Identification of blood-brain barrier membrane protein interacting with exosome derived from breast cancer cell line (MCF-7) in human

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Cancer-derived exosome circulating in the blood stream have the potential to interact with vascular endothelial cells. A recent report has shown that the internalization of brain-metastatic-breast cancer cell line derived exosome into brain capillary endothelial cells was involved in the migration of cancer cells across the blood-brain barrier (BBB) (Nat Commun. 2015;6:6716.). However, the mechanism of interaction between cancer-derived exosome and brain capillary endothelial cells still remains unknown. The purpose of the present study was to identify the candidate(s) of cancer-derived exosome-interacting proteins expressed on the human brain vascular endothelial cells (hCMEC/D3 cells). An exosome enriched fraction was isolated from the conditioned medium of MCF-7 cells, a human breast cancer cell line, by ExoQuick TC. The MCF-7-derived exosome was taken up by hCMEC/D3 cells at 37 °C in a concentrative manner. The uptake was significantly reduced at 4°C. These results indicate that an energy dependent transport system could be involved in the exosome internalization into hCMEC/D3 cells. A cross-linking experiment and comprehensive proteomic analysis were employed to identify the targeted proteins on hCMEC/D3 cells. As positive control, cross-linking and proteomic identification of the transferrin interacting receptor were carried out. Holo-formed serotransferrin was labeled with a multifunctional cross-linker, Sulfo-SBED and cross-linked with its receptor protein expressed on the surface of hCMEC/D3 cell suspension at 4 °C. Proteomic analysis with data dependent acquisition mode succeeded to identify transferrin receptor protein 1 as the transferrin-interacting protein. The cross-linking of MCF-7 derived exosome and hCMEC/D3 cell suspension were performed in the same way. The exosome-interacting proteins were identified by comprehensive quantitative proteomics, known as SWATH™ method. SWATH analyses identified five cell adhesion proteins and one cell junction protein. In conclusion, six candidates of exosome-interacting proteins on hCMEC/D3 cells were identified by the combined methods of cross-linking and comprehensive proteomics.