Structure of influenza viruses, connected with influenza life cycle

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Received:20.1.2015 / Accepted:9.3.2015 / Published: 1.4.2015

Influenza represents one of the biggest threats to the global population [1] and is considered as the one of the potential most dangerous pandemic agents. Not surprisingly, the World Health Organization (WHO) initiated the Global Influenza Program (GIP), which provides to member states strategic guidance, technical support and coordination of activities necessary for improving the preparedness to combat with effects of seasonal (or pandemic) influenza, which may represent danger to the health and lives of global population [2, 3]. According to WHO is seasonal influenza responsible for several million cases and almost half a million deaths annually [4]. Aim of this article is provide an overview of the structure of influenza virus and linking of the individual structures in the life cycle of influenza virion.

Keywords: Influenza virus; highly pathogenic avian influenza; pandemic; virion structure

1. Introduction

Social, economic and environmental impacts of annual seasonal epidemics are considerable, it is almost impossible to estimate what would happen in the case of a pandemic. Economic losses associated with Highly pathogenic avian influenza (HPAI) in the US were estimated on tens to hundreds millions of dollars [5]. In Europe the situation wasn´t better. In the Netherlands HPAI outbreak lasted two months, affecting 255 poultry farms and more than 30 million domestic fowl had to be killed [6].

Impact of influenza pandemics are known from the history of the 20th century, when three big influenza pandemics were described: Spanish Flu (1918), the Asian flu (1957) and Hong Kong-Flu (1968) [7]. The Spanish flu is regarded as the biggest in history, the number of victims was estimated on 50 million [8]. In the 21st century, two epidemics with pandemic potential (avian and swine flu) were recorded [9, 10]. Recently new subtypes such as H7N7 and H7N2 [11], H9N2 [12], H7N9 [13] are able to cause human infection. Many researches dealing that there is significant emergence of another pandemic. The question is when and where it will come and how much serious it will be [14-16].

Currently we are better prepared for the fight against epidemics representing by improved prevention, diagnosis and therapy [17-19]. On the other hand risk of extremely rapid spread of the pandemic increase because of the globalization, airplane transport and growth of population [20]. For this reason it is necessary to search new possibilities in the fields of prevention, treatment and diagnosis.

2. Structure of influenza virion

Influenza virions (Fig. 1) are enveloped, the capsid of the virus may be spherical or filamentous. The genome of influenza viruses is a linear, segmented and formed by (-) ssRNA. Influenza genom was encoded in 1976 [21, 22]. Till 2001, eight genome segments (Influenza A and B), and ten proteins, encoded by them, were described: nucleoprotein (NP), haemagglutinin (HA), neuraminidase (NA), proteins of polymerase

complex (PB1 PB2 and PA), matrix proteins (M1 and M2) and non-structural proteins (NS1 and NS2) [23]. In 2001, mitochondrial protein PB1-F2 [24] was described and up till 2012 six other proteins were found: PB1-N40 [25], PA-X [26] and NS3 [27], M42 [28], PA-N155 [29] and PA-N182 [29].

Total size of the Influenza A genome is 13.5 kbp, the size of different genome segments varies between 890 and 2341 bp [30]. Most of influenza A and B proteins are enclosed by lipid bilayer membrane, only three of them constitute an exceptions – two of them has antigenetic characters: trimer HA and tetramer NA. The third one, called M2, is integrated into the membrane and serves as a ion channel [31].

All of the segments of vRNA are associated with polymerase complex and nucleoprotein (NP), and they form ribonucleoproteins (RNP), which are responsible for transcription and replication of influenza [32, 33]. Structure of the native RNP was described by Arranz et al. [34].

Influenza proteins, coded by relevant segments:

- PA, PB1, PB2, PB1-F2 transcripts are on 1st, 2nd, and 3rd segments. PA, PB1, PB2 forms polymerase complex, PB1-F2 is a product of an alternative open reading frame.
- HA (Haemagglutinin) transcript is on the 4th segment.
- NP (Nucleoprotein) transcript is on the 5th segment.
- NA (Neuraminidase) transcript is on the 6th segment.
- M1 (Matrix) transcript is on the 7th segment.
- M2 (M2 ion channel) transcript is on the 7th segment, splicing of M1 transcript.
- NS1 (Non-structural protein) transcript is on the 8th segment.
- NS2 (NEP Nuclear export protein) transcript is on the 8th segment, and is formed by splicing of NS1 transcript.

2.1 Viral polymerase complex (PB1, PB2 and PA)

All of segments of influenza genome are assembled into complexes, containing RNA, polymerase complex and nucleoprotein. These complexes are characterised as ribonucleoproteins (RNPs) and represent minimal transcription and replication equipment of influenza virus.

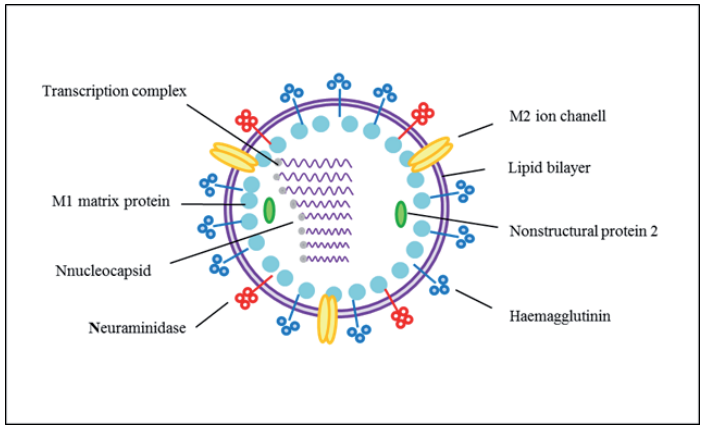

Figure 1. Scheme of influenza life cycle

Viral polymerase formed complementary RNA (cRNA) during replication. cRNA is used for synthesis of new vRNA copies [35, 36]. Molecular mechanisms of transcription and replication is not fully understood, but recent studies suggest that transcription can be implemented by cis-acting RNA polymerase, while replication by trans-acting RNA polymerase [37]. Viral polymerase contains two alkaline polymerase proteins (PB1, PB2) and one acidic protein PA, assembled into structure of trimer, where C end of PA binds N end of PB1 and C end of PB1 binds N end of PB2 [34, 35]. PB1 represents active side for binding of 5´and 3´ terminal ends of vRNA and cRNA [38]. PA and PB2 play the key roles in initiation of transcription process, binding and cleavage of the host pre-mRNA [37]. Many of influenza strains expresses the PB1-F2 protein, which is transcribed from an alternative open reading frame (+1 ORF) PB1. PB1-F2 protein is involved in the induction of apoptosis of host cells, reacts with PB1, influences activity of the polymerase complex, and participates to the viral pathogenesis of some influenza virus strains [39].

2.2 Haemagglutinin

Haemagglutinin (HA) is trans-membrane glycoprotein (size 13.5 nm, molecular weight 76 kDa). HA is target molecule for neutralising antibodies. Primary function of HA is initiation of infection, and interaction with sialic acid receptor of host cell [33, 40]. Interaction of HA with sialic acid receptor leads to virus entry into the host cell, release of viral RNA and viral replication. Currently, the most widespread HA subtypes of influenza A in the human population are H1 and H3. Exceptionally avian HA subtypes can cause disease in humans: H5 [41, 42]; H7 [43] and H9 [44].

HA monomers are synthesized separately as precursor HA0, which are proteolytically cleaved into two sub-unites (HA1 and HA2) [45, 46]. After cleavage of HA0, three cleaved HA structures form the mushroom-shaped trimer [47]. Top of the mushroom consists of antiparallel β-sheet region HA1 subunits and lower part of mushroom consists of three spirally twisted α-helices (HA2) [48]. HA1 subunit (receptor binding site) allows binding of the virus to host cell receptors [47]. Human HA preferentially recognize α-2,6 glycosidic bond on receptors, whereas avian viruses prefer α-2,3 [33, 47]. Preference of human or avian type of receptor is given by number of aminoacids in HA.

2.3 Nucleoprotein

Nucleoprotein (NP) is part of transcription equipment, and is bond with viral envelope by M1 protein [37]. NP is RNA binding protein, forms NP-RNA complex, and poses the template for transcription and replication [49]. NP has the ability to polymerize in trimeric structure, formed by NP monomers connected together through loops and pockets of neighbouring NPs [32, 50]. Although NP is considered phylogenetically as conserved protein, influenza B NP (unlike type A) is tetramer [51]. Most important functions of the NP are: covering vRNA, facilitating NP´s folding into structures dsRNP [52]. NP also interacts with PB1 and PB2 subunits of viral polymerase [33, 37]. Structure of NP as a trimeric complex consisting of head, body and tail was described using surface plasmon resonance (SPR) with high-resolution [53].

2.4 Neuraminidase

Neuraminidase (NA) represents second surface antigen of influenza, and it is involved in releasing of newly formed virions out of host cell. Neuraminidase has to cleave sialic acid from the surface of host cells enzymatically, before releasing of new virions [40]. NA is an enzyme with hydrolytic activity, cleaving the glycosidic bond between the sialic acid (N-acetylneuraminic acid) and D-galactosamin or D-galactose, which represents HA receptor on host cell surface [54, 55]. NA is plugged during the penetration of the virus through the mucin layer of the mucosa, budding of the virus and releasing of the virus from the host cells [56]. In 2012 NA mutation D151G of A/Tanzania/205/2010 strain, which allows NA to assume the function of HA and mediate the binding of host cell receptor, was described [57]. In 2013 Hooper et al. designed mutation G147R, which resulted in take-over of all HA functions. This mutation was developed under laboratory condition but can occurs also by naturally way [55].

Antibodies against neuraminidase prevents the spread of infection between cells, but they do not have neutralizing activity [58]. NA, as well as HA, undergo to antigenic drift, which may resulted in occurrence of resistance to neuraminidase inhibitors (NAIs) [59]. Substitution of arginine (R) on the position 292 by lysine (K) R292K is manifested as resistance to Oseltamivir and Zanamivir [59, 60]. Another mutation, which brings resistance to Oseltamivir, is H274Y [61].

2.5 Matrix proteins (M1 and M2)

The seventh segment of vRNA encodes the matrix protein (M1) and the ion channel protein (M2). M1 protein forms structured layer under the viral membrane, and forms a bridge between the viral envelope and the core (vRNP). M2 is a multifunctional membrane protein forming a proton channel [62]. The process of viral entry into the host cell and release of the RNP requires coordinated action of M2 and M1 proteins [36, 62]. After entry of virus into the host cell and release of virion out of endosome, the activity of the M2 ion channel increases,

thereby increase the flow of positively charged moleucules, which results in acidification. Acidification of the internal environment of the virus, leads to the disruption of the bond between HA and M1 and uncoating of viral particles, followed by merging of HA with the endosomal membrane, RNP transport close to the nucleus, and beginning of viral RNA synthesis [30].

2.6 Nonstructural proteins (NS1 and NS2)

The eighth segment of type A influenza virus encodes two proteins, known as non-structural proteins (NS1 and NS2) [63]. These proteins are produced by alternative mRNA splicing [64]. Both proteins play key role in replication. Due to this fact they are considered as targets for development of new drugs. NS1 is a multifunctional protein, and is important for escaping out of the host immune system [65]. NS1 blocks synthesis of α/β interferons [66, 67]. NS1 is RNA binding protein, which is involved in regulation of many cell processes: inhibition of host mRNA polyadenylation, inhibition of export of polyadenylated host mRNA, inhibition splicing of mRNA and inhibition of interferon-mediated anti-viral response [68-70]. NS1 reduces both synthetic as well as pulmonary proinflammatory cytokines [71]. NS2 was firstly described as a part of purified viral particles and in the nucleus of infected eukaryotic cells [67, 72]. NS2 is also known as NEP and is involved in the transport of RNA and polymerase protein complex during replication, comparing to NS1, which is less described [73]. A number of studies showed NS2 participation in the regulation of viral RNA replication [74, 75].

3. Replication

Influenza viruses are replicated in the columnar epithelial cells of the respiratory tract [76] and are spread via respiratory secretions in small aerosol particles, generated during sneezing, coughing, and speaking [77]. The incubation period is 1-4 days. H1N1 and H3N2 subtypes (influenza A) and influenza B currently circulate in human population [4], the prevalence of these three flu strains may vary in time or geographically within countries, between countries or continents during one flu season [76].

The initial step of influenza infection is binding of virion to the host cell surface. Interactions between host and pathogen are mediated by HA antigen (on the side of virus) and sialic acid receptor (on the side host cell). After the successful attachment of the virus to the receptor, membrane fusion occurs and new envelope is formed around the virion [71]. New compartment (endosome) is formed in the next step, thereafter host cell begins to digest the endosome. Decrease of pH (< 6.0) leads to the activation of HA [78-80]. HA trimer becomes unstable and is partially unfolded [33, 81]. Inner content of virion is released into host cell cytoplasm after membrane fusion. Viral RNA (vRNA) polymerase complex and RNA-dependent RNA polymerase are transported into the cytoplasm of the host cell [55, 78]. Subsequently polymerase complex is transported into the host cell nucleus where the RNA dependent polymerase make positive complementary cRNA which is exported into the cytoplasm and translated, or

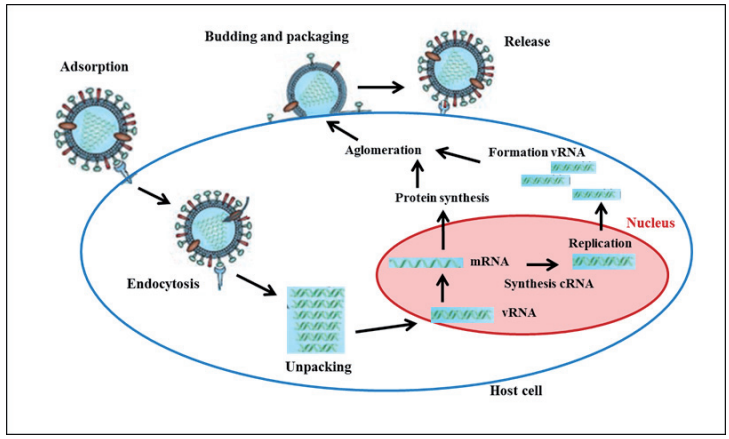

Figure 2. Scheme of influenza life cycle

remains in the nucleus. Influenza viruses are not capable to encode apparatus to produce 5'cap on its own mRNA. 5'cap is cleaved from the host mRNA, and thereafter bonded to viruses

mRNA [78]. Newly synthesized viral proteins are transported using the Golgi apparatus to the cell surface, or transported to the nucleus where they bind vRNA and contribute to assembling of new virions. Synthesized RNA contains a lot of uncorrected errors (one nucleotide for each 10kbp) which leads to the fact, that almost each virus contains mutation [36].

Other viral proteins have a wide range of functions, such as cleavage of cellular mRNA to obtain nucleotides for the synthesis of vRNA or inhibition of translation of host mRNA. vRNA and synthesized viral proteins are assembled into shape of new virions inside the host cell. Thereafter budding unit (in which RNP is inserted) is formed on the surface of the host cell, which is covered by HA and NA antigens on the surface [40]. In order to leave of newly formed virions out of the host cell, sialic acid receptors must be enzymatically cleaved (this receptor was used to bind the virus in early infection by HA) [24, 82]. The death of the host cell occurs after the release of newly replicated virions.

Acknowledgments

The study was financially supported by FLUMED IP A 4.4.

Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform requirements“ for biomedical papers.

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