

Analysis of Extracellular Vesicle Marker Levels in Blood Samples from Patients with Neurodegenerative Diseases Using Exosome ELISA

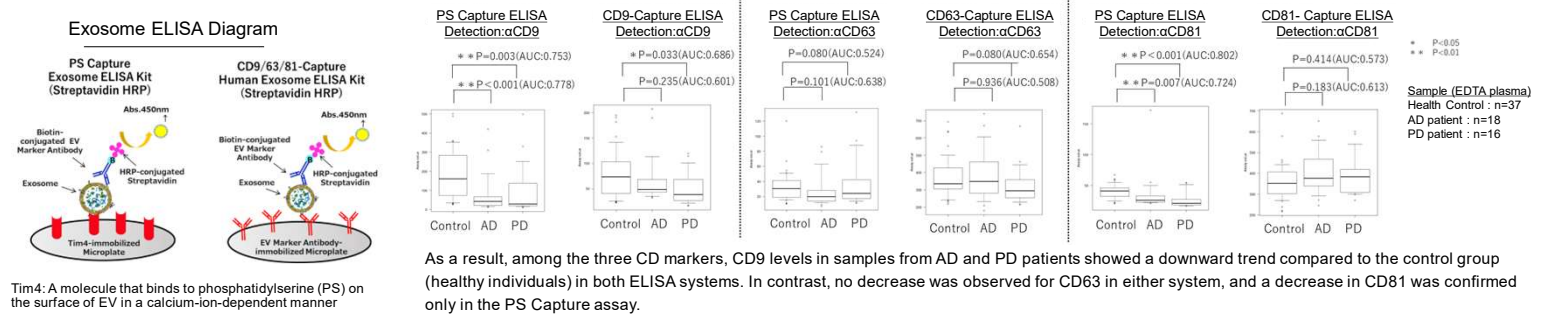
ONaoko Imawaka 1, Ryo Ukekawa1, Takahiro Nishibu1 1 Fujifilm Wako Pure Chemical Corporation, Biotechnology Center

1. Abstract

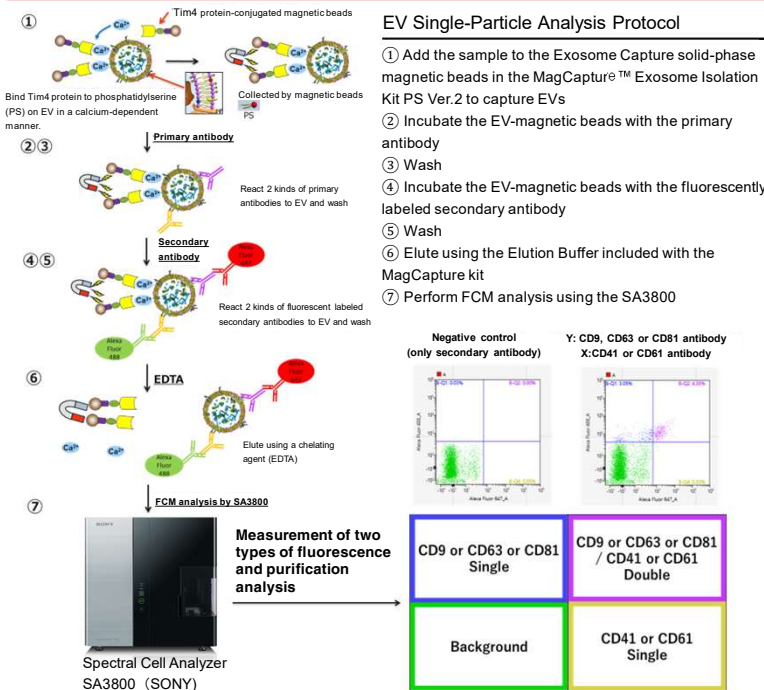
Extracellular vesicles (EVs), including exosomes, are membrane-bound vesicles that contain cell-derived nucleic acids, proteins, lipids, and other components. Since secreted EVs circulate in bodily fluids such as blood, they are expected to serve as biomarkers in the field of neurodegenerative diseases—including Alzheimer's disease (AD) and Parkinson's disease (PD), for which minimally invasive early diagnostic methods are in demand. In this study, to monitor changes in blood EV levels in neurodegenerative diseases, we measured the levels of EV markers CD9, CD63, and CD81 in blood samples from healthy individuals and patients with neurodegenerative diseases using a PS Capture ELISA—which captures EVs via Tim4, a protein that binds to phosphatidylserine on the EV surface in a calcium-dependent manner—and an antibody sandwich ELISA, which captures EVs using EV marker antibodies. The results confirmed that, among the three types of EV markers, CD9 was significantly reduced in plasma samples from AD and PD patients in both ELISA systems. Since a decrease in the amount of CD9-positive EVs was suggested in the blood of patients with neurodegenerative diseases, we investigated the relationship with platelet-derived EVs as part of the characterization of CD9-positive EVs. Single-particle analysis of EVs using FCM revealed that approximately 90% of platelet marker CD41-positive EVs and CD61-positive EVs co-express CD9. Furthermore, PS Capture ELISA and antibody sandwich ELISA showed that not only CD9 levels but also CD41 and CD61 levels in EVs in the plasma of patients with neurodegenerative diseases tended to be lower than in healthy individuals. These results suggest that platelet-derived EVs may be reduced in the blood of patients with neurodegenerative diseases.

2. EV Marker Measurement by Exosome ELISA

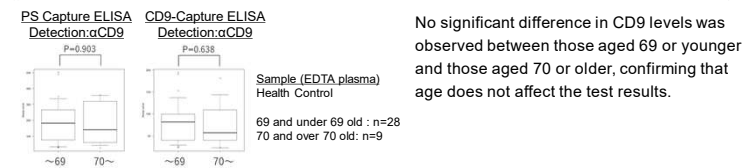
We measured the levels of CD9, CD63, and CD81 in plasma samples from healthy individuals and patients with neurodegenerative diseases (AD and PD) using a Tim4-based PS affinity ELISA (PS Capture ELISA) and an antibody sandwich ELISA (CD9/CD63/CD81-Capture ELISA). In addition, measurements were calculated using purified EVs derived from COLO201 cells (CD9/CD63/CD81-positive EVs) as ELISA standards.



3. Single-particle analysis of PS-positive EVs



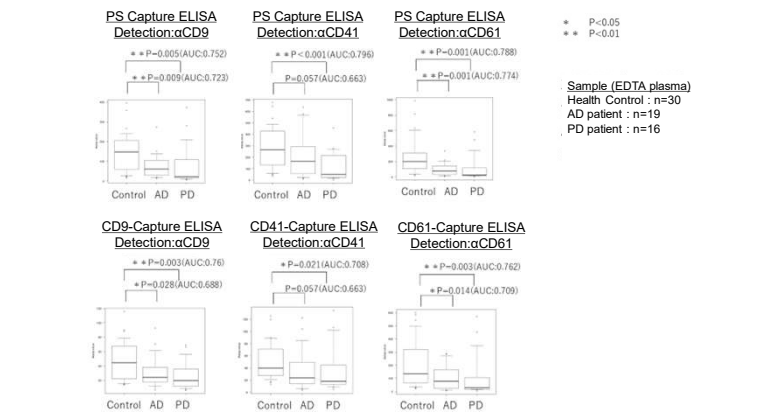
[Reference] Comparison of CD9 marker levels between healthy subjects aged 69 and under and those aged 70 and over



4. Measurement of platelet markers using exosome ELISA

We measured the levels of the EV marker CD9 and the platelet markers CD41 and CD61 in plasma samples from healthy individuals and patients with neurodegenerative diseases (AD and PD) using PS Capture ELISA and antibody sandwich ELISA.

In addition, purified EVs derived from COLO201 cells were used as ELISA standards for CD9 measurement, while purified EVs derived from EDTA plasma from healthy individuals (CD41/CD61-positive EVs) were used for CD41 and CD61 measurements.

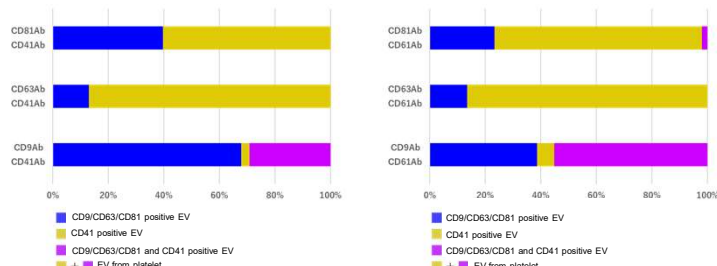


As a result, samples from patients with AD and PD showed a tendency toward lower levels of CD41 and CD61, as well as CD9, compared to healthy individuals.

5. Summary

These results indicate that many platelet-derived EVs co-express CD9, and that levels of CD9, as well as the platelet markers CD41 and CD61, are reduced in plasma samples from patients with AD and PD, suggesting a possible reduction in platelet-derived EVs in the blood of these patients. Regarding the association between platelets and neurodegenerative diseases, previous reports have indicated that platelets are involved in the pathophysiological mechanisms and progression of AD, and that abnormalities in the morphology and function of platelets are observed in PD patients; it is therefore conceivable that this may also affect EV secretion. Moving forward, we plan to investigate the levels of platelet-derived EV markers in other diseases to assess their utility as biomarkers for neurodegenerative diseases.

We examined the co-expression of the EV markers CD9, CD63, and CD81, as well as the platelet markers CD41 and CD61, in PS-positive EVs using EDTA-treated plasma from healthy individuals via single-particle analysis by FCM. The proportion of EVs expressing only the EV markers CD9, CD63, and CD81 is shown in blue; the proportion of EVs expressing only the platelet markers CD41 and CD61 is shown in yellow; and the proportion of EVs co-expressing these markers is shown in purple.



The results showed that while approximately 90% of platelet-derived CD41-positive EVs and CD61-positive EVs (yellow + purple) co-expressed CD9 (purple), co-expression of CD63 and CD81 was limited to a few percent or less, confirming that many platelet-derived PS-positive EVs express CD9.