BROAD-SPECTRUM THERAPEUTICS FOR VIRAL DISEASES: A MEDICAL COUNTERMEASURE PLATFORM FOR EMERGING THREATS

A Novel Class Of Small Molecule Inhibitors Of Pikfyve Potently Suppresses Encephalitic Alphavirus Infections

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Venezuelan equine encephalitis virus (VEEV) is one of several encephalitis alphaviruses that cause human infections. While typically transmitted via mosquitos, VEEV retains infectivity as an aerosol and is considered a major biothreat agent. There is an urgent need for the development of countermeasures to prevent or treat these infections.

We have previously discovered that members of the Numb-associated kinases (NAKs) family are required for effective VEEV infection and are candidate targets for broad-spectrum antivirals. While screening small molecule derivatives of NAK inhibitors for anti-VEEV activity, we identified a novel isothiazolo[4,3-b]pyridine-based inhibitor, RMC-113, which potently suppressed replication of encephalitis alphaviruses, but showed no inhibition of NAKs. RMC-113 demonstrated activity against VEEV TC-83 (vaccine strain), VEEV Trinidad donkey (TrD, wild type strain), and Eastern equine encephalitis virus (EEEV) in human astrocytes (U87-MG cells), with EC50 values of 0.2 µM, 0.5 µM, and 3.5 µM, respectively, and no apparent toxicity. RMC-113 also demonstrated potent activity against unrelated viruses from the coronavirus, flavivirus, and filovirus families. Passaging VEEV (TC-83) in U-87 MG cells for ten times in the presence of RMC-113 revealed no phenotypic resistance, as measured by plaque assays, whereas escape mutants emerged upon passaging VEEV under the nonstructural protein 2 (nsP2) inhibitor ML336. To pinpoint the specific stage(s) of the VEEV life cycle that RMC-113 suppresses, we conducted time-of-addition experiments revealing suppression of viral titer at stages corresponding to viral entry, replication/transcription, and assembly/egress. Using a trans-replication system, we confirmed that RMC-113 inhibits VEEV (TrD) viral RNA transcription and replication. In a neurovascular unit model composed of human primary brain endothelial cells, astrocytes and pericytes, RMC-113 treatment suppressed VEEV (TrD)-induced blood brain barrier (BBB) injury as measured by permeability FITC-dextran assay.

To identify the target of RMC-113, we first measured its binding to human kinases via KINOMEscan and ProQinase analyses. Both screens showed high affinity of RMC-113 to the lipid kinases PIKfyve and PIP5K2C, but not the anticipated NAKs. In vitro and cell-based kinase assays confirmed the high affinity of RMC-113 to PIKfyve and PIP5K2C. Suppression of PIKfyve, but not PIP5K2C, expression by siRNAs inhibited VEEV replication in U87-MG cells, as measured by plaque assays. Notably, overexpression of PIKfyve, but not PIKfyve kinase-dead mutant or PIP5K2C, reversed the anti-VEEV effect of RMC-113, validating PIKfyve as the molecular target mediating the antiviral effect of RMC-113. Lastly, multiple RMC-113 derivatives demonstrated comparable or improved antiviral activity that largely correlated with their anti-PIKfyve activity.

Together, our studies discovered PIKfyve as a novel target for broad anti-encephalitic alphavirus treatment contributing to our understanding of virus-host interactions in these infections and pathogeneses. Moreover, these studies proposed attractive lead small molecules with anti-PIKfyve activity, which not only suppress encephalitis alphaviral infections but also protect the BBB integrity. Our current structure-activity relationship effort aims at improving the pharmacokinetics properties of RMC-113 derivatives to advance them into in vivo studies. If successful, this work will deliver a novel class of potent inhibitors to combat multiple biothreat agents, with a high barrier to resistance.

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