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ORIGINAL ARTICLE

Isolation, identification and evolution analysis of a novel subgroup of avian leukosis virus isolated from a local Chinese yellow broiler in South China

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Abstract Avian leukosis virus (ALV) causes high mortality associated with tumor formation and decreased fertility, and results in major economic losses in the poultry industry worldwide. Recently, a putative novel ALV subgroup virus named ALV-K was observed in Chinese local chickens. In this study, a novel ALV strain named GD14LZ was isolated from a Chinese local yellow broiler in 2014. The proviral genome was sequenced and phylogenetically analyzed. The replication ability and pathogenicity of this virus were also evaluated. The complete proviral genome sequence of GD14LZ was 7482 nt in length, with a genetic organization typical of replication-competent type C retroviruses lacking viral oncogenes. Sequence analysis showed that the *gag*, *pol* and *gp37* genes of GD14LZ have high sequence similarity to those of other ALV strains (A–E subgroups), especially to those of ALV-E. The *gp85* gene of the GD14LZ isolate showed a low sequence similarity to those other ALV strains (A–E subgroups) but showed high similarity to strains previously described as ALV-K. Phylogenetic analysis of *gp85* also suggested that the GD14LZ isolate was related to ALV-K strains. Further study showed that this isolate replicated more slowly and was less pathogenic than other ALV strains. These results indicate that the GD14LZ isolate belongs to the novel subgroup ALV-K and probably arose by recombination of ALV-K with endogenous viruses with low replication and pathogenicity. This virus might have existed in local Chinese chickens for a long time.

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Introduction

Avian leukosis viruses (ALVs), members of the genus *Alpharetrovirus* of the family *Retroviridae*, are classified into 10 subgroups from A to J based on their host range, virus envelope interference, and cross-neutralization patterns [1]. Members of only six of these subgroups (A–E and J) infect chickens. Subgroup E is an endogenous virus that has little or no pathogenicity, while the other subgroups are exogenous ALVs that induce neoplastic diseases of different pathotypes and other reproduction problems in chickens [2]. Subgroups A, B and J include mainly exogenous ALVs that infect chickens in the field, while subgroups C and D have rarely been reported [3]. In the 1990s, ALV-A, B, C, and D were largely eradicated from the breeding flocks of most international breeding companies [4]. ALV-J was first isolated from commercial meat-

type chickens in 1988 in the United Kingdom but has since spread to other countries, causing enormous economic losses in the poultry industry worldwide [3]. However, in recent years, many international breeding companies have claimed that ALV-J has been eliminated from their breeding flocks (mainly white broilers) by very strict eradication programs [5]. In contrast, to date, programs have rarely, if ever, been implemented for the control of ALV infections on chicken farms in China. During the past decade, ALV-A, ALV-B and ALV-J strains have been reported to infect different flocks, especially the native chickens breeds, which suggests that ALV infections in China are extremely complex [4, 6–8].

Several strains were reported in the native Chinese chicken breed "luhua" in 2012. Comparison of the amino acid (aa) sequence of the gp85 envelope protein with those of all six subgroups known to infect chickens, showed relatively low sequence similarity (77.7–84.6 % aa sequence identity) to the subgroup comprising the ALV-A to E strains, although the lowest similarity was with subgroup J (<40 % aa sequence identity). In contrast, the exogenous ALV strain TW-3593 isolated from indigenous chicken breeds (TCCs) in Taiwan [9] and several fowl glioma-inducing viruses (FGV) reported in Japanese local chickens showed high homology in gp85 (>90 % aa sequence identity). Therefore, it was proposed that these similar strains represent a new subgroup, designated ALV-K [10, 11]. Recently, more and more ALV-K strains have been isolated from Chinese indigenous breed chickens [12].

In this study, we investigated the exogenous ALV strain GD14LZ, which was isolated in South China in 2014 from the plasma samples of local yellow broiler chickens using DF-1 cell culture and ALV p27 antigen detection. Interestingly, comparsions of the nucleotide (nt) and amino acid (aa) sequences of *gp85* with reference ALV strains available in GenBank showed that the GD14LZ strain was closely related to the ALV-K isloates but showed relatively low similarity to members of subgroup A-E and J. Furthermore, the LTR of GD14LZ exhibited high sequence similarity to the endogenous virus strain ev-1, the length of which is only 274 bp and inconsistent with that of an exogenous virus. We also found that this ALV isolate replicated slowly relative to the exogenous virus strains GD08 (ALV-A) and NX0101 (ALV-J) and showed low pathogenicity in infected specific-pathogen-free (SPF) white leghorn chickens. In this study, we determined the complete genome sequence of the novel ALV strain GD14LZ to clarify the evolutionary relationships to other known strains of subgroups A to E and J.

Materials and methods

Virus isolation and identification

To estimate the infection status of ALV, a total 120 of plasma samples were collected from Chinese local yellow broilers in South China in October 2014. Whole blood was collected in sterile 2-ml tubes containing 1 % sodium heparin (Jianyang, Guangzhou, China) and the tubes were inverted several times to aviod clotting. The DF-1 chicken fibroblast cell line was used for ALV culture [13]. DF-1 cells (kept in our laboratory) were grown in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Shanghai, China) supplemented with 10 % fetal bovine serum (FBS; Invitrogen) at 37 °C under 5 % CO_2. For virus isolation, plasma samples were incubated on DF-1 cell monolayers in 24-well culture plates after centrifugation at 6,000×*g* for 10 min; uninfected DF-1 cells were used as a negative control. The culture supernatant containing the virus stocks was harvested 7 days later. After three blind passages of infected cells, the supernatant and cell samples were stored at −80 °C for further investigation. After three freeze-thaw cycles, the supernatant samples in each well (described previously) were examined for ALV group-specific p27 antigen using an Avian Leukosis Virus Antigen Test Kit (IDEXX, Yuanheng Laboratories, Beijing, China) as described previously [14]. For subgroup verification, positively infected DF-1 cells were selected as templates for *env* gene amplification using a primer pair (shown in Table 1)

Table 1 Primers for PCR amplification of GD14LZ genomic proviral DNA

Primer[a]	Sequence (5′-3′)	Fragment size (bp)
Env-F	GGATGAGGTGACTAAGAAAG	2200
Env-R	CCATCAACCCAGGTGCACACCAATG	
A-F	TGTAGTCAAATAGAGCCAGAGG	3450
A-R	ACCAGCTTTTGAACATCCCACA	
B-F	GTATGTAGCACCCGTAGG	2022
B-R	ACAAGACCAGGACACCAAT	
C-F	GGATGAGGTGACTAAGAAAG	2454
C-R	TGAAGCCTTCTGCTTCATTCAG	

[a] F and R represent upstream and downstream primers, respectively

designed to amplify a highly conserved region common to all ALV subgroups.

Genomic DNA amplification and sequencing

For further amplification and to obtain the complete proviral genome for cloning, three pairs of overlapping primers were designed based on the sequences of TW-3593 and JS11C1 vailable in GenBank (shown in Table 1). Polymerase chain reaction (PCR) tests were carried out with genomic DNA extracted from infected DF-1 cell as a template. PCR amplification of sequences was performed using Premix LA Taq polymerse (TaKaRa, Dalian, China) in a 50-μl PCR volume containing 5 μl of total template, 25 μl of 2× Premix LA Tap buffer (TaKaRa) and 1 μM forward and reverse primers. The thermocycling profiles for PCR amplification included an initial denaturation for 5 min at 95 °C, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 3 min) for 30 cycles, and a final prolonged extension at 72 °C for 10 min. The PCR products were separated by 1 % agarose gel electrophoresis, purified using an Omega Gel Extraction kit (Omega Bio-tek) and cloned into the PMD-19T vector. The resulting construct was then used to transform *Escherichia coli* DH5α cells (TaRaKa). The DNA from positive clones was sequenced directly (GENEWIZ, Suzhou, China), and each fragment was sequenced three times independently.

Sequence aligenment and analysis

The full-length proviral genome sequence of the GD14LZ isolate was assembled using DNAStar version 7.0, and multiple sequence alignment was performed with Clustal X (BioEdit version 7.0). The transcriptional regulatory elements in the non-coding regions of the genome were analyzed using Softberry (Softberry, Mount Kisco, NY, USA). Phylogenetic analysis was carried out using a Clustal W alignment and the neighbor-joining method with 1,000 bootstrap replicates using MEGA version 5.0. Nucleotide and deduced amino acid sequence similarity searches were performed using the National Center for Biotechnology Information BLAST (Basic Local Alignment Search Tool) program at GenBank. The sequences obtained in this study have been submitted to GenBank (accession number KU605774). The ALV reference strains (with origin and accession numbers) available in GenBank that were used in this study are shown in Table 2.

Replication of the GD14LZ isolate in DF-1 cells

The titers of the GD14LZ strain are presented as $TCID_{50}$ ml^{-1} and were measured using the Reed-Muench method by ELISA. DF-1 cells were plated (approximately 10^6 cells) in each 60-mm dish 1 day before infection with 0.1 ml of GD14LZ virus at a concentration of approximately $10^{3.8}$ $TCID_{50}$ ml^{-1}. An exogenous ALV subgroup

Table 2 Avian leukosis virus strains used in this study

No.	Subgroup	Isolate	Origin	Acession no.	No.	Subgroup	Isolate	Origin	Accession no.
1	A	RSA-A	France	M37980	19	J	HPRS-103	UK	Z46390
2	A	MQNCSU	USA	DQ365814	20	J	ADOL-7501	USA	AY027920
3	A	RAV-1	USA	M19113	21	J	GD1109	China	JX254901
4	A	A46	USA	DQ412726	22	J	SD07LK1	China	FJ216405
5	A	B53	USA	DQ412727	23	J	NHH	China	HM235668
6	A	MAV-1	USA	L10922	24	J	NX0101	China	AY897227
7	A	SDAU09C1	China	HM452339	25	J	HN0001	China	AY897219
8	A	SDAU09C3	China	HM452340	26	FGV	FGV	Japan	AB112960
9	A	SDAU09E1	China	HM452341	27	FGV(variant)	Oki-009	Japan	AB669433
10	B	RSV-Schmidt-RuppinB	USA	AF052428	28	FGV(variant)	Sp-40	Japan	AB617819
11	B	RSV-2	USA	M14902	29	FGV(variant)	Km-5892	Japan	AB682778
12	C	RSV-PragueC	USA	J02342	30	Proposed new subgroup/K	GDFX0601	China	KP686142
13	D	RSV-Schmidt-RuppinD	USA	D10652	31	Proposed new subgroup/K	GDFX0602	China	KP686143
14	E	ev-1	USA	AY013303	32	Proposed new subgroup/K	GDFX0603	China	KP686144
15	E	ev-3	USA	AY013304	33	Proposed new subgroup/K	TW3593	China	HM582658
16	E	RAV-0	USA	M12172	34	Proposed new subgroup/K	JS11C1	China	KF746200
17	E	ALVE-B11	Canada	KC610517					
18	E	SD0501	China	EF467236	35	Proposed new subgroup/K	GD14LZ	China	KU605774

Isolates 1 to 34 represent different subgroups of avian leukosis virus strains available in GenBank; Isolate 35 is the strain identified in this study

A strain (strain GD08, kindly provided by Professor Weusheng Cao at South China Agricultural University, P. R. China) and a subgroup J strain (strain NX0101, kindly provided by Professor Zhizhong Cui at ShanDong Agricultural University, P. R. China) were used as controls. The infections were carried out in the presence of 1 % FBS at 37 °C under 5 % CO_2 and harvested (approximately 400 μl/plate) on days 1, 2, 3, 4, 5, 6 and 7 postinfection. The supernatant was replaced with an equal volume of DMEM after each collection. After three freeze-thaw cycles, the harvested samples were examined for ALV group-specific p27 antigen (ELISA) to determine the replication kinetics. Each sample was tested independently three times.

Animal experiment

Ten specific-pathogen-free (SPF) white leghorn chicks (aged 1 day) were inoculated intraperitoneally [15] with approximately 0.3 ml of virus strain GD14LZ at a concentration of $10^{3.8}$ $TCID_{50}$ ml^{-1}. Another 10 chicks were injected with 0.3 ml of uninfected cell culture supernatant as a negative control. All chicks were re-inoculated at 5 days of age [15]. Two groups of chicks were housed alone in two negative-pressure isolators and provided with food and water *ad libitum*. To monitor the infection status (viremia level) of the chicks, whole blood samples were collected at 8, 9, 10, and 11 weeks postinfection. Total RNA was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). The chickens were then euthanized and monitored for gross or microscopic tumors at 11 weeks postinfection.

Statistical analysis

The significance of the differences between the trials was analyzed by Student's *t*-test using GraphPad Prism (version5.0) software (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Virus isolation and identification of exogenous ALVs

No visible morphological changes or cytopathic effects (CPE) were observed in infected DF-1 cells, indicating that infection with cytopathic subgroup B, C and D ALVs could be excluded [16]. ELISA analysis of plasma samples from 120 chickens revealed several that tested positive for group-specific antigen p27, while uninfected DF-1 cells tested negative. DF-1 cells are resistant to endogenous subgroup ALV-E viruses [17]; therefore, these results demonstrated that the isolates belonged to the exogenous, non-subgroup E viruses. Comparisons of the *env* sequences of different ALV subgroup reference strains showed low nucleotide sequence similarity to exogenous ALVs from subgroups A, B, C, D and J (Table 2).

Sequence analysis of the complete proviral genome of GD14LZ

To further clarify the subgroup of the exogenous ALV, the complete proviral genome of the GD14LZ isolate was amplified using three pairs of overlapping primers (shown in Table 1). The full-length proviral genome of ALV isolate GD14LZ was 7,482 bp longwith a genetic organization typical of replication-competent type C retroviruses lacking viral oncogenes (5′-LTR -leader-*gag-pol-env*- 3′-LTR). The sequence of the ALV isolate GD14LZ has been submitted to GenBank (accession number KU605774). A schematic diagram of the genome structure of GD14LZ and comparsion with other ALV strains from GenBank are shown in Figure 1. In a comparison with reference ALVs from GenBank, the complete genome of GD14LZ was most closely related to TW-3593, GDFX0601, GDFX0602, and GDFX0603 (95.9–99 % identity) and also showed at least 95.9 % identity to ALV-E (with the exception of the *gp85* gene, which exhibited only 78.8 % identity). The *gag*, *pol* and *gp37* gene sequences of the GD14LZ strain were well conserved, sharing over 92.6 % nt and aa sequence identity with the reference ALVs, with the exception of the *gp37* gene of ALV-J, which showed only 11.9 nt and 36.4 % aa identity (Fig. 1). The 3′ UTR of this isolate exhibited high sequence similarity (>98.9 %) to ALV-E, TW-3593, GDFX0601, GDFX0602, and GDFX0603, but showed relatively low similarity to ALV-J, and JS11C1 (< 43.6 %). Also, the 3′ UTR of the virus isolate had the E (XSR) element deletion, but the E(XSR) could be found in certain strains of RSV and ALV-J [18]. The 5′-leader sequence of the GD14LZ isolate included three ATG codons and short open reading frames, which were also conserved in all ALVs.

The LTR region of this virus isolate was only 274 bp in length, which is consistent with the length of LTRs of endgenous viruses, but not with those of exogenous viruses. Furthermore, the sequence of this region in the GD14LZ isolate showed only 39.4 %, 43.2 %, 34.3 %, 37.7 % and 36.9 % overall nucleotide sequence identity with subgroups ALV- A, B, C, D and J, respectively. In contrast, the LTR of the GD14LZ strain shared the highest identity (98.5 %), and was most closely related to ALV-E (Fig. 1). Within the LTR of the GD14LZ isolate, the U3 region (only 175 bp) also showed low identity to ALV-A, ALV-B, ALV-C and ALV-J (data not shown). In contrast, the U3 sequence was most similar to the U3 region of *ev-1*

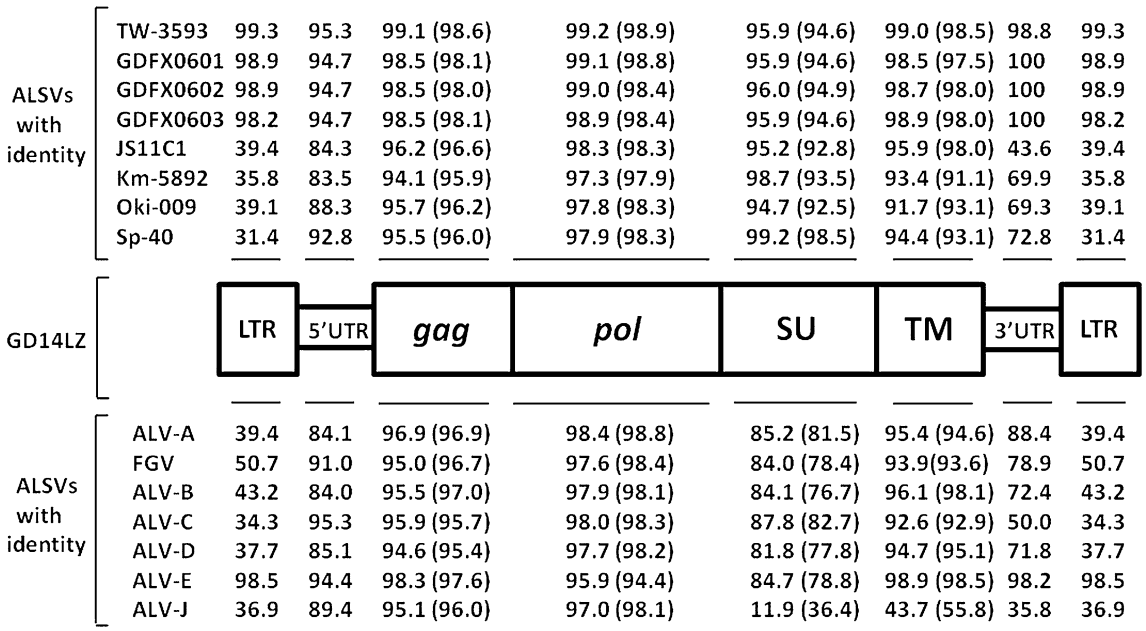

Fig. 1 Comparison of the proviral genome sequence of the GD14LZ isolate the with those of other avian leukosis sarcoma viruses. Boxes, the genomic structure of the ALV GD14LZ isolate; lines above, viral sequences from the putative new subgroup of ALVs showing similarity to those regions of the GD14LZ strain (percentage identity); lines below, viral sequences from all known subgroups of ALVs, ALV-A (RSA), ALV-B (RSV-SR-B), ALV-C (RSV-C), ALV-D (RSV-SR-D), ALV-E (ev-1), ALV-J (HPRS-103) and FGV prototype showing the percentage nucleotide (outside the brackets) and amino acid (inside the brackets) sequence identity in the corresponding regions of the GD14LZ strain

loci, with only two single nucleotide substitutions corresponding to nonsense mutations at positon 87 (G to A) and at position 118 (T to G) (Fig. 2). The transcriptional regulatory elements of the GD14LZ strain identified in the U3 region were similar to *ev*-1, including only the first of two CArG boxes ($CC(A/T)_6GG$), which are considered a characteristic of endogenous virus LTRs in general [19, 20]. In contrast, pentanucleotide repeat element (PRE) boxes (GGTGG) were absent. However, the Y box (AATTG), TATA box (TATT/ATAA) and polyadenylation signal (AATAAA), which are well conserved in exogenous [21, 22] and endogenous viruses, were also present in the GD14LZ isolate. The CCAAT enhancer box, which is usually present within the first 20 bp of the U3 region in exogenous viruses, was not present in *ev*-1 or GD14LZ. However, like *ev* LTRs, the U3 region of GD14LZ contained the CCAAT-like motif. Although the location of these sites was different (position 105 to 113; TGACGCAAG), the consensus motif 5′-T(T/G)NNG(C/T)AA(T/G), which constitutes a functional enhancer box, was identical (Fig. 2) [21, 23, 24].

The GD14LZ *gp85* gene showed less than 87.8 % nt and 82.7 % aa sequence identity to the reference strains of subgroups A to E and only 11.8 % nt and 36.4 % aa to ALV-J, indicating that the GD14LZ *gp85* gene is unique among the ALV subgroups (Fig. 1). The GD14LZ *gp85* gene shared the greatest identity (>95 %) with JS11C1, TW-3593 and FGV variants sp-40, Km-5892 and Oki-009. Phylogenetic analysis of the gp85 amino acid sequences revealed that GD14LZ belonged to a single clade with JS11C1, TW-3593, GDFX0601, GDFX0602, GDFX0603, Sp-40, Oki-009 and Km-5892 and showed a distant phylogenetic relationship to other existing ALV subgroup reference strains (Fig. 3).

Growth kinetics in DF-1 cells and pathogenicity in SPF chickens of the GD14LZ strain

The influence of the E-like LTRs on GD14LZ replication was evaluated *in vitro* using DF-1 cells, which are commonly used as host cells for ALV proliferation. DF-1 cells were infected with GD08, NX0101 and GD14LZ. As shown in Figure 4, culture supernatants of cells infected with the GD08 (ALV-A) and NX0101 (ALV-J) strains had higher viral titers from day 2 to day 7 postinfection than those infected with GD14LZ. These results showed that the GD14LZ strain replicated more slowly than the GD08 (ALV-A) and NX0101 (ALV-J) strains in DF-1 cells. Furthermore, to evaluate pathogenicity *in vivo*, one-day-old SPF chicks were inoculated intraperitoneally with the GD14LZ strain. The infection status of infected chicks was determined by RT-PCR of RNA extracted from whole blood samples at 8, 9, 10, 11 weeks postinfection. As expected, most of the infected chickens were viremic by

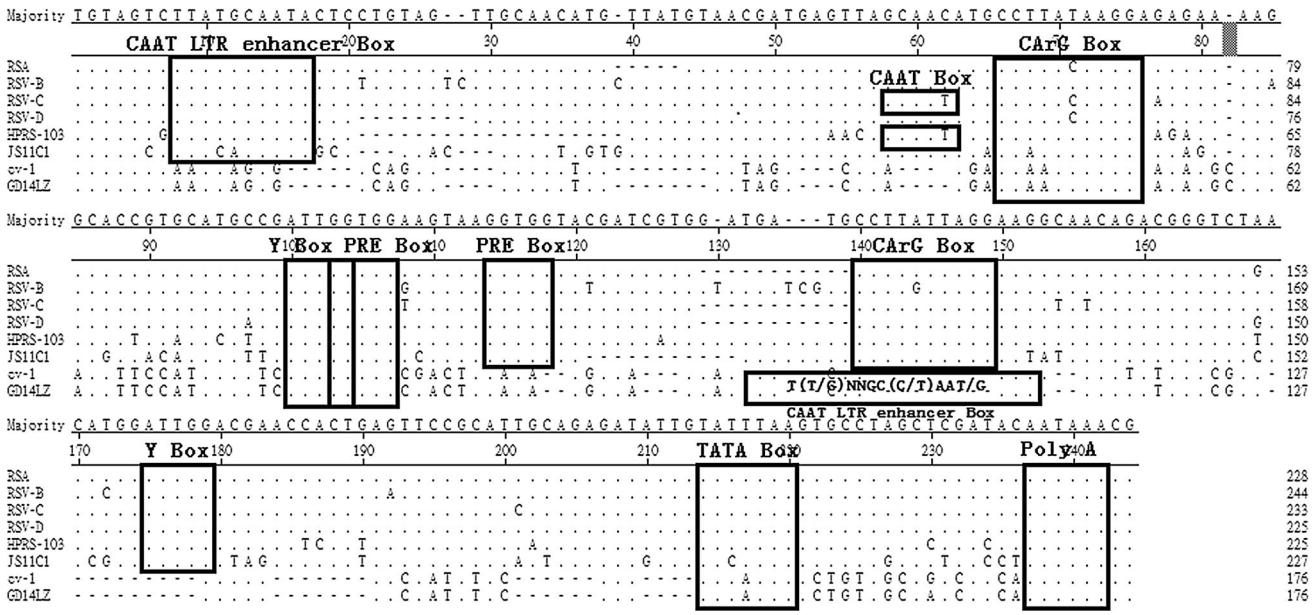

Fig. 2 Comparsion of the U3 region of the LTRs of the GD14LZ isolate and other ALVs, Dots indicate identical nucleotides, and nucleotides that differ are identified. Dashes (-) indicated gaps in the alignment. The locations of putative transcription regulatory elements are indicated in boxes and labeled

the time the study was terminated, and all were negative at 9 weeks postinfection. In addition, no significant lesions or tumors were observed when chickens were euthanized at 11 weeks postinfection. Negative control chickens had no infection or tumors.

Discussion

The GD14LZ strain was isolated from a Chinese local yellow broiler in South China in 2014. The complete genome of GD14LZ strain was most closely related to TW-3593, GDFX0601, GDFX0602, and GDFX0603 (95.9 % to 99 %). The LTRs, *gag, pol,* and *gp37* of GD14LZ showed high sequence similarity to endogenous ALVs (ALV-E). However, *gp85* of the GD14LZ strain showed low similarity to members of other subgroups of ALV. Phylogenetic analysis of the amino acid sequence of the *gp85* gene revealed that GD14LZ clustered with JS11C1, TW-3593, GDFX0601, GDFX0602, GDFX0603, Sp-40, Oki-009 and Km-5892, indicating a distant phylogenetic relationship to other existing ALV-subgroup reference strains (Fig. 3). As the ALV subgroups are determined based on the GP85 envelope protein [25–27], we propose that the GD14LZ isolate might be classified as a member of a new subgroup of ALVs (named ALV-K). Of course, this will need further confirmation. Viral envelope interference and cross-neutralization patterns will be investigated.

Fowl glioma is histopathologically characterized by multiple nodular astrocytic growths with disseminated non-suppurative encephalitis [28, 29]. This disease is caused by fowl glioma-inducing virus (FGV), which belongs to subgroup A of avian leukosis virus (ALV-A). FGV infections are common in Japanese fowl flocks (Chabo bantam) [30–32]. Based on the amino acid sequences of gp85, several FGV variants (Oki-009, Sp-40 and Km-5892) are present in the same clade as the GD14LZ isolate, while the FGV prototype is clustered in the other clade (Fig. 3). The FGV variants (Oki-009, Sp-40 and Km-5892) might be recombined with different FGV subgroups or derived from different origins, as described previously [32].

Due to the low sequence similarity (mainly in the LTRs 31.4 %-50.7 % and 3′-UTR 43.6 %-78.9 %) between GD14LZ, FGV prototype, FGV variants, and the JS11C1 strain (Fig. 1), it can be ruled out that the GD14LZ isolate is an FGV variant. Like the TW-3593, GDFX0601, GDFX0602 and GDFX0603 strains, the GD14LZ strain contains E-like LTRs of only 274 bp. Furthermore, all of the putative regulatory elements in the ALV-E strains were present in the GD14LZ strain, such as the LTRs of all endogenous viruses, the CCAAT enhancer box, one CArG box and one PRE box (Fig. 2). Moreover, the Y box, TATA box and the polyadenylation signal were conserved. The low or absent oncogenicity of ALV-E is believed to depend on the weak enhancer properties of the LTRs of the viral genome [1]. The CCAAT/enhancer elements possess enhancer activity, and deletions in this region decrease LTR promoter function by 20- to 200-fold [33, 34]. A previous study has suggested that LTRs from endogenous viruses may be responsible for the lower viral transcription

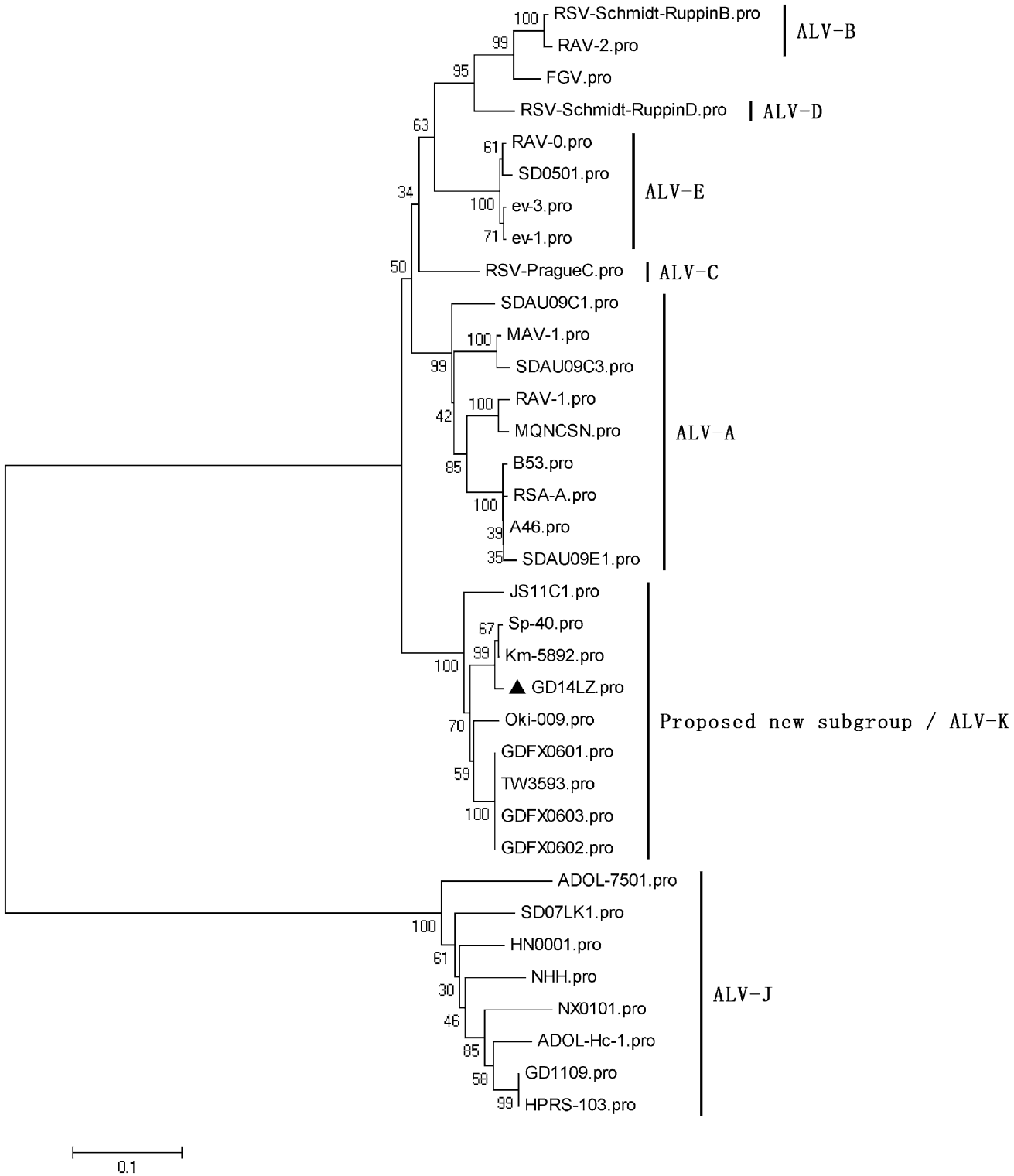

Fig. 3 Phylogenetic tree of gp85 glycoprotiens. Sequences from GenBank were aligned with the GD14LZ isolates (labeled "▲") using Clustal W. The phylogenetic tree was prepared using MEGA version 5.0

rate [22]. In addition, the backbones of three recombinant ALV-E viruses (PDRC-1039, PDRC-3246 and PDRC-3249) isolated from a contaminated Marek's disease vaccine, contained a part of the envelope gene of ALV-A without tumor-inducing ability in white-leghorn-type chickens [35]. Thus, we evaluated the replication ability

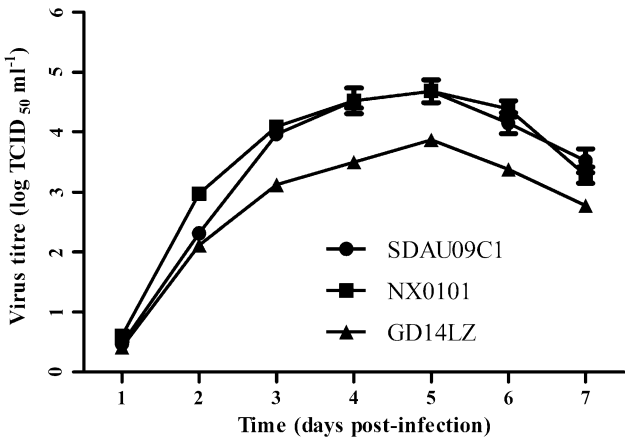

Fig. 4 Replication of the GD14LZ, ALV-A (GD08) and ALV-J (NX0101) strains in DF-1 cells. Growth curves were generated by determining viral titers at different intervals and expressing them as $TCID_{50}$ ml^{-1}. Data represent the mean ± SD of three independent experiments. Student's *t*-test revealed significant differences among the three viruses

and pathogenicity of the GD14LZ strain. As expected, our results showed that GD14LZ replicates more slowly in DF-1 cells than the GD08 (ALV-A) and NX0101 (ALV-J) strains and does not induce tumor formation in SPF chickens. All of these characteristics are consistent with those of recombinant viruses with E-like LTRs.

Interestingly, all the ALV-K strains were isolated from the same regions of East Asia (Japan, the Chinese mainland and Taiwan) [11]. The Japanese Chabo bantam originated from Thailand, Vietnam, Taiwan and China and was introduced through trade in the seventeenth to nineteenth centuries [36]. It is possible that the putative new subgroup ALVs isolated from these places share a common ancestor. The TW-3593-like ALVs identified in TCC flocks in Taiwan [9] are considered to be unique to this region. More interestingly, the GDFX0601, GDFX0602, and GDFX0603 strains, which are similar to the GD14LZ strain, were also isolated from local yellow chickens in South China. All of these data indicated that the unique ALV strains TW-3593, GD14LZ, GDFX0601, GDFX0602 and GDFX0603 share the same ancester. Taken together, these results suggest that the GD14LZ-like ALVs may have existed in Chinese local chicken flocks for a long time. We propose that the presence of E-like LTRs and the eradication programs implemented in local Chinese chicken flocks could explain why these viruses were not reported until recently.

In summary, the GD14LZ isolate has an endogenous-virus backbone, and contains a *gp85* gene from ALV-K. The isolate represents a new subgroup of ALV viruses that do not induce tumors in SPF chickens and replicate at a relatively slow rate in DF-1 cells. Molecular characterization and analysis of the evolution of the complete GD14LZ genome suggest that this isolate probably arose by recombination of ALV-K with endogenous viruses and belongs to a new subgroup (ALV-K) that has been present in Chinese local chickens for a long time. The findings of the present study will benefit ALV eradication programs in Chinese local chickens.

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Compliance with ethical standards

Competing interests The authors declare that have no competing interests.

Ethics statement The animal experiment was carried out in accordance with the institutional and national guidelines for the use and care of laboratory animals. Use of animals in this study was approved by the South China Agricultural University Committee of Animal Experiments (approval ID: 201004152).

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