate that while displaying no significant cytotoxicity, griffithsin is a potent inhibitor of MERS-CoV infection. Griffithsin also inhibits entry into host cells of particles pseudotyped with the MERS-CoV spike protein, suggesting that griffithsin inhibits spike protein function during entry. Spike proteins have a dual function during entry, they mediate binding to the host cell surface and also the fusion of the viral envelope with host cell membrane. Time course experiments show that griffithsin inhibits MERS-CoV infection at the binding step. In conclusion, we identify griffithsin as a potent inhibitor of MERS-CoV infection at the entry step.

1. Introduction

Coronaviruses belong to the *Coronaviridae* family. They are enveloped viruses with a large single stranded, positive-sense RNA genome and infect both humans and animals. Until 2003, human coronaviruses were responsible for only mild respiratory diseases, mainly common colds. This changed with the emergence of a highly pathogenic coronavirus associated with a severe respiratory syndrome, namely the severe acute respiratory syndrome coronavirus (SARS-CoV). In 2012, a new deadly human coronavirus emerged in the Arabian Peninsula, Middle East respiratory syndrome coronavirus (MERS-CoV) responsible for severe pneumonia that can be associated with additional clinical symptoms such as vomiting, diarrhea or renal failure (Alhogbani, 2016; Zaki et al., 2012). The MERS-CoV epidemic is still ongoing, and so far more than 1700 laboratory-confirmed cases have been diagnosed with a mortality rate of approximately 35%. There are neither clinically approved antivirals nor vaccines available against MERS-CoV and therapeutic options for MERS-CoV infections are limited to palliative and supportive care.

The MERS-CoV spike protein is a main determinant of virus entry into host cells as it mediates both binding to the DPP4 (dipeptidyl peptidase 4) receptor and fusion of the viral envelope with host cell membrane (Millet and Whittaker, 2014; Raj et al., 2013). It is a type I fusion protein and is highly glycosylated with 19 predicted N-glycosylation sites. One option to block MERS-CoV infection is to take advantage of the highly glycosylated nature of its spike protein by using lectins in order to inhibit MERS-CoV entry and thus virus propagation. Griffithsin (GRFT) is a 121 amino acid long lectin that was isolated from the red marine alga *Griffithsia* sp. Griffithsin has been shown to have antiviral activity against HIV-1 within the picomolar range (Mori et al.,

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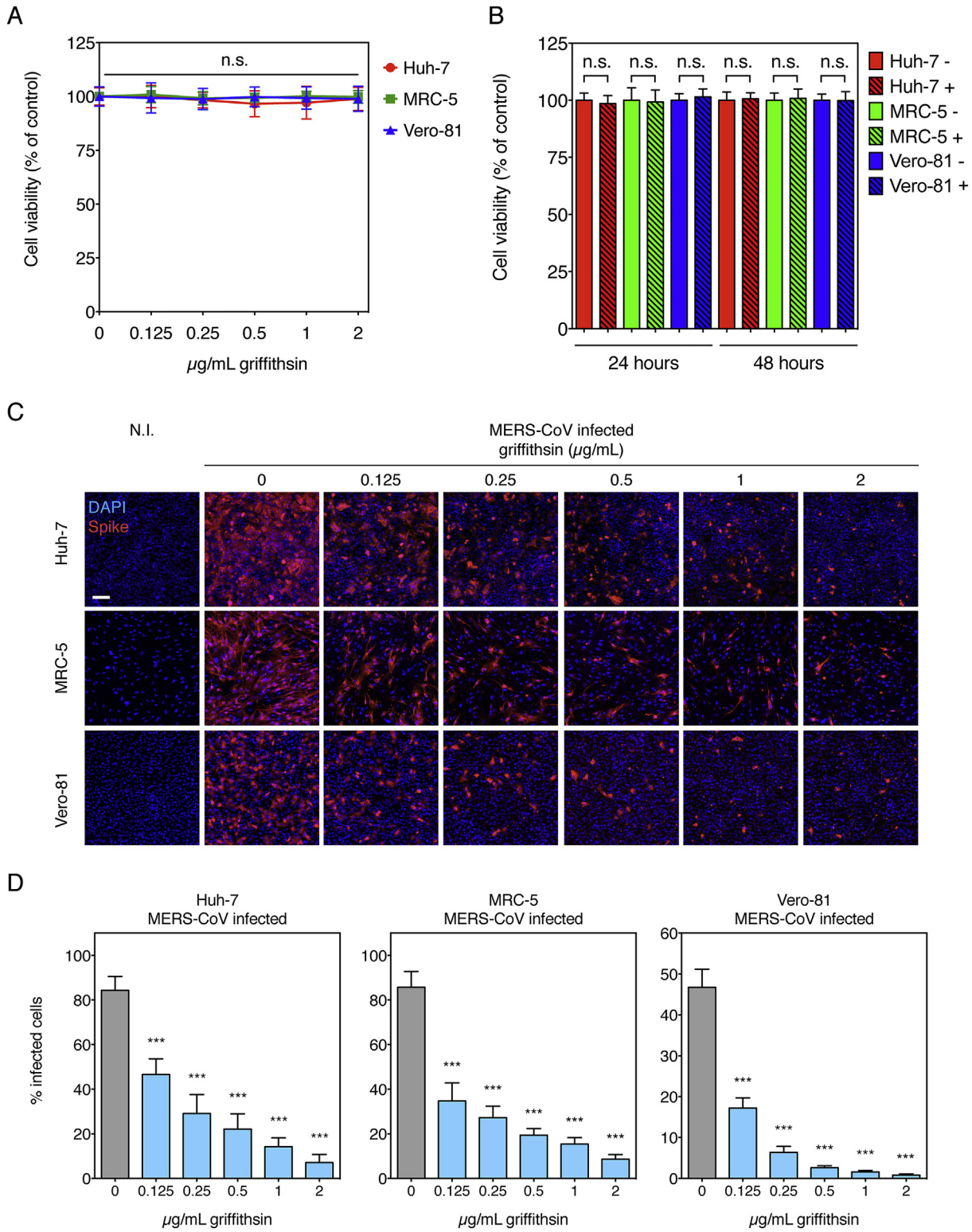

Fig. 1. Effect of griffithsin on cell viability and MERS-CoV infectivity. Griffithsin cell viability dose-response **(A)** and effect after extended incubation times **(B)**. Huh-7, MRC-5, and Vero-81 cells were treated with increasing concentrations of griffithsin **(A)** or with 0 μg/mL (−) or 1 μg/mL (+) of the lectin **(B)**. For **(A),** after 2 h of incubation, supernatants were replaced with medium without griffithsin and cells were incubated at 37 °C for 7 h. In **(B)**, cells were left treated with griffithsin for 24 h or 48 h. Cell viability was measured using a luminescence-based ATP quantitation assay. Results are expressed as average of cell viability (% of 0 μg/mL control), with error bars representing SD from the average of three independent experiments. Data were statistically analyzed using one-way ANOVA test **(A)** or using a two-tailed Student *t*-test **(B)**, with the following convention for *p*-value significance: not significant (n.s.), $p > 0.05$. **(C)** Dose-response analysis of griffithsin on MERS-CoV infection. Immunofluorescence assay of MERS-CoV-infected Huh-7, MRC-5, and Vero-81 cells in presence of increasing concentrations of griffithsin. Cells were infected with MERS-CoV strain EMC/2012 at an m.o.i. of 10, in presence of increasing amounts of griffithsin for 2 h at 37 °C. PBS was used for the non-treated control condition. Cells were washed with PBS and the inoculum replaced with cell growth medium. The cells were incubated at 37 °C 5% CO_2 for an additional 7 h. Cells were then fixed, immunolabeled for MERS-CoV S, and stained for nuclei (DAPI). N.I.: non infected control. **(D)** Quantification of MERS-CoV-positive cells in presence of increasing concentration of griffithsin. For each condition, five 10 × objective fields were randomly acquired and analyzed for total number of