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Helicases as antiviral drug targets

Key Messages

1. We have demonstrated for the first time that the helicase of a ribonucleic acid virus, the SARS coronavirus (SARS-CoV), is a valid target for drug development.
2. Using high throughput screen and chemical synthesis, several lead compounds targeting the SARS-CoV helicase have been identified. We have shown that these compounds can inhibit SARS-CoV helicase activity and viral growth in cell culture systems. These compounds can potentially be used to target other viruses.

Introduction

SARS is a serious type of pneumonia that was first recognised in late 2002 and shown to be caused by the SARS coronavirus (SARS-CoV).[1,2] We have previously reported on the purification and characterisation of the SARS-CoV helicase.[3] We have assessed a number of drugs for their ability to inhibit SARS-CoV helicase activities and viral growth.[4,5]

In order to validate the drug target, we generated drug-resistant SARS-CoV strains. Since a compound can inhibit SARS-CoV replication by either interfering directly with the helicase or with a cellular target, it is necessary to verify whether the drugs are indeed targeting the helicase. If virus mutants resistant to an inhibitory compound can be selected, it is likely that the target of the inhibitor is a viral process. Drug targets can be identified by defining the gene in which the mutation conferring the resistant phenotype has occurred. To determine the molecular target, we selected resistant viruses in the presence of increasing concentrations of a SARS-CoV inhibitor—bananin—identified in this study.[5] We then identified the nature of the mutation that confers bananin-resistance. In parallel, we expanded the screen for SARS-CoV helicase inhibitors and synthesised several bananin derivatives with SARS-CoV–inhibiting effects.

Methods and results

This study was conducted from June 2004 to May 2006.

Confirmation of helicase as valid drug target

The SARS-CoV were cultured with FRhK-4 in a 96-well plate in the presence of bananin. The released viruses in the culture medium were collected and used to infect a fresh batch of FRhK-4 cells. After three passages, selected SARS-CoV were cultured in the presence of increasing amounts of bananin. Several independently bananin-resistant strains were then isolated. Their growth in the presence of bananin were compared to that of the wild type of SARS-CoV to confirm their drug resistance. We then identified the nature of the mutation that conferred bananin-resistance. Briefly, viral ribonucleic acid (RNA) from resistant strains was prepared as described previously.[3] The helicase genes were cloned as reverse transcription–polymerase chain reaction (PCR) products. Clones were verified using restriction enzyme digestion and subjected to sequencing analysis. Sequencing confirmed mutations in the SARS-CoV helicase. Back titration (Fig 1) and real-time PCR showed that in the presence of 50 or 100 μM of bananin, mutant clones exhibit 4 to 6 times higher resistance than the wild type virus.

To investigate whether the helicase gene is responsible for drug resistance, we first sequenced the helicase genes of all drug-resistant strains, and found that they all contained a point mutation in the beginning of the helicase domain, causing Ser259, a potent phosphorylation site, to change into leucine.

We established cell lines expressing either wild types or mutants. These cell lines were infected with wild-type SARS-CoV in the presence of different concentrations of bananin. The supernatants were collected and back titrated on fresh cells. Virus RNA was also extracted and real-time PCR was performed to monitor viral replication. Mutant helicase expression can rescue wild-type SARS-CoV replication in the presence of bananin, indicating that helicase is the authentic target for bananin in vivo (Fig 2).

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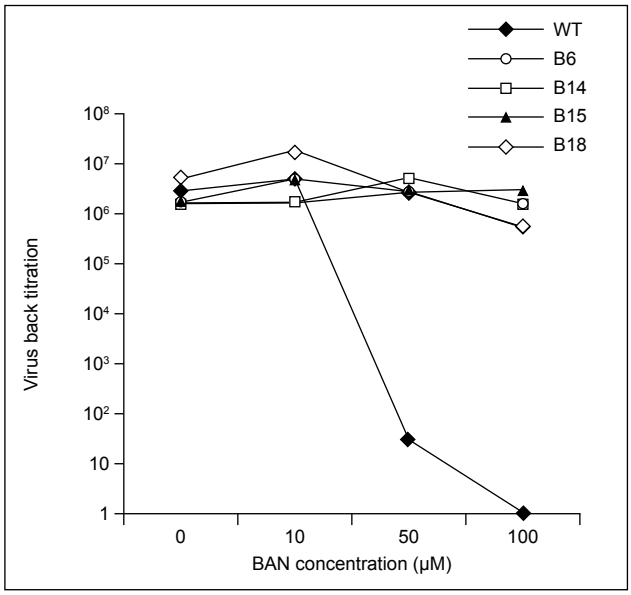

Fig 1. Bananin-resistance of selected virus strains (SCV)
SCV were cultured on FRhK-4 cells in the presence of 100 μM bananin. After several passages, 11 resistant strains were picked from independent clones. Titration of several virus clones including B6, B14, B15, B15, and wild-type virus control (WT) were carried out on FRhK-4 cells in the presence of different concentration of BAN to verify drug resistance

Screen for more SARS-CoV helicase inhibitors

We used a chemical library of 50 240 structurally diverse small molecule compounds in a cell-based assay to screen for small molecule compounds that curb the infectivity of the virus. In the primary screening using high concentrations of each compound, we identified 1003 compounds that protect cells from SARS-CoV–induced cell death. These compounds were re-tested and the concentration of selected compounds was lowered during a secondary screening. A total of 108 compounds retained consistent and significant protective effects against SARS-CoV–induced cell death. To identify compounds that inhibit the SARS-CoV helicase, we then screened the active compounds against the polynucleotide stimulated ATPase activity of SARS-CoV helicase. Seven compounds exhibited SARS-CoV helicase inhibitory activity.[4]

Synthesis of bismuth complexes against SARS-CoV

We have previously demonstrated that bismuth compounds can effectively inhibit SARS-CoV growth in cell culture. A series of bismuth complexes were designed and synthesised, including bismuth porphyrin complexes, bismuth macrocyclen complexes, bismuth 12-crown-4 complex, bismuth bipyridine complex, bismuth phenanthroline complex, Bi(NTA), Bi(EDTA) and $Bi(AHA)_3$. The newly synthesised compounds were tested for their activities against SARS helicase. Among these complexes, we found that the two bismuth porphyrin complexes and red blood cell exhibited the best inhibition activity in the in-vitro experiments.

In summary, we used reverse genetic methods to identify natural targets for the SARS-CoV inhibitor

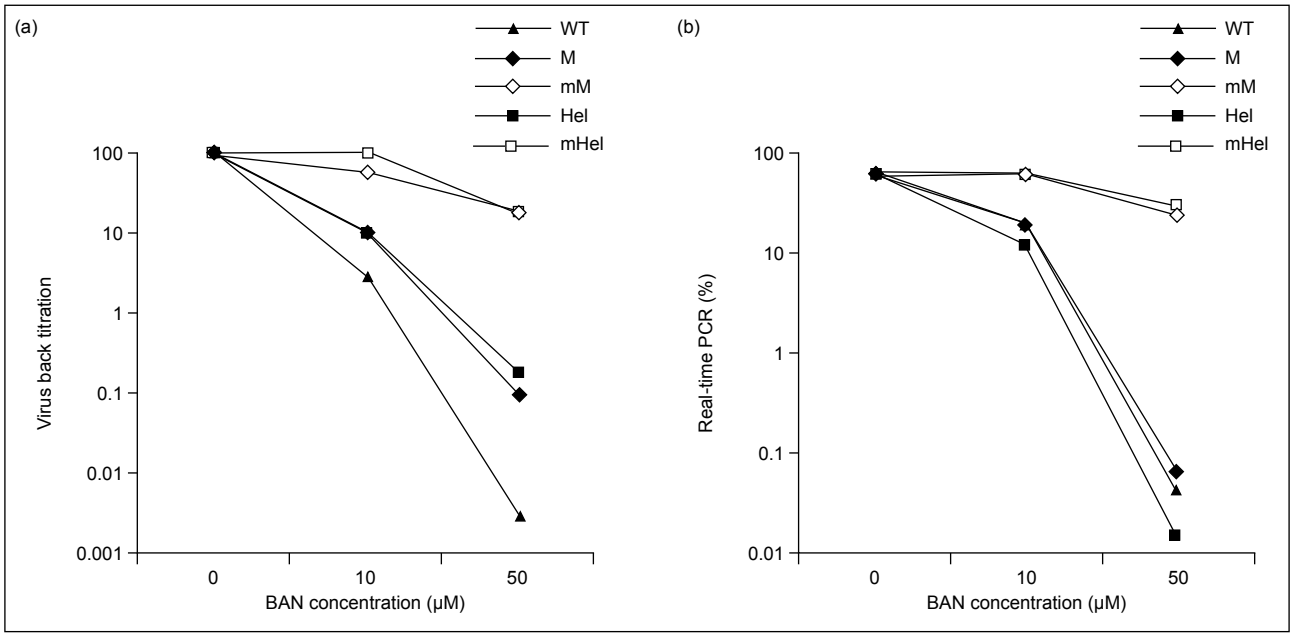

Fig 2. Expression of mutant helicase or M protein can rescue wild type selected virus strains (SCV) replication in the presence of BAN
Wild-type FRhK-4 cells (WT) or FRhK-4 cells stably expressing wild type M (M), mutant M bearing Ala68/Val and Arg124/Trp (mM), wild-type helicase (Hel) or mutant helicase bearing Ser259/Leu (mHel) were infected with wild-type SCV in the presence of 0, 10, 50 μM BAN. (a) Back titration or (b) Q real time–polymerase chain reaction (PCR) showed SCV can replicate in FRhK-4 cells with expression of mutant M or helicase in the presence of BAN. Virus titres (a) or helicase copies/β-actin (b) in absence of BAN were set as 100%. Data in (b) were derived from three independent experiments

bananin. We have found functional mutations in helicase proteins in bananin-resistant strains. The mutant protein expressed in cultured cells can rescue wild-type SARS-CoV replication in the presence of bananin. In addition, we have identified 108 compounds that can inhibit SARS-CoV growth, seven of which exhibited inhibitory activity against the SARS-CoV helicase.[4] Furthermore, bismuth complexes with an IC_{50} (median inhibition concentration, ie concentration that reduces the effect by 50%) lower than micromolar concentration and displaying extremely low toxicities towards normal cells have been synthesised. Further development, including testing in animal models, of the lead compounds developed in this study is worthwhile. These compounds also have the potential to be effective against other viruses.

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