Original Article

Three-dimensional Culture of Human Airway Epithelium in Matrigel for Evaluation of Human Rhinovirus C and Bocavirus Infections*

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Abstract

Objective Newly identified human rhinovirus C (HRV-C) and human bocavirus (HBoV) cannot propagate *in vitro* in traditional cell culture models; thus obtaining knowledge about these viruses and developing related vaccines are difficult. Therefore, it is necessary to develop a novel platform for the propagation of these types of viruses.

Methods A platform for culturing human airway epithelia in a three-dimensional (3D) pattern using Matrigel as scaffold was developed. The features of 3D culture were identified by immunochemical staining and transmission electron microscopy. Nucleic acid levels of HRV-C and HBoV in 3D cells at designated time points were quantitated by real-time polymerase chain reaction (PCR). Levels of cytokines, whose secretion was induced by the viruses, were measured by ELISA.

Results Properties of bronchial-like tissues, such as the expression of biomarkers CK5, ZO-1, and PCK, and the development of cilium-like protuberances indicative of the human respiration tract, were observed in 3D-cultured human airway epithelial (HAE) cultures, but not in monolayer-cultured cells. Nucleic acid levels of HRV-C and HBoV and levels of virus-induced cytokines were also measured using the 3D culture system.

Conclusion Our data provide a preliminary indication that the 3D culture model of primary epithelia using a Matrigel scaffold *in vitro* can be used to propagate HRV-C and HBoV.

Key words: 3D cell culture; Human airway epithelium (HAE); Human rhinovirus C; Human bocavirus; Propagation

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INTRODUCTION

Non-influenza respiratory viruses such as parainfluenza virus, human rhinovirus, human metapneumovirus, respiratory syncytial virus (RSV), and human coronavirus affect people of all age groups, and they can cause mild to severe respiratory illnesses from common colds to severe respiratory disease[1-2]. The human rhinovirus C (HRV-C) species was discovered in 2006[3], and it is

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more virulent than HRV-A and HRV-B species, which are associated with severe illnesses such as pneumonia, acute wheezing, and exacerbation of asthma in infancy[4]. Human bocavirus (HBoV) was identified in 2005 from human nasopharyngeal aspirates of patients with respiratory tract illness[5]. Acute wheezing was the most common clinical symptoms observed in HBoV-infected patients[6]. Infections caused by these two viruses pose a substantial disease burden to young children; however, the biological properties and pathogenesis of HRV-C and HBoV have not been fully clarified. The ability of HRV-C and HBoV to propagate in traditional monolayer (2D) cellular culture or animals represents the bottleneck that limits related studies.

Traditional 2D cellular culture is a common platform to study infectious disease[7]; however, this method cannot mimic the physiological complexity of *in vivo* microenvironments, which might restrict the propagation of HRV-C and HBoV. The development of suitable cell culture models is thus promising for studying the life cycle of infectious viruses and their interactions with the host. Previously, studies have shown that HRV-C15 and HRV-C11 strains propagate in fully differentiated human nasal or bronchial epithelial cells[8], and HRV-C15 and HRV-C41 strains propagate in the differentiated human sinus epithelial cells[9-11]. Additionally, HRV-C2, HRV-C7, HRV-C12, HRV-C15, and HRV-C29 types were all capable of mediating productive infection in reconstituted 3D human airway epithelia[12]. Human trachea epithelial cells were previously cultured in an air-liquid interface (ALI), and they formed a pseudostratified epithelium, in which HBoV propagated[13]. These studies suggest that novel 3D tissue-like cell culture systems are suitable for studying HRV-C and HBoV.

Matrigel basement membrane matrix is a commercial cell culture medium consisting of a gelatinous protein mixture secreted by Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells. It is rich in extracellular matrix (ECM) components, and used widely for 3D cell culture[14]. Cells cultured in Matrigel show many differences in gene and protein expression, survival, proliferation, differentiation, and metabolism, compared to those cultured by traditional 2D culture methods[15-18]. Cells grown in 2D and 3D cultures also respond differently to chemical drugs or radiation[19-20]. Thus, 3D cell culture is the third model that bridges the gap between traditional cell culture and animal models[21-23].

Although it has been demonstrated that viruses (HBoV, infectious bronchitis virus, avian influenza viruses, RSV, and HRV-C) propagate well in 3D cultured cells using ALI methodologies[24-27], this strategy is time-consuming (approximately 15 days). In the current study, we constructed a 3D cell culture model using Matrigel as the scaffold and measured the propagation abilities of HRV-C and HBoV using this platform. The results of this study showed that both HRV-C and HBoV propagate in the 3D Matrigel system, whereas they could not be detected in 2D cultures.

MATERIALS AND METHODS

2D Cell Culture

Primary human airway epithelial (HAE) cells were obtained from the Chinese Center for Disease Control and Prevention. Cells were cultured in BEGM media (Lonza, Walkersville, MD). HAE cells were used from passage 1 to passage 5 for all experiments, with passage 1 defined as the thawed cells from the nitrogen canister.

Trypsin-EDTA (0.25%) was used to remove cells from culture flasks for sub-culturing. Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. Two million cells were seeded in 75 cm^2 culture flasks and sub-cultured at 80% confluence.

3D Culture

The 3D culture system using Matrigel as a scaffold was prepared according to the manufacturer's protocol. Briefly, the mixture of Matrigel (BD Biosciences, Baltimore, MD) solution, thawed in an ice box, and cell suspension (1×10^4 cells/insert, 200 µL/insert) was added to the insert (12-Transwell plate), or pre-plated cells in the membrane of the insert, using cooled pipette tips, and then covered with a Matrigel layer. These samples and Matrigel only-coated plates, were incubated at 37 °C for 30-45 min for solidification. Further, the cells were plated on the surface of the Matrigel layer, for Matrigel only-coated inserts, and then 3D culture medium was added to all inserts and wells; medium was changed every 2 days. Cells in the inserts of 12-well uncoated plates were used as 2D controls. The morphology of cells at day 7 was observed using a reverse phase-contrast microscope.