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The Role of Autophagy-Mediated Dengue Virus Antibody-Dependent Enhancement Infection of THP-1 Cells

Liming Jiang[a, b, c] Qiangming Sun[a, b]

[a]Institute of Medical Biology, Chinese Academy of Medical Sciences, and Peking Union Medical College, Kunming, PR China; [b]Yunnan Key Laboratory of Vaccine Research & Development on Severe Infectious Diseases, Kunming, PR China; [c]School of Marine Sciences, Ningbo University, Ningbo, PR China

Keywords

Antibody-dependent enhancement · Dengue virus · Autophagy · THP-1

Abstract

Background: Antibody-dependent enhancement (ADE) of dengue virus (DENV) infection is identified as the main risk factor of severe dengue diseases. The underlying mechanisms leading to severe dengue fever remain unclear. ***Methods:*** THP-1 cells were treated with an autophagy inducer (rapamycin) or inhibitor (3-methyladenine [3-MA]) and infected with DENV and DENV-ADE. In order to investigate the expression profile of autophagy-related genes in DENV-ADE and DENV direct infection of THP-1 cells, the PCR array including 84 autophagy-related genes was selected to detect the expression of related genes, and then heat map and clustergram were established by analysis software to compare the expression differences of these genes between the DENV-ADE and DENV direct infection. ***Results:*** Autophagy-inducing complex related genes ATG5 and ATG12 were upregulated, and autophagosomes were also observed by transmission electron microscopy among DENV-ADE- and DENV-infected THP-1 cells, which indicated that autophagy was involved in dengue infection. The results show that 3-MA has a significant inhibitory effect on ATG12 in THP-1 cells; on the contrary, the expression of ATG12 was upregulated in THP-1 cells that were treated with rapamycin. The autophagy-related genes ESR1, INS, BNIP3, FAS, TGM2, ATG9B, and DAPK1 exhibited significant differences between DENV-ADE and DENV direct infection groups. ***Conclusion:*** In the present study, an additional mechanism of autophagy was inhibited by the autophagy inhibitor (3-MA) in DENV- and DENV-ADE-infected THP-1 cells. Our finding provided a clear link between autophagy and antibody-enhanced infection of DENV. © 2020 S. Karger AG, Basel

Introduction

Dengue viruses (DENVs) are transmitted by mosquitoes and cause dramatic public health issues in subtropical and tropical areas of the world, causing >50 million people to be infected annually, especially in Latin America and Southeast Asia [1]. Although the majority of these

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Qiangming Sun
Institute of Medical Biology, Chinese Academy of Medical Sciences & Peking Union Medical College (CAMS & PUMC)
935 Jiao Ling Road, Kunming, Yunnan Province 650118 (China)
qsun@imbcams.com.cn

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infections are relatively mild febrile diseases or asymptomatic, significant consequences can develop possibly leading to the life-threatening dengue shock syndrome (DSS) or dengue hemorrhagic fever (DHF) [2, 3].

There are 4 antigenically related but distinct serotypes termed DENV-1, DENV-2, DENV-3, and DENV-4 of DENVs, and each serotype comprises many distinct genotypes [4]. The mechanisms of DENV pathogenesis, particularly severe dengue fever of DHF/DSS, remain unclear with an increasing number of controversial and contradictory findings [5]. The antibodies directed against a specific DENV serotype have lifelong protective effect on this specific DENV serotype; however, they do not cross-protect against the other serotypes [6, 7]. Furthermore, data from numerous clinical and in vitro experiments showed that the DSS/DHF phenomenon mainly comes from secondary infection of the heterologous serotype of DENVs [8–10]. Mechanistically, antibody-dependent enhancement (ADE) may be involved in the immunopathogenesis of severe dengue fever of DSS and DHF. In addition, the pre-existing heterotypic antibody via Fc-receptors triggers ADE among heterotypic DENV infection [8, 11, 12]. Autophagy is an evolutionarily ancient pathway that has been conserved in almost all eukaryotic cells under the conditions of nutrient starvation, environmental stresses, and infection by a pathogen. Autophagosomes are formed from portions of the cytoplasm or small cells that were sequestered into double-membrane vesicles and fuse with the lysosome to form an autolysosome [13, 14]. The autophagic process induced by many RNA viruses in mammalian cells promotes their infection through the membrane scaffold for RNA replication among poliovirus, mouse hepatitis virus, DENVs, and rhino viruses [15–17]. Our previous study showed that increased autophagy promoted viral RNA replication in DENV-ADE-infected K562 cells [18].

In this study, THP-1 cells were used to establish a DENV-ADE and DENV direct infection model. Then, the QIAGEN chip was used to detect the difference of expression of autophagy-related genes. The analysis software of heat map and clustergram was used to compare the expression profile of autophagy-related genes between DENV-ADE and DENV direct infection.

Materials and Methods

Cell Culture and Viruses

Human acute monocytic leukemic (THP-1) cells and *Aedes albopictus* mosquito (C6/36) cells were kept in the Institute of Medical Biology, Chinese Academy of Medical Sciences. Both cells were cultured in the RPMI-1640 medium (1640, BI) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin and cultured in an incubator at 37 or 28°C in a humidified atmosphere containing 5% CO_2.

DENV serotype 3 (DENV-3) was isolated from a severe dengue fever patient in Xishuangbanna Dai Autonomous Prefecture People's Hospital in 2013, and it was propagated in C6/36 cells. DENV-3 was confirmed by genome sequencing (gb: KR296743). Written consent was obtained from dengue fever patients. The study protocol was approved by the Institutional Ethics Committee (Institute of Medical Biology, Chinese Academy of Medical Sciences, and Peking Union Medical College) in accordance with the Declaration of Helsinki for Human Research of 1974 (last modified in 2000).

DENV-3 Direct and DENV-3-ADE Infection of THP-1 cells

For the experimental group of DENV, THP-1 cells were incubated with DENV-3 at a multiplicity of infection of 5 (MOI 5) and then incubated in a CO_2 incubator for 24 h. For the experimental group of DENV-ADE, it was performed as described previously [18]. DENV-2 anti-prM glycoprotein monoclonal antibody (AB41473; Abcam) was selected to establish the DENV-3-ADE model. In brief, 5 MOI of DENV-3 was mixed with the antibody dilutions (1:64) and placed at 37°C for 1 h with 5% CO_2 to allow the formation of an immune complex. The DENV-antibody immune complex was then added into 2×10^5 THP-1 cells in 24-well plates and incubated for 24 h at 37°C with 5% CO_2. Then, the cells were washed 3 times with PBS to remove the remaining DENV-antibody complex followed by suspending in 1.5 mL of the maintenance medium per well for an additional time. THP-1 cells cultured in the RPMI-1640 medium (1640, BI) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin served as the mock group. For treatment of cells, THP-1 cells were incubated with 9-mM autophagy inhibitor 3-methyladenine (3-MA; Sigma-Aldrich) and 150-nM autophagy inducer rapamycin (Sangon) for 24 h.

RNA Extraction and Autophagy Genes Detection

Protected RNA present in the THP-1 supernatant was extracted according to the manufacturer's protocol of the QIAGEN RNA kit (Qiagen, Hilden, Germany #330231). Contaminating genomic DNA was removed by treatment with RNase-free DNase I (QIAGEN). The extracted RNA was used for ATG12 quantification and QIAGEN chip detection through quantitative PCR analysis. Real-time PCR of the sample and standard was conducted using SYBR Green I Mastermix (Applied Biosystems) on a Bio-Rad CFX96 thermocycler. Amplification conditions were 95°C for 30 s and 40 cycles of 95°C for 5 s, 55°C for 20 s, and 72°C for 30 s.

Observation of Autophagy by TEM

THP-1 cells were observed by TEM at 24 h after infection or treatment by DENV or DENV-ADE and 3-MA or rapamycin, respectively. The sample was placed in 2.5% glutaraldehyde (0.2 mol/L sodium cacodylate buffer preparation) for 2 h, and then ultrathin slices were observed through the Hitachi transmission electron microscope H-7650. The magnification rate was 500 times.

Autophagy PCR Arrays and Data Analysis

The expression profile of autophagy-related genes was detected using the RT^2 profiler™ PCR array (human autophagy, QIAGEN)

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Table 1. Detailed information of QIAGEN chip autophagy genes and functions

Po	Ref seq	Symbol	Description
A01	NM_005163	AKT1	V-akt murine thymoma viral oncogene homolog 1
A02	NM_017749	AMBRA1	Autophagy/beclin-1 regulator 1
A03	NM_000484	APP	Amyloid beta (A4) precursor protein
A04	NM_031482	ATG10	ATG10 autophagy related 10 homolog (*S. cerevisiae*)
A05	NM_004707	ATG12	ATG12 autophagy related 12 homolog (*S. cerevisiae*)
A06	NM_017974	ATG16L1	ATG16 autophagy related 16-like 1 (*S. cerevisiae*)
A07	NM_033388	ATG16L2	ATG16 autophagy related 16-like 2 (*S. cerevisiae*)
A08	NM_022488	ATG3	ATG3 autophagy related 3 homolog (*S. cerevisiae*)
A09	NM_052936	ATG4A	ATG4 autophagy related 4 homolog A (*S. cerevisiae*)
A10	NM_178326	ATG4B	ATG4 autophagy related 4 homolog B (*S. cerevisiae*)
A11	NM_178221	ATG4C	ATG4 autophagy related 4 homolog C (*S. cerevisiae*)
A12	NM_032885	ATG4D	ATG4 autophagy related 4 homolog D (*S. cerevisiae*)
B01	NM_004849	ATG5	ATG5 autophagy related 5 homolog (*S. cerevisiae*)
B02	NM_006395	ATG7	ATG7 autophagy related 7 homolog (*S. cerevisiae*)
B03	NM_024085	ATG9A	ATG9 autophagy related 9 homolog A (*S. cerevisiae*)
B04	NM_173681	ATG9B	ATG9 autophagy related 9 homolog B (*S. cerevisiae*)
B05	NM_004322	BAD	BCL2-associated agonist of cell death
B06	NM_001188	BAK1	BCL2-antagonist/killer 1
B07	NM_004324	BAX	BCL2-associated X protein
B08	NM_000633	BCL2	B-cell CLL/lymphoma 2
B09	NM_138578	BCL2L1	BCL2-like 1
B10	NM_003766	BECN1	Beclin 1, autophagy related
B11	NM_001196	BID	BH3 interacting domain death agonist
B12	NM_004052	BNIP3	BCL2/adenovirus E1B 19-kDa interacting protein 3
C01	NM_004346	CASP3	Caspase 3, apoptosis-related cysteine peptidase
C02	NM_001228	CASP8	Caspase 8, apoptosis-related cysteine peptidase
C03	NM_004064	CDKN1B	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)
C04	NM_000077	CDKN2A	Cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)
C05	NM_000086	CLN3	Ceroid lipofuscinosis, neuronal 3
C06	NM_001908	CTSB	Cathepsin B
C07	NM_001909	CTSD	Cathepsin D
C08	NM_004079	CTSS	Cathepsin S
C09	NM_003467	CXCR4	Chemokine (C-X-C motif) receptor 4
C10	NM_004938	DAPK1	Death-associated protein kinase 1
C11	NM_018370	DRAM1	DNA-damage regulated autophagy modulator 1
C12	NM_178454	DRAM2	DNA-damage regulated autophagy modulator 2
D01	NM_004836	EIF2AK3	Eukaryotic translation initiation factor 2-alpha kinase 3
D02	NM_182917	EIF4G1	Eukaryotic translation initiation factor 4 gamma, 1
D03	NM_000125	ESR1	Estrogen receptor 1
D04	NM_003824	FADD	Fas (TNFRSF6)-associated via death domain
D05	NM_000043	FAS	Fas (TNF receptor superfamily, member 6)
D06	NM_000152	GAA	Glucosidase, alpha; acid
D07	NM_007278	GABARAP	GABA(A) receptor-associated protein
D08	NM_031412	GABARAPL1	GABA(A) receptor-associated protein-like 1
D09	NM_007285	GABARAPL2	GABA(A) receptor-associated protein-like 2
D10	NM_004964	HDAC1	Histone deacetylase 1
D11	NM_006044	HDAC6	Histone deacetylase 6
D12	NM_004712	HGS	Hepatocyte growth factor-regulated tyrosine kinase substrate
E01	NM_001017963	HSP90AA1	Heat shock protein 90-kDa alpha (cytosolic), class A member 1
E02	NM_006597	HSPA8	Heat shock 70-kDa protein 8
E03	NM_002111	HTT	Huntingtin
E04	NM_000619	IFNG	Interferon, gamma
E05	NM_000618	IGF1	Insulin-like growth factor 1 (somatomedin C)
E06	NM_000207	INS	Insulin
E07	NM_001145805	IRGM	Immunity-related GTPase family, M
E08	NM_005561	LAMP1	Lysosomal-associated membrane protein 1

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Table 1 (continued)

Po	Ref seq	Symbol	Description
E09	NM_181509	MAP1LC3A	Microtubule-associated protein 1 light chain 3 alpha
E10	NM_022818	MAP1LC3B	Microtubule-associated protein 1 light chain 3 beta
E11	NM_001315	MAPK14	Mitogen-activated protein kinase 14
E12	NM_002750	MAPK8	Mitogen-activated protein kinase 8
F01	NM_004958	MTOR	Mechanistic target of rapamycin (serine/threonine kinase)
F02	NM_003998	NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B cells 1
F03	NM_000271	NPC1	Niemann-Pick disease, type C1
F04	NM_002647	PIK3C3	Phosphoinositide-3-kinase, class 3
F05	NM_002649	PIK3CG	Phosphoinositide-3-kinase, catalytic, gamma polypeptide
F06	NM_014602	PIK3R4	Phosphoinositide-3-kinase, regulatory subunit 4
F07	NM_006251	PRKAA1	Protein kinase, AMP-activated, alpha 1 catalytic subunit
F08	NM_000314	PTEN	Phosphatase and tensin homolog
F09	NM_130781	RAB24	RAB24, member RAS oncogene family
F10	NM_000321	RB1	Retinoblastoma 1
F11	NM_005873	RGS19	Regulator of G-protein signaling 19
F12	NM_003161	RPS6KB1	Ribosomal protein S6 kinase, 70 kDa, polypeptide 1
G01	NM_000345	SNCA	Synuclein, alpha (non-A4 component of amyloid precursor)
G02	NM_003900	SQSTM1	Sequestosome 1
G03	NM_000660	TGFB1	Transforming growth factor, beta 1
G04	NM_004613	TGM2	Transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)
G05	NM_153015	TMEM74	Transmembrane protein 74
G06	NM_000594	TNF	Tumor necrosis factor
G07	NM_003810	TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10
G08	NM_000546	TP53	Tumor protein p53
G09	NM_003565	ULK1	Unc-51-like kinase 1 (*C. elegans*)
G10	NM_014683	ULK2	Unc-51-like kinase 2 (*C. elegans*)
G11	NM_003369	UVRAG	UV radiation resistance-associated gene
G12	NM_017983	WIPI1	WD repeat domain, phosphoinositide interacting 1
H01	NM_001101	ACTB	Actin, beta
H02	NM_004048	B2M	Beta-2-microglobulin
H03	NM_002046	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
H04	NM_000194	HPRT1	Hypoxanthine phosphoribosyltransferase 1
H05	NM_001002	RPLP0	Ribosomal protein, large, P0
H06	SA_00105	HGDC	Human genomic DNA contamination
H07	SA_00104	RTC	Reverse transcription control
H08	SA_00104	RTC	Reverse transcription control
H09	SA_00104	RTC	Reverse transcription control
H10	SA_00103	PPC	Positive PCR control
H11	SA_00103	PPC	Positive PCR control
H12	SA_00103	PPC	Positive PCR control

at 24 h. In brief, the following components were mixed in a 5-mL tube or a multichannel reservoir: 550 μL of 2 × SuperArray PCR master mix, 102 μL of the diluted first-strand cDNA synthesis reaction, and 448 μL of ddH_2O. The cocktails were added to the PCR array. Real-time PCR detection was performed under the following thermal cycling conditions: 95°C for 10 min; 40 cycles of 95°C for 15 s and 60°C for 1 min. The results were analyzed by the $^{\Delta\Delta}$Ct method. The included autophagy genes and their function information are listed in Table 1.

Results

The Enhancement of DENV-ADE Infection Was Dependent on the Final Concentration of Anti-DENV prM Antibody

The direct infection model was established in THP-1 cells through incubating with DENV-3 at an MOI of 5. Meanwhile, DENV-3 and anti-prM antibody (AB41473; Abcam) were selected to establish the DENV-3-ADE model. The enhancement of DENV-ADE infection was

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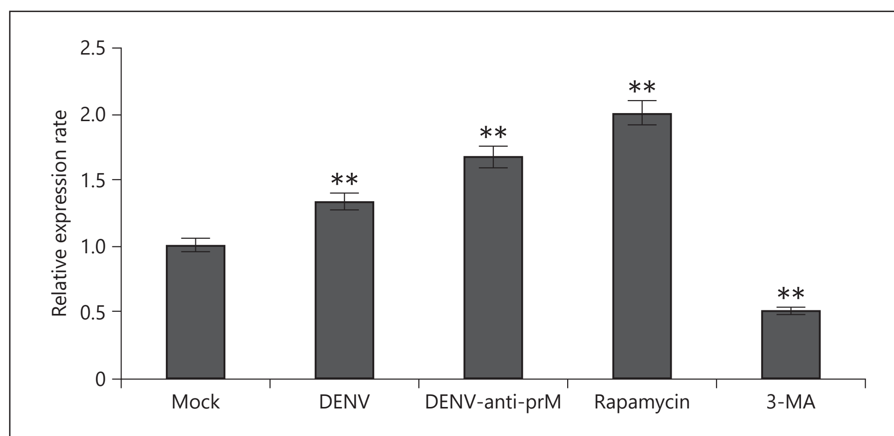

Fig. 1. ATG12 expression in THP-1 cells infected or treated with DENV, DENV-anti-prM (DENV-ADE), rapamycin, and 3-MA. ** $p < 0.01$. DENV, dengue virus; ADE, antibody-dependent enhancement; 3-MA, 3-methyladenine.

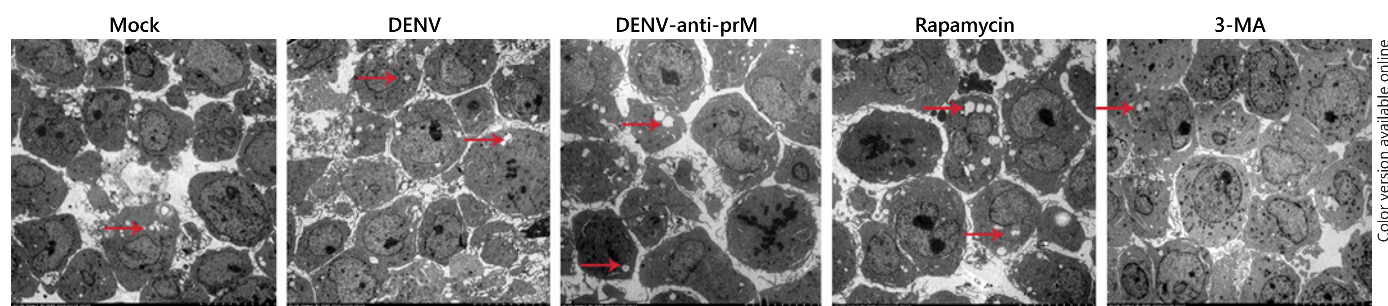

Fig. 2. Observation of autophagosomes in THP-1 cells infected or treated with DENV, DENV-anti-prM (DENV-ADE), rapamycin, and 3-MA by TEM. The red arrow indicates white vesicles. DENV, dengue virus; ADE, antibody-dependent enhancement; 3-MA, 3-methyladenine.

dependent on the final antibody concentration. Anti-DENV-2 prM antibody exhibited an enhancement peak at an antibody dilution of 1/64, and this was concordant with the results found in Fcγ R-expressing K562 cells [18].

Autophagy Complex-Related Gene ATG12 in THP-1 Cells

The expression of the autophagy-related gene ATG12 of THP-1 cells was increased in the DENV, DENV-ADE, and rapamycin treatment groups 24 h after infection or treatment. On the contrary, it was downregulated in the 3-MA group 24 h after treatment. The expression of ATG12 in THP-1 cells was detected by RT-PCR. The results showed that 3-MA had a significant inhibitory effect on ATG12 expression in THP-1 cells (Fig. 1).

Autophagosome Was Detected in THP-1 Cells

To further confirm that autophagy could be induced by DENV infection, double-membrane autophagosome-like vesicles were observed through TEM in the THP-1 cells. The double-membrane vesicle defined as an autophagosome vesicle was found in autophagic effected THP-1 cells. A similar double-membrane vesicle (autophagosome vesicle) was promoted and easily observed in DENV-anti-prM, DENV direct infection, and rapamycin-treated THP-1 cells (Fig. 2); the autophagy process was blocked by the autophagy inhibitor 3-MA in THP-1 cells (Fig. 2).

Global Analysis of Autophagy Gene Expression Patterns

To explore the expression patterns of autophagy relevant genes in THP-1 cells during the DENV direct infection and DENV-ADE infection, global cellular autophagy relevant gene expression patterns were compared with those of the control group by applying the PCR Array Excel data analysis template (located at: www.SABiosciences.com/pcrarraydataanalysis.php). Subsequently, 3 groups of differentially expressed autophagy genes were summarized. Then, we used QIAGEN online software to draw the autophagy-related gene cluster analysis chart (Fig. 3), and

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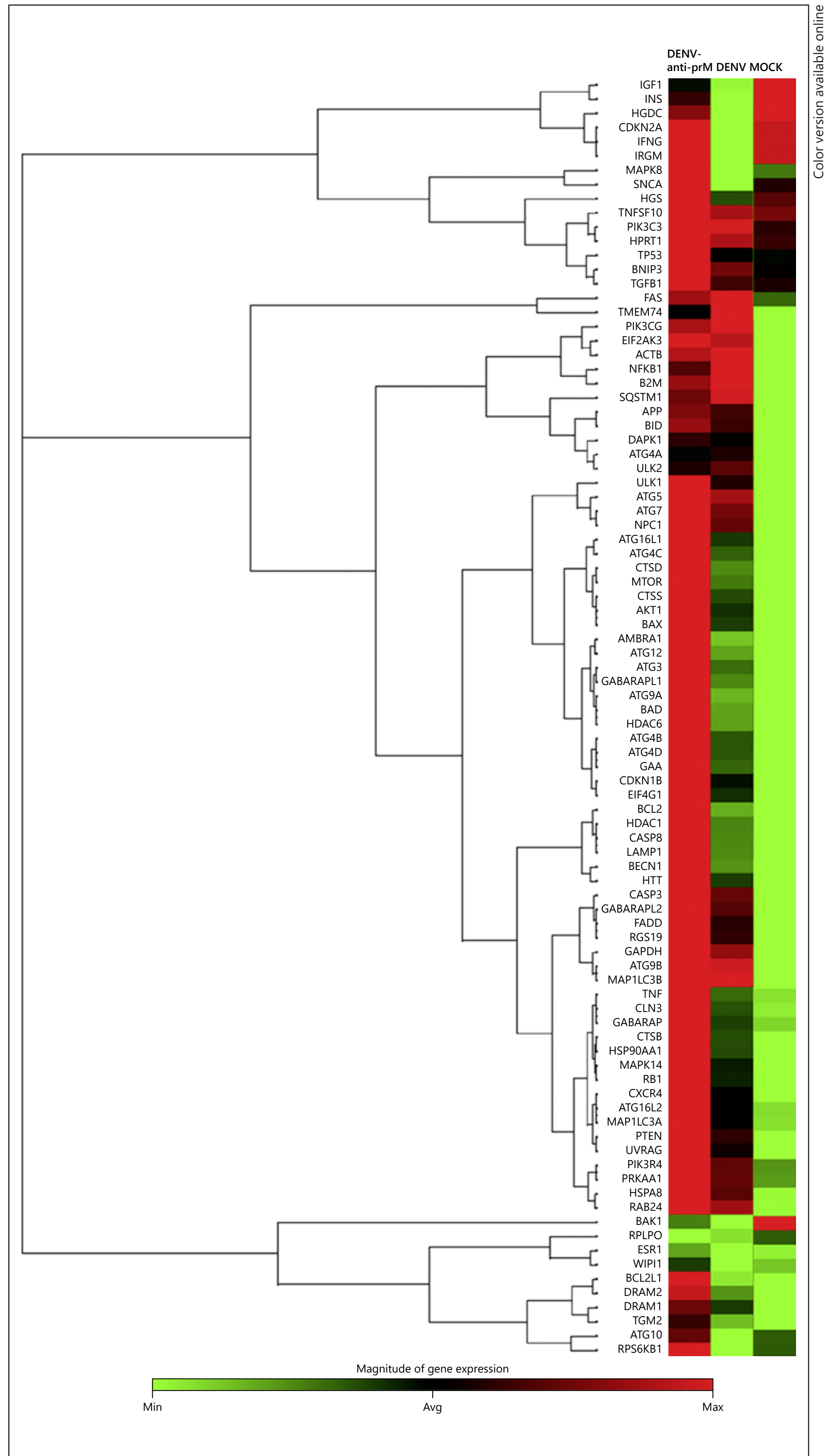

Fig. 3. Hierarchical clustering of autophagy-related genes differentially expressed in DENV and DENV-ADE groups 24 h after infection compared to control samples. DENV, dengue virus; ADE, antibody-dependent enhancement.

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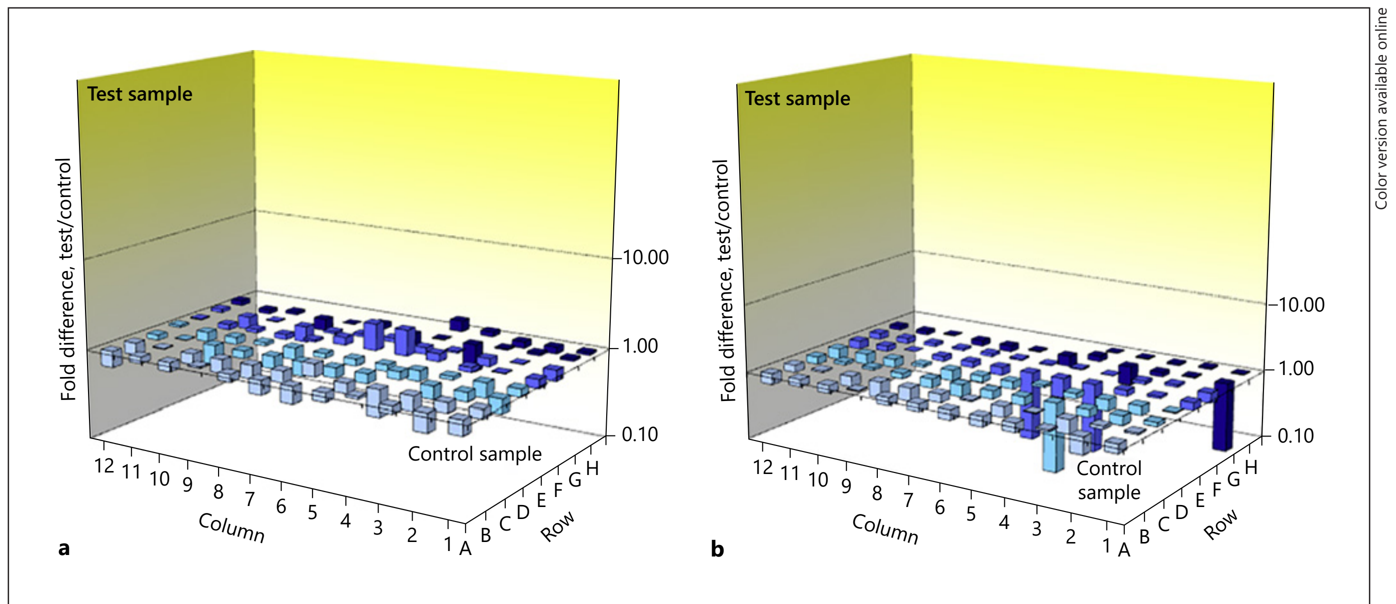

Fig. 4. a 3D map showing differentially expressed autophagy-related genes between DENV infection and noninfected THP-1 cells. **b** 3D map showing differentially expressed autophagy-related genes between DENV-ADE infection and uninfected THP-1 cells. DENV, dengue virus; ADE, antibody-dependent enhancement.

this figure can intuitively show the expression patterns of 84 genes. The expression profile of autophagy genes was changed after DENV-3 direct and DENV-3-ADE infection of THP-1 cells (Fig. 3).

The hierarchical cluster of the differentially expressed autophagy genes was generated by the QIAGEN online analysis site in THP-1 cells infected with DENV and DENV-ADE. The hierarchical cluster provided the expression differences between the selected groups of each gene in the array layout. The results showed that the autophagy genes were significantly increased among the DENV-ADE group compared with DENV directly infected THP-1 cells, and there were obvious differences in the autophagy gene expression pattern between DENV- and DENV-ADE-infected THP-1 cells. Besides, the upregulation and downregulation of autophagy gene expression were significantly different in the THP-1 cells after 24-h culture (Fig. 3).

3D Display of the Results of Autophagy PCR Array

Through the online analysis template, located at www.ABiosciences.com/pcrarraydataanalysis.php, the results of 3D display of the autophagy chip were generated by the PCR Array Data analysis tool. 3D map of autophagy-related genes showed the different expression profiles in DENV-ADE- and DENV-infected THP-1 cells, and THP-1 cells of the basic culture medium were set as the control group (Fig. 4).

The Gene Expression of DENV- and DENV-ADE-Infected THP-1 Cells

Only the ESR1 gene was downregulated in the group of DENV directly infected THP-1 cells; nevertheless, 6 genes including INS, BNIP3, FAS, TGM2, ATG9B, and DAPK1 were upregulated among the group of DENV-ADE. It is worth noting that the expression of INS had a distinct differential expression in the DENV-ADE group. These results suggested that autophagy-regulated gene expression was facilitated by DENV and DENV-ADE infection of THP-1 cells, and autophagy enhancement promoted the replication of DENV in THP-1 cells (Fig. 4).

Discussion

THP-1 cell line was widely used for the research of the infection mechanism of DENV-ADE and DENV. The autophagy was initiated under the condition of starvation or pathogen infection [14]. The regulation mechanism between autophagy and viral infection is different between different viruses [9, 15, 18–24].

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It has been reported that DENV infection can mediate autophagy [25, 26]. Previous studies suggested that DENV uses autophagy for DENV replication in the AG129 mouse model and the THP-1 cell line, but not in the U937 cell line [27–31]. The degradation of lipid droplets was also increased during DENV-2 infection [32, 33].

For the function of autophagy in DENV-ADE infection, DAK and ATG5-ATG12 were activated and inhibited the production of IFN and then induced the expression of IL-10, thus inhibiting the IFN-mediated antiviral pathway [34, 35]. In this study, we demonstrated another possible mechanism of autophagy induced by rapamycin, DENV, and DENV-ADE. Our finding provided a clear link between autophagy and DENV-ADE infection. Given the importance of DENV-ADE for life-threatening DSS or DHF, the results of this study increased our knowledge about the complex relationship between severe dengue fever and autophagy and provided useful information for development of vaccine and therapeutic drugs.

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Statement of Ethics

The patients provided written consent for participation in the study and for publication of the results. Ethical approval was obtained from the Institutional Ethics Committee (Institute of Medical Biology, Chinese Academy of Medical Sciences, and Peking Union Medical College, IMB. No. 2013R00081), and the study was conducted in accordance with the Declaration of Helsinki for Human Research of 1974 (last modified in 2000).

Conflict of Interest Statement

The authors declare no conflicts of interest.

Funding Sources

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Author Contributions

L.M.J. and Q.M.S. conceived and designed the study and wrote the manuscript, L.M.J. performed the experiments and analyzed the data, and Q.M.S. oversaw the laboratory work. All authors read and approved the final manuscript.

Availability of Data and Material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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