Methods: We performed quantitative proteomics mass spectrometry in paired 20 FFPE biopsy breast cancer samples consist of non-responsive and responsive groups to chemotherapy. For verification of enriched biomarkers and biological pathways, ten human breast cancer cell lines enrolled and verified biological functions through molecular biology-driven assays, including RNAI, cell-titer-glo luminescent assay, mitochondrial membrane potential assay (MMPA), IF, exosome uptake assay, time-lapse live cell imaging system, and the 3D tumor spheroid-based function assays. Machine learning analysis using caret recursive feature elimination to select and apply them to an independent cohort with 50 FFPE biopsy samples to discover the most optimal combination of immunohistochemical biomarkers to predict chemo-responsiveness.

Results: A total of 6,424 proteins were identified and 254 were confirmed to be significantly altered proteins related to chemotherapeutic response. From the patient group with chemo-resistance, we featured 56 upregulated proteins considerably in six closely related subcellular organelles concerning transcellular transportation system based on domain knowledge for text-mining and public network databases for network analysis.
14 intracellular exosomal transportation markers were identified to control chemo-sensitivity through siRNA array panel assay. Live cell images showed changed exosomal trafficking in cell lines manipulated by candidate markers. Through the verification step by machine learning analysis, we selected five markers and applied them to an independent cohort with FFPE biopsy samples to discover the most optimal combination of immunohistochemical biomarkers to predict chemo-responsiveness.

Conclusions: The present study provides the first evidence to identify a predictive biomarker for chemotherapeutic response based on in-depth proteomics. The newly discovered biomarkers and biological evidence can provide the novel insight to overcome chemo-resistance in breast cancer.

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Funding: This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea.

Disclosure: All authors have declared no conflicts of interest.