



# Global Transcriptomic Analysis of the Interactions between Phage $\varphi$ Abp1 and Extensively Drug-Resistant *Acinetobacter baumannii*

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**ABSTRACT** *Acinetobacter baumannii* is a growing threat, although lytic bacteriophages have been shown to effectively kill *A. baumannii*. However, the interaction between the host and the phage has not been fully studied. We demonstrate the global profile of transcriptional changes in extensively drug-resistant *A. baumannii* AB1 and the interaction with phage  $\varphi$ Abp1 through RNA sequencing (RNA-seq) and bioinformatic analysis. Only 15.6% (600/3,838) of the genes of the infected host were determined to be differentially expressed genes (DEGs), indicating that only a small part of the bacterial resources was needed for  $\varphi$ Abp1 propagation. Contrary to previous similar studies, more upregulated rather than downregulated DEGs were detected. Specifically,  $\varphi$ Abp1 infection caused the most extensive impact on host gene expression at 10 min, which was related to the intracellular accumulation phase of virus multiplication. Based on the gene coexpression network, a middle gene (*gp34*, encoding phage-associated RNA polymerase) showed a negative interaction with numerous host ribosome protein genes. In addition, the gene expression of bacterial virulence/resistance factors was proven to change significantly. This work provides new insights into the interactions of  $\varphi$ Abp1 and its host, which contributes to the further understanding of phage therapy, and provides another reference for antibacterial agents.

**IMPORTANCE** Previous research has reported the transcriptomic phage-host interactions in *Escherichia coli* and *Pseudomonas aeruginosa*, leading to the detailed discovery of transcriptomic regulations and predictions of specific gene functions. However, a direct relationship between *A. baumannii* and its phage has not been previously reported, although *A. baumannii* is becoming a rigorous drug-resistant threat. We analyzed transcriptomic changes after  $\varphi$ Abp1 infected its host, extensively drug-resistant (XDR) *A. baumannii* AB1, and found defense-like responses of the host, step-by-step control by the invader, elaborate interactions between host and phage, and elevated drug resistance gene expressions of AB1 after phage infection. These findings suggest the detailed interactions of *A. baumannii* and its phage, which may provide both encouraging suggestions for drug design and advice for the clinical use of vital phage particles.

**KEYWORDS**  $\varphi$ Abp1, *Acinetobacter baumannii*, RNA-seq, bacteriophage, transcriptome

**A***cinetobacter baumannii* is responsible for numerous health care-associated infections and burn and wound infections (1, 2). As a Gram-negative opportunistic pathogen, *A. baumannii* was recently listed as one of the six most dangerous pathogens due to its multiple resistance to antibiotics (2, 3). In addition, more *A. baumannii* strains

**Citation** Yang Z, Yin S, Li G, Wang J, Huang G, Jiang B, You B, Gong Y, Zhang C, Luo X, Peng Y, Zhao X. 2019. Global transcriptomic analysis of the interactions between phage  $\varphi$ Abp1 and extensively drug-resistant *Acinetobacter baumannii*. *mSystems* 4:e00068-19. <https://doi.org/10.1128/mSystems.00068-19>.

**Editor** Sergio Baranzini, University of California, San Francisco

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**Received** 31 January 2019

**Accepted** 29 March 2019

**Published** 16 April 2019

were found to be resistant to all known antibiotics, which has alerted people to find an alternative arsenal (4, 5).

In the middle of the 1910s, the bacteriophage (phage) was suggested to have a positive outcome in the treatment of human infections (6, 7). During the following decades, increasing evidence has shown the feasibility of phage therapy to treat drug-resistant bacterial infections (8, 9). Indeed, not only has active bacteriophage been applied directly in the clinic under prudent observation (10), but also new phage-derived potential antimicrobial agents have been identified and certified (11, 12). However, phage-host interactions have not been fully studied.

Recent interest in bacteriophages has been triggered by increasing antimicrobial resistance, omics development of phage-host studies, and the screening of new antibacterial agents from phage-derived gene products (13, 14). Like all viruses, the phage relies heavily on host metabolism and must take over host processes to complete a productive infection (15, 16). Therefore, the course of phage infection is a complex struggle between the virus and the bacterial host (17, 18). However, current knowledge of phage-host interactions is based largely on a small number of *Escherichia coli* phages (19–21), *Pseudomonas aeruginosa* phages (22), and phages with less clinical importance (23), whereas insight into the phage infection courses in multidrug-resistant strains remains quite limited. Thus, an understanding of phage interactions with *A. baumannii* is essential for the development and application of phage therapy.

In previous reports, we screened a lytic *A. baumannii* phage, named  $\varphi$ Abp1, from hospital sewage against a clinically isolated extensively drug-resistant (XDR) *A. baumannii* strain, AB1 (XDR-AB1), in our burn ward (12).  $\varphi$ Abp1 was proven to be a qualified candidate for the treatment of both systemic and local XDR *A. baumannii* infections in mouse models (9). Moreover,  $\varphi$ Abp1 gene product 50 (*gp50*), endolysin, was reported to exhibit marked lytic activities against 48 clinically isolated XDR *A. baumannii* strains with different multilocus sequence typing (MLST) types (8), implying that *gp50* can be an excellent anti-infection agent against XDR *A. baumannii*. However, the interactions between  $\varphi$ Abp1 and its host are unclear, which limits its clinical application in the future. Therefore, we need an in-depth understanding of the interactions between  $\varphi$ Abp1 and its host.

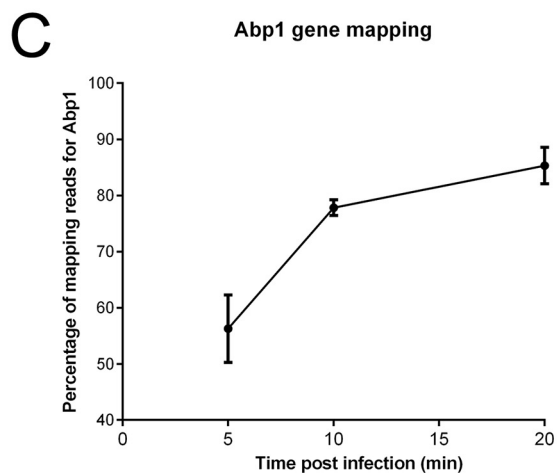
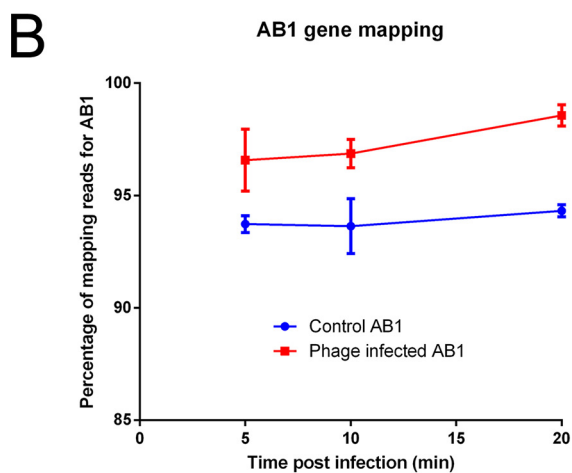
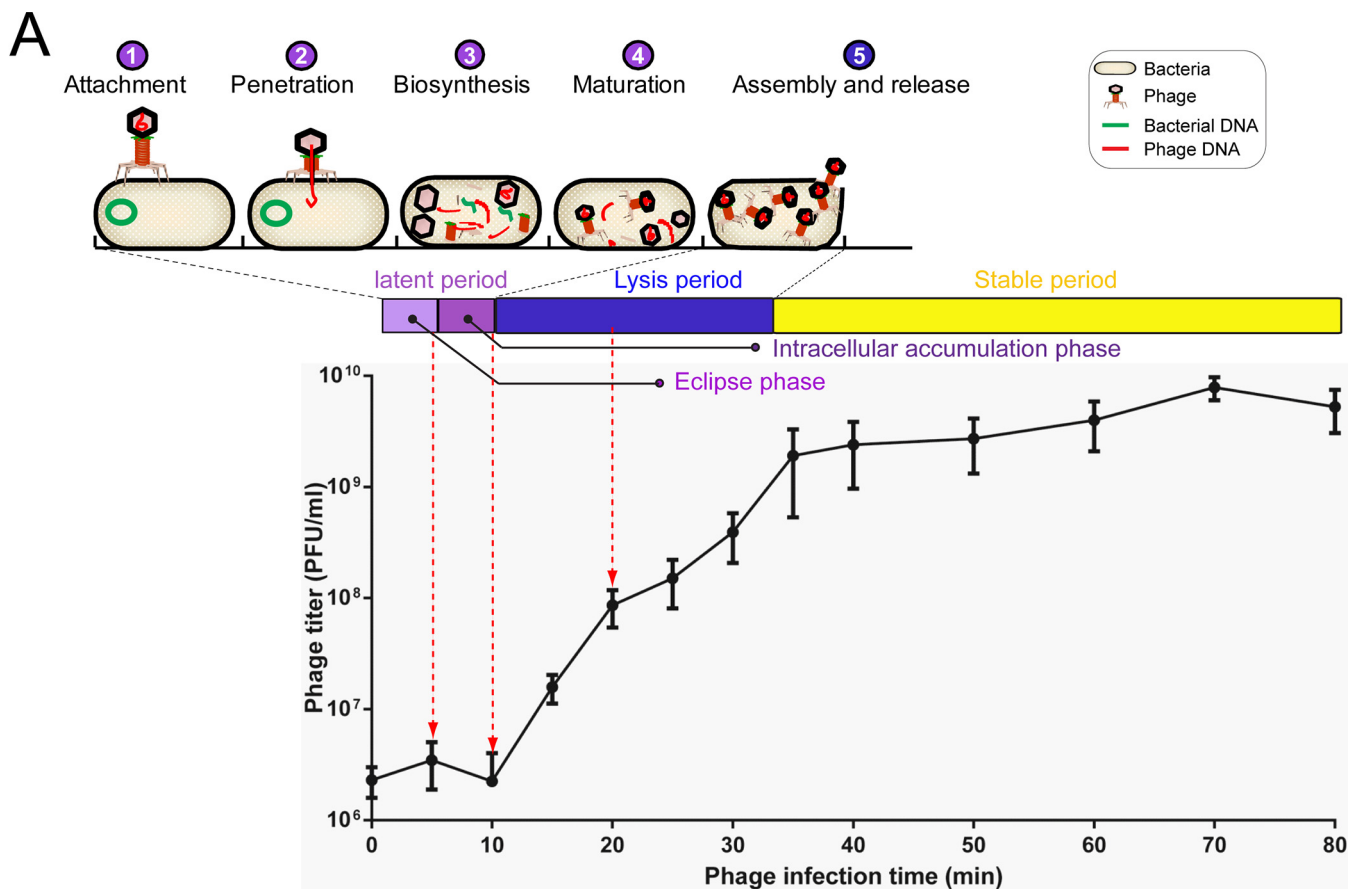
In this work, RNA sequencing (RNA-seq) was performed to investigate the interactions between  $\varphi$ Abp1 and its host at three time points representing each infection stage. Analyses of gene expression patterns, differentially expressed genes (DEGs), and coexpression during infection were performed. This work aimed to draw new insight into the interactions between  $\varphi$ Abp1 and its host, to establish a general understanding of  $\varphi$ Abp1-based anti-*A. baumannii* phage therapy, and to provide more options for antibacterial agents.

## RESULTS

**Experimental design of RNA-seq after  $\varphi$ Abp1 infection.** First, we investigated the resistance of the host strain AB1 to 20 antibiotics commonly used clinically (see Table S1 in the supplemental material). The results showed susceptibility to polymyxin B only, affirming the grim antibiotic resistance situation and tremendous clinical importance of  $\varphi$ Abp1.

Three sampling time points were selected for subsequent RNA sequencing according to the life cycle of  $\varphi$ Abp1. AB1 cultures infected with  $\varphi$ Abp1 at 5 min (eclipse phase), 10 min (intracellular accumulation phase), and 20 min (lysis period), as well as three AB1 cultures free of phage at the corresponding time points as control groups, were collected for RNA-seq analysis (Fig. 1A). RNA-seq was repeated three times for each group.

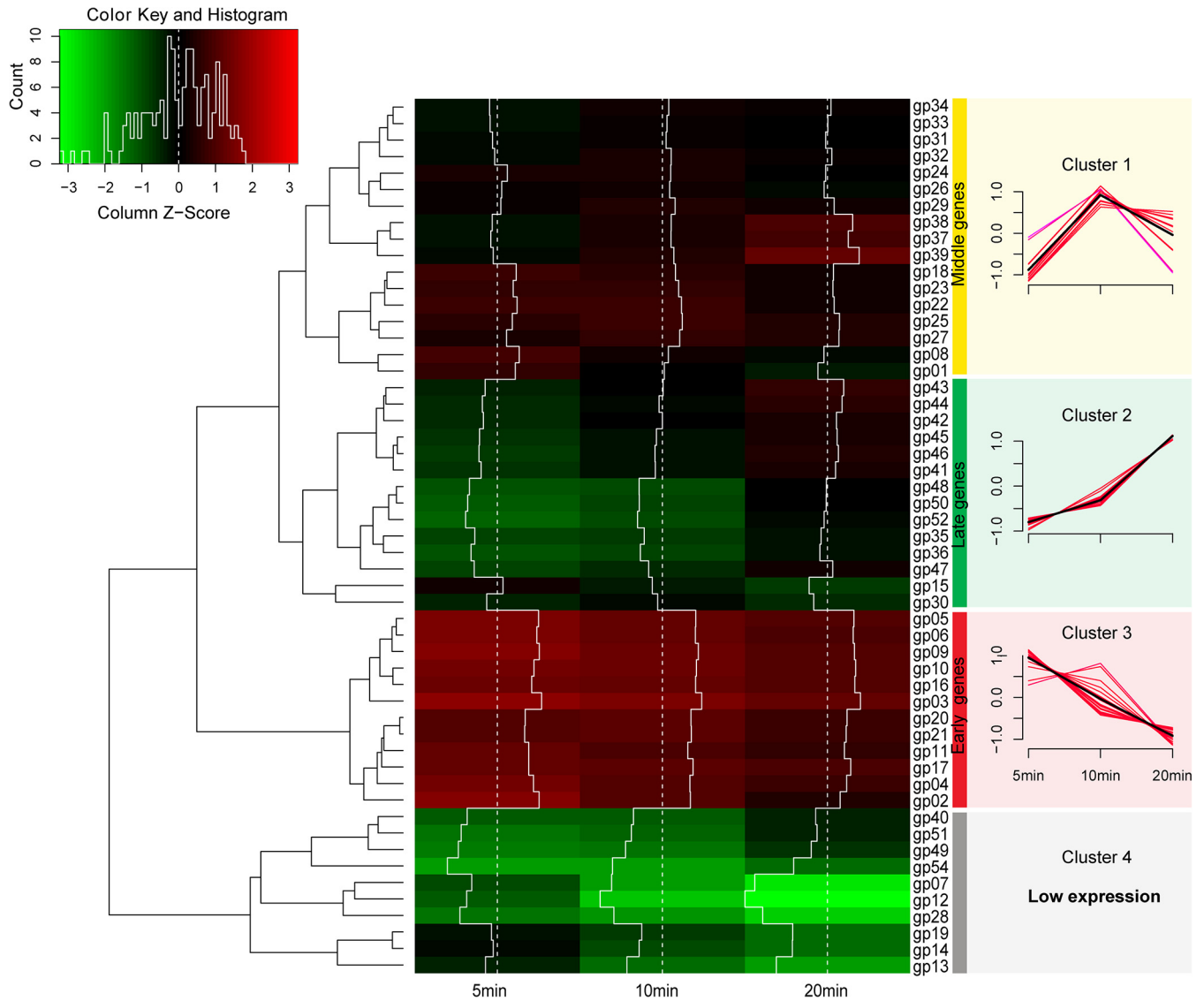
**$\varphi$ Abp1 takes over the transcriptional resources of host cells.** *A. baumannii* strain ATCC 17978 (GenBank accession no. [NZ\\_CP018664.1](#)) and *Acinetobacter* phage  $\varphi$ Abp1 (GenBank accession no. [NC\\_021316.1](#)) were applied as the reference genomes. RNA-seq analysis generated an average of 11.8 million or 12.3 million reads in bacterial cultures infected with or without phage, respectively. The RNA-seq reads were aligned to both



**FIG 1** Life cycle annotation, one-step growth curve, and mapping of  $\phi$ Abp1. (A) The phage multiplication stages in a life cycle were divided into attachment, adsorption, penetration, biosynthesis, maturation, and assembly and release. The one-step growth curve of  $\phi$ Abp1 is from 0 to 80 min, which includes 3 periods: a latent period (divided into an eclipse phase and an intracellular accumulation phase), a lysis period, and a stable period. Red dotted arrows indicate the time points of sample collection. (B and C) Percentages of RNA-seq reads mapping to the reference AB1 (B) and  $\phi$ Abp1 (C) genomes at different infection time points.

the AB1 and  $\phi$ Abp1 genomes in a strand-specific manner. The proportion of reads mapping to the AB1 genome stayed above 92%, indicating that ATCC 17978 is a proper reference for AB1 annotation (Fig. 1B). For phage consideration, the proportion of reads mapping to the phage genome increased from 56.3% (5 min) to 85.3% (20 min) (Fig. 1C), suggesting a process during which  $\phi$ Abp1 took over the transcriptional resources of the host cells.

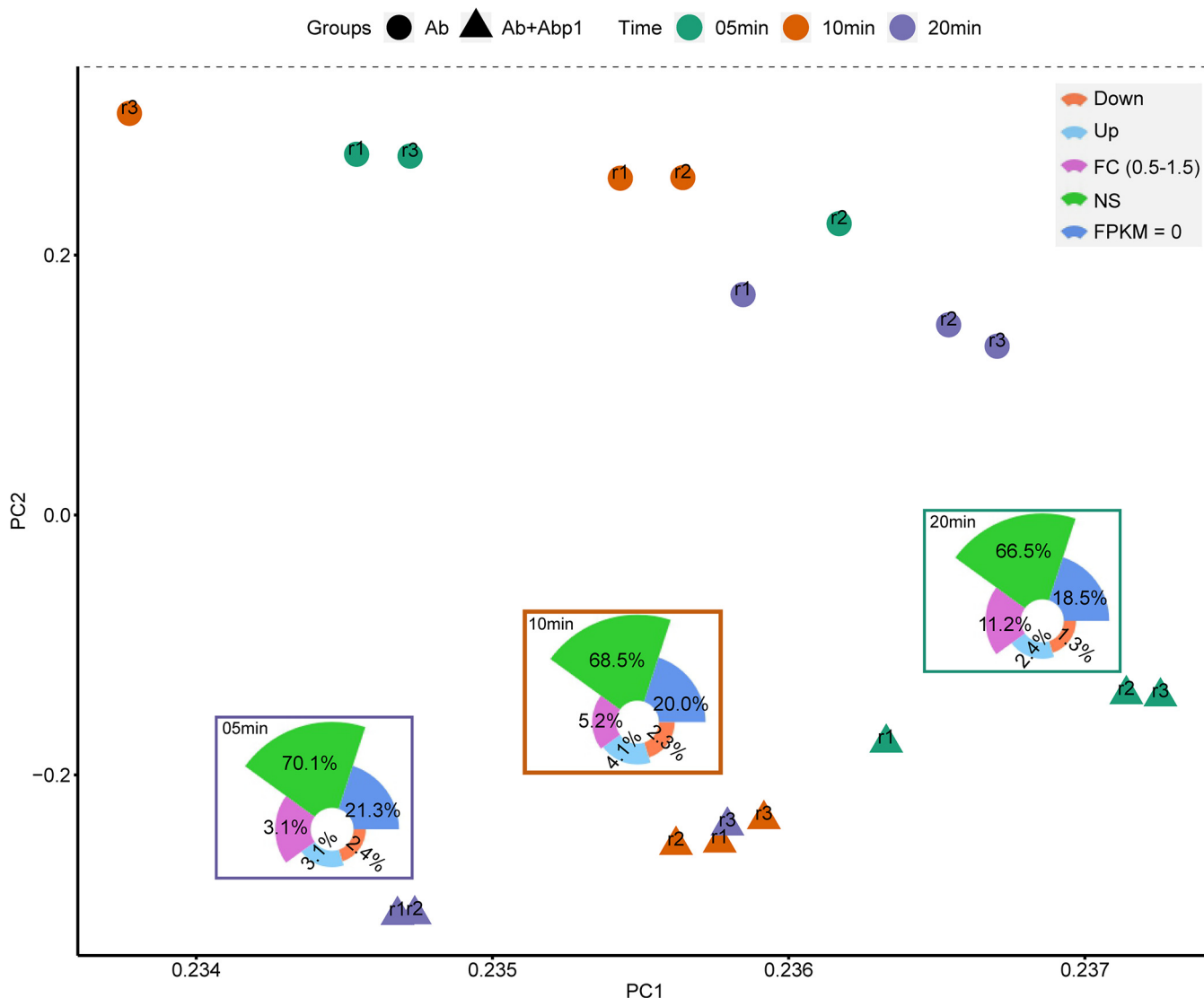
Next, 54 phage genes were clustered by hierarchical cluster analysis based on their transcriptional levels (fragments per kilobase of transcript sequence per million base



**FIG 2** Transcriptomic profile of  $\phi$ Abp1 genes in the host cells. (Left) Hierarchical cluster heat map of  $\phi$ Abp1 genes; (right) expression level of each cluster of  $\phi$ Abp1 genes. Based on the FPKM values of genes, hierarchical cluster analysis was performed using ward.D2 and Minkowski methods. A total of 54  $\phi$ Abp1 genes (gp01 to gp54) were clustered into 4 clusters (clusters 1 to 4).

pairs sequenced [FPKM values) into 4 clusters of genes, including middle genes (cluster 1, at 10 min) (Fig. 2), late genes (cluster 2, at 20 min), early genes (cluster 3, at 5 min), and low-expression genes (cluster 4).

**$\phi$ Abp1 infection induces more activation than inhibition of AB1 genes.** On the basis of the FPKM values of all 3,838 AB1 genes, PCA (principal-component analysis) showed that the greater distance between points suggested a greater difference in AB1 gene expression (Fig. 3), suggesting that the gene expression of the phage infection group changed far more than that of the phage-free group. According to the expression levels, genes with fold change (FC) values of  $>1.5$  and  $q$  values of  $<0.05$  were defined as DEGs. Totals of 3.7% (145/3,838), 6.4% (244/3,838), and 5.5% (211/3,838) DEGs were found 5 min, 10 min, and 20 min after  $\phi$ Abp1 infection, respectively. Contrary to previous similar studies (22), more upregulated DEGs than downregulated DEGs were detected in our study. Approximately 68% of the AB1 genes were expressed stably during  $\phi$ Abp1 infection (nonsignificant [NS] [ $q > 0.05$ ]), implying that only a small part of the bacterial resources was needed for  $\phi$ Abp1 propagation. The highest upregulation rate of the host genes was at 10 min (156; 4.1%).

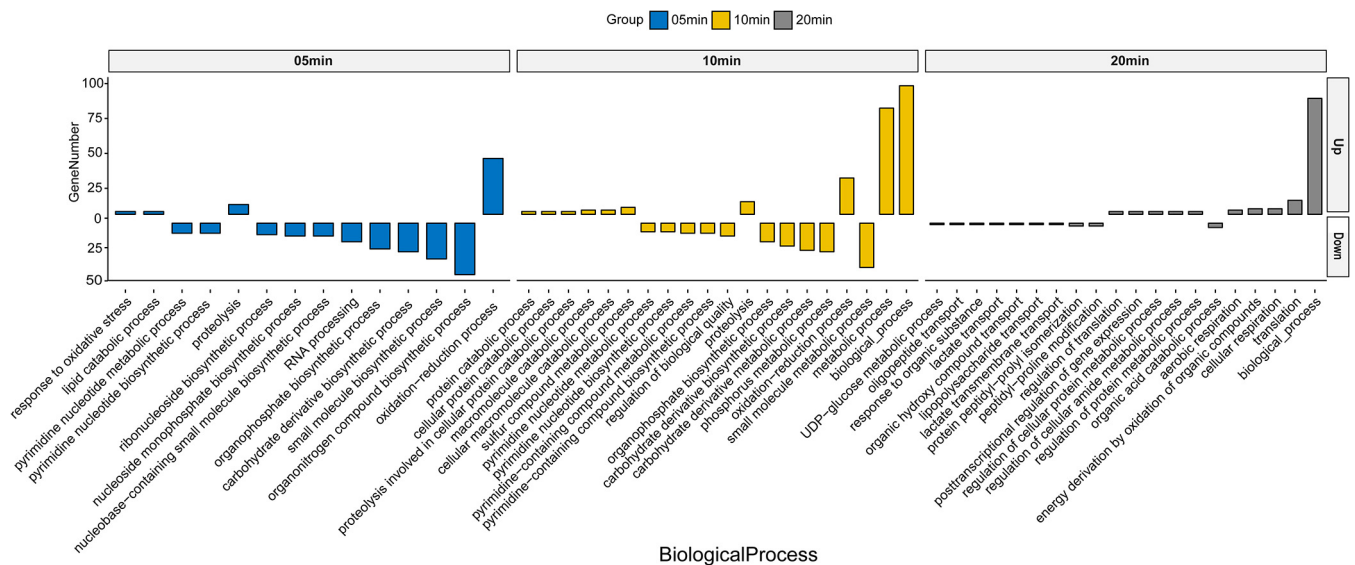


**FIG 3** PCA plot of the expression of AB1 genes. The principal-component analysis was performed according to the expression levels of AB1 genes in phage-infected groups (Ab+Abp1) and phage-free groups (Ab). A greater distance between points suggests a greater difference in AB1 gene expression in those 6 samples. Down, downregulated DEGs; Up, upregulated DEGs; NS, nonsignificantly changed genes.

**Functional analysis reveals the step-by-step control of  $\phi$ Abp1 on host genes.**

The host DEGs were grouped into the eclipse phase (5 min), intracellular accumulation phase (10 min), and lysis process (20 min) according to phage infection time points and underwent gene ontology (GO) analysis. The biological function enrichment of GO results showed that DEGs had a remarkable function classification among the three stages. The upregulated genes mainly involved stress reactions (such as oxidation-reduction processes and proteolysis) in the eclipse and intracellular accumulation phases (5 and 10 min), metabolic processes (10 min), and translation processes (20 min) (Fig. 4). The downregulated DEGs mainly included host biosynthetic processes (5 and 10 min), nucleic acid metabolic processes (5 and 10 min), and material transport processes (20 min) (Fig. 4). The detailed expression data on specific genes (>1.5-fold;  $q$  values of <0.05) are presented in Table S2.

KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment suggested that the downregulated genes of AB1 were significantly enriched in the pathways related to nucleic acid complements, such as purine metabolism and pyrimidine metabolism (Fig. 5). The upregulated genes were enriched in a wide range of KEGG



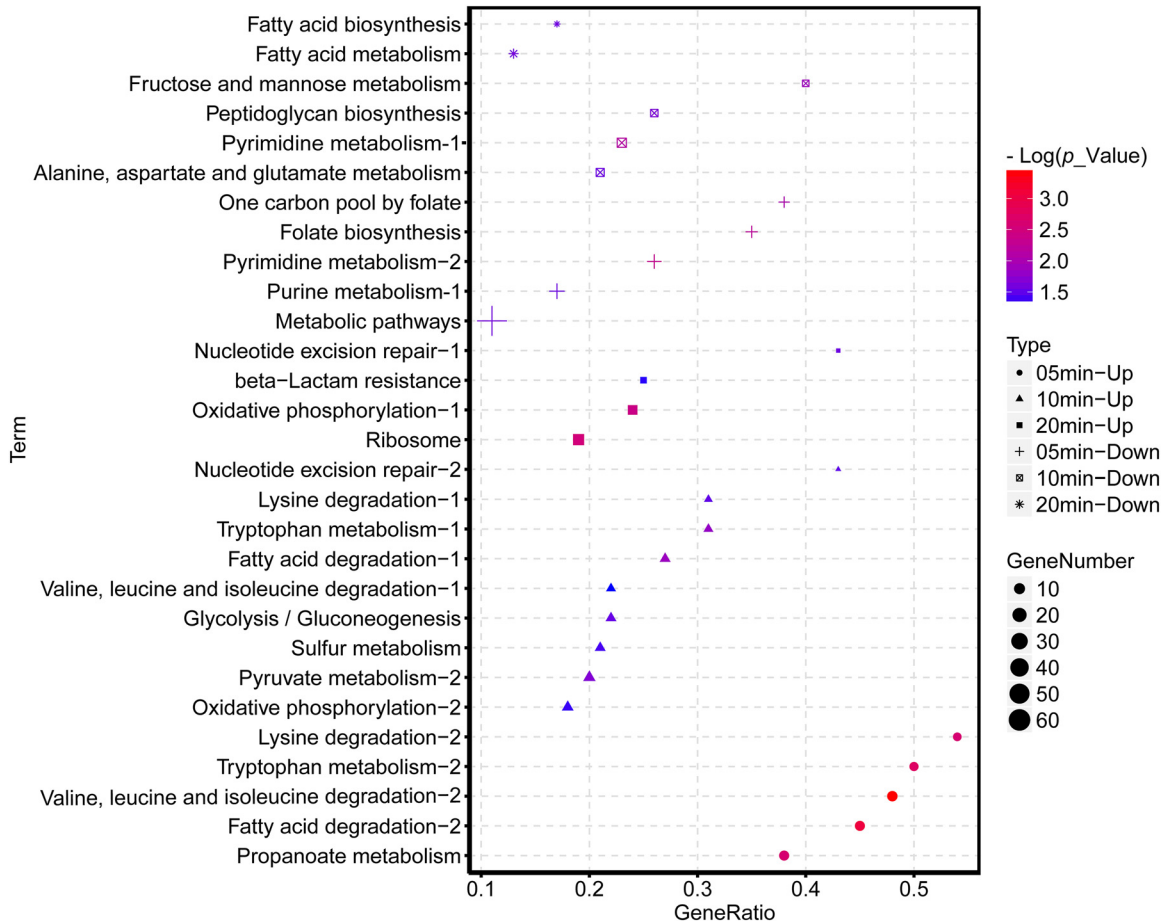
**FIG 4** GO analysis for the biological processes of host DEGs (up- and downregulated genes). GO terms enriched from upregulated (Up) and downregulated (Down) genes are shown.

pathways, among which the most remarkably enriched pathways were the multiple amino acid pathways at 10 min and the ribosome pathway at 20 min.

**Phage-host interaction network analysis.** To screen genes with vital roles in the regulation of host gene expression,  $\varphi$ Abp1-AB1 interaction networks based on gene coexpression analysis were constructed. First, we classified  $\varphi$ Abp1 genes into early (*gp01* to *gp21* [*gp01-21*]), middle (*gp22-34*), and late (*gp35-54*) genes according to the gene clustering in Fig. 3 and the sequential expression of the  $\varphi$ Abp1 phage gene. A total of 944 coexpression relationships between 240 host genes and 24 phage genes were screened by gene coexpression network analysis, including 49 negative correlations and 895 positive correlations. The results formed a network structure centered on the phage genes *gp01*, *gp08*, *gp13*, and *gp34*, indicating that these four genes may play a central role in interacting with the host genes (Fig. 6). Furthermore, the network also includes 3 subnetworks centered on *gp12*, *gp02*, and the *gp03-gp04-gp05-gp06-gp09* cluster. The expression patterns of *gp01*, *gp02*, *gp08*, *gp12*, and *gp34* were validated by real-time quantitative PCR (RT-qPCR) (Table S3, part I). Among these central genes, *gp34* was annotated as a phage-associated RNA polymerase, and other central phage genes were all early phage genes with unknown annotations (Table S4). Thus, in the negative regulation network, the results showed that *gp34* plays a core role.

**Effects of  $\varphi$ Abp1 infection on host resistance and virulence.** From a clinical perspective, it is important to consider the alterations in *A. baumannii* virulence and antibiotic resistance genes after  $\varphi$ Abp1 infection. The expression of 24 virulence factors, including type II/V/VI secretory systems (*hcp* and *tssE*), porin (*carO*), and pili (*ompR*), and the expression of 21 resistance factors, including efflux pumps (*adeK*, *mdfA*, and *rs02660*) and beta-lactamases, were selected according to previous reports (24) and statistically analyzed by their differentially expressed patterns. The results showed that the expression of virulence factors was mainly inhibited after  $\varphi$ Abp1 infection, but the drug resistance genes of the host were mainly activated (Fig. 7). The genes with significantly changed expression included 8 virulence factors and 9 resistance factors after  $\varphi$ Abp1 infection ( $P < 0.05$ ). It is noteworthy that 3 efflux pump-related genes appeared to be upregulated more than 1.5-fold at 20 min, suggesting that phage infection may alter the virulence and resistance of the host. All these host genes (resistance and virulence genes) were selected for further RT-qPCR validation. The validation result is consistent with the RNA-seq results (Table S3, part II). These genes should be paid attention in future studies of  $\varphi$ Abp1 therapy and phage-derived antibacterial products.





**FIG 5** KEGG categories of host DEGs (up- and downregulated genes) enriched at selected points after  $\phi$ Abp1 infection. The shape of the point indicates the time points. The enrichment  $P$  value of each pathway was normalized as negative  $\log(P$  value) and is shown as a color gradient. The number of genes enriched in each pathway is represented by the size of the points.

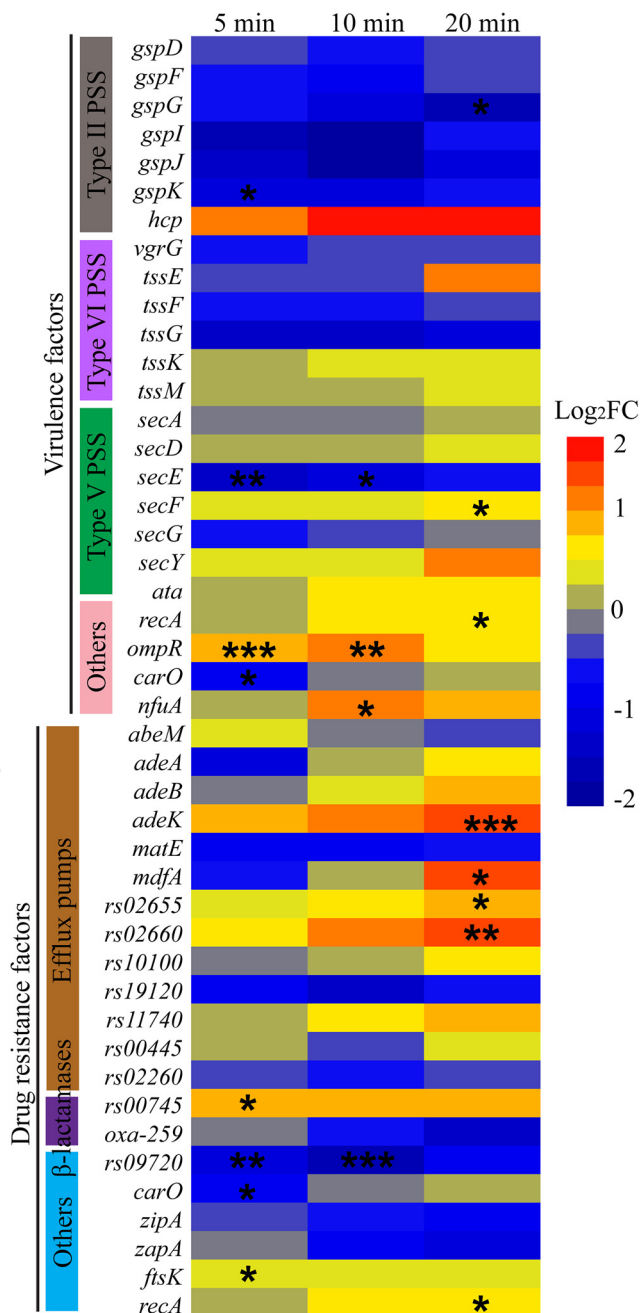
## DISCUSSION

In this study, we progressed the transcriptional scheme of  $\phi$ Abp1 infection of AB1 and illustrated the interaction during  $\phi$ Abp1 predation in the host strain. Our results demonstrated that the expression of  $\phi$ Abp1 genes does not follow the standard lytic phage pattern. In the early infection stage after  $\phi$ Abp1 entered the cell (5 min), short and slight expression of the  $\phi$ Abp1 early genes was observed, with minimal impacts on the host. Next, during the middle stage (10 min), the expression of all  $\phi$ Abp1 middle genes peaked, as  $\phi$ Abp1 started to hijack cellular resources by regulating an increasing number of host DEGs. Finally, after most host cells were lysed, until phage-resistant mutants emerged and/or until lysogenic conversion occurred at the late stage of infection (80 min), a balance of phage-host interactions was achieved. Moreover, the majority of the host genes were constitutively expressed throughout the course of infection. However, upregulated host DEGs significantly overwhelmed downregulated DEGs during all stages of infection, indicating precise control by  $\phi$ Abp1 rather than simply forbidding host genes, as reported previously (19, 22, 25).

Phages take over host resources to propagate, including the manipulation of host proteins, molecular processes, cellular pathways that are related to transcription and translation, signal transduction, and metabolism (26). GO analysis in our work indicated that the host DEGs were regulated in a very precise way, including the irritation of stress reactions in the early infection stages. The GO analysis indicates a quick and thorough response from AB1 against  $\phi$ Abp1 infection, which has also been reported for the phage-host interaction between  $\phi$ R1-37 and *Yersinia enterocolitica* (23), and metabolic







**FIG 7** Expression of AB1 genes related to virulence and drug resistance after phage infection. The warm colors indicate upregulation, while the cold colors indicate downregulation. The asterisks indicate  $q$  values, where “\*”, “\*\*”, and “\*\*\*” indicate  $q$  values of  $<0.05$ ,  $<0.01$ , and  $<0.001$ , respectively. PSS indicates pili and secretion systems.

network, suggesting the weak regulatory role of the phage late genes, as was also reported in our previous study (22).

GO and KEGG plus networking analyses together indicate a strong and precise phage control. Despite the rigorous challenge from the host stress response, the host material biosynthetic and transfer processes were all halted, while the reassembly of viral protein was promoted, which is consistent with other reports (30). These results suggest that the takeover and shutoff of host AB1 gene expression by  $\phi$ Abp1 was step-by-step rather than all of a sudden.

From the perspective of treating *A. baumannii* infections, it is crucial to know

whether this remedy induced changes different from those of other antibiotics or whether this remedy induced changes in virulence and antibiotic resistance of *A. baumannii*. Previous reports have focused on transcriptomic changes when *A. baumannii* was treated with antibiotics, including imipenem (31, 32), colistin (33), doripenem (33), amikacin (31), and meropenem (31). The numbers of DEGs induced by antibiotics were 28 by amikacin (31), 417 by imipenem-meropenem (31) (88/68 in an earlier study [32]), and 400 by colistin (33). However, in our studies, the application of phage  $\phi$ Abp1 caused changes in significantly more DEGs (600/3,838; 15.6%) during the treatment process, affirming thoroughly and elaboratively controlled predation and killing by  $\phi$ Abp1 in AB1 infection rather than just a simple lysing action.

In addition to spreading virulence and antibiotic resistance markers among bacteria, bacteriophages were also proven to promote the expression and/or induction of virulence/resistance traits in infected cells (34) of *Enterococcus faecalis* (35), *Pseudomonas aeruginosa* (36), *Bacillus anthracis* (21), and *Escherichia coli* (37). All these examples correspond to prophages, while there was practically no research regarding the effect of lytic predation on host virulence and antibiotic resistance. In our study, we have proven that  $\phi$ Abp1 lytic predation promoted a general inhibition of the expression of virulence factors. However, the drug resistance genes of AB1 were partially activated after infection with  $\phi$ Abp1. The molecular mechanism of virulence/resistance alteration remains to be further studied. Previous papers all report good results from phage application, including excellent lysis ability and zero side effects (38–40). Our observation of activated drug resistance gene expression is a contraindication to previous studies, which might cause researchers to use caution in phage applications. Thus, this study may be a hint to study the influence of lytic phages on antibiotic resistance/virulence phenotypes in their hosts during treatment.

This transcriptomic study has its inherent shortcomings. For the whole phage predation and lysis process, we are still unable to elucidate the translation changes, especially when *gp34* significantly inhibited ribosome expression. Moreover, due to the lack of other validation experiments, some descriptions and discussions remain to be further studied.

In conclusion, we provide a general description of a global phage-host transcriptome interaction that grounds further research aimed at elucidating the indicated interactions between  $\phi$ Abp1 and XDR-AB1. Despite previous similar observations (22), we discovered new stage-dependent inhibition of host genes by  $\phi$ Abp1, a novel ribosome-centered *gp34* negative control, and partially elevated antibiotic resistance expression during lytic infection. Overall, in-depth analysis of the mechanism of host gene expression shutoff performed by the phage, as well as knowledge of the precise control by  $\phi$ Abp1, is pivotal for research on novel antibacterial compounds and the development for phage therapy.

## MATERIALS AND METHODS

**Bacterium preparation, antibiotic susceptibility testing, and phage preparation.** AB1 was isolated from a burn patient in the burn ward of Southwest Hospital during routine bacteria monitoring. The susceptibility of AB1 to various antibiotics (listed in Table S1 in the supplemental material) (Oxoid, Hampshire, UK) was determined by the Kirby-Bauer (KB) method according to previously reported procedures (4). The MIC assay was conducted with the Vitek 2 compact automated ID/AST instrument system (bioMérieux, Craonne, France) according to the manufacturer's procedures (41). The antibiotic susceptibility results were interpreted according to Clinical and Laboratory Standards Institute criteria (42).

$\phi$ Abp1 was previously screened against AB1 from hospital sewage at the sewage management center of Southwest Hospital (12).  $\phi$ Abp1 particles were collected and purified using the CsCl gradient ultracentrifugation method (9). Both AB1 and  $\phi$ Abp1 were stored in our laboratory at  $-80^{\circ}\text{C}$  in glycerol. AB1 was inoculated aerobically at a 1:100 dilution in Luria-Bertani (LB) medium (Oxoid, Hampshire, UK) at  $37^{\circ}\text{C}$  overnight before the study.

**One-step growth curve.** Phage multiplication in the host can be divided into five processes: (i) attachment, (ii) penetration, (iii) biosynthesis, (iv) maturation, and (v) assembly and release. To determine the phage infection stage for sampling, a one-step growth curve of  $\phi$ Abp1 was measured as previously reported (9). Briefly, for one-step growth experiments, AB1 cells were infected with  $\phi$ Abp1 (MOI [multiplicity of infection] of 0.1) after a 5-min adsorption and centrifuged for 30 s at  $13,000 \times g$ . Unadsorbed phage was removed from the supernatant by washing twice with LB medium. The infected bacterial pellets were then resuspended in 5 ml LB medium, and the cultures were grown at  $37^{\circ}\text{C}$  with

shaking at 160 rpm. Samples were taken at 5- or 10-min intervals (up to 80 min). The number of  $\phi$ Abp1 particles was immediately determined using the double-layer agar plaque method. Experiments were carried out in triplicate.

**Total RNA extraction.** A total volume of 10 ml AB1 culture (optical density at 600 nm [OD<sub>600</sub>] of 0.6) was infected with phage  $\phi$ Abp1 at an MOI of 10, while an equal volume of an uninfected AB1 culture served as the negative control. Six samples for RNA isolation were taken (1 ml) from the noninfected/infected culture at three time points postinfection (5, 10, and 20 min), with three biological duplications. RNA extraction was performed using the SV total RNA isolation system (Promega, Madison, WI, USA). RNA quantity and quality checks were performed using a Bioanalyzer (Agilent, Santa Clara, CA, USA) and the RNA 6000 Nano kit (Agilent, Santa Clara, CA, USA).

**RNA sequencing.** For RNA sequencing, total RNA from all 18 samples was depleted of rRNA using the Ribo-Zero rRNA removal kit for Gram-negative bacteria (Epicentre, Madison, WI, USA). The cDNA libraries were constructed and sequenced on an Illumina HiSeq 2500 sequencer (Illumina, San Diego, CA, USA), using paired-end 2- by 150-bp reads. The raw data and processed bam files were deposited in the Gene Expression Omnibus (GEO) database.

**Bioinformatic analyses.** RNA sequence reads were aligned to the *A. baumannii* strain ATCC 17978 (GenBank accession no. [NZ\\_CP018664.1](https://ncbi.nlm.nih.gov/nuccore/NZ_CP018664.1)) and *Acinetobacter* phage  $\phi$ Abp1 (GenBank accession no. [NC\\_021316.1](https://ncbi.nlm.nih.gov/nuccore/NC_021316.1)) sequences using Bowtie2 (<http://bowtie-bio.sourceforge.net/bowtie2/index.shtml>). RNA-seq data analysis was performed with Tophat (<http://ccb.jhu.edu/software/tophat/index.shtml>) and Cufflinks (<https://cole-trapnell-lab.github.io/cufflinks/cuffdiff/index.html>). Gene expression values were determined by the expected number of fragments per FPKM and the false discovery rate (FDR) (*q* value). DESeq was used to calculate differentially expressed genes (DEGs) between the two groups. Genes with a fold change value (FC) of  $\geq 1.5$  and a *q* value of  $\leq 0.05$  were considered to be DEGs. GO (gene ontology) term enrichment of DEGs was performed with Blast2GO software (BioBam) based on Wallenius' non-central hypergeometric distribution. KOBAS (<http://kobas.cbi.pku.edu.cn/>) was used for KEGG pathway analysis. The analysis and visualization of gene coexpression networks were achieved by using Cytoscape 3.4.0 (<https://cytoscape.org/>).

**RT-qPCR validation of RNA-seq results.** Real-time quantitative PCR (RT-qPCR) analysis was further performed to validate the RNA-seq results. Seven AB1 (virulence- or drug resistance-related) genes and five  $\phi$ Abp1 (core regulatory) genes were selected for RT-qPCR validation. RT-qPCR was performed using SYBR Premix Ex Taq II (TaKaRa Bio, Dalian, China). The primers used in this study are listed in Table S5 in the supplemental material. The 16S rRNA gene was selected as the reference gene for normalization.

**Data availability.** The raw data and processed files were deposited in the NCBI GEO database with accession no. [GSE117396](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE117396).

## SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/mSystems.00068-19>.

**TABLE S1**, DOCX file, 0.02 MB.

**TABLE S2**, DOCX file, 0.04 MB.

**TABLE S3**, DOCX file, 0.02 MB.

**TABLE S4**, DOCX file, 0.01 MB.

**TABLE S5**, DOCX file, 0.02 MB.

## ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (grant no. 81571896, 81772073, and 31801037); the Foundation of State Key Laboratory of Trauma, Burns and Combined Injury (grant no. SKLZZ201708); and the Technological Innovation Plan in Major Fields of Southwest Hospital, Key Projects (grant no. SWH2016ZDCX2001). Xia Zhao is supported by the Youth Science Foundation of Army Medical University (grant no. 2017XQN01) and the National Natural Science Foundation of China Youth Science Fund (grant no. 31801087).

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