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Identification of actin network proteins, talin-1 and filamin-A, in circulating extracellular vesicles as blood biomarkers for human myalgic encephalomyelitis/ chronic fatigue syndrome

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Abstract

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a serious, debilitating disorder with a wide spectrum of symptoms, including pain, depression, and neurocognitive deterioration. Over 17 million people around the world have ME/CFS, predominantly women with peak onset at 30–50 years. Given the wide spectrum of symptoms and unclear etiology, specific biomarkers for diagnosis and stratification of ME/CFS are lacking. Here we show that actin network proteins in circulating extracellular vesicles (EVs) offer specific non-invasive biomarkers for ME/CFS. We found that circulating EVs were significantly increased in ME/CFS patients correlating to C-reactive protein, as well as biological antioxidant potential. Area under the receiver operating

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characteristic curve for circulating EVs was 0.80, allowing correct diagnosis in 90–94% of ME/CFS cases. From two independent proteomic analyses using circulating EVs from ME/CFS, healthy controls, idiopathic chronic fatigue, and depression, proteins identified from ME/CFS patients are involved in focal adhesion, actin skeletal regulation, PI3K-Akt signaling pathway, and Epstein-Barr virus infection. In particular, talin-1, filamin-A, and 14–3-3 family proteins were the most abundant proteins, representing highly specific ME/CFS biomarkers. Our results identified circulating EV number and EV-specific proteins as novel biomarkers for diagnosing ME/CFS, providing important information on the pathogenic mechanisms of ME/CFS.

Keywords

ME/CFS; circulating EV; non-invasive biomarkers; actin network proteins

Introduction

ME/CFS is a serious and complex debilitating disease with a wide spectrum of symptoms, including muscle pain and neurocognitive deterioration that occurs following ME/CFS development (2015; Gallagher et al., 2004; Twisk, 2014, 2018). The diagnosis of ME/CFS is based on clinical symptoms that include a broad spectrum of disease severity from mild to debilitating. Conclusive ME/CFS non-invasive diagnosis is thus difficult for clinicians to achieve using the current ME/CFS diagnostic methods and subjective symptoms, and although immunological abnormalities including impaired calcium ion channel (Brenu et al., 2013; Brenu et al., 2014; Brenu et al., 2011; Cabanas et al., 2019; Jason et al., 2009; Montoya et al., 2017), dis-regulation of the neuronal-immunological system (G and Maes, 2014; Komaroff et al., 2018), abnormalities of metabolism (Nagy-Szakal et al., 2018; Naviaux et al., 2016), and has been recognized as an important contributor to ME/CFS, the pathogenic mechanisms are not fully understood. Potential ME/CFS biomarkers that have been proposed include changes in autonomic nervous function (Van Cauwenbergh et al., 2014), circulating cytokines (Broderick et al., 2010; Moneghetti et al., 2018; Yang et al., 2019), Epstein-Barr (EB) virus (Loebel et al., 2017), energy metabolism (Castro-Marrero et al., 2013; Mikirova et al., 2012), oxidative stress (Maes et al., 2011), and sleep-wake cycle (Togo and Natelson, 2013), but additional biomarkers are needed to distinguish ME/CFS from other diseases associated with fatigue, such as idiopathic chronic fatigue (ICF) and depression. Current ME/CFS therapies, cognitive behavior therapy and graded exercise treatment, are not fully effective (Cleare et al., 2015). The discovery of objective ME/CFS biomarkers, as well as ME/CFS pathogenic mechanisms including ME/CFS etiology, represent a critical breakthrough long-awaited in the field of ME/CFS (Lloyd and Meer, 2015).

Extracellular vesicles (EVs) are released from damaged or stressed cells with cellular content, such as proteins, and circulate in the bloodstream (Yanez-Mo et al., 2015). EVs are thus recognized as non-invasive biomarkers for a variety of diseases (Yanez-Mo et al., 2015). Furthermore, EVs contribute to disease pathogenesis via their function in cell-to-cell communications and delivering EV contents from the cell or origin to target cells, resulting in modulation of cell signaling in target cells (Eguchi and Feldstein, 2018; Yanez-Mo et al.,

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2015). Circulating EV number and EV composition can be used for diagnosis of human diseases, including chronic liver diseases (Eguchi et al., 2019; Shah et al., 2018) as well as for metabolic status (Kobayashi et al., 2018).

MATERIALS AND METHODS

Subjects and study design

The study was approved by the ethics committees of Kansai University Welfare of Science (Approval No. 09–06) and Osaka City University Graduate School of Medicine (Approval No. 2151), and was conducted in accordance with the Declaration of Helsinki. All subjects, ME/CFS patients (n=99), ICF patients (n=6), depression patients (n=8), and healthy individuals (n=56) provided written informed consent for participation in the study before enrolment. Healthy individuals who were confirmed not to have abnormal results on any major clinical laboratory tests (hemoglobin, CRP, albumin, triglycerides, glucose, AST, ALT, or cholesterol, etc.), BMI 30 and <17, subjective sleep problems, problems in daily life by fatigue, and shift worker. Out of 56 recruited healthy individuals three were excluded from the analysis, as they did not meet the inclusion criteria. ME/CFS, ICF, and depression patients who visited the outpatient clinic of Osaka City University hospitals and B clinic were randomly enrolled into the study. ME/CFS and ICF were diagnosed based on the 1994 Center for Disease Control clinical criteria (Fukuda criteria)(Fukuda et al., 1994) and Canadian of Consensus Case Definition (Carruthers et al., 2011) by specialists at the Osaka City University hospitals and B clinic. Depression was assessed using a structured clinical interview for DSM-IV. Exclusion criteria were as follows: 1) neuro-inflammatory or immune disorders diagnosed by clinical laboratory tests and magnetic resonance imaging; 2) any active medical condition that could explain the presence of chronic fatigue; 3) presence of any diagnosable illness that relapsed or was not completely resolved, such as some types of malignancy or chronic cases of hepatitis B or C virus infection; 4) alcohol or other substance abuse; 5) severe obesity as defined by a body mass index 30 kg/m^2; 6) pregnancy; or 7) lactation. The presence of major depressive disorder, fibromyalgia, or somatoform disorder was not a criterion for exclusion. Abdominal discomfort syndrome was not assessed. Psychiatric disorders associated with CFS symptoms were diagnosed by psychiatrists at Osaka City University Hospitals and B clinic. Blood was collected with or without anticoagulant for plasma or serum, respectively. CRP was measured using serum by LSI Medience (Tokyo, Japan).

Flow cytometry analysis of circulating EVs

EVs were counted using calcein-AM via flow cytometry, as described before(Kobayashi et al., 2018). Briefly, plasma was incubated for 30 min at room temperature with final 4 μg/ml of Calcein-AM (Life Technologies, Carlsbad, CA). EV acquisition was performed using the BD LSRII Flow Cytometer System (BD Biosciences, San Jose, CA) or BD Cant II (BD Biosciences, Tokyo, Japan) for validation and data were analyzed using FlowJo software (TreeStar, Ashland, OR). Gating parameters were defined using ultraviolet 2.5 μm Alignflow alignment beads (Life Technologies) and negative controls. Forward and side scatter parameters were plotted on logarithmic scales to best cover a wide size range. Single staining controls were used to check fluorescence compensation settings and to set up

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positive regions. EVs were identified using a forward-scatter analysis. EV number was counted using 2.5 μm Alignflow alignment beads (Life Technologies) as the size standards.

EV size determination

Circulating EVs were ultracentrifuged at 100,000×*g* for 60 min at 10°C and suspended in PBS. For dynamic light-scattering analysis, entire size was measured by Zetasizer Nano ZS90 (Malvern, Worcestershire, UK) and averages were taken from 3 healthy individual or 5 ME/CFS patients. For transmission electron microscopy, EVs were adhered to 100-mesh Formvar and carbon-coated grids for 5 min at room temperature. Grids were washed once with water, stained with 1% uranyl acetate (Ladd Research Industries, Williston, VT) for 1 min, dried and viewed using a JEOL JEM-1400Plus transmission electron microscope (JEOL, Peabody, MA). Images were captured using a Gatan OneView digital camera (Gatan, Pleasanton, CA).

Oxidative stress

Both oxidation and anti-oxidation activities were measured simultaneously in serum(Fukuda et al., 2016). Briefly, oxidative activity was assessed by measuring d-ROMs (Diacron International, Grosseto, Italy) and anti-oxidative activity by measuring the Biological Antioxidant Potential (BAP) (Diacron International) using an AU480 automated analyzer (Beckman Coulter, Tokyo, Japan).

Analysis of protein composition in circulating EVs via nanoLC-MS/MS

Circulating EVs were isolated from equal amount of plasma via qEV (Izon Science, Cambridge, MA) according to the instructions from the manufacturer. Briefly, the same amount of plasma from each patient/individual was applied on the qEV column and corrected EVs as fractions. For the first group (CFS and HC; n=3 each), EVs were concentrated via Amicon ultra 3 K centrifugal filter (Millipore, Burlington, MA), followed by alkylation with iodoacetamide and trypsinization using the same amount of proteins among samples. Samples were separated using a nanoLC-MS/MS, Dina System (KYA Technologies, Tokyo, Japan) for nanoLC and AB SCIEX Triple TOF 5600 system (AB SCIEX, Tokyo, Japan) for MS/MS at Oncomics Co. (Nagoya, Japan). For the second group (CFS, ICF, and depression who were between the ages of 20 and 40: n=4 each), EVs were precipitated with trichloroacetic acid, followed by reduction, alkylation with iodoacetamide and trypsinization using the same amount of proteins among samples. Samples were separated using nanoLC-MS/MS, EASY-nLC 1200 (Thermo Fisher Scientific, Tokyo, Japan) and Q Exactive Plus (Thermo Fisher Scientific) at APRO Science Institute (Tokushima, Japan). Data from the first and second groups were analyzed using Scaffold4 (Proteome Software, Portland, OR) against the SwissProt database at APRO Science Institute. Quantitative value (normalized total spectra) on Scaffold4 was converted to log scale, and then was used for Heat map using Heatmapper software (Babicki et al., 2016). Protein interactions were generated using STRING v10.5 software (Szklarczyk et al., 2015) selecting MCL clustering and confidence levels for network edges based on experiments and databases. KEGG pathway was generated on STRING software.

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Statistics

All data are expressed as mean ± standard error of the mean (SEM) unless otherwise indicated. Differences between groups were compared using the Mann-Whitney test or Kruskal-Wallis test. Correlations of EV number with CRP, BAP, and d-Roms were determined using the Spearman rank-sum test. For statistical analysis on proteomics, we converted quantitative values (normalized total spectra), which are automatically generated on Scafolld4 proteome software, to log scale, then, calculated significance by Mann-Whitney test in an initial cohort (two groups: 3 healthy individuals and 3 ME/CFS patients) or by Kruskal-Wallis test in a second subsequent independent cohort (three groups: 4 ME/CFS patients, 4 ICF patients, and 4 depression patients). Statistical analyses were performed using SPSS version 22.0J software (SPSS Japan, Tokyo, Japan). The statistical analyses for the ROC curve were performed using Prism Software (Graph Pad, La Jolla, CA).

RESULTS

Current evidence from our own and other laboratories has led us to investigate whether the number of circulating EV is increased in ME/CFS. A first group (ME/CFS 1) included 33 healthy controls (HC) and 39 ME/CFS patients diagnosed with ME/CFS based on the 1994 Center for Disease Control clinical criteria(Fukuda et al., 1994), matched with age, gender, weight, and BMI (Supplementary table 1). Circulating EVs were stained with calcein to count the intact circulating EVs (Kobayashi et al., 2018), excluding contaminated proteins in plasma and quantifying the calcein-positive circulating EVs based on the intensity of unstained circulating EVs via flow cytometry (Fig. 1A). The dot plot of gated population is shown in supplementary figure 1. The circulating EV number was significantly increased in ME/CFS patients ($P < 0.001$) (Fig. 1B). To confirm those results, we recruited 30 more ME/CFS patients (ME/CFS 2), excluding smokers and obese individuals (Supplementary table 1). Circulating EV number was also significantly increased in ME/CFS patients ($P < 0.001$) (Fig. 1B) and the mean circulating EV number was similar between ME/CFS 1 and ME/CFS 2 (Fig. 1B). An increased circulating EV number in ME/CFS was further validated in an additionally recruited 20 HC and 30 ME/CFS patients ($P < 0.001$) (Supplementary Fig 1B). Circulating EVs consisted of small EVs (diameter, <100 nm) and large EVs (diameter, 100–1000 nm) in both ME/CFS patients and HC as determined via dynamic light scattering analysis (Fig. 1C) and in ME/CFS patients via transmission electron microscopy (TEM) (Fig. 1D). Circulating EV number correlated significantly with serum c-reactive protein (CRP) levels ($\rho = 0.442$, $P = 0.0007$) (Fig. 1E), and this correlation persisted even in cases with CRP levels within the normal range (0 to 0.1) ($\rho = 0.310$, $P = 0.032$) (Supplementary Fig. 1C) and BAP as antioxidant potential, ($\rho = -0.314$, $P = 0.007$) (Fig. 1F), two known markers that are increased in ME/CFS(Fukuda et al., 2016). To determine specificity and sensitivity, we determined the ROC curve using circulating EV number, CRP, d-Roms, and BAP levels. Area under ROC curve (AUC) of circulating EV number in ME/CFS 1 was 0.802 (95%CI 0.70–0.90; $P < 0.0001$) (EVs versus CRP: $P < 0.001$; EVs versus BAP: $P < 0.05$), significantly higher than the AUC of CRP levels (0.641; 95%CI 0.49–0.79), AUC of d-Roms levels (0.540; 95%CI 0.41–0.67) and AUC of BAP levels (0.710; 95%CI 0.59–0.83;

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$P = 0.002$) (Fig. 1G). The ME/CFS 2 group was diagnosed with ME/CFS in 90–94% using several cutoff values the from AUC of EV number (Supplementary table 2).

Increased levels of circulating EV number with an AUC of 0.802 for ME/CFS diagnosis led us to further investigate whether assessing EV cargo would lead to the discovery of specific EV signatures in ME/CFS patients that may represent novel biomarkers for this disease. Purified circulating EVs from three HC and three ME/CFS patients (Supplementary table 3) were ascertained in terms of the EV proteome by nano liquid chromatography tandem-mass spectrometry (nanoLC-MS/MS) analysis (Supplementary Fig 2A). A total of 124 proteins were present in EVs from both ME/CFS patients and HC, but a significant number of proteins was present only in EVs from ME/CFS patients, while 22 proteins were present only in EVs from HC (Fig. 2A, supplementary table 4). Furthermore, 75 proteins were significantly changed in EVs from ME/CFS patients compared to those from HC ($P < 0.05$), including 66 up-regulated proteins including actin network proteins, such as talin-1, filamin-A, actin, myosin-9, vinculin, gelsolin, and tubulin, and 9 down-regulated proteins (Fig. 2B, supplementary table 5). Notably, 63 up-regulated proteins made a cluster in protein-protein interactions (Fig. 2C) relating to several pathways, focal adhesion, regulation of the actin cytoskeletal, phosphoinositide-3-kinase (PI3K)-Akt signaling pathway, and EB virus infection (Fig. 2C and 2D, supplementary table 6).

We further explored whether identified proteins in EVs from ME/CFS can distinguished from ICF and depression two condition also associated with fatigue. The number of circulating EVs was similar among the three groups (Supplementary Fig 3A). Purified circulating EVs from four ICF, four depression, and four ME/CFS patients (Supplementary table 7) were ascertained for the EV proteome by nanoLC-MS/MS analysis (Supplementary Fig 3B). A total of 579 proteins were present in EVs from ME/CFS, ICF, and depression patients. A significant number of proteins (176 of 579 proteins) were present only in EVs from ME/CFS patients, while 20 and 32 proteins were present only in EVs from ICF and depression patients, respectively (Fig. 3A, supplementary table 8). In addition, 134 proteins were significantly changed in EVs from ME/CFS patients compared to those from ICF and depression patients ($P < 0.05$) and there were 111 up-regulated and 23 down-regulated proteins (Fig. 3B, supplementary table 9). Notably, actin network proteins such as talin-1, filamin-A, actin, myosin-9, vinculin, gelsolin, and tubulin, were also significantly up-regulated in EVs from ME/CFS patients compared to those from ICF and depression (Fig. 3B). Of the 111 up-regulated proteins, 105 were associated with core protein-protein interactions (Fig. 3C) and involved in focal adhesion, regulation of the actin cytoskeleton, PI3K-Akt signaling pathway, and EB virus infection (Fig. 3C and 3D, supplementary table 10).

A specific circulating EV profile, from two independent experiments using ME/CFS and HC or ME/CFS, ICF, and depression allowed us to identify specific proteins in blood EVs for ME/CFS diagnosis. From the list of significantly changed proteins in EVs from ME/CFS patients, 31 proteins were identified as common proteins in EVs from ME/CFS patients, including 30 up-regulated and 1 down-regulated protein (supplementary table 11). Of the 31 identified proteins in EVs, 29 formed a cluster in protein-protein interactions (Fig. 4A), involved in regulation of the actin cytoskeleton, focal adhesion, PI3K-Akt signaling

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pathway, and EB virus infection (Fig. 4B, supplementary table 12). The 12 most abundant proteins in EVs were closely matched in the two independent experiments and part of the actin network protein family (talin-1, filamin-A, actin, actinin, vinculin, gelsolin, and integrin) (Fig. 4C and 4D) and 14–3-3 family proteins (Fig. 4C). In particular, the top three proteins in EVs, talin-1, filamin-A, and actin, were exactly the same in two independent experiments (Fig. 4C). These results revealed that actin network proteins in EVs are novel non-invasive biomarkers for ME/CFS diagnosis.

DISCUSSION

This is the first report to find that actin network proteins including talin-1 and filamin-A in circulating EVs can be used for specific ME/CFS diagnosis, distinguishing from ICF and depression. We also showed that the number of circulating EVs was significantly increased in ME/CFS compared to healthy controls, confirming the findings recently reported (Castro-Marrero et al., 2018). Current potential biomarkers reported with AUC 0.7–0.8 for ME/CFS diagnosis include peripheral blood mononuclear cell gene expression (Frampton et al., 2011), plasma neuropeptide Y for symptom severity (Fletcher et al., 2010a), natural killer cell function (Fletcher et al., 2010b), cytokines in women (Fletcher et al., 2009), metagenomic or metabolomics (Nagy-Szakal et al., 2018), and blood pressure/peripheral pulse characteristics (Allen et al., 2012; Frith et al., 2012), which are also changed with orthostatic disturbance and heart diseases. Overall the current evidence based on many ME/CFS studies measuring cytokines suggest that changes in circulating cytokines do not seem to explain the core characteristic of ME/CFS (Blundell et al., 2015). We discovered new pathways regulating actin cytoskeletal and focal adhesion, and detected several other pathways (PI3K-Akt signaling pathway, and EB virus infection) that have been reported in ME/CFS studies (Navaneetharaja et al., 2016). Actin network proteins, talin-1, actin, alpha-actinin-1, vinculin, gelsolin, and integrin are essential proteins for cytoskeletal connections and ion channels connections resulting in effect on intracellular signaling (Dalghi et al., 2018; Sasaki et al., 2014), indicating that this profile may be matched to a wide variety of ME/CFS symptoms, including muscle weakness/pain, endocrine disorder, and brain inflammation. Actin network proteins also play important roles in the skeletal muscle as follows: 1) talin 1 regulates the stability of myotendinous junctions through the vinculin-talin-integrin system in skeletal muscle (Conti et al., 2008); and 2) human serum gelsolin is mainly derived from skeletal muscle (Kwiatkowski et al., 1988). Our results suggest that skeletal muscle damage may be involved in ME/CFS pathology, since ME/CFS patients lose mobility with progression of the disease and graded exercise is one of the treatments proven to have an impact on these patients (Pietrangelo et al., 2018). Actin network proteins including talin, vinculin filamin, actin, and actinin, also have an important role in cardiac myocyte and heart function (Zemljic-Harpf et al., 2009), thus the results in this study may additionally reflect a potential abnormality of heart function in ME/CFS patients. Furthermore, we also detected 14–3-3 family proteins that are associated with virus infections including EB virus. Interestingly, EB virus components were detected in a large cohort of ME/CFS patients (Pedersen et al., 2019). EB virus components trigger systemic autoimmune diseases (Draborg et al., 2016) and deficient EBV-specific B- and T-cell response were observed in ME/CFS patients (Loebel et al., 2014). This evidence led us to

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hypothesize that EVs may be involved in ME/CFS progression or etiology through EB virus infection and subsequent triggering of autoimmune disease in skeletal muscles, although future studies will need to investigate this hypothesis further.

CONCLUSION

In conclusion, we revealed that circulating EV levels are significantly increased in ME/CFS patients. These EVs contain a specific protein cargo, particularly actin network proteins and 14–3-3 family proteins, which represent novel-specific ME/CFS biomarkers and can distinguish this condition from ICF and clinical depression, which are two highly challenging differential diagnoses in the clinical arena. Future studies including larger cohorts that would allow for matching the various conditions by key variables such as age and gender as well as external validation studies are warranted.

The novel findings of this study may open new windows to reveal ME/CFS pathogenic mechanisms and may aid in the development of better ME/CFS biomarkers and effective therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ME/CFS	myalgic encephalomyelitis/chronic fatigue syndrome
EVs	extracellular vesicles
CRP	C-reactive protein
BAP	biological antioxidant potential
d-Roms	diacron-reactive oxygen metabolites
AUC	area under the receiver operating characteristic curve
ICF	idiopathic chronic fatigue

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- Circulating EV number was increased in ME/CFS patients correlating to CRP and BAP
- AUROC for circulating EVs was 0.802 allowing correct diagnosis in 90–94% of ME/CFS
- Proteins in actin skeletal regulation and EB virus infection were identified in ME/CFS patients
- Talin-1, filamin-A and 14–3-3 proteins were the most abundant proteins representing highly specific ME/CFS

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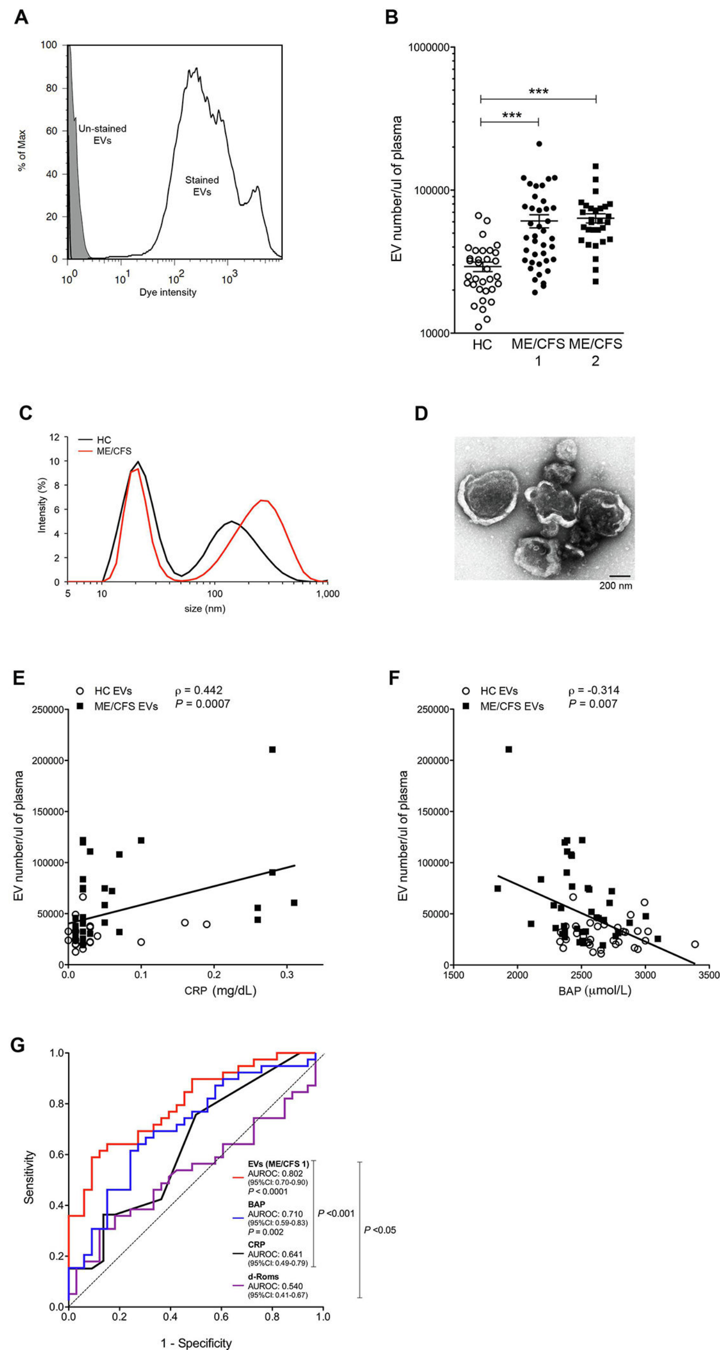

Figure 1. Circulating EVs are diagnostic factor in ME/CFS patients correlating to CRP and BAP.
A-D) Characterization of circulating EVs from ME/CFS patients, first group (ME/CFS 1) and second group (ME/CFS 2), or healthy controls (HC) via flow cytometry. **A)** Histograph of unstained or stained circulating EVs from ME/CFS patient. **B)** Quantification of calcein-positive circulating EVs. **C)** Dynamic light scattering analysis of isolated EVs from ME/CFS patients (ME/CFS) or HC. **D)** Transmission electron microscopy of isolated EVs from ME/CFS patients. **E)** Dot plot of circulating EVs and CRP level. **F)** Dot plot of

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circulating EVs and BAP level. **G)** ROC curve with circulating EVs, CRP level, BAP level, and d-Roms level. HC: healthy individual. Values represent mean ± SEM. ***P < 0.001.

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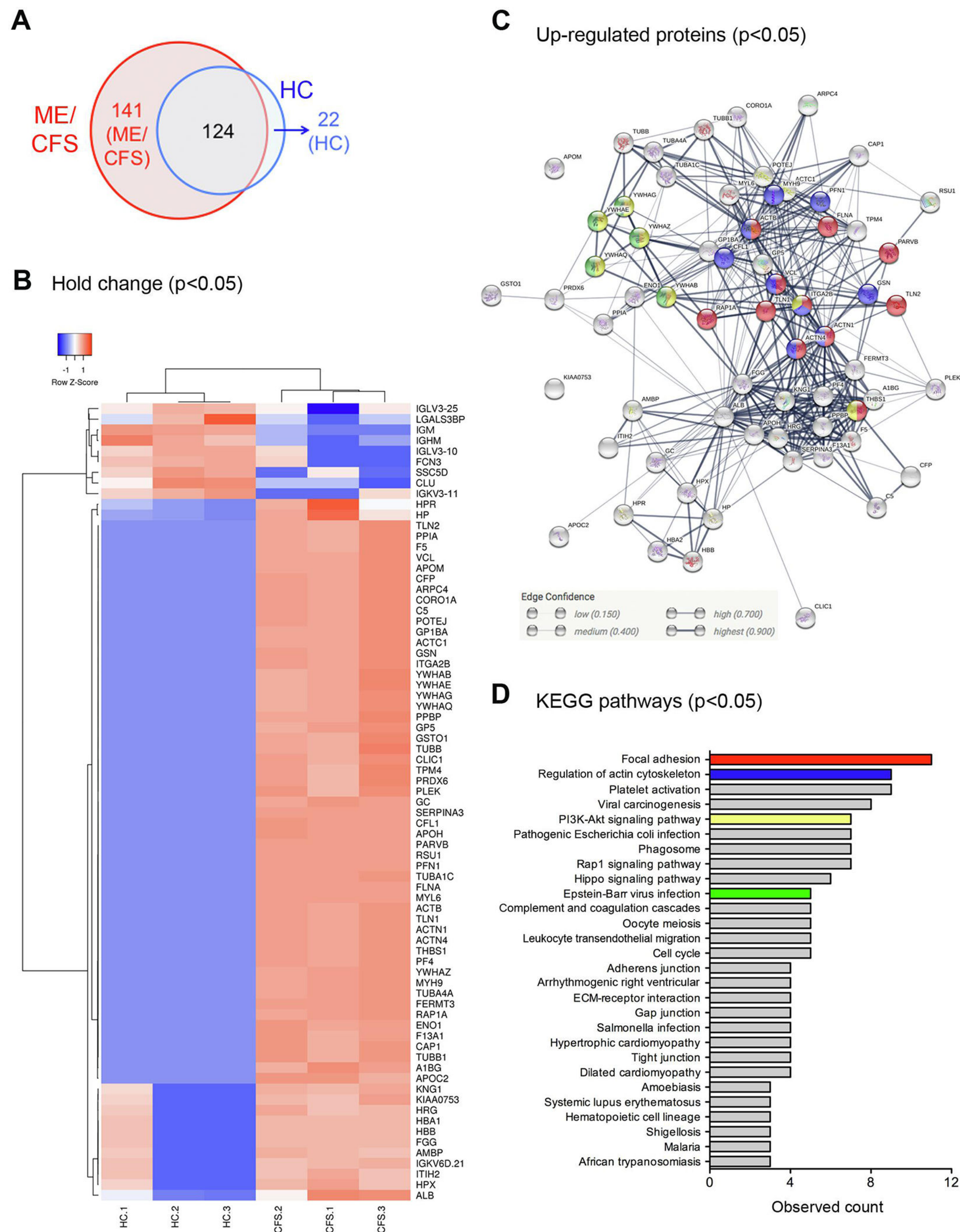

Figure 2. Detected proteins in circulating EVs from ME/CFS and HC via proteome analysis.
A) Detected protein number. **B)** Heat map analysis of significantly changed proteins between ME/CFS and HC (p<0.05). **C)** Protein interaction using significantly up-regulated proteins in ME/CFS patients (p<0.05). The colors correspond to figure D. **D)** KEGG pathway for significantly up-regulated proteins in ME/CFS patients. Arrhythmogenic right ventricular, Arrhythmogenic right ventricular cardiomyopathy.

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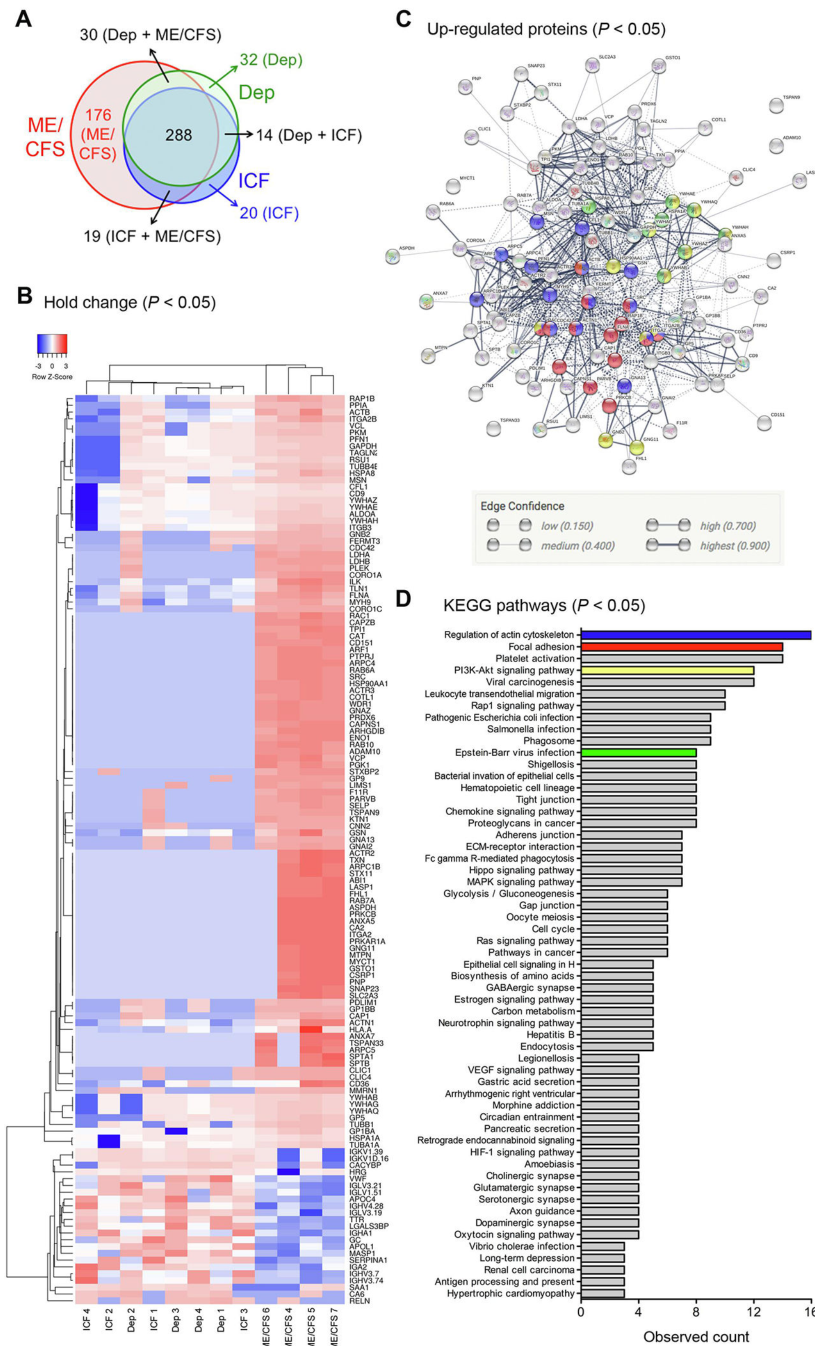

Figure 3. Proteins detected in circulating EVs from ME/CFS, ICF, and depression via proteome analysis.

A) Detected protein number. **B)** Heat map analysis of significantly changed proteins between ME/CFS and HC (p<0.05). **C)** Protein interaction using significantly up-regulated proteins in ME/CFS patients (p<0.05). The colors correspond to figure D. **D)** KEGG pathway in significantly up-regulated proteins in ME/CFS patients. ICF, idiopathic chronic fatigue; Arrhythmogenic right ventricular, Arrhythmogenic right ventricular

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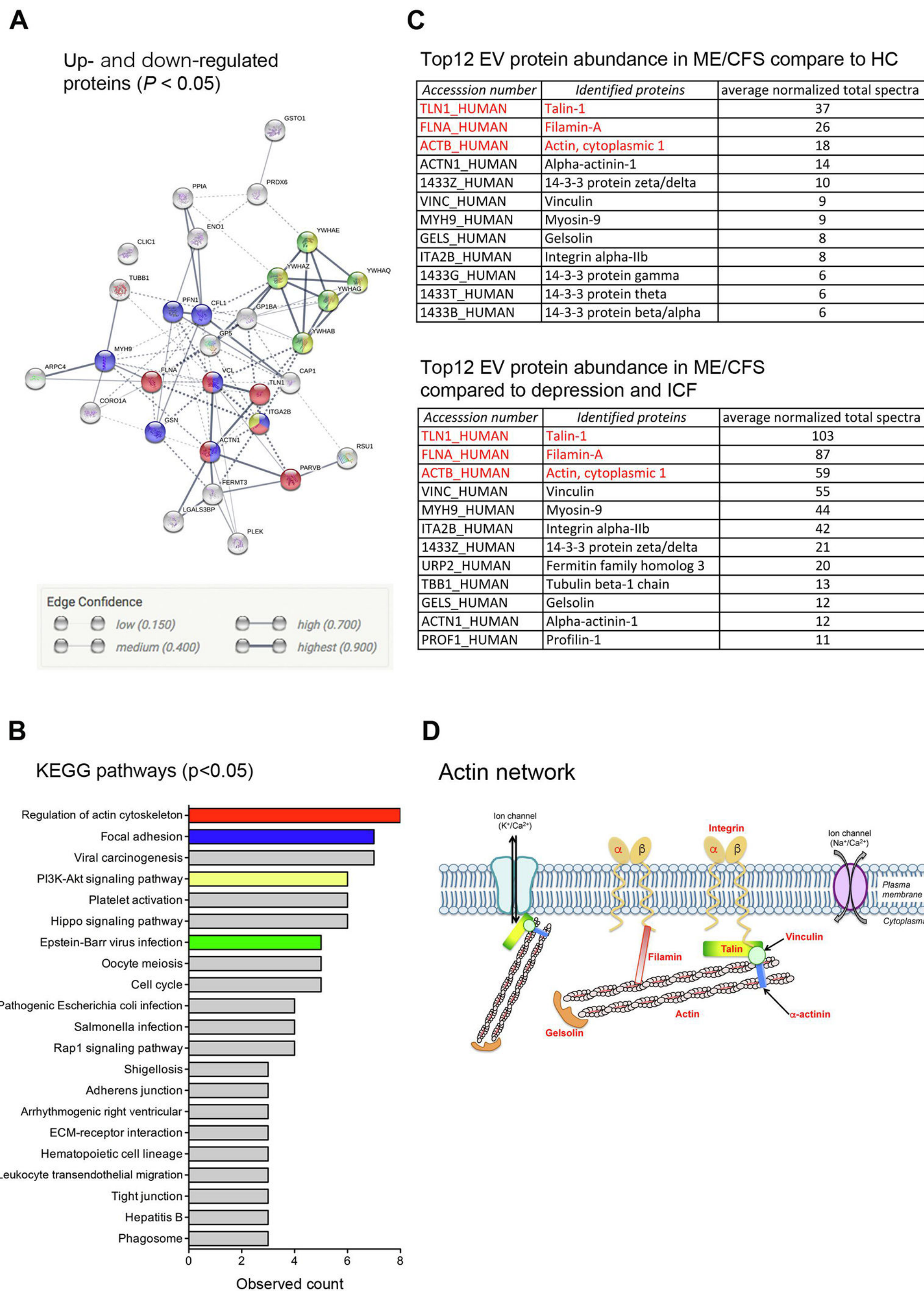
cardiomyopathy; Epithelial cell signaling in H, Epithelial cell signaling in Helicobacterpylori infection.



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Top12 EV protein abundance in ME/CFS compare to HC

Accession number	Identified proteins	average normalized total spectra
TLN1_HUMAN	Talin-1	37
FLNA_HUMAN	Filamin-A	26
ACTB_HUMAN	Actin, cytoplasmic 1	18
ACTN1_HUMAN	Alpha-actinin-1	14
1433Z_HUMAN	14-3-3 protein zeta/delta	10
VINC_HUMAN	Vinculin	9
MYH9_HUMAN	Myosin-9	9
GELS_HUMAN	Gelsolin	8
ITA2B_HUMAN	Integrin alpha-IIb	8
1433G_HUMAN	14-3-3 protein gamma	6
1433T_HUMAN	14-3-3 protein theta	6
1433B_HUMAN	14-3-3 protein beta/alpha	6

Top12 EV protein abundance in ME/CFS compared to depression and ICF

Accession number	Identified proteins	average normalized total spectra
TLN1_HUMAN	Talin-1	103
FLNA_HUMAN	Filamin-A	87
ACTB_HUMAN	Actin, cytoplasmic 1	59
VINC_HUMAN	Vinculin	55
MYH9_HUMAN	Myosin-9	44
ITA2B_HUMAN	Integrin alpha-IIb	42
1433Z_HUMAN	14-3-3 protein zeta/delta	21
URP2_HUMAN	Fermitin family homolog 3	20
TBB1_HUMAN	Tubulin beta-1 chain	13
GELS_HUMAN	Gelsolin	12
ACTN1_HUMAN	Alpha-actinin-1	12
PROF1_HUMAN	Profilin-1	11

Figure 4. Identified proteins for specific non-invasive biomarker in ME/CFS.

A) Protein interaction using significantly changed proteins from two independent proteome analyses, ME/CFS vs HC or ME/CFS vs ICF and depression. The colors correspond to figure B. **B**) KEGG pathway for significantly changed proteins from two independent proteome analyses, ME/CFS vs HC or ME/CFS vs ICF and depression. **C**) Top 12 proteins from each proteome analysis. **D**) Scheme for detected actin network proteins, with red

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letters. ICF, idiopathic chronic fatigue; Arrhythmogenic right ventricular, Arrhythmogenic right ventricular cardiomyopathy.

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