Press embargo lifted at: 12:00 a.m. on October 13, 2020

東京医科大学 TOKYO MEDICAL UNIVERSITY　東京慈恵会医科大学 THE JIKEI UNIVERSITY SCHOOL OF MEDICINE　国際スペースメディカル株式会社

Press Release

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To the media

October 12, 2020
Tokyo Medical University
Jikei University School of Medicine
International Space Medical Co., Ltd.

Identification of Prognostic Factors for COVID-19 Using Liquid Biopsy

- Expectations Not Only for Prognosis, but also New Treatment Methods and Uncovering Conditions of the Disease -

[Overview]

A joint research team analyzed exosomes and nucleic acids in blood by using a liquid biopsy method typically used for diagnosing cancer, and identified new prognostic factors concerning COVID-19. The team consisted of: Takahiro Ochiya, a professor of the Institute of Medical Science of Tokyo Medical University; Yu Fujita, an instructor for the exosome drug development study course at Jikei University School of Medicine; Kazuyoshi Kuwano, a professor for the internal medicine course and of the Department of Respiratory Medicine of Jikei University School of Medicine; Tokio Hoshina, an instructor of the Infectious Disease Department of Jikei University School of Medicine; Juntaro Matsuzaki, a researcher of University of California, San Francisco (and a visiting researcher of the Institute of Medical Science, Tokyo Medical University); and International Space Medical Co., Ltd. (president: Mitsuru Miyato). The identified exosomes and blood nucleic acids are expected to not only play a role as prognostic biomarkers, but also to help researchers develop new treatment methods and uncover conditions of the disease with the application of said biomarkers.

[Background of the Research]

A liquid biopsy is considered to be of benefit in the super-early diagnosis of cancer with minimum invasive surgery, and in the selection of anticancer agents. It is carried out by analyzing deoxyribonucleic acids (DNAs), ribonucleic acids (RNAs), and exosomes that circulate in the blood. In cancer patients and other patients who have contracted infectious diseases, these pieces of information found in blood have been reported to differ from healthy persons at an early stage of a disease. We thought that analyzing the differences in exosomes and nucleic acids in blood could be of benefit for prognoses regarding COVID-19. It is known that nucleic acids in blood in general are partially contained within exosomes, which are stable capsules that cells secrete. Exosomes are vesicles surrounded by approx. 100-nm lipid bilayers that many types of cells secrete, and are known to play important roles in cell-to-cell communication. Cells infected with bacteria and viruses, in particular, affect neighboring cells through exosomes. It has been reported in many cases, for example, that such cells contribute to the spread of viruses by enclosing the viruses in exosomes and exerting them out.

They can be a serious factor for the worsening of symptoms, while on the other hand they may reflect a normal reaction in which the body can beat a virus. In other words, analyzing the exosomes and nucleic acids of patients infected with COVID-19 can help quickly identify the response of the human body involved in the worsening of the disease. The purpose of this research was to identify new prognostic factors regarding COVID-19 with the use of liquid biopsies by analyzing the blood of patients infected with COVID-19 when they were hospitalized.

[Results and Insights Obtained from This Research]

The research team focused on 42 patients who were tested positive for COVID-19 through PCR testing and hospitalized in the Jikei University Hospital between March and May 2020. We excluded nine patients from the research who were in serious conditions based on the WHO severity scale, and focused on 31 patients who had mild symptoms at the time of their admission. We then took serums from 10 healthy persons as control samples (at Omiya City Clinic; Director: Ryo Nakagawa), and analyzed 41 samples in total. Of the 31 COVID-19 patients, nine patients saw their symptoms worsen and required respirator treatment in intensive care, and the remaining 22 were discharged with mild symptoms. Using blood samples of the 31 COVID-19 patients, (22 with mild symptoms and nine whose symptoms had worsened) and serums from the 10 healthy persons, we specifically focused on blood RNAs and exosome proteins, and conducted a comprehensive analysis by using next-generation sequencers *1) and mass spectrometry (LC-MS) *2), respectively. On the basis of the results of these analyses, we identified three different groups of early prognostic biomarkers for COVID-19 patients:

(1) Exosome proteins associated with an anti-virus response
E.g.: Exosome COPB2 and PRKCB
(2) Coagulation-related exosome proteins and RNAs
E.g.: Exosome MFAP4, ECM1, CAPN, FGG, and CD147
CDKN2B.AS1 as RNA (long-non coding RNA *3))
(3) RNA associated with liver disease
E.g.: microRNA-122-5p (microRNA *4)), SNORD33 (small nucleolar RNA *5))

Out of these markers, COPB2, an exosome protein associated with an anti-virus response (components of a COPI vesicle important for golgi-ER trafficking), had the best prognostic ability regarding the worsening of COVID-19 patients' symptoms in this research cohort (AUC=1.0, and both sensitivity and specificity = 100%). In other words, COVID-19 patients who have high levels of exosome COPB2 in their blood at the time of admission (or when they are tested positive) may be able to overcome the disease without experiencing serious events (Figure 1).

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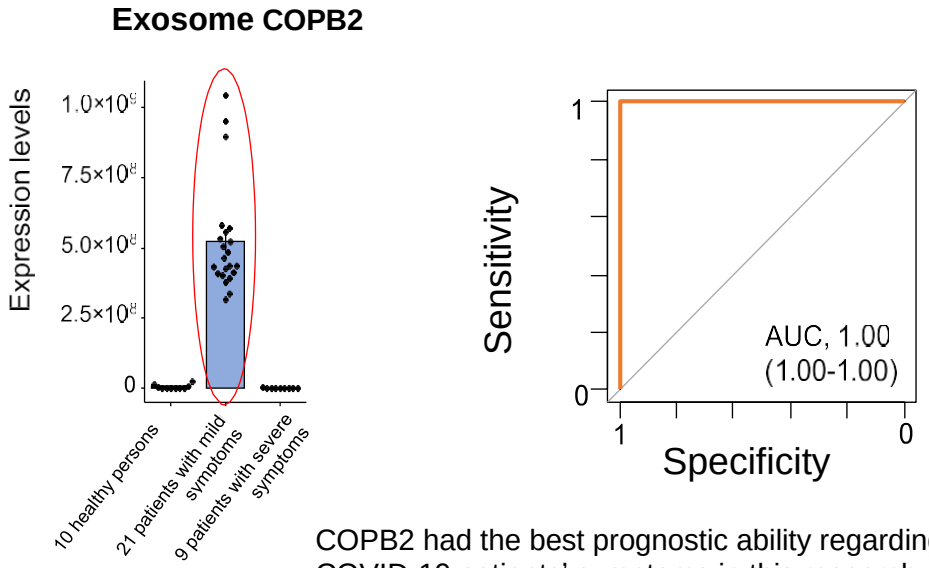
Figure 1: Early prognosis for COVID-19 using exosome COPB2

Exosome proteins related to an anti-virus response were expressed at a statistically significant level in the group of patients with mild symptoms.
COPB2 had the best result.



COPB2 had the best prognostic ability regarding the worsening of COVID-19 patients' symptoms in this research cohort (AUC=1.0, and both sensitivity and specificity = 100%).

On the other hand, the degree of liver damage and coagulability have been reported as prognostic factors for COVID-19 in previous studies with regard to (2) coagulation-related markers and (3) liver disease-related RNAs. We found that the results of this analysis reflected the clinical background of COVID-19 patients up to this time. Compared with D-dimers and hepatic enzyme ALTs which reflect coagulability measured in clinical testing, coagulation-related exosome proteins and RNAs, and liver disease-related RNAs had excellent prognostic ability.

This research on early prognoses for COVID-19 patients using liquid biopsies suggested the possibility that patients with a (1) high level of exosome proteins related to an anti-virus response such as COPB2 may not see a worsening of their condition, while patients with (2) high coagulation-related exosome proteins and RNAs or (3) high liver disease-related RNAs could see a worsening of their condition (Figure 2). This suggests that functional exosome components can also be a potential cause and mitigation for the worsening of COVID-19.

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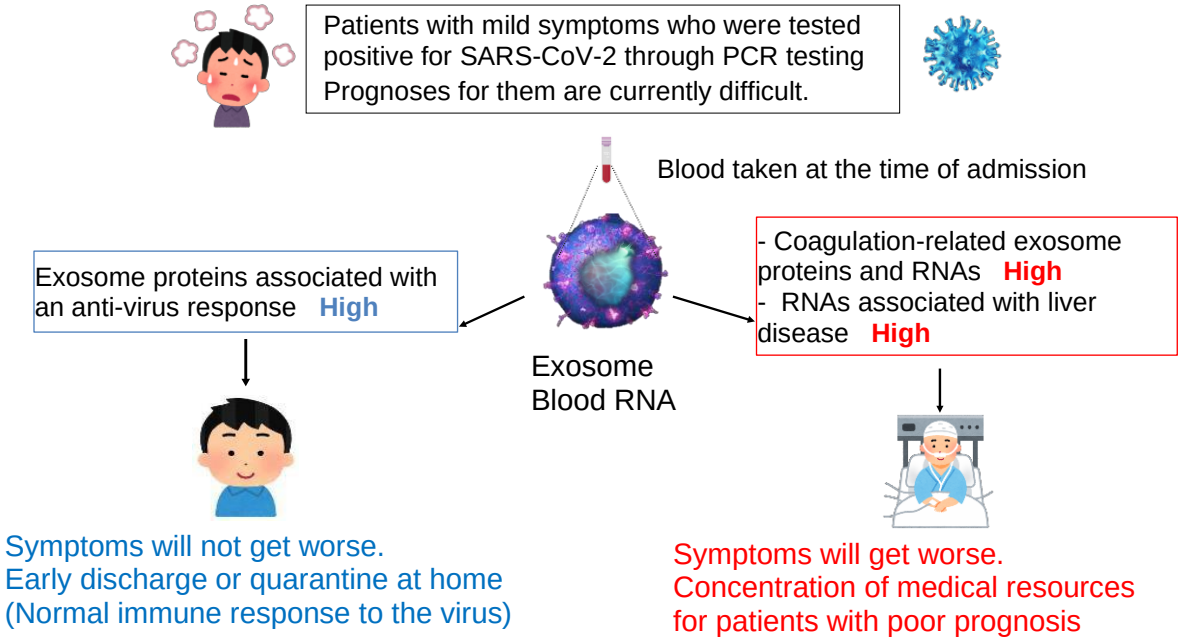
Figure 2: Early prognosis for COVID-19 patients using exosomes and blood RNAs with liquid biopsy



[Future Research Plans and Ripple Effect]

Based on the results of this research, we are currently conducting a validation analysis with the help of different patient groups. Prognoses for COVID-19 patients using liquid biopsies are expected to develop very differently than traditional humoral factors such as blood markers, cytokines, and chemokines. The important point is that exosomes circulate through blood and are incorporated into cells for gene control. In fact, there are reports that some of the above-mentioned molecules are therapeutic target molecules for SARS-CoV-2, and that they have associations with conditions of the disease. It is suggested that the factors discovered in this research are not just blood biomarkers, but also may be related to important mechanisms regarding the worsening of symptoms related to COVID-19. It is expected that supplying or eliminating the exosomes and RNAs would lead to the development of new treatment methods for COVID-19. We are currently developing diagnostic kits that focus on exosome COPB2.

[Terms]

*1) Next-generation sequencer: A highly-precise analysis method that makes it possible to determine thousands to millions of gene arrangements simultaneously. This method made analyses on the expression of various RNAs described below possible.

*2) Mass spectrometry: A method for comprehensive analysis of proteins in a test sample

*3) long-non coding RNA: Non-coding RNA (not translated into proteins) with 200 or more bases

*4) microRNA: Functional nucleic acid that becomes a micro non-coding RNA (not translated into proteins) with 20 to 25 bases

*5) small nucleolar RNA: One of the non-coding RNAs

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End

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コロナ重症化を判別

東医大 血液中のたんぱく質で

東京医科大学の落谷孝広教授らは新型コロナウイルス感染症で重症になりやすい人の判別に使えるたんぱく質を見つけた。血液中にこのたんぱく質がある人は、発症しても軽症のままだという。重症の危険がある人だけを洗い出し、治療に専念できるようになる。

研究グループは新型コロナで軽症だった患者31人の血液を採取し、血液中の「エクソソーム」と呼ぶ微粒子が含むたんぱく質を調べた。

入院後に重症化した9人と軽症のまま退院した22人を比べると、退院した全員に「COPB2」というたんぱく質があった。このたんぱく質が免疫に関わっているとみられる。

研究グループは検査技術も開発中で、数時間での判別を見込んでいる。感染者の血液中のたんぱく質を調べれば、重症になるか軽症になるかを判定できる。このたんぱく質は免疫に関わっており、治療薬の開発にも役立ちそうだ。

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コロナ重症化予測

血中候補物質特定

東京医大など

新型コロナウイルスに感染した人が重症化するかどうかを予測するのに役立つ可能性のある血液中の物質を複数特定したと、東京医大や東京慈恵医大のチームが12日、発表した。

チームの落谷孝広・東京医大教授（分子腫瘍学）は「事前に重症化する人を予測できれば、入院する患者が減り、医療崩壊を回避できる」としており、物質の有無を調べる検査キットの開発を進めている。

チームは入院時に軽症だった患者31人の血液を分析した。血中で細胞間の情報伝達を担う「エクソソーム」と呼ばれる物質を調べると、軽症で退院した22人全員から免疫に関わるたんぱく質「COPB2」が検出された。一方、後に重症化した9人からは検出されなかった。

また、肝障害に関わる「マイクロRNA」という微小物質の一種が、重症化した9人の血中から多く見つかるなどした。

（おことわり）載のコラム「坂村健の目」は今月休みます。第3木曜日掲