coupled with peptide digests of pulled down proteins and with mass spectrometry to sequence those peptides and identify targets, pull downs can be considered as the protein based equivalent of a yeast two hybrid screen.

To improve the isolation of specific binding partners, pull down methods have been developed involving the use of cross linking, of large scale tissue lysates, and of spin columns. Appropriate methods of sample preparation for mass spectrometry based identification of interacting proteins have been developed as well, including specialized gel staining techniques, band excision, and in gel tryptic digestion. Data interpretation and most commonly encountered problems are, for example, discussed in Current Protocols in Cell Biology, "UNIT 17.5 Protein Protein Interactions Identified by Pull Down Experiments and Mass Spectrometry," Adam Brymora, Valentina A. Valova, and Phillip J. Robinson, Published Online: 1 May 2004 DOI: 10.1002/0471143030.cb1705s22, and included herein by reference.

The invention

Summary

According to a first aspect, there is provided an essentially mammalian positive-sense single stranded RNA virus classifiable as belonging to the Order: Nidovirales; Family: Coronaviridae; Subfamily: Coronavirinae; Genus: Betacoronavirus; and non-Lineage A, non-Lineage B or non-Lineage D, human betacoronavirus.

According to a second aspect, there is provided a nucleic acid, preferably a cDNA, obtainable from a virus as defined herein.

According to a third aspect, there is provided a vector comprising a nucleic acid as defined herein.

According to a fourth aspect, there is provided a cell comprising a virus as defined herein, a nucleic acid as defined herein or a vector as defined herein.

According to a fifth aspect, there is provided a proteinaceous molecule encoded by a nucleic acid as defined herein.

According to a sixth aspect, there is provided an antigen comprising a proteinaceous molecule as defined herein.

According to a seventh aspect, there is provided an antibody specifically directed against an antigen as defined herein.

According to an eighth aspect, there is provided a method for identifying a viral isolate as a MERS-CoV comprising reacting said viral isolate or a component thereof with a nucleic acid as defined herein and/or with an antibody as defined herein.

According to a ninth aspect, there is provided a method for virologically diagnosing a MERS-CoV infection of a mammal comprising determining in a sample of said mammal the presence of a viral isolate or component thereof by reacting said sample with a nucleic acid as defined herein or an antibody as defined herein.

According to a tenth aspect, there is provided a method for serologically diagnosing a MERS-CoV infection of a mammal comprising determining in a sample of said mammal the presence of an antibody specifically directed against a Betacoronavirus, preferably Lineage C virus or component thereof by reacting said sample with a proteinaceous molecule or fragment thereof as defined herein or an antigen as defined herein.

According to an eleventh aspect, there is provided a diagnostic kit for diagnosing a MERS-CoV infection comprising a virus as defined herein, and/or a nucleic acid as defined herein, and/or a proteinaceous molecule as defined herein, and/or an antigen as defined herein and/or an antibody as defined herein.

According to a twelfth aspect, there is provided use of a virus as defined herein, and/or a nucleic acid as defined herein, and/or a vector as defined herein, and/or a cell as defined herein, and/or a proteinaceous molecule or fragment thereof as defined herein, and/or an antigen as defined herein, and/or or an antibody as defined herein for the production of a pharmaceutical composition, preferably for the production of a pharmaceutical composition for the treatment or prevention of a Betacoronavirus, preferably Lineage C virus infection, preferably a human infection, preferably an infection with a MERS-Cov, or for the production of a pharmaceutical composition for the treatment or prevention of atypical pneumonia and/or renal failure, preferably wherein said atypical pneumonia and/or renal failure is a human disease.

According to a thirteenth aspect, there is provided a pharmaceutical composition comprising a virus as defined herein, and/or a nucleic acid as defined herein , and/or a vector as defined herein , and/or a cell as defined herein , and/or a proteinaceous molecule or fragment thereof as defined herein , and/or an antigen as defined herein , and/or or an antibody as defined herein .

According to a fourteenth aspect, there is provided a pharmaceutical composition as defined herein for use in the treatment or prevention of a Betacoronavirus, preferably Lineage C virus, preferably MERS-CoV, infection.

According to a fifteenth aspect, there is provided a pharmaceutical composition as defined herein for use in the treatment or prevention of atypical pneumonia and/or renal failure.

According to a sixteenth aspect, there is provided a pharmaceutical composition as defined herein for use in the treatment or prevention of a MERS-CoV infection in a mammal, preferably wherein said mammal is a rabbit.

According to a seventeenth aspect, there is provided a pharmaceutical composition as defined herein, and/or a virus as defined herein, and/or a nucleic acid as defined herein, and/or a vector as defined herein, and/or a cell as defined herein, and/or a proteinaceous molecule or fragment thereof as defined herein, and/or an antigen as defined herein, and/or an antibody as defined herein, for use in the in vivo determination of MERS-CoV infection, preferably for determination of MERS-CoV-DPP4 interaction, in a mammal, preferably wherein said mammal is a rabbit.

According to a eighteenth aspect, there is provided a pharmaceutical composition as defined herein, and/or a virus as defined herein, and/or a nucleic acid as defined herein, and/or a vector as defined herein, and/or a cell as defined herein, and/or a proteinaceous molecule as defined herein, and/or an antigen as defined herein, and/or an antibody as defined herein, for use in the in vivo determination of MERS-CoV infection, preferably for determining protection against MERS-CoV-infection, in a mammal, preferably wherein said mammal is a rabbit.

According to a nineteenth aspect, there is provided a proteinaceous substance having been provided with a proteinaceous molecule as defined herein and additionally comprising at least a fragment of an N-terminal dipeptidyl peptidase protein.

According to a twentieth aspect, there is provided a container provided with a virus as defined herein, and/or a nucleic acid as defined herein, and/or a vector as defined herein, and/or a cell as defined herein, and/or a proteinaceous molecule as defined herein, and/or an antigen as defined herein, and/or an antibody as defined herein and/or a pharmaceutical composition as defined herein and/or a substance as defined herein.

According to a twenty-first aspect, there is provided a method of identifying a candidate modulator as an agent that modulates the function of a dipeptidyl peptidase, said method comprising: a. providing a substance as defined herein in the presence and absence of said candidate modulator under conditions permitting binding of a first fragment derived from a virus with a second fragment derived from a peptidase protein; b. measuring binding of said first to said second fragment, wherein a decrease or increase in binding in the presence of said candidate modulator, relative to binding in the absence of said candidate modulator, identifies said candidate modulator as an agent that modulates the function of a dipeptidyl peptidase.

According to a twenty-second aspect, there is provided a method of identifying a candidate antiviral agent as an agent that modulates the binding of a virus to dipeptidyl peptidase, said method comprising: a. providing a substance as defined herein in the presence and absence of said candidate antiviral agent under conditions permitting binding of a first fragment derived from a virus with a second fragment derived from a peptidase protein; b. measuring binding of said first to said second fragment, wherein a decrease or increase in binding in the presence of said antiviral agent, relative to binding in the absence of said candidate modulator, identifies said antiviral agent as an agent that modulates the function of a dipeptidyl peptidase.

According to a twenty-third aspect, there is provided use of a fragment of a viral protein as an agent that modulates the function of an N-terminal dipeptidyl peptidase.

According to a twenty-fourth aspect, there is provided use of a fragment of an N-terminal dipeptidyl peptidase as an agent that modulates the function of a viral protein.

According to a twenty-fifth aspect, there is provided use of an inhibitor of N-terminal dipeptidyl peptidase cell-surface expression on a cell for inhibition of replication of a virus in said cell.

Description

The invention provides an isolated essentially mammalian positive-sense single stranded RNA virus classifiable as belonging to the Order: Nidovirales; Family: Coronaviridae; Subfamily: Coronavirinae; Genus: Betacoronavirus; and non-Lineage A, non-Lineage B or non-Lineage D, human betacoronavirus. The invention also provides a human virus (the prefix human stands for the virus classified as capable of infecting humans where a bat virus is capable of infecting bats), preferably isolated or isolatable from humans, having a receptor binding domain (RBD) capable of binding to a dipeptidyl peptidase 4 (DPP4), no such isolates have been deposited or in any other way made available to the art until now. In particular, the invention provides an isolated essentially mammalian positive-sense single stranded RNA virus classifiable as belonging to the Order: Nidovirales; Family: Coronaviridae; Subfamily: Coronavirinae; Genus: Betacoronavirus; Lineage C human betacoronavirus. The invention also provides an isolated essentially mammalian positive-sense single stranded RNA virus classifiable as belonging to the Order: Nidovirales; Family: Coronaviridae; Subfamily: Coronavirinae; Genus: Betacoronavirus; Lineage: C and isolatable from humans, and components thereof. Until now, no Betacoronavirus isolates have

been isolated from humans that were then classified as belonging to Lineage: C of Betacoronavirus. In particular, the invention provides a Lineage: C human Betacoronavirus having a receptor binding domain (RBD) capable of binding to a dipeptidyl peptidase 4 (DPP4). In particular, no such isolates have been deposited or in any other way made available to the art until now. In a preferred embodiment, a virus according to the invention is isolated or isolatable from a human. In particular, the invention provides a new previously undescribed Coronavirus isolated from cases of unexplained disease in September 2012 and identified herein as belonging to a newly recognized and previously undescribed species of human Corona Virus (HCoV), herein identified as HCoV-SA1 or HCoV EMC or Middle East Respiratory Syndrome-Coronavirus (MERS-CoV). In particular the specific nucleic acid and/or amino acid sequences of the MERS-CoV genome and sequences encoding (parts of) viral proteins and antigenic polypeptides are provided, as demonstrated by phylogenetic analyses. Further, the invention relates to diagnostic means and methods, prophylactic means and methods and therapeutic means and methods to be employed in the diagnosis, prevention and/or treatment of disease, in particular of respiratory disease, in particular of mammals, more in particular in humans, most in particular specific for MERS-CoV. It particularly also relates to an isolated virus and its receptor. The invention also provides identification of N-terminal dipeptidyl peptidase as virus receptor and uses thereof, identification of the receptor binding domain of MERS-CoV mapping to a 231-residue region 2 in the spike protein that efficiently elicits neutralizing antibodies identification and uses thereof and dipeptidyl peptidase 4 receptor determinants of respiratory MERS-coronavirus infection, and uses thereof. The invention in particular provides specific diagnostics of MERS-CoV, sub-unit compositions of S1-MERS CoV protein

14. A method for serologically diagnosing a MERS-CoV infection of a mammal comprising determining in a sample of said mammal the presence of an antibody specifically directed against a Betacoronavirus, preferably Lineage C virus or component thereof by reacting said sample with a proteinaceous molecule or fragment thereof according to claim 9 or an antigen according to claim 10.

15. A diagnostic kit for diagnosing a MERS-CoV infection comprising a virus according to anyone of claims 1 to 5, and/or a nucleic acid according to claim 6, and/or a proteinaceous molecule according to claim 9, and/or an antigen according to claim 10 and/or an antibody according to claim 11.

16. Use of a virus according to any one claims 1 to 5, and/or a nucleic acid according to claim 6, and/or a vector according to claim 7, and/or a cell according to claim 8, and/or a proteinaceous molecule or fragment thereof according to claim 9, and/or an antigen according to claim 10, and/or or an antibody according to claim 11 for the production of a pharmaceutical composition, preferably for the production of a pharmaceutical composition for the treatment or prevention of a Betacoronavirus, preferably Lineage C virus infection, preferably a human infection, preferably an infection with a MERS-Cov, or for the production of a pharmaceutical composition for the treatment or prevention of atypical pneumonia and/or renal failure, preferably wherein said atypical pneumonia and/or renal failure is a human disease.

17. A pharmaceutical composition comprising a virus according to any one claims 1 to 5, and/or a nucleic acid according to claim 6, and/or a vector according to claim 7, and/or a cell according to claim 8, and/or a proteinaceous molecule or fragment thereof according to claim 9, and/or an antigen according to claim 10, and/or or an antibody according to claim 11.

18. A pharmaceutical composition according to claim 17 for use in ~~method for~~ the treatment or prevention of a Betacoronavirus, preferably Lineage C virus, preferably MERS-CoV, infection~~, comprising providing a mammal, preferably a human, with a pharmaceutical composition according to claim 17~~.

19. A pharmaceutical composition according to claim 17 for use in ~~method for~~ the treatment or prevention of atypical pneumonia and/or renal failure ~~comprising providing an individual with a pharmaceutical composition according to claim 17~~.

20. A pharmaceutical composition according to claim 17 for use in ~~method for~~ the treatment or prevention of a MERS-CoV infection in ~~comprising providing~~ a mammal ~~with a pharmaceutical composition according to claim 17~~, preferably wherein said mammal is a rabbit.

21. A ~~method for in vivo determining MERS-CoV infection, preferably for determining MERS-CoV-DPP4 interaction, comprising providing a mammal with a~~ pharmaceutical composition according to claim 17, and/or ~~with~~ a virus according to any one claims 1 to 5, and/or ~~with~~ a nucleic acid according to claim 6, and/or ~~with~~ a vector according to claim 7, and/or ~~with~~ a cell according to claim 8, and/or ~~with~~ a proteinaceous molecule or fragment thereof according to claim 9, and/or ~~with~~ an antigen according to claim 10, and/or ~~with~~ an antibody according to claim 11, for use in the in vivo determination of MERS-CoV infection, preferably for determination of MERS-CoV-DPP4 interaction, in a mammal, preferably wherein said mammal is a rabbit.

~~22. A method for in vivo determining MERS-CoV infection, preferably for~~

22. A ~~determining protection against MERS-CoV infection, comprising providing a mammal with a~~ pharmaceutical composition according to claim 17, and/or ~~with~~ a virus according to any one claims 1 to 5, and/or ~~with~~ a nucleic acid according to claim 6, and/or ~~with~~ a vector according to claim 7, and/or ~~with~~ a cell according to claim 8, and/or ~~with~~ a proteinaceous molecule according to claim 9, and/or ~~with~~ an antigen according to claim 10, and/or ~~with~~ an antibody according to claim 11, for use in the in vivo determination of MERS-CoV infection, preferably for determining protection against MERS-CoV-infection, in a mammal, preferably wherein said mammal is a rabbit.

23. A proteinaceous substance having been provided with a proteinaceous molecule according to claim 9 and additionally comprising at least a fragment of an N-terminal dipeptidyl peptidase protein.

24. A substance according to claim 23 wherein said proteinaceous molecule comprises an ectodomain of a spike protein, said ectodomain preferably derived from the S1 region of a coronavirus.

25. A substance according to claim 23 or 24 above wherein said peptidase protein is a dipeptidyl peptidase 4 (DPP4), preferably human DPP4.

26. A substance according to claims 23, 24 or 25 having been subjected to crystallization.

27. A container provided with a virus according to any one claims 1 to 5, and/or a nucleic acid according to claim 6, and/or a vector according to claim 7, and/or a cell according to claim 8, and/or a proteinaceous molecule according to claim 9, and/or an antigen according to claim 10, and/or ~~or~~ an antibody according to claim 11 and/or a pharmaceutical composition according to claim 17 and/or a substance according to anyone of claims 23 - 26.

28. A method of identifying a candidate modulator as an agent that modulates the function of a dipeptidyl peptidase, said method comprising:

a. Providing a substance according to anyone of claims 23 to 26 in the presence and absence of said candidate modulator under conditions permitting binding of a first fragment derived from a virus with a second fragment derived from a peptidase protein.
b. Measuring binding of said first to said second fragment, wherein a decrease or increase in binding in the presence of said candidate modulator, relative to binding in the absence of said candidate modulator, identifies said candidate modulator as an agent that modulates the function of a dipeptidyl peptidase.

29 A method of anyone of claim 28 wherein said first and/or said second fragment is detectably labeled, preferably with a moiety selected from the group consisting of a radioisotope, a fluorophore, a quencher of fluorescence, an enzyme, and an affinity tag.

30 A method of identifying a candidate antiviral agent as an agent that modulates the binding of a virus to dipeptidyl peptidase, said method comprising: