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PHYLOGENETIC ANALYSIS OF NEURAMINIDASE GENE OF INFLUENZA A(H3N2) VIRUSES ISOLATED IN UKRAINE IN 2013–2014 SEASON

Influenza remains a serious problem for the world. The accumulation of genetic changes (mutations) in influenza viruses leads to changes in their antigenic properties, the emergence of strains resistant to antiviral drugs and the emergence of new epidemics. The ***aim*** *of recent study was to perform the phylogenetic analysis of neuraminidase gene of influenza A(H3N2) viruses isolated in Ukraine in 2013–2014 season.* ***Methods.*** *Molecular genetics, phylogenetic and statistical methods were used for this study.* ***Results.*** *High (94%) genetic similarity of Ukrainian isolates and isolates from other countries of 2013–2014 epidemic seasons demonstrates the stability of viral populations in Ukraine. The allocation of Ukrainian viruses to three different clusters points to different ways of their spread to Ukraine. The analysis of NA sequences revealed novel amino acid substitutions Y155F, D251V, S315G to the majority of Ukrainian isolates. None of virus isolates of season 2013–2014 in Ukraine contained an E119D mutation in NA sequence which is associated with oseltamivir resistance.* ***Conclusions.*** *The results of our work showed that influenza viruses isolated in 2013–2014 epidemic season had not specific mutations, associated with resistance to antiviral drugs such as oseltamivir.*

Key words: neuraminidase, mutation, isolate, phylogenetic analysis.

Influenza viruses are the main cause of respiratory diseases in humans. Despite significant scientific achievements of recent decades influenza becomes a serious problem for the entire world. The most dangerous phenomenon is the emergence of complications and chronic disease exacerbation after carried flu. The influenza virus surface proteins hemagglutinin and neuraminidase are high variable, that's why virus could omit the host immune system [1]. Variability of influenza viruses is caused by the changes in gene sequences that occur due to mutations. In turn, the accumulation of genetic changes in viruses leads to changes in their antigenic properties [2].

The investigation of genetic changes among all influenza strains is necessary to reveal that some RNA segments of A (H3N2) influenza virus are originated from

viruses affecting different hosts. The phylogenetic analysis applied to new influenza isolates allows to monitoring the rate and direction of virus variations practically in real time. Furthermore, the comparative analysis of their protein sequences allows to reveal point amino acid substitution providing the mechanism of virus adaptation to human immune system. Sequences of surface antigens – hemagglutinin (HA) and neuraminidase (NA) – are usually used for genetic analyses. Aminoacid substitution E119D of NA gene leads to development of resistance to antiviral drug oseltamivir (Tamiflu). Therefore, a comparative phylogenetic analysis of NA sequences of A (H3N2) influenza viruses circulating in Ukraine during epidemic season 2013–2014 was the aim of the study.

Materials and methods

Nasal-throat washes taken from influenza-affected individuals from the different regions of Ukraine during outbreak were used in the study. In total, 673 samples were analyzed using real-time polymerase chain reaction (RT-PCR) in 2013–2014 seasons. Preparation of reaction mixture for PCR was made according to recommended protocol "WHO. CDC protocol of realtime RT-PCR for swine influenza A(H1N1) revision 1" [3].

Sequencing of influenza viruses A (H3N2) genes, isolated in our laboratory, was performed in the World Influenza Center in London using the technology of RNA-SEQ, which allows sequencing coding and noncoding mRNA (http://www.crick.ac.uk/research/science-technology-platforms/advanced-sequencing/).

Neuraminidase (NA) gene sequences of influenza isolates A/Ukraine/77/2014 , (EPI_ISL_163129), A/Ukraine/218/2014 (EPI_ISL_162152), A/Ukraine/710/2013 (EPI_ISL_154035), A/Ukraine/728/2013 (EPI_ISL_154036), A/Dnipro/227/2014 (EPI_ISL_162105), A/Dnipro/229/2014 (EPI_ISL_162160), A/Dnipro/232/2014 (EPI_ISL_162107), A/Dnipro/234/2014 (EPI_ISL_162108), A/Dnipro/235/2014 (EPI_ISL_162109), A/Kharkov/201/2014 (EPI_ISL_163099), A/Kharkov/203/2014 (EPI_ISL_162113), A/Zhitomir/286/2014 (EPI_ISL_162157), A/Zhitomir/290/2014 (EPI_ISL_162159); A/Ukraine/6004/2013 (EPI_ISL_154034) and A/Ukraine/6161/2014 (EPI_ISL_163128), which were received from Ukrainian surveillance system; vaccine strain A/Texas/50/2012 (EPI_ISL_170149), reference strains A/Perth/16/2009 (EPI_ISL_87516), A/Victoria/361/2011 (EPI_ISL_158723), A/Stockholm/18/2011 (EPI_ISL_93712), A/Hong Kong/146/2013 (EPI_ISL_176514) and A/Samara/73/2013 (EPI_ISL_143568) and isolates from different countries were selected to perform phylogenetic comparisons. Phylogenetic analysis was performed using MEGA 5 software [4].

Sequences of influenza viruses from other countries were received from Genbank (http://www.ncbi.nlm.nih.gov/Genbank) and GISAID resource (http://platform.gisaid.org/), using BLAST (Basic Local Alignment Search Tool) analysis (http://www.ncbi.nlm.nih.gov/ Blast.cgi). Sequences were aligned using ClustalW algorithm. Phylogenetic trees were built by the nearest neighbor joining method [5] applying Kimura 2-parameter model [6]. Evolutional distances were calculated in terms of the number of base substitutions per site. A bootstrap technique with 1000

replications was used to test statistical validity of received data [7]. Nucleotide sequences were translated into amino acid sequences using MEGA 5 software [4].

Findings

In this study we compared nucleotide sequences encoding surface antigens of influenza viruses NA proteins because they are highly prone to mutation-related changes. The high similarity of HA and NA genes (94%) was observed for viruses isolated on the different continents already at the stage of BLAST system-assisted search and was confirmed by data obtained by other authors [8, 9]. Taking into account the considerable number (exceeding 1000) of available sequences of influenza viruses, the geographic location and date of material sampling from a sick person were chosen as the main criteria for search and selection.

The comparison of NA genes of influenza isolates demonstrated the high genetic similarity equal to 94%.

All influenza viruses A(H3N2) isolated during 2013–2014 outbreak season were found to be allocated to the subcluster 3C of the Victoria/208 cluster and retained mutation S367N and K369T, which attributed to cluster 3, and also contained mutations L81P and N402D (deletion/loss of glycosylation site [10]), which referred to subcluster 3C.

Phylogenetic data are presented in Fig. 1.

All discovered isolates from this season were genetically related to vaccine strain A/Texas/50/2012. According to the certain differences the isolates were divided into 3 genetic groups. Groups 1 and 2 are characterized by common substitution D93G (asparadic acid is replaced by glycine) for isolates from epidemic season 2012–2013. Also substitution E221D (glutamic acid is replaced by asparadic acid) has selected in this groups, compared with the previous season isolates.

Group 1 – viruses with substitution I392T. This group contained isolates from Kharkov – A/Kharkov/203/2014, which were the closest to isolate from Norway and were selected substitution V396I (valine is replaced by isoleucine), and A/Dnipro/234/2014 with substitution L338S (leucine is replaced by serine). Dnepropetrovsk's isolate was the most related to strains from Turkey and France (fig. 1).

Group 2 – contained viruses with mutation V412I (valine is replaced by isoleucine). Four Ukrainian isolates belong to this group. Strain A/Dnipro/227/2014 acquired substitution Q5K (glutamine is replaced by lysine). A/Moscow/137/2013 was the most similar to our isolate, both selected mutation S332F (serine is replaced by phenylalanine). Other three isolates A/Ukraine/710/2013, A/Ukraine/728/2013 i A/Dnipro/235/2014 carried mutations S44P and P45S. Those strains probably were introduced to the country in result of one entry during epidemic season. Strain A/Ukraine/728/2013 also confer mutation G40D (glycine is replaced by asparagines acid), and A/Dnipro/235/2014 – K75R (lysine is replaced by arginine). In general, this group was more numerous and contained isolates from the European region only.

Group 3 – is the largest group. Mainly Ukrainian isolates from different districts belonged to this group. Sequences of this group were more similar to reference strain A/Athens GR/112/2012 than to the newest reference viruses. Mutations

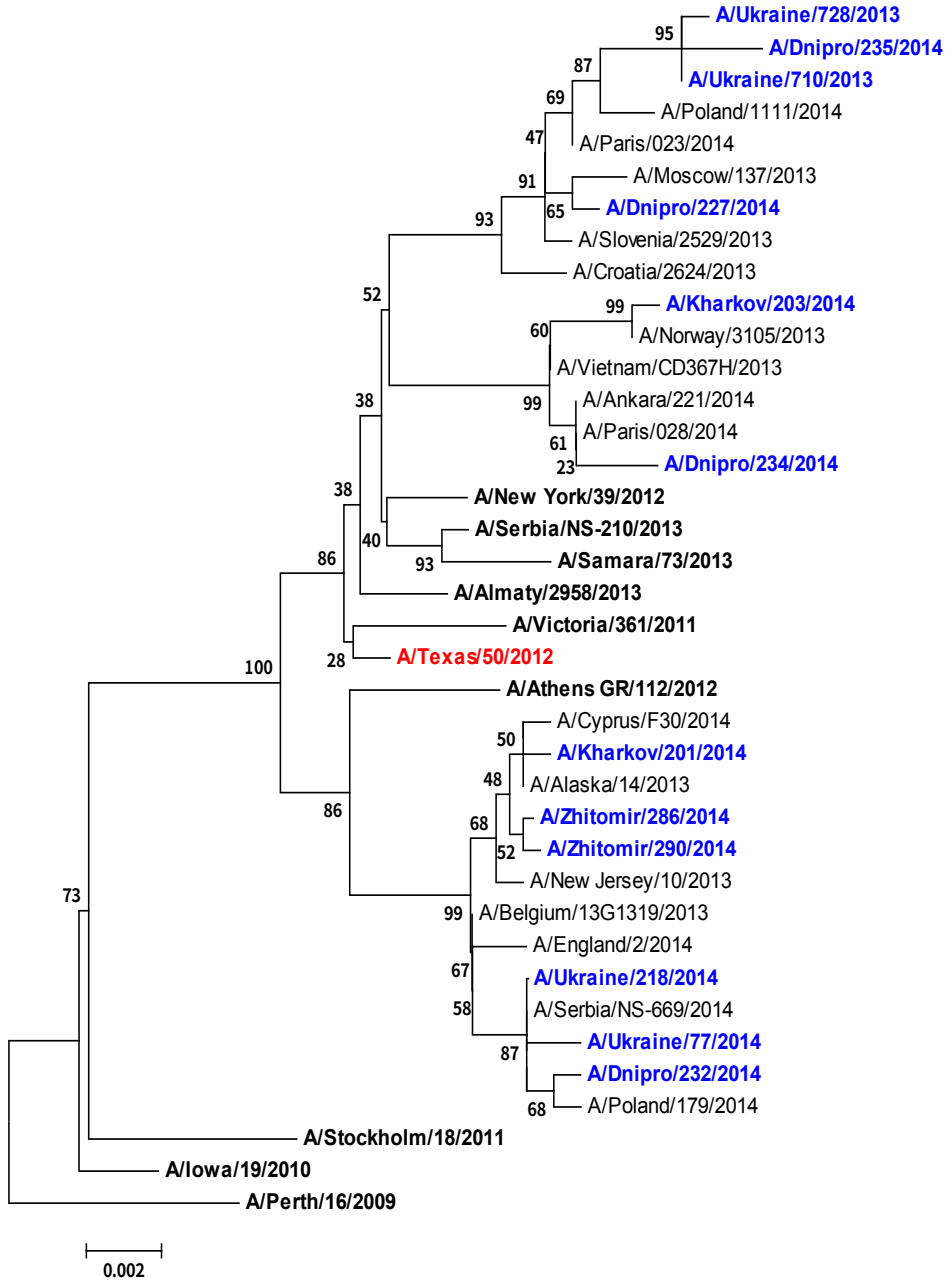

Fig. 1. Molecular phylogenetic analysis of NA nucleotide sequences for influenza viruses A(H3N2), NJ method Kimura 2-parameter model

Y155F, D251V and S315G were new. The third group was quite heterogeneous and contained strain from Alaska, USA and mainly from European country. Strain A/Kharkov/201/2014 was the closest to isolates from Cyprus and Alaska. Isolates A/Zhitomir/286/2014 and A/Zhitomir/290/2014 are placed next to each other on the phylogenetic tree (fig. 1). Even little group indicated the multiply entering of circulating influenza viruses to the Ukrainian territory. Isolates A/Ukraine/77/2014,

A/Dnipro/232/2014 and viruses from Poland and Serbia gained substitution I312T. Influenza isolate from Dnipropetrovsk and Poland also conferred mutation T434I.

In general, variability rate of NA genes was lower than in the HA genes in outbreak season 2013–2014. All discovered isolates had not conferred mutation associated with reduce susceptibility to oseltamivir.

Influenza viruses A (H3N2) from 2013–2014 outbreak season were genetically different. They were allocated in the subcluster 3C of cluster Victoria/208 and were related to vaccine strain A/Texas/50/2012.

According to the results there were identified 8 different pathways of influenza viruses' penetration on the territory of Ukraine.

The high (94%) genetic similarity observed of Ukrainian isolates to viruses isolated during epidemic season 2013-2014 in other countries. The allocation of Ukrainian viruses to three different clusters points to different ways of their spread to Ukraine. The analysis of NA sequences revealed novel amino acid substitutions Y155F, D251V, S315G to the majority of Ukrainian isolates. None of virus isolates of season 2013-2014 in Ukraine no contained E119D mutation in NA sequence associated with oseltamivir resistance.

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ФІЛОГЕНЕТИЧНИЙ АНАЛІЗ ГЕНУ НЕЙРАМІНІДАЗИ ВІРУСІВ ГРИПУ А(H3N2), ВИДІЛЕНИХ В УКРАЇНІ В СЕЗОНІ 2013–2014 рр.

Реферат

Метою *даної роботи було проведення філогенетичного аналізу генів нейрамінідази вірусів грипу А(H3N2), виділених в Україні в 2013–2014 сезоні.* ***Методи.*** *В роботі були використані методи молекулярної генетики, філогенії та математичної статистики.* ***Результати.*** *Висока (94%) генетична подібність українських ізолятів, виділених під час епідемічного сезону в інших країнах свідчить про стабільність вірусної популяції в Україні. Розміщення українських ізолятів вірусів грипу у трьох різних кластерах свідчить про різні шляхи занесення вірусів на територію України. Аналіз послідовностей NA виявив у більшості українських ізолятів нові заміщення амінокислот у положеннях Y155F, D251V, S315G. Жоден із досліджуваних ізолятів вірусу грипу не містив в послідовності NA мутації E119D, яку асоціюють із резистентністю до озельтамівіру. Більшість випадків респіраторних захворювань людини спричинені вірусами. Поверхневі глікопротеїни вірусу грипу є високо варіабельними, внаслідок чого*

здатні уникати впливу імунної системи господаря. Дослідження генетичних змін серед всіх штамів вірусів грипу є важливим для визначення походження сегментів РНК вірусів грипу A(H3N2) від одного чи декількох різних господарів. Застосування філогенетичного аналізу дозволяє проводити моніторинг рівня та напрямку мінливості вірусів грипу практично у реальному часі. Більш того, порівняння амінокислотних послідовностей робить можливим виявлення початку нових заміщень та спостерігати механізми адаптації вірусу до імунної системи людини. Зазвичай для філогенетичного аналізу використовують послідовності поверхневих антигенів – гемаглютиніну (HA) та нейрамінідази (NA). Метою нашої роботи було провести філогенетичний аналіз послідовностей NA вірусів грипу A(H3N2), виділених в Україні в 2013–2014 сезоні.

Ключові слова: вірус грипу, грип, філогенетичний аналіз.

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ФИЛОГЕНЕТИЧЕСКИЙ АНАЛИЗ ГЕНА НЕЙРАМИНИДАЗЫ ВИРУСОВ ГРИППА A(H3N2), ВЫДЕЛЕННЫХ В УКРАИНЕ В СЕЗОНЕ 2013–2014 гг.

Реферат

Целью *представленной работы было проведение филогенетического анализа генов нейраминидазы вирусов гриппа A(H3N2), выделенных к Украине в 2013–2014 сезоне.* ***Методы.*** *В работе были использованы методы молекулярной генетики, филогении и математической статистики.* ***Результаты.*** *Высокая (94%) генетическая схожесть украинских изолятов, выделенных в эпидемический сезон в других странах, свидетельствует о стабильности вирусной популяции в Украине. Расположение украинских изолятов вирусов гриппа в трех различных кластерах свидетельствует о различных путях попадания вирусов на территорию Украины. Анализ последовательностей NA выявил в большинстве украинских изолятов новые замещения аминкислот в положениях Y155F, D251V, S315G. Ни один из исследованных изолятов вируса гриппа не имел в последовательности NA мутации E119D, с которой ассоциируют резистентность к осельтамивиру. Большинство случаев респираторных заболеваний человека вызваны вирусами. Поверхностные гликопротеины вируса гриппа являются высоко вариабельными, вследствие чего способны избегать воздействия иммунной системы хозяина. Исследование генетических изменений среди всех штаммов вирусов гриппа является важным для определения происхождения сегментов РНК вирусов гриппа A(H3N2) от одного или нескольких разных хозяев. Применение филогенетического анализа позволяет проводить мониторинг уровня и направления изменчивости вирусов гриппа практически в реальном времени. Более того, сравнение аминокислотных*

последовательностей делает возможным выявление начала новых замещений и наблюдать механизмы адаптации вируса к иммунной системе человека. Обычно для филогенетического анализа используют последовательности поверхностных антигенов – гемагглютинина (HA) и нейраминидазы (NA). Целью нашей работы было провести филогенетический анализ последовательностей NA вирусов гриппа А (H3N2), выделенных в Украине в 2013–2014 сезоне.

Ключевые слова: вирус гриппа, грипп, филогенетический анализ.

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