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研究課題名（和文）　Host responses in whole blood of children infected with respiratory syncytial virus

研究課題名（英文）　Host responses in whole blood of children infected with respiratory syncytial virus

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　2,700,000円

研究成果の概要（和文）：仙台医療センターと宮城県立こども病院で合計39名の小児の研究参加の同意を得ることが出来た。対象患者から鼻咽頭スワブおよび血液を採取しRNAを抽出後、鼻咽頭検体でPCRを行った結果、30例からウイルスが検出され9例は陰性だった。陽性となったウイルスの内訳はRSウイルス19例であった。RSウイルスが検出された19名では12名が軽症群、7名が重症群に属していた。 次世代シーケンシングおよびプロテオミクス解析による血液中の遺伝子発現や遺伝子間・タンパク質間の相互作用に関しては現在進行中である。

研究成果の概要（英文）：A total of 39 children at Sendai Medical Center and Miyagi Children's Hospital were enrolled in the study. Nasopharyngeal swabs and whole blood samples were collected from patients followed by total RNA isolation and purification. PCR was performed on nasopharyngeal swabs and identified 19 RSV positive samples. Clinical data were obtained and patients were grouped into mild and severe groups. Among the 19 children infected with RSV, 12 were mild while 7 were severe. Whole blood transcription profiling by next-generation sequencing and proteomics analysis are currently ongoing.

研究分野：　Virology

キーワード：　RSV　blood　host response

１．研究開始当初の背景 Background of the beginning of the research

Acute respiratory tract infections due to viral and bacterial pathogens are major causes of illness and death in children worldwide. The leading cause of acute lower respiratory tract infection is respiratory syncytial virus (RSV), which affects 33.8 million children younger than 5 years of age and responsible for 4 million hospital admissions and 200,000 deaths worldwide (Legand A. et al. Future Virol. 2013). To date, there is no vaccine for RSV and the treatment option that is specific to the virus is limited. This can be due to the limited understanding of host cellular responses during RSV infection. In addition, one of the major challenges for clinicians is the difficulty in predicting which children infected with RSV will develop a serious condition that requires hospital admission and which children will have mild symptoms based on physical examination and limited availability of diagnostic tools.

Previous studies have been conducted using microarrays and proteomics to identify host genes and proteins that are affected during RSV infection (Janssen R et al. J Virol. 2007; Hastie M et al. Mol Cell Proteomics. 2012). However, these studies utilized in vitro cell lines and laboratory-adapted A2 strain of RSV, which do not reflect the clinical setting. Microarray studies using whole blood from children with acute respiratory infections suggest the possible use of blood RNA transcriptional profiles to differentiate between children infected with RSV and other pathogens and to distinguish between mild and severe forms of pneumonia (Mejias et al. PLoS Med. 2013; Brand et al. PLoS ONE 2015). However, these studies identified host factors based only from transcriptome data, which might miss out other host factors due to inherent limitations of microarray method. In addition, the timing and expression patterns of proteins and genes are different. To date, there are limited data on the integrated transcriptome and proteome analyses of whole blood from children with RSV infection.

２．研究の目的 Purpose of the study

This study aimed to identify the host responses in whole blood of children infected with RSV (Fig. 1). Specifically, this study aimed to identify host transcripts and proteins which increased or decreased in children with RSV infection. This study aimed to identify host factors in blood samples that can distinguish severe and mild forms of RSV infection. And this study aimed to integrate proteome and transcriptome data to generate patient-host factor network in understanding RSV disease severity and pathogenesis.

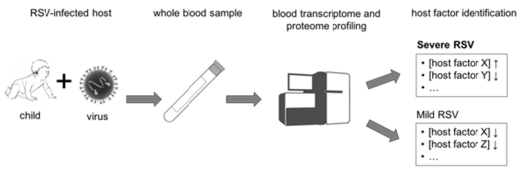
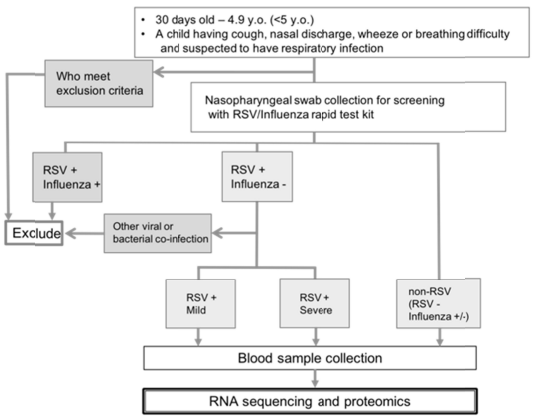


Figure 1. Identification of host factors in blood of children with severe RSV infection.

３．研究の方法 Method of the study

This is a hospital-based prospective case comparison study on identifying host RNA transcripts and proteins in whole blood of children who are infected with RSV. The flow of the study is shown in Fig. 2. Approval from ethics committee were obtained at Tohoku University Graduate School of Medicine, Sendai Medical Center, and Miyagi Children's Hospital. Children, younger than five years old with acute respiratory infection including coughing, nasal discharge, wheezing, or breathing difficulties were recruited in the study. Children with documented chronic heart disease, chronic lung, liver, or kidney disease, immunodeficiency, genetic disease including Down syndrome, prematurity (<36 weeks), or undergoing steroid



treatment within 2 weeks before clinical presentation were excluded in the study.

Figure 2. Flow diagram of the study.

Severity of RSV disease were assessed based on the Global Respiratory Severity Score, which is comprised of the following parameters: general appearance, wheezing, rales, retractions, cyanosis, lethargy, poor air movement, oxygen saturation, and respiratory rate (Caserta et al. JID 2017). A score of <3.5 is mild while a score of ≥3.5 is severe.

Patient information was collected using questionnaire and chart review. Chest X-ray results and laboratory results were also collected. Nasopharyngeal swabs were collected from each subject for RSV/influenza rapid diagnostic test and PCR. Alternatively, nasal aspirate or nasal wash was collected. The remaining nasal samples were stored at 4℃ and tested by PCR for confirmatory tests. About 1 mL of peripheral blood samples were collected from each subject. Blood collection was performed together during the routine blood sampling of the hospital. Blood samples were collected in blood RNA tubes, which contain anticoagulants and stabilizers and stored at 4℃.

Total RNA extraction was performed on nasal samples followed by cDNA synthesis, and PCR assay to confirm the presence of RSV, influenza virus, rhinovirus, human metapneumovirus, and adenovirus. For blood samples, to total RNA and protein isolation was performed. To identify host genes that are affected during RSV infection, total RNA in blood samples were analyzed using RNA sequencing (RNA-seq) technology. RNA-seq utilizes next-generation sequencing (NGS) technology, which allows a more sensitive and unbiased method in identifying and quantifying gene expression compared to microarray experiments. To identify host proteins that are affected during RSV infection, total protein will be analyzed using mass spectrometry.

RNA transcript and protein abundance level will be normalized and compared with controls. Host factors with at least two-fold increase or decrease in abundance level will be identified using hierarchical clustering method based on likelihood ratio test while controlling the FDR at 1%.

Network analysis will be used in identifying subnetworks of host factors, which might be missed out by clustering analysis especially on data points with less dramatic changes in expression pattern. An advantage of using this method is the ability to interrogate the network and identify subnetwork of host factors associated with mild or severe RSV. The list of significantly altered genes and proteins found in subnetworks of each patient will be combined to generate a patient-host factor network. Since each patient will be categorized according to degree of disease severity, the network will be interrogated to identify subnetworks of host factors that are associated with mild or severe cases. The freeware Cytoscape (www.cytoscape.org) will be used to visualize and analyze the network. Network statistical analysis, network topology and structure analyses will be performed using the different application programs in the Cytoscape software.

4．研究成果 Research result

We enrolled a total of 39 children at Sendai Medical Center and Miyagi Children's Hospital. Whole blood samples and nasopharyngeal swabs were collected from patients followed by total RNA and protein isolation and purification.

PCR was performed on nasopharyngeal swabs and identified 19 RSV, 7 rhinovirus, 2 human metapneumovirus, 1 influenza B virus, 1 adenovirus, and 9 negative samples. Clinical data were obtained and patients were grouped into mild and severe groups according to the Global Respiratory Severity Score (Fig. 3). Of the 39 children, 29 belonged to the mild group while 10 belonged to the severe group. Among the 19 children who were infected with RSV, 12 had mild RSV while 7 had severe RSV.

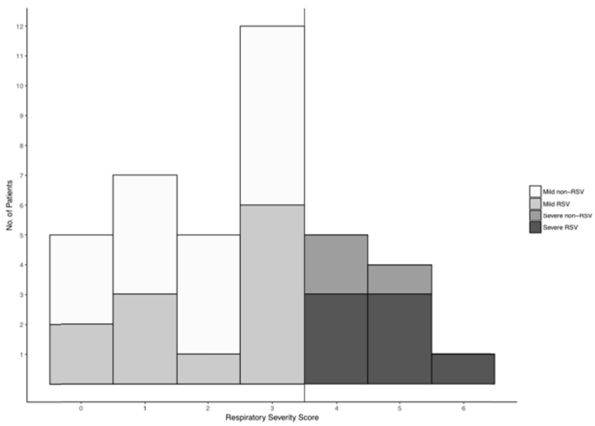


Figure 3. Distribution of children based on Global Respiratory Severity Score.

From 1 mL of whole blood, the mean total RNA yield is 1.6 ug/mL. Quality of isolated RNA was evaluated using Bioanalyzer and the mean RNA Integrity Number (RIN) obtained is 7.2, which indicates high quality of isolated RNA (Fig. 4). Next-generation sequencing and proteomics analysis are currently ongoing.

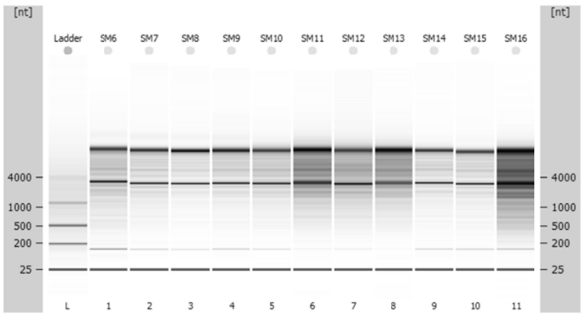


Figure 4. Bioanalyzer results showing high quality of isolated RNA. Two dark bands represent 28S and 18S ribosomal RNA.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕　（計　0　件）

〔学会発表〕　（計　0　件）

〔図書〕　　（計　0　件）

〔産業財産権〕

○出願状況（計　0　件）

○取得状況（計　0　件）

〔その他〕
ホームページ等

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