RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

ISSN 2221-1896 (PRINT) www.roavs.com ISSN 2223-0343 (ONLINE)

Interaction between mouse hepatitis virus membrane protein and heat shock protein70 (HSP70)

Yanfen Wang[1], Chunping Li[2], Yanhua Feng[2], Yanhui Li[2], Haichun Ma[2]

[1]The second Hospital of Jilin University Changchun 130041, Jilin Province, PR China, [2]The First Hospital of Jilin University, Changchun 130021, Jilin Province, PR China

Abstract

The membrane (M) protein of mouse hepatitis virus (MHV) is a major structural component of virions, which seems to be a multifunctional protein involved in viral replication and budding. However, how M protein interacts with host protein remains largely elusive. To identify cellular proteins that interact with the M protein and to elucidate the possible involvement of M protein in virus replication and budding, yeast two-hybrid system assay was used. HSP70, a heat shock protein were identified as a novel interaction partner of N protein by yeast two-hybrid system. The direct interaction M protein with HSP70 was confirmed by confocal microscopy analysis. To the best of our knowledge, this is the first report that MHV-M protein interacts with the heat shock protein70 (HSP70) within host cells, which might suggest that HSP70 protein may play a role in mouse hepatitis virus assembly, which enhance our understanding of the molecular mechanisms of MHV assemble.

Key word: Mouse Hepatitis Virus, Membrane Protein, Hsp70 Protein, Yeast Two-Hybrid System

Introduction

MHV is a member of the family *Coronaviridae*, which represent a significant ubiquitous group of viral pathogens that infect both humans and animals, causing respiratory, gastro-intestinal, and neurologic diseases. MHV, a group II coronavirus, is a natural pathogen of mice, normally infecting the liver, gastrointestinal tract, and central nervous system, causing a wide range of disease, including hepatitis, gastroenteritis, and acute and chronic encephalomyelitis (Ye et al., 2007; Rose and Weiss, 2009; Rose et al., 2010). Therefore, probing the infection mechanism at the molecular level is of utmost significance, since it is the foundation for discovering and developing anti-MHV drugs or vaccine.

Like other known coronaviruses, MHV is an enveloped virus containing three outer structural proteins, namely the membrane (M), nucleocapsid (N), and spike (S) proteins (Lai and Cavanagh, 1997; Versteeg et al., 2007). The M protein is the most abundant viral protein in coronaviruses which is produced throughout infection and is an important multifunctional protein. Membrane (M) protein accounts for about 40% of total protein, consisting of 224 - 225 amino acids. M protein was predicted to span the membrane three times and display a short ectodomain and a large endodomain on the basis of M proteins of MHV and SARS (De et al., 1998; Kuo and Masters, 2002; He et al., 2004). It is believed that the C-terminal endodomain interacts with N and S proteins (De et al., 1998), which plays an mportant role in the formation as well as assembly of the virus. The protein-protein interaction between *Coronaviridae* N and M proteins has been reported on mouse hepatitis virus and transmissible gastroenteritis (Narayanan and Makino, 2001; Kuo and Masters, 2002). However, the host cellular proteins interaction with the M protein in MHV has not been reported so far.

In this sudy, the yeast two-hybrid and confocal microscopy techniques were employed to study the interactions between M and host proteins in MHV, and elucidate the mechanism of molecular replication of MHV.

Corresponding Author: Haichun Ma, The first hospital of Jilin University, Changchun 130021, Jilin Province, PR China

Materials and Methods

The strain of *Saccharomyces cerevisiae* used in this study was AH109 and Y187 from Clontech (USA). Yeast cells were cultured at 30°C either in a complete YPD medium (1% yeast extract, 1% peptone and 2% glucose) or in a synthetic defined (SD) medium supplemented with required essential nutrients. Plates contained 2% agar. Transformation of yeast cells was performed by the lithium acetate. *Eherichiacoli* KC8 was used for general cloning. Beta-galactosidase assays were carried out according to the CLONTECH Matchmaker manual (PT3024-1, Clontech, USA).

For bait construction, the full-length M gene of the MHV (MHV-A59) was PCR-amplified from a genomic construct of clone, and cloned into the pMD18-T vector (Takara, China). The full-length M gene was subjected to DNA sequencing, and the inserts were verified against the corresponding region of the MHV coronavirus. The full-length M gene was excised from the pMD18-T – M construct using the restriction enzymes EcoRI and BamHI, and ligated into the pGBKT7 vector to generate an N-terminal in frame fusion with the GAL4 activation domain (BD), and the resultant plasmid was named as pGBKT7-M. For mammalian cell expression, the full-length M gene and HSP70 were subcloned into the pCMV-Myc vector fluorescence vector pEGFP-N1 and pDsRed-N1, respectively. All DNA manipulations were performed as described by Sambrook et al (Sambrook and Russell, 2001). All constructs were verified by restriction digestion and sequencing.

Yeast two-hybrid screening was performed according to the instructions of the manufacturer using the pretransformed MATCHMAKER rat liver cDNA library (Clontech). In brief, pGBK-T7-M was transformed into yeast strain AH109. The transformants containing bait plasmid were mated with the pre-transformed human brain cDNA library. Candidates for two-hybrid interaction were initially selected on SD (-His, -Leu, -Trp) medium and further confirmed on SD (-Ade, -His, -Leu, -Trp) medium containing X-gal. The plasmid DNA was isolated from the positive clones and sequenced according to the instructions of the manufacturer. To further confirm the interaction between M and HSP70, Y187 and AH109, each of which contained pGAD-T7-HSP70 and pGBK-T7-M, respectively, were mated and tested using SD (-Ade, -His, -Leu, -Trp) medium containing X-gal.

A human cancer cell line, 293T (kidney carcinoma), was maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Hyclone) & 1% penicillin/ streptomycin (Invitrogen). Transient transfections were performed using Lipofectamine plus reagent (Invitrogen) or Welfect (Wellgene) according to the recommendations of the manufacturers.

Vero cells were grown on coverslips in a 6-well chamber and simultaneously transfected with the recombinant fluorescence plasmids pEGFP-M and pDsRed-HSP70. After 24 h transfection, the cells were washed with PBS three times and fixed in 4% paraformaldehyde for 20 min at room temperature. The coverslips were then washed with PBS and mounted. Intracellular localization of the M protein and HSP70 was observed under a Leica confocal microscope (Germany).

Results

To identify potential host cell targets of M protein, its entire open reading frame encoding 225 amino acids was inserted into a bait plasmid and a rat liver library of prey plasmids was screened by the yeast two-hybrid system. Among six million clones, 8 formed colonies on the SD (-Ade, -His, -Leu, -Trp) plates. X-gal filter assay showed that 5 of 8 clones were both histidine and LacZ-positive. They were reexamined in a bait dependency test, which reduced the number of positive clones to nine. Finally, sequence analysis and database searching revealed HSP70 were found as target protein (Fig. 1).

To further confirm the interaction between M and HSP70 proteins, the localization patterns of the M protein and HSP70 were investigated in Vero cells using confocal microscopy technology. pEGFP-M and pDsRed-HSP70 were transfected simultaneously into Vero cells. As shown in Figure 2, HSP70 and M protein mainly were localized in the cytoplasm. The merged image revealed that the M protein and HSP70 co-localized in the cytoplasm of Vero cells indicating that M protein interacts with HSP70 in cells.

Fig. 1: Yeast two-hybrid analysis of the interaction of M & HSP70
1: pGBKT7-M+pGADT7, 2: pGBKT7 + pGADT7-X, 3: pGBKT7+pGADT7-HSP70, 4: Positive control: pGBKT7-53+ pGADT7-T; 5: Negative control: pGBKT7-Lam+ pGADT7-T

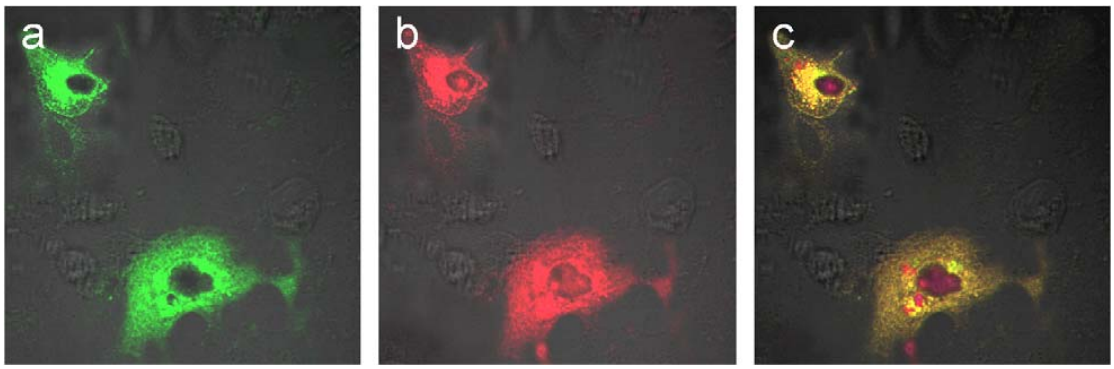

Fig. 2: Co-localization of the MHV-N protein and RACK1
pEGFP-M (green) and pDsRed-HSP70 (red) were co-transfected into Vero cells. After 24 h, cells were fixed, mounted, and the localization of the proteins was observed with a Leica confocal microscope. As shown, the M protein and HSP70 were colocalized in the cytoplasm. A: pEGFP-M; B: pDsRed-HSP70; C: Merge

Discussion

In the present study, by employing a series of biochemical and biophysical methods, we have firstly reported that MHV-M protein has a specific binding to rat HSP70. Eukaryotic Hsp70s are highly abundant cytosolic and nuclear molecular chaperones that play essential roles in various aspects of protein homeostasis, including protein synthesis, transportation, degradation, and folding (Hightower et al., 1991; Soti and Pal et al., 2005). Transient interactions between Hsp70 and unfolded polypeptides maintain its substrates in soluble, intermediate folded states and prevent protein misfolding and aggregation. Moreover, the intermediate substrates are subsequently folded into their native forms by the machinery of the Hsp70 chaperone (Levy et al., 1995; Freeman al., 1996; Minami et al., 1996). This is accomplished, in part, by the help of a subclass of proteins known as cochaperones, which modulate the chaperone function of Hsp70 and its association with other partner proteins. Therefore, we presume that HSP70 plays a crucial role during the folding, maturation and secretion of M protein, which helped virus assembly and replication.

We have identified an interaction between M, a membrane protein and HSP70, an intracellular host protein. The knowledge of protein-protein interactions of the assembly and replication factor M is of importance to understand the role of M during the infection process. However, the molecular significance of the interaction between M and HSP70 is largely unknown; elucidation of these questions will depend on further studies. Moreover, the disruption of interaction between M and HSP70 proteins using RNA interference technology may provide further clues to the specific function of M and HSP70 protein.

In conclusion, our data have shown for the first time both *in vitro* and *in vivo* that the nucleocapsid protein of MHV has a high binding affinity to rat HSP70. This current research contributes useful data that will shed light on the molecular mechanism of M assembly and provide valuable clues for mutagenic studies in disrupting virion assembly and replication and developing antiviral agents.

References

De, H.C.A., Kuo, L., Masters, P.S., Vennema, H. and Rottier, P.J. 1998. Coronavirus particle assembly: primary structure requirements of the membrane protein. *Virology Journal*, 72: 6838-6850.

Freeman, B.C. and Morimoto, R.I. 1996. The human cytosolic molecular chaperones hsp90, hsp70 (hsc70) and hdj-1 have distinct roles in recognition of a non-native protein and protein refolding. *EMBO Journal*, 15: 2969–2979.

He, R., Dobie, F., Ballantine, M., Leeson, A., Yan, L. & Batien, N. 2004. Analysis of multimerization of SARS coronavirus nucleocapsid protein. *Biochemistry Biophysics Research Communication*, 316: 476-483.

Hightower, L.E. 1991. Heat shock, stress proteins, chaperones, and proteotoxicity. *Cell*, 66: 91-197.

Kuo, L. and Masters, P.S. 2002. Genetic evidence for a structural interaction between the carboxy termini of the membrane and nucleocapsid proteins of mouse hepatitis virus. *Journal of Virology*, 76:4987-4999.

Lai, M. & Cavanagh, D. 1997. The molecular biology of coronaviruses. *Advances in Virus Res*, 48: 1-100.

Levy, E.J., McCarty, J., Bukau, B. and Chirico, W.J. 1995. Conserved ATPase and luciferase refolding activities between bacteria and yeast Hsp70 chaperones and modulators. *FEBS Letter*, 368: 435–440.

Minami, Y., Hohfeld, J., Ohtsuka, K. and Hartl, F.U. 1996. Regulation of the heat-shock protein 70

reaction cycle by the mammalian DnaJ homolog, Hsp40. *Journal of Biological Chemistry*, 271:19617–19624.

Narayanan, K. and Makino, S. 2001. Cooperation of an RNA packaging signal and a viral envelope protein in coronavirus RNA packaging. *Journal of Virology*, 75: 9059-9067.

Rose, K.M. and Weiss, S.R. 2009. Murine coronavirus cell type dependent interaction with the type I interferon response. *Viruses*, 1: 689-712.

Rose, K.M., Elliott, R., Martinez-Sobrido, L., Garcia-Sastre, A. and Weiss, S.R. 2010. Murine coronavirus delays expression of a subset of interferon-stimulated genes. *Journal of virology*, 84:5656-5669.

Sambrook, J. and Russell, D.W. 2001. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press.

Soti, C. and Pal, C. 2005. Molecular chaperones as regulatory elements of cellular networks. *Current Opinion in Cell Biology*, 17: 210-215.

Versteeg, G.A., Bredenbeek, P.J., van den Worm, S.H.E. and Spaan, W.J.M. 2007. Group 2 coronaviruses prevent immediate early interferon induction by protection of viral RNA from host cell recognition. *Virology*, 361: 18-26.

Ye, Y., Hauns, K., Langland, J.O., Jacobs, B.L. and Hogue, B.G. 2007. Mouse hepatitis coronavirus A59 nucleocapsid protein is a type I interferon antagonist. *Journal of virology*, 81: 2554-2563.