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Brief Communication

Ultrastructural fingerprints of avian influenza A (H7N9) virus in infected human lung cells

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ABSTRACT

In this study, we investigated the ultrastructural modifications induced by influenza A (H7N9) virus in human lung epithelial cells. One particular characteristic of H7N9 viral infection is the formation of numerous M1-associated striated tubular structures within the nucleus and the cytoplasm, which have only previously been observed for a limited number of influenza A viruses, notably the 2009 pandemic (H1N1) virus.

Introduction

In March 2013, a novel avian influenza A (H7N9) virus that infected humans was identified in China (H.-N. Gao et al., 2013; R. Gao et al., 2013). Infection of poultry with influenza A subtype H7 viruses occurs worldwide, but the introduction of this subtype into the human population, and the resulting fatal cases, has not been observed previously (Belser et al., 2013). The cases occurred in an initial wave ($n=133$) from February to May 2013, and since October 2013 a second wave of human cases has been occurring. As of 28 January 2014, the case fatality rate of all confirmed cases is 22%, but many cases are still hospitalized (WHO report, 28 January 2014). Over a very short period of time, intensive surveillance and research efforts have provided a first overview of this new influenza outbreak, notably in terms of clinical findings, epidemiology, pathogenicity and possible antiviral-resistance markers (Belser et al., 2013; Gao et al., 2013a, 2013b; Richard et al., 2013; Mok et al., 2013). The genotype of these H7N9 influenza viruses isolated from humans may have originated in China by reassortment of H9N2 viruses with duck viruses carrying H7 and N9 genes (D. Liu et al., 2013; Q. Liu et al., 2013; Van Ranst and Lemey, 2013). While there is a putative risk of H7N9 spread from person to person, with acquisition of several markers of adaptation to non-avian hosts or virulence in PB2, PB1-F2, M1 and NS1 viral proteins (D. Liu et al., 2013; Q. Liu et al., 2013), only a few studies have yet started to investigate the cellular biology of the H7N9 virus, to identify underlying mechanisms of adaptation.

Recently, we have revisited electron microscopy (EM) studies in infected cells (Anisimova et al., 1980; Ciampor, 1972; Terrier et al., 2012) and we have shown that influenza A viruses induce a major remodeling of the host cell ultrastructure and the formation of diverse viral structures depending on the subtype/strain and the genomic composition of the viruses (Terrier et al., 2012). Our data suggest that each influenza A virus strain could be associated with a specific cellular fingerprint, which possibly correlates with the functional properties of its viral components (Terrier et al., 2012).

In this study, we examined the ultrastructural modifications induced by influenza A (H7N9) virus in cultured human lung epithelial cells. With the help of immunogold-labeling EM and confocal microscopy (CM), we identified an abundant accumulation of M1-associated

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striated tubular structures in the nucleus and the cytoplasm, that have only been observed with a limited number of influenza strains, notably the 2009 pandemic (H1N1) virus (Goldsmith et al., 2011; Terrier et al., 2012).

Results and discussion

Human lung epithelial A549 cells were mock-infected or infected with influenza virus strain A/Anhui/1/2013 (H7N9) at a multiplicity of infection of 1 and incubated for 24 h. Cells were then fixed and embedded for standard EM or anti-M1 immunogold labeling EM, in Epon or Lowicryl resin, respectively, as previously described (Terrier et al., 2012).

EM of the ultrathin sections revealed numerous zones of viral budding, with the accumulation of electron dense material near to the plasma membrane (Fig. 1A and B). The morphology of the released viral particles was heterogeneous with spheroidal and filamentous shapes. The average size and density of the surface glycoprotein spikes (data not shown) were in agreement with those previously measured for several other influenza A viruses (Moulès et al., 2011).

We then investigated the host cell ultrastructure modifications induced by H7N9 infection, and compared with mock-infected cells (Fig. 1D versus 1C). One of the most noticeable observations for the H7N9 infected cells was the extensive remodeling of the nucleolar compartment, which we have previously reported as a common feature for influenza A viruses (Terrier et al., 2012).

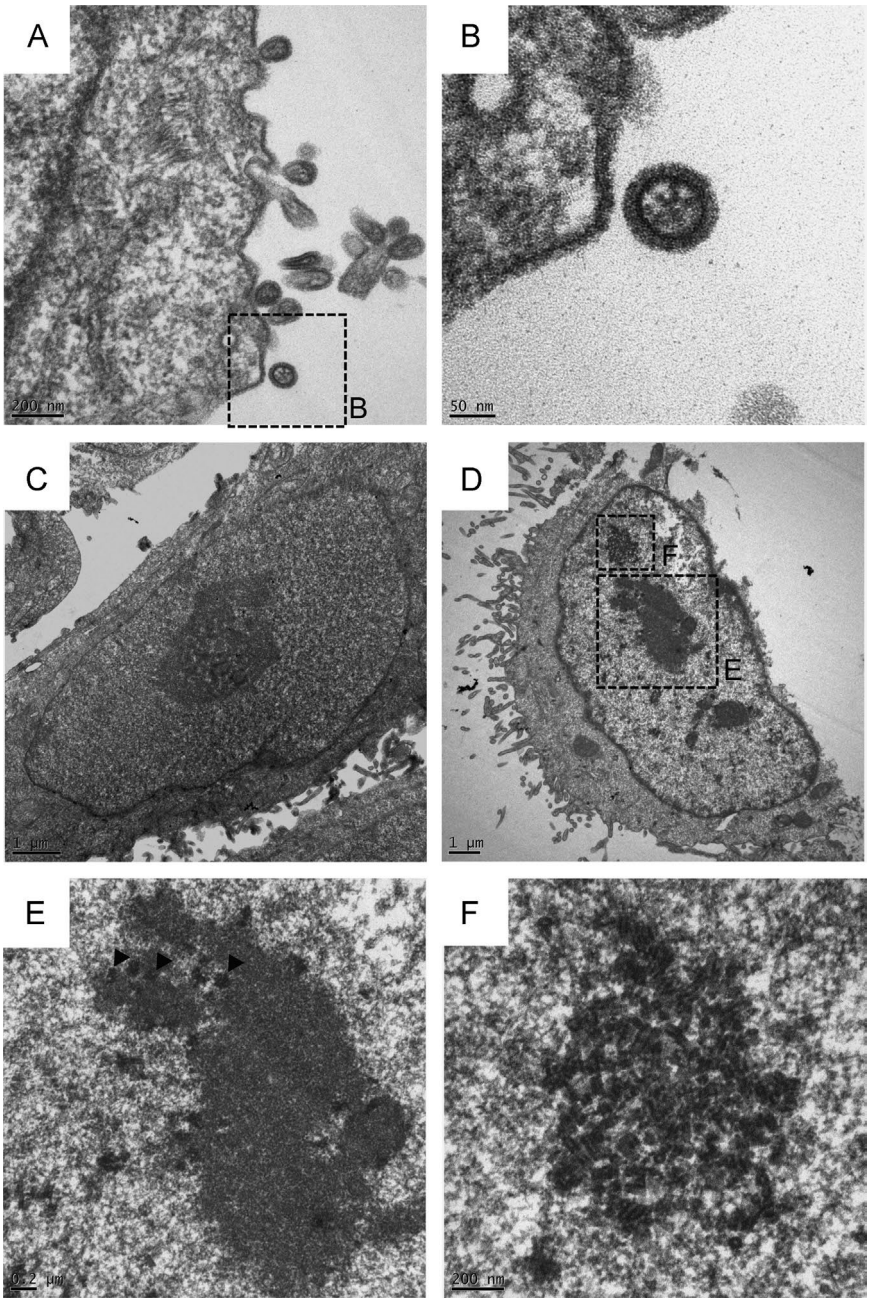

Fig. 1. Ultrathin EM section of A549 human lung cells infected with avian influenza A (H7N9) virus. (A) Pleomorphic viral particles visible at budding regions. (B) Glycoprotein spikes and viral genome segments. (C) Non-infected cell. (D) Influenza A H7N9 infected cell. The disruption of nucleolar compartments and the formation of virally induced structures are clearly visible. (E) Detailed view of H7N9-induced disruption of the nucleolar compartments. Numerous electron dense round-shaped bodies were observed inside or in the proximity of the nucleoli (black arrowheads). (F) Detailed view of virally-induced striated structures in the nucleus.