

# Study of the activation of the PI3K/Akt pathway by the motif of $\sigma$ A and $\sigma$ NS proteins of avian reovirus

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## Abstract

The present study was conducted to determine whether avian reovirus (ARV) activates the phosphatidylinositol 3-kinase-dependent Akt (PI3K/Akt) pathway according to the PXXP or YXXXM motifs of  $\sigma$ A and  $\sigma$ NS proteins. Gene splicing by overlap extension PCR was used to change the PXXP or YXXXM motifs of the  $\sigma$ A and  $\sigma$ NS genes. Plasmid constructs that contain mutant  $\sigma$ A and  $\sigma$ NS genes were generated and transfected into Vero cells, and the expression levels of the corresponding genes were quantified according to immunofluorescence and Western blot analyses. The Akt phosphorylation (P-Akt) profile of the transfected Vero cells was examined by flow cytometry and Western blot. The results showed that the  $\sigma$ A and  $\sigma$ NS genes were expressed in the Vero cells, and P-Akt expression in the  $\sigma$ A mutant groups (amino acids 110–114 and 114–117) was markedly decreased. The results indicated that the  $\sigma$ A protein of ARV activates the PI3K/Akt pathway via the PXXP motif. The results of this study reveal the mechanisms by which ARV manipulates the cellular signal transduction pathways, which may provide new ideas for novel drug targets.

## Keywords

Avian reovirus, PI3K/Akt pathway,  $\sigma$ A protein,  $\sigma$ NS protein, PXXP motif

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## Introduction

Avian reovirus (ARV) is one of the most important avian viruses, causing clinical diseases in poultry worldwide and resulting in severe economic losses.<sup>1,2</sup> ARV-affected flocks commonly suffer viral arthritis or tenosynovitis, runting-stunting syndrome (RSS), enteric disease, immunosuppression and malabsorption syndrome.<sup>2–5</sup> In the early stages of ARV infection, ARV activates the phosphatidylinositol 3-kinase-dependent Akt (PI3K/Akt) signalling axis in Vero cells<sup>6</sup> – a pathway that is associated with cell survival, proliferation, migration, differentiation and apoptosis.<sup>7,8</sup>

PXXP or YXXXM/YXXM motifs are present in a number of viral and cellular proteins involved in PI3K signaling and form extended helices that bind to SH domains on the p85 subunit of PI3K.<sup>9–15</sup> Therefore, as our previous research review<sup>16</sup> showed that various amino acid sequence have PXXP/YXXXM/YXXM motifs,  $\sigma$ A,  $\sigma$ NS,  $\mu$ A,  $\mu$ B and  $\mu$ NS of ARV were speculated to be involved in PI3K signalling. The results

showed that  $\sigma$ A and  $\sigma$ NS-expressing cells had higher P-Akt levels than the pcAGEN-expressing cells and that in the cells expressing other proteins (i.e.  $\mu$ A,  $\mu$ B and  $\mu$ NS), pretreatment with the PI3K inhibitor LY294002 inhibited Akt phosphorylation in  $\sigma$ A- and  $\sigma$ NS-expressing cells.<sup>16</sup> These results indicate that the  $\sigma$ A and  $\sigma$ NS proteins can activate the PI3K/Akt pathway.

Mutant the PXXP motif of NS1 protein from Influenza A virus or mutant the YXXM motif of envelope protein of avian leukosis virus results in loss of PI3K/Akt pathway activation.<sup>15,17</sup> According to amino

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acid sequence analysis of the  $\sigma A$  and  $\sigma NS$  genes, the PXXP and YXXXM motifs are conserved between different ARV strains. The aim of the current study was to determine whether the  $\sigma A$  and  $\sigma NS$  proteins affect the activation of the PI3K/Akt pathway *in vivo* via the PXXP or YXXXM motifs. To accomplish this objective, we mutated the PXXP or YXXXM motifs of  $\sigma A$  and  $\sigma NS$  genes. Plasmid constructs containing mutant  $\sigma A$  and  $\sigma NS$  genes were generated and transfected into Vero cells, and the expression levels of the  $\sigma A$  and  $\sigma NS$  genes were quantified according to immunofluorescence and Western blot analysis. The Akt phosphorylation (P-Akt) profiles of the transfected Vero cells were examined by flow cytometry and Western blot analysis.

## Materials and methods

### Plasmids and primers

The plasmids  $\sigma A$ -pcAGEN and  $\sigma NS$ -pcAGEN were generated by our lab.<sup>16</sup> Nine pairs of primers were designed to mutate the PXXP or YXXXM motifs of the  $\sigma A$  and  $\sigma NS$  genes (Table 1). The primer sequences are presented in Table 1. The red colours represent the mutant bases. The complete  $\sigma A$  gene (1248 bp) was amplified using the primers  $\sigma A$ -F (5'-gatgatctcgaggcaccatggcgcgtgccatatacagac-3') and  $\sigma A$ -R (5'-atcgcggccgttaggcggtaaaagtggctagaac-3'), and the complete  $\sigma NS$  gene (1101 bp) was amplified using the primers  $\sigma NS$ -F (5'-gatgatctcgaggcaccatggacaacaccgtcgtgtt-3') and

$\sigma NS$ -R (5'-atcgcggccgttagccatcctagctggagagac-3'). The underlined text indicates Kozak sequences, and the italicized text represents restriction sites (XhoI and NotI). All primers were synthesized by Takara (Dalian, PR China).

### RNA extraction and RT-PCR amplification of the mutant $\sigma A$ and $\sigma NS$ genes

Genomic RNA was extracted from 200  $\mu$ l of ARV using an EasyPure viral DNA/RNA kit (Transgen, Beijing, PR China) according to the protocol suggested by the manufacturer.

RT-PCR was performed using an RNA LA PCR kit (Takara). Gene splicing by overlap extension PCR was used to mutate  $\sigma A$  and  $\sigma NS$  genes. To amplify the  $\sigma A$ -M1 gene ( $\sigma A$  gene mutant 1), the PCR protocol consisted of three rounds of PCR amplification. The first PCR amplification used  $\sigma A$ -F and  $\sigma A$ -M1-R primers, with  $\sigma A$ -pcAGEN as the template; the second PCR amplification used  $\sigma A$ -M1-F and  $\sigma A$ -R primers, with  $\sigma A$ -pcAGEN as the template. The PCR products of these two amplifications were obtained via gel extraction. The third PCR amplification used  $\sigma A$ -F and  $\sigma A$ -R primers with the gel extraction purification product of the first and second PCR used as the template. The PCR product was then obtained via gel extraction.

We used the same method to amplify the other mutant genes:  $\sigma A$ -M2,  $\sigma A$ -M3,  $\sigma A$ -M4,  $\sigma A$ -M5,  $\sigma A$ -M6,  $\sigma NS$ -M1,  $\sigma NS$ -M2 and  $\sigma NS$ -M3.

**Table 1.** Primer sequences.

Mutant gene	Primer name	Sequence (5'-3')	Amino acids	Amino acid sequence $\rightarrow$ mutated sequence
$\sigma A$ -M1	$\sigma A$ -M1-F	caacggctgaagcagcctatccaggt	55–58	PXXP $\rightarrow$ AXXA
	$\sigma A$ -M1-R	acctggataggctgcttcagccgttg		
$\sigma A$ -M2	$\sigma A$ -M2-F	atgtccaagagtctgcactccac	65–69	YXXXM $\rightarrow$ FXXXXA
	$\sigma A$ -M2-R	gtggagtgcagactcttggaaacat		
$\sigma A$ -M3	$\sigma A$ -M3-F	atgctgccgctgcccgcgtatcagccagc	110–114	PPXXP $\rightarrow$ AAXXA
	$\sigma A$ -M3-R	gctggctgatacgcggcggcaggggagcat		
$\sigma A$ -M4	$\sigma A$ -M4-F	atcctccccctgccgcgctatcaggcagc	114–117	PXXP $\rightarrow$ AXXA
	$\sigma A$ -M4-R	gctgcctgatacgcggcggcaggggagggat		
$\sigma A$ -M5	$\sigma A$ -M5-F	acatggctgtgaggctgatg	200–203	PXXP $\rightarrow$ AXXA
	$\sigma A$ -M5-R	catcagcctcaacagccatgt		
$\sigma A$ -M6	$\sigma A$ -M6-F	atctcgatcagcaggcacgtgct	207–211	YXXXM $\rightarrow$ FXXXXA
	$\sigma A$ -M6-R	agcacgtgcctgctgatgaaat		
$\sigma NS$ -M 1	$\sigma NS$ -M1-F	gctcgagtgggcgtattgact	159–162	PXXP $\rightarrow$ AXXA
	$\sigma NS$ -M1-R	agtcaatagcgcactcgcagc		
$\sigma NS$ -M 2	$\sigma NS$ -M2-F	tcctttcatgcttgacgcagta	179–183	YXXXM $\rightarrow$ FXXXXA
	$\sigma NS$ -M2-R	tactgctcaagcatgaaagga		
$\sigma NS$ -M 3	$\sigma NS$ -M3-F	cggtcgtactcgtcttctt	333–336	PXXP $\rightarrow$ AXXA
	$\sigma NS$ -M3-R	aagaagcaggtacgaccg		

### Recombinant plasmid construction

The mutant  $\sigma A$  and  $\sigma NS$  gene products were cloned into pMD18-T cloning vectors (Takara) according to the manufacturer's instructions. The constructed recombinant plasmids were designated  $\sigma A$ -M1-pMD18T,  $\sigma A$ -M2-pMD18T,  $\sigma A$ -M3-pMD18T,  $\sigma A$ -M4-pMD18T,  $\sigma A$ -M5-pMD18T,  $\sigma A$ -M6-pMD18T,  $\sigma NS$ -M1-pMD18T,  $\sigma NS$ -M2-pMD18T and  $\sigma NS$ -M3-pMD18T. The plasmids were digested with XhoI and NotI enzymes (Takara) and were then ligated into the corresponding sites of pcAGEN expression vectors before being transformed into competent *Escherichia coli* cells (DH5 $\alpha$ ). Positive colonies, which were designated  $\sigma A$ -M1-pcAGEN,  $\sigma A$ -M2-pcAGEN,  $\sigma A$ -M3-pMD18T,  $\sigma A$ -M4-pcAGEN,  $\sigma A$ -M5-pcAGEN,  $\sigma A$ -M6-pcAGEN,  $\sigma NS$ -M1-pcAGEN,  $\sigma NS$ -M2-pcAGEN and  $\sigma NS$ -M3-pcAGEN, were identified by PCR and double digestion and sequenced by Invitrogen (Guangzhou, PR China).

### Expression of $\sigma A$ and $\sigma NS$ proteins

Plasmids (pcAGEN,  $\sigma A$ -pcAGEN,  $\sigma NS$ -pcAGEN,  $\sigma A$ -M1-pcAGEN,  $\sigma A$ -M2-pcAGEN,  $\sigma A$ -M3-pMD18T,  $\sigma A$ -M4-pcAGEN,  $\sigma A$ -M5-pcAGEN,  $\sigma A$ -M6-pcAGEN,  $\sigma NS$ -M1-pcAGEN,  $\sigma NS$ -M2-pcAGEN and  $\sigma NS$ -M3-pcAGEN) were extracted using a plasmid mini kit (Omega Bio-Tek, Norcross, GA). Vero cells were seeded onto 6- or 24-well cell culture plates and were then transfected with 2.5 or 0.5  $\mu$ g of the appropriate expression vectors using Lipofectamine<sup>®</sup> 3000 transfection reagent (Invitrogen) according to the manufacturer's instructions.

### Immunofluorescence and Western blot analysis to quantify protein expression

After the cells were seeded onto 24-well plates and allowed to adhere, Vero cells were transfected with various plasmids for 6 h and were then washed three times with PBS. The cells were then fixed with cold methanol for 10 min at room temperature, washed three times with PBS and blocked for 1 h in 10% normal goat serum (Abcam, Cambridge, UK) in PBS containing 0.5% Triton X-100 (Sigma-Aldrich, St Louis, MO). The cells were then incubated with primary Abs against ARV<sup>18</sup> overnight at 4°C. Following three 5-min washes with PBST, the cells were incubated with fluorescently labelled secondary Abs (Alexa Fluor; Abcam) for 60 min at room temperature.

Vero cells seeded onto six-well plates were harvested, and their cell lysates were used for Western blot analysis to detect  $\sigma A$  and  $\sigma NS$  protein expression, as described by Xie et al.<sup>18</sup> Proteins were visualized using an enhanced chemiluminescence reagent

(Bio-Rad, Hercules, CA) and were detected using a Bio-Rad ChemiDoc MP Imaging System.

### Flow cytometry and Western blot analysis of P-Akt expression

For flow cytometry and Western blot analyses of P-Akt expression, Vero cells seeded onto six-well plates were harvested following transfection with the corresponding plasmids for 6 h. The cells were then subjected to flow cytometry and Western blot analysis to detect P-Akt expression.

For flow cytometry analysis, the transfected cells were detached from the culture plates via incubation with Accutase (Sigma-Aldrich) for 5 min. Then, the cells were washed with PBS and fixed and permeabilized by incubation with Cytofix/Cytoperm solution (BD Biosciences, Franklin Lakes, NJ) at 4°C for 20 min. Next, the cells were washed twice with FACS buffer (0.5% BSA, 0.01% sodium azide in DPBS) and then incubated at 4°C for 30 min with a rabbit Ab (Cell Signaling Technology, Danvers, MA) against P-Akt. After this incubation, the cells were washed twice with FACS buffer and then incubated at 4°C for 30 min with goat anti-rabbit IgG conjugated to Alexa Fluor<sup>®</sup> 488 (Abcam). After staining, the cells were washed twice with FACS buffer and re-suspended in FACS buffer for analysis. The samples were then analysed via flow cytometry (Beckman Coulter, Brea, CA).

Vero cells seeded onto six-well plates were harvested, and their cell lysates were subjected to Western blot analysis to detect P-Akt expression, as described by Wang et al.<sup>19</sup>

All flow cytometry and Western blot analyses were repeated three times.

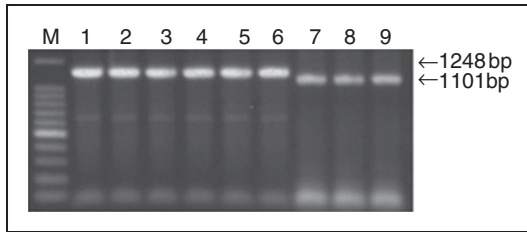
## Results

### Recombinant plasmid construction

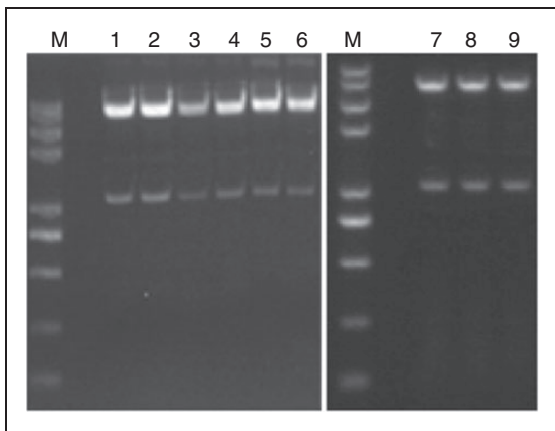
The recombinant plasmids  $\sigma A$ -M1-pcAGEN,  $\sigma A$ -M2-pcAGEN,  $\sigma A$ -M3-pMD18T,  $\sigma A$ -M4-pcAGEN,  $\sigma A$ -M5-pcAGEN,  $\sigma A$ -M6-pcAGEN,  $\sigma NS$ -M1-pcAGEN,  $\sigma NS$ -M2-pcAGEN and  $\sigma NS$ -M3-pcAGEN were first subjected to PCR amplification (Figure 1) and double digestion (Figure 2). Then, DNA sequencing was performed to ensure that the recombinant plasmids contained intact mutant  $\sigma A$  and  $\sigma NS$  genes, validating that the recombinant plasmids were successfully constructed.

### $\sigma A$ and $\sigma NS$ protein expression

As shown by the immunofluorescence and Western blot analyses, the  $\sigma A$  and  $\sigma NS$  proteins were expressed in



**Figure 1.** Identification of the recombinant plasmids by PCR. Lane M: 100-bp DNA ladder; Lane 1:  $\sigma$ A-M1-pcAGEN; Lane 2:  $\sigma$ A-M2-pcAGEN; Lane 3:  $\sigma$ A-M3-pcAGEN; Lane 4:  $\sigma$ A-M4-pcAGEN; Lane 5:  $\sigma$ A-M5-pcAGEN; Lane 6:  $\sigma$ A-M6-pcAGEN; Lane 7:  $\sigma$ NS-M1-pcAGEN; Lane 8:  $\sigma$ NS-M2-pcAGEN; Lane 9:  $\sigma$ NS-M3-pcAGEN.



**Figure 2.** Identification of the recombinant plasmids by double digests. Lane M: Trans2K Plus II DNA Marker; Lane 1:  $\sigma$ A-M1-pcAGEN; Lane 2:  $\sigma$ A-M2-pcAGEN; Lane 3:  $\sigma$ A-M3-pcAGEN; Lane 4:  $\sigma$ A-M4-pcAGEN; Lane 5:  $\sigma$ A-M5-pcAGEN; Lane 6:  $\sigma$ A-M6-pcAGEN; Lane 7:  $\sigma$ NS-M1-pcAGEN; Lane 8:  $\sigma$ NS-M2-pcAGEN; Lane 9:  $\sigma$ NS-M3-pcAGEN.

the Vero cells 6 h after transfection with the corresponding plasmids ( $\sigma$ A-pcAGEN,  $\sigma$ A-M1-pcAGEN,  $\sigma$ A-M2-pcAGEN,  $\sigma$ A-M3-pMD18T,  $\sigma$ A-M4-pcAGEN,  $\sigma$ A-M5-pcAGEN,  $\sigma$ A-M6-pcAGEN,  $\sigma$ NS-pcAGEN,  $\sigma$ NS-M1-pcAGEN,  $\sigma$ NS-M2-pcAGEN and  $\sigma$ NS-M3-pcAGEN; Figures 3 and 4). Cells that were not transfected and transfected with pcAGEN served as negative control cells (Figure 3a and b and Figure 4, lanes 1 and 2).

#### $\sigma$ A and $\sigma$ NS proteins activate the PI3K/Akt signalling pathway

Figure 5 shows that  $\sigma$ A-M3- and  $\sigma$ A-M4- expressing cells had the lowest levels of P-Akt compared to  $\sigma$ A-pcAGEN,  $\sigma$ A-M1-pcAGEN,  $\sigma$ A-M2-pcAGEN,  $\sigma$ A-M5-pcAGEN and  $\sigma$ A-M6-pcAGEN. However, these cells had the same levels of P-Akt as negative control and pcAGEN-expressing cells. Figure 6 shows the same

results as Figure 4. After mutation at amino acids 110–114 (PPXXP→AAXXA) and 114–117 (PXXP→AXXA), the  $\sigma$ A gene lost the capacity to increase P-Akt expression in Vero cells.

The  $\sigma$ NS-M1-,  $\sigma$ NS-M2- and  $\sigma$ NS-M3-expressing cells had similar P-Akt levels compared to  $\sigma$ NS-pcAGEN, but these cells had increased levels of P-Akt expression compared to negative control cells and pcAGEN-expressing cells (data not shown). The mutations at amino acids 159–162 (PXXP→AXXA), 179–183 (YXXXM→FXXXA) and 333–336 (PXXP→AXXA) did not affect the capacity of the  $\sigma$ NS gene to increase P-Akt expression in Vero cells.

#### Discussion

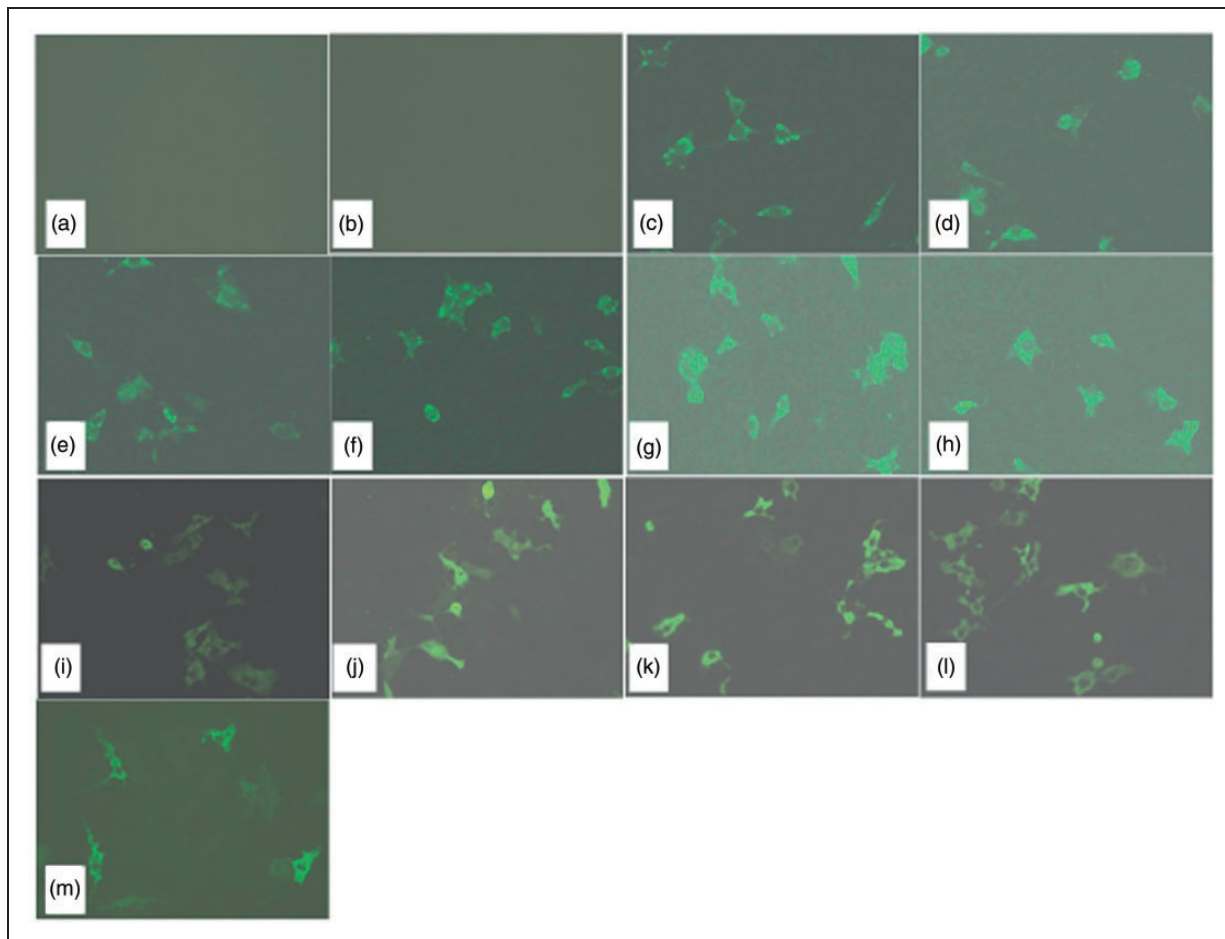
The PI3K/Akt pathway is involved in numerous cellular processes, such as cell proliferation, differentiation and survival.<sup>7,8</sup> PI3K is activated by the binding of autophosphorylated tyrosine kinase receptors or non-receptor tyrosine kinases to the SH2 and SH3 domains of its regulatory/adaptor subunit p85.<sup>19</sup> This binding is mediated by PXXP and YXXXM/YXXM motifs and activates the PI3K/Akt pathway.<sup>9–15</sup> As reported by Shin,<sup>15</sup> the Influenza A virus NS1 protein activates the PI3K/Akt pathway by direct interaction with the p85 subunit of PI3K (SH2-binding motif YXXXM and SH3-binding motif PXXP).

The S2-encoded protein  $\sigma$ A, a component of the inner core shell, displays anti-IFN activity by preventing the activation of the dsRNA-dependent protein kinase PKR; this activity is likely linked to its capacity to bind and sequester dsRNA.<sup>20,21</sup> The non-structural protein  $\sigma$ NS is encoded by the ARV S4 genome segment. As a non-structural RNA-binding protein that accumulates in viral factories of ARV-infected cells,  $\sigma$ NS is a likely candidate for playing key roles in RNA packaging and replication.<sup>22</sup>

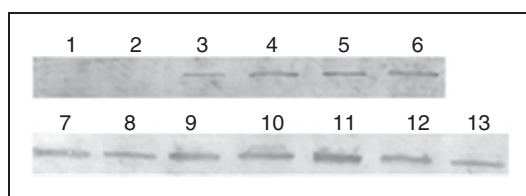
Our previous works showed that the  $\sigma$ A and  $\sigma$ NS proteins of ARV activate the PI3K/Akt pathway.<sup>16</sup> According to amino acid sequence analysis of the  $\sigma$ A and  $\sigma$ NS genes,  $\sigma$ A contains four PXXP and two YXXXM motifs, and  $\sigma$ NS contains one YXXXM and two PXXP motifs. Mutation of the PXXP motif of NS1 protein from influenza A virus or mutation of the YXXM motif of envelope protein from avian leukosis virus will result in loss of PI3K/Akt pathway activation.<sup>15,17</sup> We speculate that the mutant PXXP and YXXXM motifs for the  $\sigma$ A and  $\sigma$ NS genes of ARV may also affect the activation of the PI3K/Akt pathway.

Gene splicing by overlap extension PCR was used to change the PXXP or YXXXM motifs of the  $\sigma$ A and  $\sigma$ NS genes and to construct the mutant  $\sigma$ A and  $\sigma$ NS recombinant plasmids. The mutant  $\sigma$ A and  $\sigma$ NS





**Figure 3.** IFA analysis of the expression of recombinant plasmids in transfected Vero cells. (a) Negative control, (b) pcAGEN, (c)  $\sigma$ A-M1-pcAGEN, (d)  $\sigma$ A-M2-pcAGEN, (e)  $\sigma$ A-M3-pcAGEN, (f)  $\sigma$ A-M4-pcAGEN, (g)  $\sigma$ A-M5-pcAGEN, (h)  $\sigma$ A-M6-pcAGEN, (i)  $\sigma$ A-pcAGEN, (j)  $\sigma$ NS-M1-pcAGEN, (k)  $\sigma$ NS-M2-pcAGEN, (l)  $\sigma$ NS-M3-pcAGEN, (m)  $\sigma$ NS-pcAGEN.



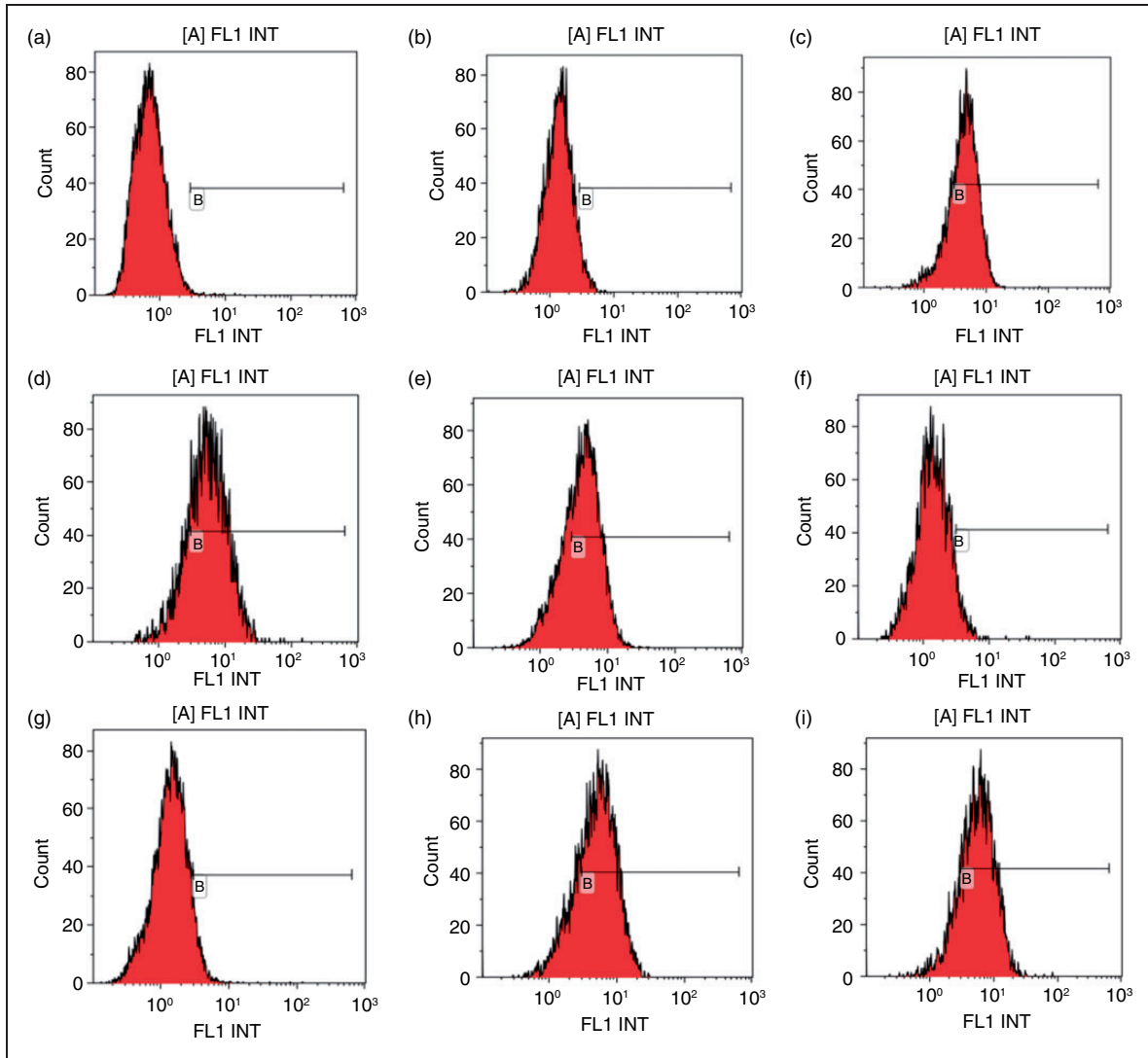
**Figure 4.** Western blot analysis of gene expression. Lane 1: Negative control; Lane 2: pcAGEN; Lane 3:  $\sigma$ NS-M1-pcAGEN; Lane 4:  $\sigma$ NS-M2-pcAGEN; Lane 5:  $\sigma$ NS-M3-pcAGEN; Lane 6:  $\sigma$ NS-pcAGEN; Lane 7:  $\sigma$ A-M1-pcAGEN; Lane 8:  $\sigma$ A-M2-pcAGEN; Lane 9:  $\sigma$ A-M3-pcAGEN; Lane 10:  $\sigma$ A-M4-pcAGEN; Lane 11:  $\sigma$ A-M5-pcAGEN; Lane 12:  $\sigma$ A-M6-pcAGEN; Lane 13:  $\sigma$ A-pcAGEN.

recombinant plasmids were transfected into Vero cells. The P-Akt profile of the transfected Vero cells was examined by flow cytometry and Western blot analysis.

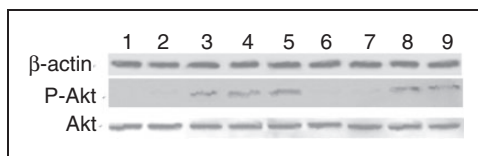
In this study, after amino acids 110–114 (PPXXP→AAXXA) and 114–117 (PXXP→AAXXA) were

mutated, the  $\sigma$ A gene lost its capacity to improve P-Akt expression in Vero cells, and the  $\sigma$ A protein of ARV activated the PI3K/Akt pathway via the PXXP motif. PXXP motif of the  $\sigma$ A protein of ARV is involved in PI3K signalling, and the mutant PXXP motif of  $\sigma$ A affects PI3K/Akt pathway activation. These results are similar to those of a previous study.<sup>16</sup> However, whether this activation was a result of the  $\sigma$ A protein binding to the p85 subunit via interactions with PXXP motifs, as occurs in other viruses,<sup>11,15</sup> requires further study.

We also demonstrated that the mutations at amino acids 159–162 (PXXP→AAXXA), 179–183 (YXXXM→FXXXA) and 333–336 (PXXP→AAXXA) did not affect the capacity of the  $\sigma$ NS gene to increase P-Akt expression in Vero cells. The  $\sigma$ NS protein may activate the PI3K/Akt pathway via more than one motif, other motifs or  $\sigma$ NS interaction with the P85 subunit according to the middle protein. These hypotheses must be further studied.



**Figure 5.** Flow cytometry analysis of P-Akt expression levels. (a) Negative control, (b) pcAGEN, (c)  $\sigma$ A-pcAGEN, (d)  $\sigma$ A-M1-pcAGEN, (e)  $\sigma$ A-M2-pcAGEN, (f)  $\sigma$ A-M3-pcAGEN, (g)  $\sigma$ A-M4-pcAGEN, (h)  $\sigma$ A-M5-pcAGEN, (i)  $\sigma$ A-M6-pcAGEN.



**Figure 6.** Western blot analysis of P-Akt expression levels. Lane 1: Negative control; Lane 2: pcAGEN; Lane 3:  $\sigma$ A-pcAGEN; Lane 4:  $\sigma$ A-M1-pcAGEN; Lane 5:  $\sigma$ A-M2-pcAGEN; Lane 6:  $\sigma$ A-M3-pcAGEN; Lane 7:  $\sigma$ A-M4-pcAGEN; Lane 8:  $\sigma$ A-M5-pcAGEN; Lane 9:  $\sigma$ A-M6-pcAGEN.

Moreover, the relationship between the p110 catalytic subunit of PI3K and the PXXP or YXXXM/YXXM motifs of the  $\sigma$ A/ $\sigma$ NS genes will be further studied, and these research results will be published in the future.

Although the exact mechanisms by which PI3K/Akt regulates ARV replication and other biological functions of PI3K/Akt in virus infection remain uncharacterized, our study reveals the mechanism underlying PI3K/Akt activation and adds a novel aspect to the functions of the  $\sigma$ A and  $\sigma$ NS proteins of ARV.

#### Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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