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Original Article

Analysis of respiratory syncytial virus fusion protein from clinical isolates of Korean children in palivizumab era, 2009–2015☆

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ABSTRACT

Introduction: Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infections among infants and young children. The fusion (F) protein of RSV is a major target for monoclonal antibodies and vaccine candidates. We analyzed sequence polymorphisms of the RSV F protein and investigated palivizumab-resistance mutation in clinical isolates from Korean children in post-palivizumab era.

Methods: A review of pediatric patients with RSV infections in Korea from September 2009 to April 2015 was conducted. We performed RSV *F* gene sequence analysis on positive clinical samples and compared to reference sequences, A2 and 9320.

Results: RSV *F* gene data were obtained from 60 patients (30 RSV-A and 30 RSV-B), of whom 15 (10 RSV-A and 5 RSV-B) received palivizumab. The nucleotide and amino acid identities of the *F* gene sequence were conserved between RSV isolates and reference strains. There was no significant difference between isolates from patients who received and did not receive palivizumab. One or more amino acid changes were observed in all RSV-A and 26 RSV-B isolates. Twenty-five variations in RSV-A and 17 in RSV-B were noted. One variation within antigenic site II was noted in a RSV-A isolate; D263N with unknown significance was found in a patient without palivizumab prophylaxis. N276S variation adjacent to antigenic site II was observed in 27 RSV-A isolates. However, no known palivizumab-resistant mutations were found in either RSV-A or RSV-B isolates.

Conclusions: The RSV *F* gene was highly conserved and no known palivizumab-resistant mutants were found in Korean circulating strains.

1. Introduction

Respiratory syncytial virus (RSV) is the most important respiratory pathogen of infancy and early childhood, and a major cause of hospitalization for acute lower respiratory tract infections (ALRI) globally [1]. In the recent study, it was estimated that globally 33.1 million episodes of RSV-associated ALRI occurred in children younger than 5 years in 2015. RSV was associated with about 28% of all ALRI episodes and 13–22% of all ALRI mortality in young children [2]. Currently, there is no effective treatment for RSV-associated ALRI and only palivizumab is used for prophylaxis in high risk patients. However, several RSV vaccines and therapeutic agents are currently being developed and investigated in clinical trials [1,3,4].

RSV encodes 11 proteins. The attachment glycoprotein (G) and the fusion glycoprotein (F) are the two major surface glycoproteins, the most important epitopes for neutralization. RSV F protein mediates RSV binding to cellular receptors, allowing entry into host cells. RSV F protein is required for infection and can initiate infection in the absence of G protein [5]. RSV F protein is the preferred target for vaccines and antiviral agents [1,4].

Palivizumab is a humanized monoclonal antibody binding to antigenic site II of RSV F protein [5]. It is currently approved in more than 83 countries since 1998 [3,6]. RSV F protein is known as highly

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conserved. However, RNA viruses are highly mutable, and are highly adaptive to selective pressure. RSV cultured with palivizumab developed *F* gene mutations, and was resistant to palivizumab prophylaxis in cotton rats [7–9]. Palivizumab resistant RSV strains have also been found in clinical isolates [10–12]. In Korea, palivizumab was introduced in 2004. It was approved by Korean public health insurance in 2006 and formally used in high-risk preterm infants since then [13].

Limited information regarding the molecular epidemiology of the RSV *F* gene and palivizumab-resistant RSV is available in Korea [14]. The objective of this study was to conduct molecular analysis of RSV *F* genes of clinical isolates collected from 2009 to 2015 and investigate whether palivizumab resistant RSV mutant isolates were circulating among Korean children with or without receiving palivizumab.

2. Methods and materials

2.1. Patients and clinical samples

Pediatric patients diagnosed with RSV infection at Samsung Medical Center, Seoul, Korea from September 2009 to April 2015 were identified. RSV detection was performed by multiplex PCR assay (Seeplex® RV7 Detection Kit, Seegene, Seoul, Korea). Stored RSV positive nasopharyngeal aspirate specimens that were retained after RSV diagnosis were used for RSV *F* gene sequence analysis.

Clinical data including demographic characteristics, underlying diseases, palivizumab prophylaxis and management of RSV infection were collected for patients with obtainable RSV *F* gene sequence data. This study was approved by the Institutional Review Board of Samsung Medical Center.

2.2. Molecular analysis of the F gene

The reference strains RSV-A A2 and RSV-B 9320 (GenBank accession numbers, M74568.1 and AY353550, respectively) were used as positive controls. RSV RNA was isolated from nasopharyngeal aspirate specimens. The extracted viral RNA was reverse-transcribed into cDNA. The RSV *F* gene was then amplified as described previously [12,15]. Sequence analyses for RSV *F* gene were performed and results were compared with reference sequences. The detailed experimental methods are described in the supplementary material (Supplementary methods).

2.3. Phylogenetic analysis

Phylogenetic analysis of RSV *F* gene sequences was performed with reference strains (A2 and 9320), and analyzed together with 36 *F* gene sequences identified from GenBank which were identified during our study period (2009–2015) (Supplementary Table 1). The phylogenetic trees were constructed using the neighbor-joining method and the topologic accuracy of the tree was evaluated by using 2000 bootstrap replicates. Nucleotide and amino acid identities were estimated by calculating pairwise distances (p-distance) with MEGA6 software using the maximum-composite likelihood and Poisson correction models, respectively. Multiple sequence alignment was performed using Clustal W in MEGA6 and BioEdit (version 7.2.5). The rates of synonymous (dS) and non-synonymous substitutions (dN) were evaluated by using the Nei-Gojobori model in MEGA6. The number of nucleotide differences per sequence between reference strains and *F* gene sequences of RSV isolates was identified.

2.4. Identification of palivizumab-resistant RSV isolates

Polymorphism was defined as variation from amino acid change that appeared most frequently at that position in reference sequences [15]. Palivizumab-resistant RSV was defined when known *F* gene mutation with confirmed resistant phenotype to palivizumab was detected [12,15]. Sequence variant was defined as a known polymorphism unrelated to palivizumab resistance [15]. To identify mutants or sequence variants, F protein sequences of RSV isolates were compared to reference sequences RSV-A2 and RSV 9320. Polymorphisms at other neutralizing epitopes of RSV F protein as well as palivizumab binding site (antigenic site II, the position of amino acid 258-275) were also assessed for clinical RSV isolates.

3. Results

3.1. Patient characteristics and RSV isolates

During the study period, RSV-A infections were identified more frequently. However, RSV-B infection was predominant in 2010–2011 and 2013–2014 seasons. Sixty RSV positive nasopharyngeal samples were available for genotypic analysis (30 RSV-A and 30 RSV-B). During 2011–2012 season, only RSV-A isolates ($n = 14$) were analyzed. During 2013–2014 season, 19 of 20 analyzed isolates were RSV-B. Most of analyzed RSV isolates were detected during the epidemic period in Korea (Supplementary figures).

Patient characteristics are summarized in Table 1. Forty (66.7%) patients had underlying diseases. RSV associated mortality was not observed in patients included in this study.

3.2. Molecular characteristics of F gene in RSV isolates

Of 60 RSV isolates available for *F* gene sequencing, 55 (25 RSV-A and 30 RSV-B) were used for phylogenetic analysis and molecular characterization. Five RSV-A isolates were excluded from phylogenetic analysis due to limited *F* gene sequence data. In RSV-A isolates, a 1500-bp region (positions 151-1650 in *F* gene of A2) was determined and 22 unduplicated sequences were obtained. In RSV-B isolates, a 1446-bp region (positions 151-1596 in *F* gene of 9320) was determined and 26 unduplicated sequences were obtained. Comparing sequences of 48 distinct RSV isolates, RSV *F* gene (positions 151-1596) was conserved, sharing 77.7% nucleotide and 88.6% amino acid sequence identities. RSV *F* gene phylogenetic trees are shown in Fig. 1. In 22 RSV-A isolates, the nucleotide p-distance range for A2 was 0.0470–0.0544 (mean 0.0505; median 0.0507). The range of nucleotide p-distance for 9320 was 0.0000–0.0386 (mean 0.0250, median 0.0321) in RSV-B isolates.

Nucleotide and amino acid identities (mean ± SD, %) of *F* gene sequences between RSV isolates and reference strains were as follows: 95.12 ± 0.14 and 97.28 ± 0.16, respectively, for RSV-A; 97.61 ± 1.67 and 98.96 ± 0.69, respectively, for RSV-B. RSV isolates from patients who received palivizumab and those who did not receive palivizumab showed no significant difference in nucleotide or amino acid identities.

The median number of RSV *F* gene synonymous substitutions per site between A2 and RSV-A isolates was 60 (4.00%) and that of non-synonymous substitutions was 13 (0.87%). Compared to 9320, median numbers of synonymous and non-synonymous substitutions per site in RSV-B isolates were 43 (2.97%) and 5 (0.35%), respectively. The F protein of RSV-A and RSV-B isolates was well conserved (dN/dS ratio < 1).

Table 1
Characteristics of study patients.

	Total	RSV-A	RSV-B
No. of cases	60	30	30
Sex, male (%)	39 (65.0)	21 (70.0)	18 (62.1)
Age, median, month (IQR)	11.8 (4.0–24.3)	7.7 (3.6–20.4)	15.6 (4.9–43.1)
Underlying diseases (%)[a]			
None	20 (33.3)	10 (33.3)	10 (33.3)
Prematurity	15 (25.0)	12 (40.0)	3 (10.0)
Cardiovascular disease	7 (11.7)	3 (10.0)	4 (13.3)
Hematologic-oncologic disease	12 (20.0)	2 (6.7)	10 (33.3)
Others	7 (11.7)	3 (10.0)	4 (13.3)
Palivizumab recipient (%)	15 (25)	10 (33.3)	5 (16.7)
RSV infection (%)			
Upper respiratory tract infection	15 (25.0)	3 (10.0)	12 (40.0)
Lower respiratory tract infection	45 (75.0)	27 (90.0)	18 (60.0)
Management of RSV infection (%)			
Need for oxygen therapy	18 (30.0)	11 (36.7)	7 (23.3)
Need for ICU care	9 (15.0)	5 (16.7)	4 (13.3)
Need for mechanical ventilation	6 (10.0)	4 (13.3)	2 (6.7)

IQR, interquartile range; ICU, intensive care unit.
[a] One patient had both prematurity and severe pulmonary hypertension.

3.3. Amino acid changes in RSV F protein

One or more F protein amino acid changes were observed in all (100%) RSV-A isolates and 26 (86.7%) RSV-B isolates. Twenty-five polymorphisms were identified in F protein of RSV-A isolates, of which 23 had unknown significance (Table 2). In RSV-B isolates, 17 polymorphisms were observed, of which three sequence variants were known to be palivizumab sensitive (Table 3). However, known palivizumab resistant F protein mutations were not found in RSV-A or RSV-B isolates.

RSV F protein polymorphisms observed here were also frequently found in RSV isolates identified from other studies during the same period. F protein sequence data of these isolates (n = 37; 20 RSV-A and 17 RSV-B) were compared to reference strains. No known palivizumab resistant mutants were found. Among these 20 RSV-A isolates, N105S and N276S, known palivizumab sensitive variants, were observed frequently.

3.4. Polymorphisms in neutralizing epitopes of RSV F gene

One polymorphism within antigenic site II was noted only in RSV-A F protein. D263N with unknown significance was noted in a RSV-A isolate obtained from a patient who did not receive

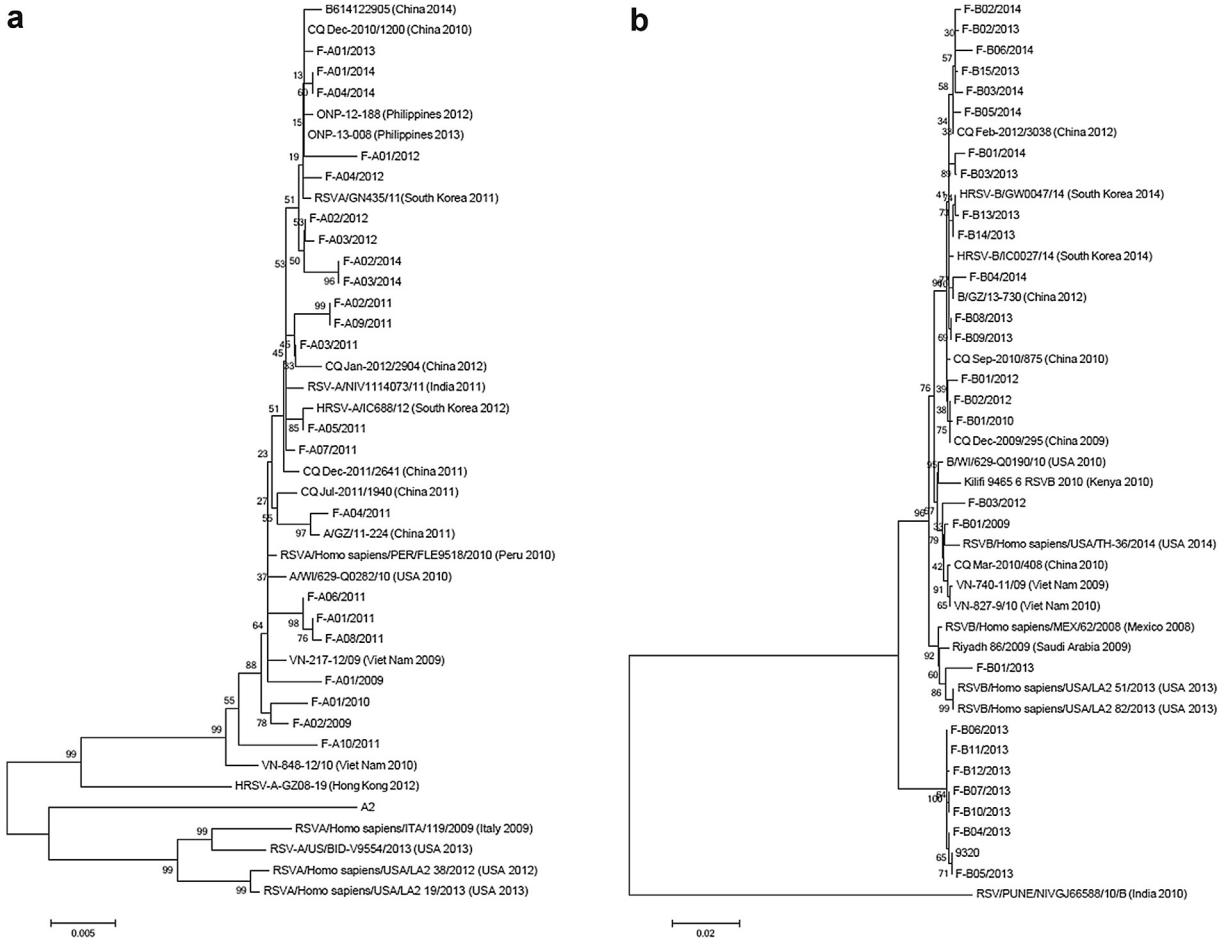


Fig. 1. Phylogenetic analysis of RSV F gene sequences. Distinct RSV F gene sequences in this study are denominated as F-(subtype A or B)(number of isolate)/(isolated season). The evolutionary history was inferred using the Neighbor-Joining method. In Fig. 1(a), the optimal tree with the sum of branch length = 0.14649023 is shown. In Fig. 1(b), the optimal tree with the sum of branch length = 0.29195279 is shown. The percentage of replicate trees in which associated taxa clustered together in the bootstrap test (2000 replicates) is shown next to branches.

Table 2
Sequence variation of F protein in RSV-A isolates.

	Region[a]	Amino acid changes in A2	Frequency in RSV-A isolates (Total N = 30)[b] (%)	Frequency in other study isolates (Total N = 20)[c] (%)
Known palivizumab sensitive	F2	N105S	29 (96.7)	15 (75.0)
	F1	N276S	27 (90.0)	14 (70.0)
Unknown significance	SP	F20L	1 (3.3)	20 (100.0)
	F2	G25S	1 (3.3)	20 (100.0)
		P102A	30 (100.0)	20 (100.0)
		T103A	30 (100.0)	20 (100.0)
	p27	Y117H	1 (3.3)	1 (5.0)
		T118I	1 (3.3)	0 (0.0)
		L119P	1 (3.3)	0 (0.0)
		A122T	30 (100.0)	20 (100.0)
		K124N	25 (83.3)	20 (100.0)
		T125N	1 (3.3)	4 (20.0)
		L129S	1 (3.3)	0 (0.0)
	F1	V139G	30 (100.0)	20 (100.0)
		V152I	30 (100.0)	19 (95.0)
		H159Q	1 (3.3)	0 (0.0)
		L178V	30 (100.0)	20 (100.0)
		D263N	1 (3.3)	0 (0.0)
		I379V	27 (90.0)	20 (100.0)
		V384I	25 (83.3)	18 (90.0)
		P389S	2 (6.7)	0 (0.0)
		M447V	24 (80.0)	20 (100.0)
		I537V	1 (3.3)	0 (0.0)
		S540A	24 (80.0)	14 (70.0)
		L541S	1 (3.3)	0 (3.3)

[a] Region in F protein. Schematic diagram of RSV F protein and neutralizing epitope sites were shown in Fig. 2.
[b] RSV-A isolates in this study.
[c] RSV-A isolates selected from GenBank. See Supplementary Table 1.

palivizumab. N276S variation adjacent to antigenic site II was observed in 27 RSV-A isolates (Fig. 2). N276S is a known palivizumab susceptible variant. The frequency of N276S was 100% in most all seasons except the 2011–2012 season (73.0%).

Table 3
Sequence variation of F protein in RSV-B isolates.

	Region[a]	Amino acid changes in 9320	Frequency in RSV-B isolates (Total N = 30)[b] (%)	Frequency in other study isolates (Total N = 17)[c] (%)
Known palivizumab sensitive	F2	F45L	3 (10.0)	12 (70.6)
	p27	A103V	17 (56.7)	6 (35.3)
	F1	Q209K	7 (23.3)	2 (11.8)
Unknown significance	F2	K65E	1 (3.3)	0 (0.0)
		T97M	22 (73.3)	17 (100.0)
	p27	L125P	1 (3.3)	3 (17.6)
		V127A	2 (6.7)	0 (0.0)
	F1	L172Q	20 (66.7)	6 (35.3)
		S173L	1 (3.3)	1 (5.9)
		S197N	26 (86.7)	17 (100.0)
		N201S	5 (16.7)	0 (0.0)
		S215F	1 (3.3)	0 (0.0)
		I291V	1 (3.3)	0 (0.0)
		D310E	1 (3.3)	0 (0.0)
		T324I	1 (3.3)	0 (0.0)
		D510N	1 (3.3)	0 (0.0)
		T529A	20 (66.7)	13 (76.5)

[a] Region in F protein. Schematic diagram of RSV F protein and neutralizing epitope sites were shown in Fig. 2.
[b] RSV-B isolates in this study.
[c] RSV-B isolates selected from GenBank. See Supplementary Table 1.

K65E, N201S and Q209K were found at site Ø in RSV-B F protein sequences. An amino acid change at 389 (antigenic site I) was observed in two RSV-A isolates (P389S) and one RSV-B strain (S389P) (Fig. 2).

4. Discussion

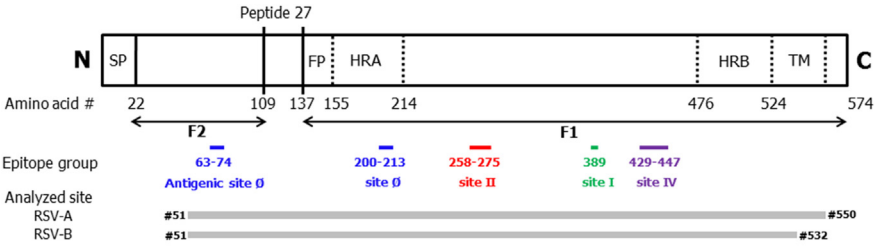
We evaluated the molecular epidemiology of RSV *F* gene and the prevalence of palivizumab resistance-conferring mutations in RSV F protein among circulating RSV isolates in Korea from 2009 to 2015. Results of the current study showed that RSV F protein was highly conserved. Any known palivizumab-resistant F protein mutations were not found in either RSV-A or RSV-B isolates. To the best of our knowledge, this is the first Korean study to investigate palivizumab resistant mutations and sequence polymorphisms of RSV F protein in post-palivizumab era.

Of 21 RSV treatments developed in the past decade, 4 of 10 vaccines and 8 of 11 therapeutic agents in active clinical trials have targeted the F protein [3,4]. Therefore, comprehensive information on the molecular epidemiology and evolutionary dynamics of RSV F protein is essential.

The highest sequence divergence between RSV-A and RSV-B is found in the G protein, on which most molecular epidemiology and genetic diversity studies have focused [16–19]. Multiple genotypes within two subgroups have been identified, but there are limited studies regarding the antigenic variation and the evolutionary patterns of RSV F protein genes [14,17,20–26]. Overall, the RSV F protein gene is considered to be highly conserved. In this study, analyzed RSV F gene sequence identity was 95.1% in RSV-A and 97.6% in RSV-B at the nucleotide level. Kim et al. [14] reported that the nucleotide identity of the RSV *F* gene was 93.5–98.7% in RSV-A and 96.5–99.2% in RSV-B during 9 consecutive seasons from 1990 to 1999 in Korea (pre-palivizumab era). This finding is similar in other countries. In Canada, the mean nucleotide identity of the F gene within a subgroup was 97% in RSV-A and 98% in RSV-B during 2006–2010 [20]. Kimura et al. studied the time-scaled, molecular evolution of the *F* gene in RSV- A [25] and RSV-B [26] using sequences from GenBank. A total of 246 sequences of RSV-A strains collected globally from 1956 to 2014 were analyzed, RSV-A showed four clusters during past 70 years [25]. In RSV-B, a total of 86 sequences from 1956 to 2013 were analyzed and RSV-B showed three clusters for 60 years [26]. However, the genetic similarity was very high and low genetic divergence was observed in both RSV-A and RSV-B strains.

Palivizumab was approved by the US in 1998 and by the European Medicines Agency in 1999. In 2004, a palivizumab resistant virus (amino acid change K272M) was selected by passage of RSV in the presence of palivizumab in cell culture, and variable resistance to palivizumab was shown in cotton rats by RSV mutants (K272M/Q, N268I) [7,8]. Palivizumab-resistant RSV (N276S, K272E) was isolated from an infant treated with palivizumab in 2010 [11]. However, Zhu et al. demonstrated that N276S in RSV-A did not confer any resistance to palivizumab by neutralization test [15].

The incidence of palivizumab-resistant RSV is variable. Four sequence changes (K272E, K272Q, S276F, and S275L) were identified in the *F* gene of RSV isolates from 93 palivizumab prophylaxis recipients. Resistance to palivizumab was confirmed by microneutralization assay, and calculated amino acid mutation frequency was 5.4% [12]. Resistance-conferring mutations within the palivizumab binding site occurred in 8.7% (2/23) of palivizumab recipients, and none of 100 non-palivizumab recipients, during 2006–2010 in Canada [20]. In a Spanish single-center analysis, one (1.9%) palivizumab resistant RSV mutant, isolated from a non-palivizumab recipient, was found during molecular characterization of F protein epitope A from 52 randomly selected specimens



RSV-A	F20L	G25S P102A T103A N105S	Y117H T118I L119P A122T K124N L129S	V139G V152I H159Q L178V	D263N	N276S	I379V V384I P389S (I) M447V (IV)	I537V S540V L541S
RSV-B		F45L K65E T97M A103V	L125P V127A	L172Q S173L S197N N201S (Ø) Q209K (Ø) S215F			I291V D310E T324I D510N	T529A

Fig. 2. Amino acid changes in different regions on the F protein of RSV isolates. At top panel of the figure, the schematic structure of RSV F protein and neutralizing epitopes are shown [27,28]. The table below showed amino acid changes in different regions in F protein of analyzed RSV isolates including important neutralizing epitopes. SP, single peptide; FP, fusion peptide; HRA, heptad-repeat A; HRB, heptad-repeat B; TM, transmembrane.

during 2013–2014 [22]. In our study, no known palivizumab resistant F protein mutations were found in either RSV-A or RSV-B isolates from 60 randomly-selected pediatric patients, including 15 palivizumab recipients, during the 2009–2014 RSV seasons.

However, several amino acid sequence variations were found in this study: 25 in RSV-A and 17 in RSV-B. Zhu et al. performed sequence analysis of 254 clinical RSV isolates from palivizumab-naïve patients during 2003–2008 [15]. Forty-six polymorphic sequence variations were observed in 39 positions at RSV-A while 34 polymorphic sequence variations were observed in 32 positions at RSV-B. Among these, N262D and S275F variations in the RSV-A F gene were resistant to neutralization at the highest palivizumab concentration tested. The most variable region reported in the F protein was p27, followed by the F2 subunit [14,21]. In this study, amino acid changes in the RSV F protein were frequently observed at p27 in RSV-A, and at F1 in RSV-B (Fig. 2). Frequently observed sequence variations in our study were also observed with high frequencies in reference strains isolated during 2008–2014 available from GenBank. Among these, N276S in RSV-A, sequence variation at adjacent to the palivizumab binding site, was observed with high frequency in both our clinical isolates (90.0%) and reference strains (70.0%). In a Canadian study, N276S sequence variant emerged at a rate of 44.4% in 2008–2009 and became the predominant RSV-A clade during 2009–2010 in palivizumab recipients and non-recipients (100%) [20]. A Japanese study analyzed RSV strains isolated from 2004 to 2013 showed that N276S sequence variant in RSV-A began to appear around 2007–2008 and was found in more than 90% after 2009 [24]. The N276S sequence variant has not been reported in RSV isolates before the use of palivizumab, the predominance of N276S may be related to some selective pressure attributable to palivizumab [21,24]. However, the frequency of N276S in RSV F protein was also increased in China during 2010–2012. It became prevalent at that time, although palivizumab was not introduced in China until then [21].

Our study has several limitations. First, because of sample availability, only limited numbers of clinical samples were included in the study. Second, a phenotypic assay was not performed to determine the palivizumab resistance of the observed sequence variations. However, sequence variants with unknown significance in our study were found in both palivizumab recipients and non-recipients, and also frequently observed in references strains.

5. Conclusions

Our study provides useful information on the current molecular epidemiology of the RSV F gene in Korea after the introduction of palivizumab. RSV-A isolates appeared to exhibit more genetic diversity than RSV-B isolates, but overall the RSV F protein was highly conserved. No known palivizumab resistant F protein mutations were found in RSV isolates, but several F protein sequence variants were observed. Genetic diversity of the RSV F protein may affect novel RSV vaccines and therapeutics in development. Therefore, a continued monitoring of the molecular evolution of RSV F gene is needed.

Conflicts of interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jiac.2019.02.013.

References

[1] Griffiths C, Drews SJ, Marchant DJ. Respiratory syncytial virus: infection, detection, and new options for prevention and treatment. Clin Microbiol Rev 2017;30:277–319.
[2] Shi T, McAllister DA, O'Brien KL, Simoes EAF, Madhi SA, Gessner BD, et al. Global, regional and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. Lancet 2017;390:946–58.
[3] Mazur NI, Martinon-Torres F, Baraldi E, Fauroux B, Greenough A, Heikkinen T, et al. Lower respiratory tract infection caused by respiratory syncytial virus: current management and new therapeutics. Lancet Respir Med 2015;3:888–900.
[4] Papadopoulos NG, Megremis S, Kitsioulis NA, Vangelatou O, West P, Xepapadaki P, et al. Promising approaches for the treatment and prevention of viral respiratory illnesses. J Allergy Clin Immunol 2017;140:921–32.
[5] McLellan JS. Neutralizing epitopes on the respiratory syncytial virus fusion glycoprotein. Curr Opin Virol 2015;11:70–5.
[6] Research and Markets Synagis (Palivizumab). Available at: http://www.researchandmarkets.com/research/sr7fkk/synagis [accessed 11 May 2016].
[7] Zhao X, Chen FP, Megaw AG, Sullender WM. Variable resistance to palivizumab in cotton rats by respiratory syncytial virus mutants. J Infect Dis 2004;190:1941–6.

[8] Zhao X, Chen FP, Sullender WM. Respiratory syncytial virus escape mutant derived in vitro resists palivizumab prophylaxis in cotton rats. Virology 2004;318:608–12.
[9] Zhao X, Sullender WM. In vivo selection of respiratory syncytial viruses resistant to palivizumab. J Virol 2005;79:3962–8.
[10] DeVincenzo JP, Hall CB, Kimberlin DW, Sánchez PJ, Rodriguez WJ, Jantausch BA, et al. Surveillance of clinical isolates of respiratory syncytial virus for palivizumab (Synagis)-resistant mutants. J Infect Dis 2004;190:975–8.
[11] Adams O, Bonzel L, Kovacevic A, Mayatepek E, Hoehn T, Vogel M, et al. Palivizumab-resistant human respiratory syncytial virus infection in infancy. Clin Infect Dis 2010;51:185–8.
[12] Zhu Q, McAuliffe JM, Patel NK, Palmer-Hill FJ, Yang CF, Liang B, et al. Analysis of respiratory syncytial virus preclinical and clinical variants resistant to neutralization by monoclonal antibodies palivizumab and/or motavizumab. J Infect Dis 2011;203:674–82.
[13] The Health Insurance Review and Assessment Service, Korea. Available at: http://www.hira.or.kr/co/search.do?searchWord=palivizumab [accessed 15 January 2018].
[14] Kim YK, Choi EH, Lee HJ. Genetic variability of the fusion protein and circulation patterns of genotypes of the respiratory syncytial virus. J Med Virol 2007;79:820–8.
[15] Zhu Q, Patel NK, McAuliffe JM, Zhu W, Wachter L, McCarthy MP, et al. Natural polymorphisms and resistance-associated mutations in the fusion protein of respiratory syncytial virus (RSV): effects on RSV susceptibility to palivizumab. J Infect Dis 2012;205:635–8.
[16] Qin X, Zhang C, Zhao Y, Zhao X. Genetic variability of subgroup A and B respiratory syncytial virus strains circulating in southwestern China from 2009 to 2011. Arch Virol 2013;158:1487–95.
[17] Tapia LI, Shaw CA, Aideyan LO, Jewell AM, Dawson BC, Haq TR, et al. Gene sequence variability of the three surface proteins of human respiratory syncytial virus (HRSV) in Texas. PLoS One 2014;9. e90786.
[18] Do LA, Wilm A, Van Doorn HR, Lam HM, Sim S, Sukumaran R, et al. Direct whole-genome deep-sequencing of human respiratory syncytial virus A and B from Vietnamese children identifies distinct patterns of inter- and intra-host evolution. J Gen Virol 2015;96:3470–83.
[19] Otieno JR, Agoti CN, Gitahi CW, Bett A, Ngama M, Medley GF, et al. Molecular evolutionary dynamics of respiratory syncytial virus group A in recurrent epidemics in coastal Kenya. J Virol 2016;90:4990–5002.
[20] Papenburg J, Carbonneau J, Hamelin ME, Isabel S, Bouhy X, Ohoumanne N, et al. Molecular evolution of respiratory syncytial virus fusion gene, Canada, 2006-2010. Emerg Infect Dis 2012;18:120–4.
[21] Xia Q, Zhou L, Peng C, Hao R, Ni K, Zang N, et al. Detection of respiratory syncytial virus fusion protein variants between 2009 and 2012 in China. Arch Virol 2014;159:1089–98.
[22] Gimferrer L, Campins M, Codina MG, Martín Mdel C, Fuentes F, Esperalba J, et al. Molecular epidemiology and molecular characterization of respiratory syncytial viruses at a tertiary care university hospital in Catalonia (Spain) during the 2013-2014 season. J Clin Virol 2015;66:27–32.
[23] Oliveira DB, Iwane MK, Prill MM, Weinberg GA, Williams JV, Griffin MR, et al. Molecular characterization of respiratory syncytial viruses infecting children reported to have received palivizumab immunoprophylaxis. J Clin Virol 2015;65:26–31.
[24] Hashimoto K, Hosoya M. Neutralizing epitopes of RSV and palivizumab resistance in Japan. Fukushima J Med Sci 2017;19:127–34.
[25] Kimura H, Nagasawa K, Tsukagoshi H, Matsushima Y, Fujita K, Yoshida LM, et al. Molecular evolution of the fusion protein gene in human respiratory syncytial virus subgroup A. Infect Genet Evol 2016;43:398–406.
[26] Kimura H, Nagasawa K, Kimura R, Tsukagoshi H, Matsushima Y, Fujita K, et al. Molecular evolution of the fusion protein (F) gene in human respiratory syncytial virus subgroup B. Infect Genet Evol 2017;52:1–9.
[27] McCarthy M, Villafana T, Stillma E, Esser MT. Respiratory syncytial virus protein structure, function and implications for subunit vaccine development. Future Virol 2014;9:753–67.
[28] Sun Z, Pan Y, Jiang S, Lu L. Respiratory syncytial virus entry inhibitors targeting the F protein. Viruses 2013;5:211–25.