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Human airway epithelial cell culture to identify new respiratory viruses: Coronavirus NL63 as a model

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A B S T R A C T

Propagation of new human respiratory virus pathogens in established cell lines is hampered by a lack of predictability regarding cell line permissivity and by availability of suitable antibody reagents to detect infection in cell lines that do not exhibit significant cytopathic effect. Recently, molecular methods have been used to amplify and identify novel nucleic acid sequences directly from clinical samples, but these methods may be hampered by the quantity of virus present in respiratory secretions at different time points following the onset of infection. Human airway epithelial (HAE) cultures, which effectively mimic the human bronchial environment, allow for cultivation of a wide variety of human respiratory viral pathogens. The goal of the experiments described here was to determine if propagation and identification of a human respiratory virus may be achieved through inoculation of HAE cultures followed by whole transcriptome amplification (WTA) and sequence analysis. To establish proof-of-principle human coronavirus NL63 (HCoV-NL63) was evaluated, and the first visualization of HCoV-NL63 virus by transmission electron microscopy (TEM) is reported. Initial propagation of human respiratory secretions onto HAE cultures followed by TEM and WTA of culture supernatant may be a useful approach for visualization and detection of new human respiratory pathogens that have eluded identification by traditional approaches.

1. Introduction

The detection of human respiratory virus pathogens can be challenging due to difficulty in obtaining high titer clinical specimens, and the inefficiency of propagating the virus in established cell lines. Viruses that replicate readily in culture and induce dramatic cytopathic effect (CPE), such as influenza viruses, respiratory syncytial viruses, adenoviruses, rhinoviruses and coronaviruses responsible for common colds (229E and OC43) were identified and characterized by electron microscopy in the 1960s. However, these efficiently propagating viruses likely represent only a fraction of the viruses that cause significant clinical disease (Iwane et al., 2004; Jartti et al., 2004; Juven et al., 2000). In recent years, viral respiratory pathogens such as human metapneumovirus (van den Hoogen et al., 2001) and human coronavirus NL63 (HCoV-NL63) (van der Hoek et al., 2004) that propagate slowly in standard cell lines have been identified using molecular approaches to amplify novel sequences from infected cell lines. However, the optimal propagation of a human respiratory virus and detection of virus-infected cells may still be a roadblock for identification of novel pathogens. A culture system which faithfully mimics human airways may alleviate some of these challenges. Therefore, human airway epithelial (HAE) cells were evaluated as a culture system for the initial propagation of a human respiratory virus, followed by visualization of the virus by transmission electron microscopy (TEM) and use of a random amplification approach to detect viral sequences.

HAE cultures are derived from primary bronchial epithelial cells isolated from the airways of human lung donors or patients undergoing lung transplantation, and have been used extensively to study the biology of respiratory epithelium (Fulcher et al., 2005). Primary bronchial epithelial cells are harvested from the inner lining of airways and are cultured on porous supports, initially submerged in

Abbreviations: HCoV-NL63, human coronavirus NL63; WTA, whole transcriptome amplification; SARS-CoV, severe acute respiratory syndrome coronavirus; CPE, cytopathic effect; TEM, transmission electron microscopy; HAE, human airway epithelium.

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medium. After the cells grow to form a confluent monolayer, the medium is removed from the apical side, creating an air–liquid interface culture. The primary cells then replicate and differentiate to recapitulate the pseudostatified epithelial morphology found in the human airway. Mature differentiated HAE cultures can be maintained for up to 2 months and contain mucus-producing goblet cells and non-ciliated and ciliated epithelial cells, which have been shown to be ideal for propagation of a wide range of human respiratory pathogens, including influenza virus (Thompson et al., 2006), parainfluenza viruses (Zhang et al., 2005), respiratory syncytial virus (Zhang et al., 2002), adenovirus (Zhang et al., 2002), and severe acute respiratory syndrome coronavirus (SARS-CoV) (Sims et al., 2006). Propagation of human viral pathogens in clinical specimens on HAE cultures may provide an optimal environment.

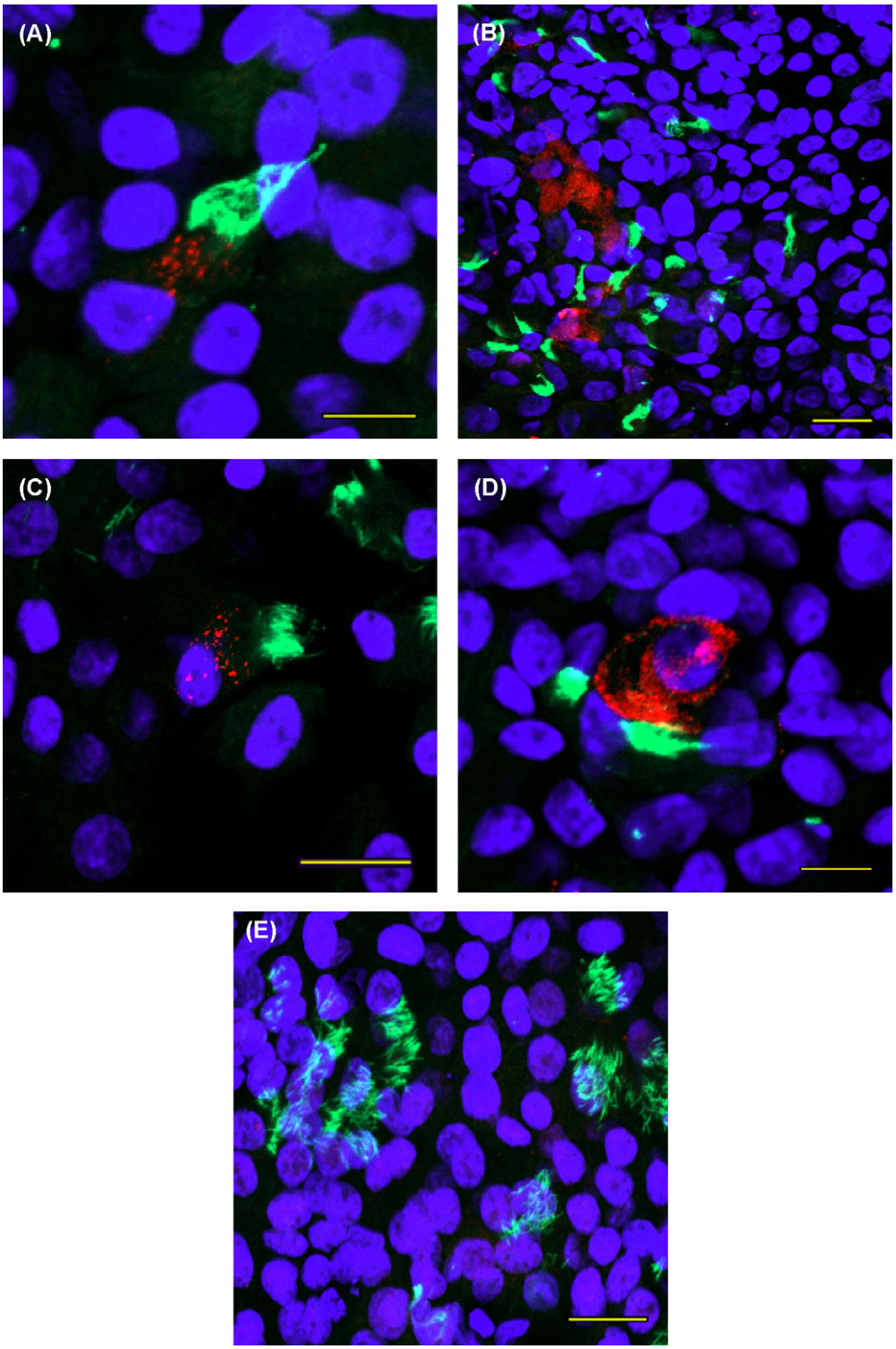

Fig. 1. Detection of HCoV-NL63 in the ciliated epithelium of HAE cultures by immunofluorescence. HCoV-NL63-infected (A–D) or mock-infected (E) HAE cultures were stained with anti-HCoV-NL63 replicase (red), anti-tubulin (green) and DAPI (blue) at 72 h post-infection and imaged by confocal microscopy. (A and D) Bar, 10 μm. (B, C and E) Bar, 20 μM.