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Extracellular Vesicles in Psychiatry Research in the Context of RDoC Criteria

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The analysis of extracellular vesicles has been accelerated because of the technological advancements in omics methods in recent decades. Extracellular vesicles provide multifaceted information regarding the functional status of the cells. This information would be critical in case of central nervous system cells, which are confined in a relatively sealed biological compartment. This obstacle is more dramatic in psychiatric disorders since their diagnosis primarily depend on the symptoms and signs of the patients. In this paper, we reviewed this rapidly advancing field by discussing definition of extracellular vesicles, their biogenesis and potential use as clinical biomarkers. Then we focused on their potential use in psychiatric disorders in the context of diagnosis and treatment of these disorders. Finally, we tried to combine the RDoC (Research Domain Criteria) with the use of extracellular vesicles in psychiatry research and practice. This review may offer new insights in both basic and translational research focusing on psychiatric disorders.

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Key Words Extracellular vesicle, Depression, Bipolar disorder, Schizophrenia, Research domain criteria.

INTRODUCTION

The cells of multicellular organisms developed various ways of communication for maintaining homeostasis and response to their inner and outer environment.[1] In addition to endocrine, paracrine, juxtacrine and autocrine signaling, the cells send signals via extracellular membrane bound structures, i.e. extracellular vesicles, and can be classified depending on their size, molecular composition and synthesis pathways.[2] The cells within central nervous system uses these extracellular vesicles for long distance communication, which allows nucleic acid (both DNA and different types RNA), protein and special lipid domain transfer between the cells.[3]

The extracellular vesicles can be classified as exosomes, which has a diameter ranging from 30 nm to 100 nm; microvesicles with diameter between 100–1000 nm; apoptotic bodies, which are released from dying cells via apoptotic process, and has a diameter between 800–5000 nm, and large oncosomes, which are released from neoplastic cells and has a diameter larger than 1 μm and can be large as 10 μm.[4,5]

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BIOGENESIS OF EXTRACELLULAR VESICLES

The exosomes are generated via multivesicular bodies (MVB) which are derived from early endosomes. Early endosomes contain lipid rafts with specific proteins e.g. tetraspanins, adhesion, fusion, and receptor transport proteins. With the intraluminal vesicle (ILV) formation, these molecules are incorporated into the exosomal membrane.[6] ESCRT, TSG-101, Alix proteins are responsible for the sorting of exosomes and after packaging, exosomes expelled to the extracellular domain via a fusion process led by Rab GTPases (Figure 1).[7]

Microvesicles are the generated through the budding of plasma membrane, which is regulated through translocation of phospholipids by floppase/flippase enzymes, organization of cytoskeleton under plasma membrane with ADP-ribosylation factor 6 (ARF6), Phospholipase D (PLD), Extracellular-signal regulated kinase (ERK) and Myosin light-chain kinase (MLCK) enzymes.[8] The molecules participating into extracellular vesicle biogenesis process are candidates for EV markers, which useful in detection and isolation of EVs (Figure 2).[9]

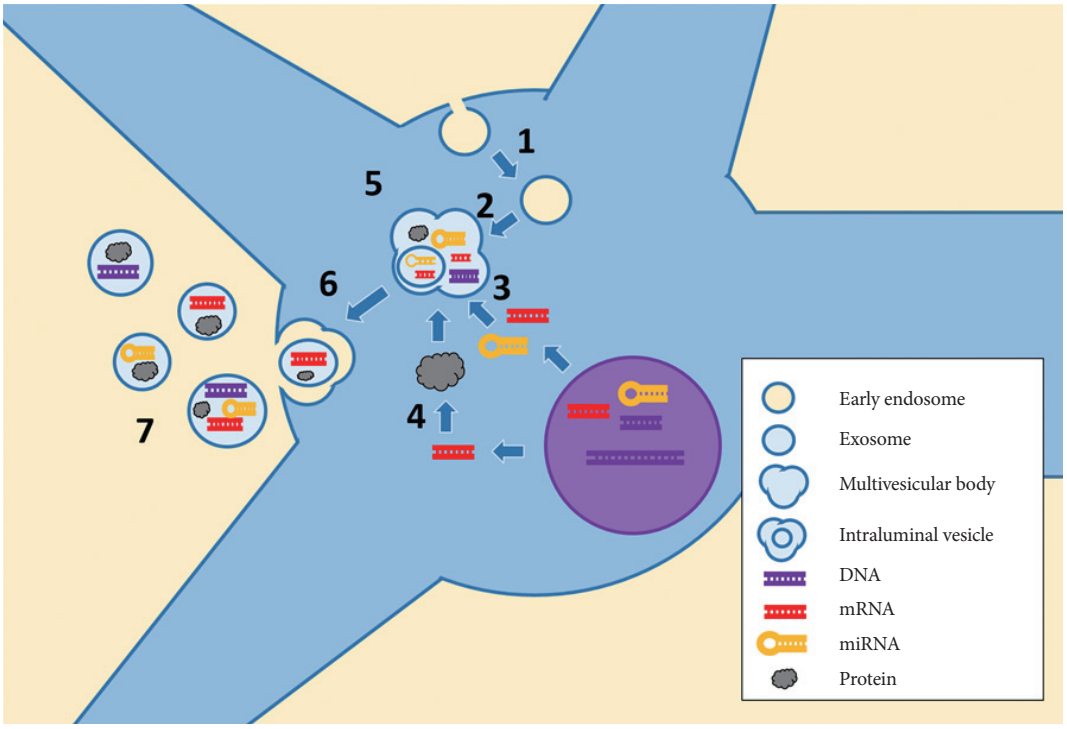

Figure 1. Exosomes Biogenesis The biogenesis of exosomes occurs within the cells through a multistep process. In this figure, the exosome formation is simplified to emphasize the important steps in this cellular process. 1. The exosome formation starts with the inward budding of plasma membranes into the cytoplasm (endocytosis), which leads to the early endosome formation. 2. Early endosomes accumulate and fuse to form multivesicular bodies (MVB). 3. Nucleic acids (mRNA, miRNA, other RNA molecules and fragments of DNA) from the nucleus are transported into the MVB, and accumulate within this membrane bound structure. 4. Some mRNAs translated to protein structures through ribosomes and these proteins are transported into the MVB. 5. The outer membrane of MVB form another internal compartment by budding into this structure. This vesicle inside of MVB is called intraluminal vesicle (ILV). Proteins and nucleic acids are sorted into the ILVs through molecular sorting mechanisms composed of proteins like ESCRT (Endosomal sorting complexes required for transport), TSG 101 (Tumor susceptibility gene), and ALIX (ALG-2-interacting protein X). 6. Exosomes expelled from the cell via exocytosis, where MVB docks with plasma membrane via Rab proteins, and unloads its exosome cargo. The contents of exosomes are diverse, which are composed of various proteins and nucleic acids. 7. Exosomes are secreted to the extracellular compartment. These membrane bound structures can travel through blood and lymphatic vessels, cerebrospinal fluid (CSF), saliva and other secretions of the body. Exosomes possess adhesion and receptor proteins on their surfaces, which allows the exosomes to bind to their specific targets.

ISOLATION OF EXTRACELLULAR VESICLES

EVs can be isolated from serum, saliva, cerebrospinal fluid (CSF), urine, breast milk, synovial fluid, effusions, semen and cell cultures.[10] The isolation of EVs requires either different centrifuge steps up to an ultracentrifuge with 100,000 g and/or mechanical filters, which are specifically designed according to diameter preferred EV subtype.[11] The EV purification and subpopulation selection can be performed with immunocapture methods.[12] The isolated EVs can be imaged with transmission and/or scanning electron microscopes. Transmission electron microscopy can be combined with immunogold method to show a specific protein within or on the EV.[13] The light microscopes with capability of super-resolution can be recruited for the imaging of EVs, particularly for exosomes, which has a diameter smaller than 100 nm.[14] The isolated EVs can be analyzed for their content of proteins, nucleic acids, lipid and carbohydrates.[15]

EXTRACELLULAR VESICLES ORIGINATING FROM CENTRAL NERVOUS SYSTEM

Since CNS is sealed by bones, connective tissues and blood-brain barriers, also contains vital centers for cognition, sensory, motor and autonomic functions, the indications for taking direct biopsies for diagnostic purposes is limited only to some life threating conditions like infection/abscess or brain tumors.[16] Many central nervous system pathologies are diagnosed with imaging modalities, including computed tomography (CT) and magnetic resonance imaging (MRI).[17] Through these imaging modalities are capable of noninvasively monitoring the organs within CNS, generally they don't any functional information regarding cellular processes.[18] The functional imaging modalities like functional MRI (fMRI) or

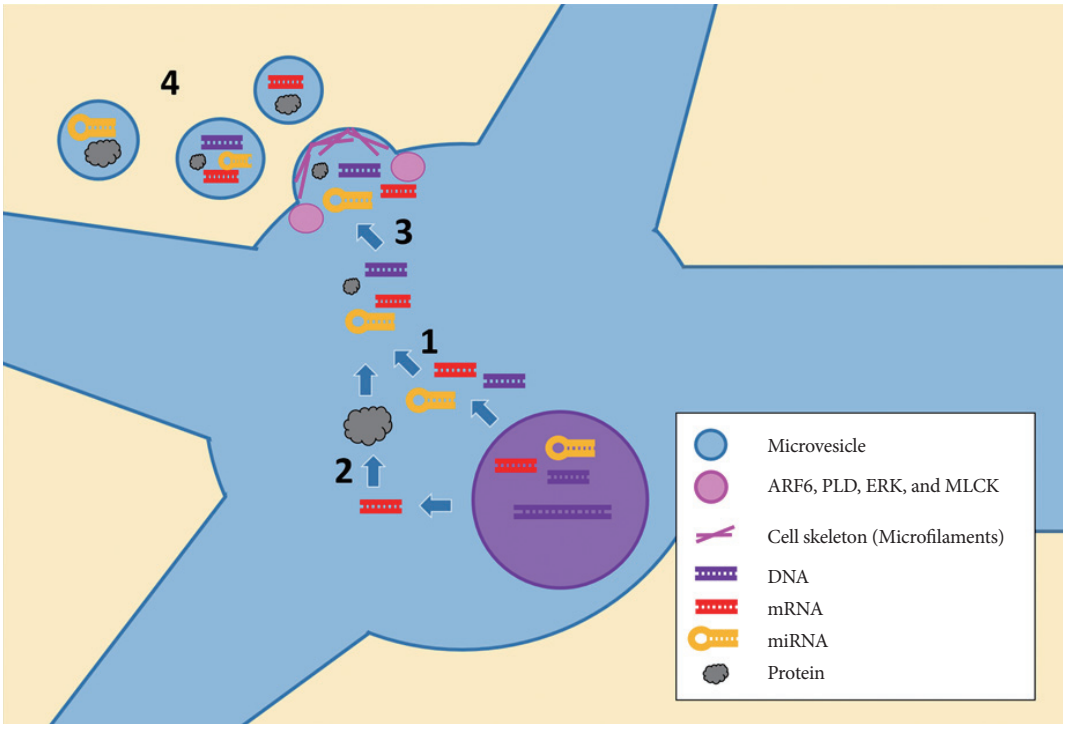

Figure 2. Microvesicle biogenesis The biogenesis of microvesicles occurs mainly on plasma membrane and adjacent cytoplasmic structures (including microfilaments and cytoplasm), through a multistep process. In this figure, the microvesicle formation is simplified to emphasize the important steps in this cellular process. 1. Nucleic acids from the nucleus are processed and transported to the cytoplasm, where they will be transported to the cytoplasmic domain. 2. Some mRNAs are translated to the proteins, which are transported to the cytoplasmic domain under the plasma membrane for packaging process. 3. Nucleic acids and proteins are transported to the plasma membrane domain, where the microvesicle formation will take place. The molecular cargo is sorted into the microvesicles through the molecular sorting machinery (e.g. ESCRT system). Enzymes and proteins e.g. ARF6 (ADP-ribosylation factor 6), PLD (Phospholipase D), ERK (Extracellular-signal regulated kinases) and MLCK (Myosin light-chain kinase) are responsible for the cell skeleton (mainly microfilament) organization. The floppase/flippase enzymes regulate the lipid domain content during the microvesicle formation process. 4. Microvesicles are secreted to the extracellular compartment. These membrane bound structures can travel through blood and lymphatic vessels, cerebrospinal fluid (CSF), saliva and other secretions of the body. Microvesicles possess adhesion and receptor proteins on their surfaces, which allows the microvesicles to bind to their specific targets.

positron emission tomography (PET) shows the metabolic activity in brain, however these techniques still suffers the resolution problem, since they can't monitor individual neuronal activity.[19] The best technique for gaining biochemical, cellular or microbiological information from CNS is obtaining CSF via lumbar puncture.[20] EVs can be collected form CSF and provide valuable information regarding the functional status of neurons and glial cells within the CNS.[21] However, lumbar puncture is still an invasive method with its possible complications e.g. hemorrhage, spinal cord/nerve root injury, even cerebral hernia.[22] In addition, the content of CSF is modified through the components of CSF- brain barrier, i.e. choroid plexus/ ependymal cells, neural and glial processes.[23] Exosomes from CNS carry molecules originating from the inner compartment, including ILV and EBV machinery, of their source cells (neurons, glial cells). In addition, these vesicles carry the inside information and messages from the CNS cells, which reside in a sealed biological compartment. It can be hypothesized, that the information within the exosomes is changed minimally during their journey from CNS to the peripheral blood. Thus, exosomes might contain more proper information regarding cellular/metabolic status within CNS when they are compared to CSF.

An alternative and relatively noninvasive way to obtain information from CNS is to collect EVs which are shed from blood brain barrier.[24] EVs from blood brain barrier contains EVs from both CNS and blood brain barrier components, i.e. endothelial cells and astrocytes.[25] The EVs from neurons and glial cells can cross blood brain barrier via transcytosis process.[26] The main limitations for obtaining EVs from peripheral blood sample are isolation of blood brain barrier derived EVs from peripheral EVs and the determination of origins of EVs derived from blood brain barrier. The areas where sealing of blood brain barrier is relatively weak (e.g. circumventricular organs) might be comparatively more direct source for exosome originating from CNS.[27] In addition, EVs with neuronal origins can be isolated from peripheral blood samples through various surface markers like NCAM or L1CAM.[28]

Exosomes with neural origins also carry GLP-anchored prion protein and Glutamate receptor GluR2/3 subunits. Since these proteins are shown in neural cell culture as neural exosomal markers, more research is needed for their use for peripheral blood neural exosome isolation.[29,30] The proteomic analysis of exosomes derived from microglia culture showed the expression of CD13 and lactate transporter MCT-1.[31] The exosomes with oligodendroglia origin has myelin components, like myelin basic protein or myelin oligodendrocyte glycoprotein.[32] Another approach for the isolation of CNS derived exosomes is the use lipidomic and proteomic profiles of these exosomes. The lipid composition of exosomes (phosphatidylserine, sphingomyelin, lyso-phosphatidylcholine, lysophosphatidylethanolamine and other families), provide information regarding the origin of exosomes.[33]

PSYCHIATRIC DISORDERS AND EXTRACELLULAR VESICLES

The isolation and biochemical analysis of EVs in psychiatric disorders can offer wide diagnostic and therapeutic opportunities, in addition to provision of valuable information regarding pathophysiology of these diseases.[34] In current clinical practice, imaging of CNS is not in routine use for the differential diagnosis of psychiatric diseases.[35] The blood tests are usually recruited for monitoring drug levels, ruling out endocrine diseases, micronutrient deficiencies or organ failures which might affect the mental status of patients.[36,37] However, blood tests are not capable to demonstrate the central nervous system biochemical and cellular properties. The information regarding the level of neurotransmitters, neural metabolic status, epigenetic and/or genetic alterations in neurons would provide valuable information for diagnosis and individualization of treatment of psychiatric disorders.

Nevertheless, the previous studies focusing on EVs in psychiatric disorders are predominantly executed in postmortem brain tissues of the patients.[38-41] The use of EVs extracted from peripheral blood samples for diagnostic purposes requires further basic research and clinical studies carried on patients diagnosed with psychiatric disorders. However, these preceding *ex-vivo* (postmortem) studies might guide the researchers to find potential targets to evaluate in patient population in order to develop biomarkers for diagnostic and/or therapeutic purposes.

In this part of the review, we will focus on some important psychiatric disorders, in which the use of EVs might be beneficial in terms of basic research, diagnosis (including secondary prevention of psychiatric disorders) and treatment. We decided to review EVs in depression, bipolar disorder, schizophrenia, and Alzheimer's disease in the context of Research Domain Criteria (RDoC), since the constructs of these criteria attempt to reflect the etiology of psychiatric diseases in different dimension, extending from the molecules to the neural circuitries and anatomical structures.[42] Although the previous studies on EV in psychiatric disorders do not cover all aspects of RDoC criteria, understanding of EV characteristics in relation to a specific RDoC construct is still important to clarify the pathogenesis and finding appropriate biomarkers for these disorders.

Depression and extracellular vesicles

Depression is a major public health problem linked with decreased functionality and may result in mortality.[43] The pathophysiology of depression is linked with increased activity in hypothalamic-pituitary-adrenal axis, hypo-connectivity in frontoparietal network, structural changes in brain, alterations in neural and glial cells, abnormal brain activity and neurotransmitter functions (involving GABA, glutamate, serotonin, epinephrine and norepinephrine).[44,45]

According to an analysis using RDoC Negative Valence Systems matrix, depression is associated with disruptions in certain neural circuits, e.g. cortico-limbic circuitry, some key genes involved in neurotransmitter metabolism (MAOA, COMT, DATI, 5HTTR, 5HTRs), glucocorticoid receptor downregulation, CRH, sex steroid, oxytocin, vasopressin upregulation, dysregulations in autonomic nervous system, hypothalamic-pituitary-adrenal axis, neuro-inflammation and extended reactivity and behavioral changes (e.g. withdrawal, sadness).[46] RDoC Negative Valence Systems matrix demonstrates different etiological pathways, which might lead to signs and symptoms of depression.

One of the components of Negative Valence Systems matrix, neuro-inflammation, occurs in depression patients within CNS and associated with the activation of microglial cells.[47] Neuro-inflammation is related to a decreased neurogenesis and increase in glutamate excitation toxicity.[48,49] The neuroinflammatory microenvironment seen in depression causes morphological and functional changes in microglia via LPS molecule. The activated microglia cells secrete higher amounts of EVs, which are loaded with proinflammatory cytokines, e.g. IL-1β, caspase-1 etc., in addition to secretion of proinflammatory cytokines (IL-6, TNF-α) via exocytosis. MAPK, JNK1/2, P38 and NFKB pathways are responsible for this functional change within ILV/MVB exosome biogenesis. The expression of various miRNA molecules e.g. miR-1202, Let7a, mir124, which have crucial functions in neurogenesis, neurotransmission (dopaminergic, GABAergic, serotonergic pathways), and ion channel regulation, are altered in several areas (prefrontal cortex, frontal cortex) of CNS.[39] Levels of these miRNAs, like miR-132 (upregulation), miR-1202 (downregula-

tion), are altered in the peripheral blood samples of patients, which are mainly carried via EVs through bloodstream and have diagnostic potential.[50]

Bipolar disorders and extracellular vesicles

The bipolar disorders are characterized with mania or hypomania, which are accompanied with major depressive attacks. Bipolar disorder pathophysiology is explained though anatomical and functional changes in brain architecture (including altered connectivity in anterior cingulate cortex, prefrontal cortex and amygdala), and neuro-inflammation.[51]

According to the RDoC positive valance system matrix, bipolar disorders are linked with abnormally elevated reward activation, and excess approach motivation, which leads to approach related hypomanic/manic symptoms.[52,53] Bipolar disorder is also linked with RDoC arousal systems matrix.[54] Anterior limbic system, amygdala and orbitofrontal cortex are associated with reward valuation, reward related decision, in addition to their association.[55] The dopaminergic neurons in midbrain (including basal ganglia) are responsible for approach motivation.[56]

The studies carried on EVs obtained from patients with bipolar disorder show concordance with RDoC matrices in terms of anatomical locations, where pathological processes take place. The brain specific miRNA-134 is responsible for the formation of dendritic spines and synapses and can be obtained via blood samples and used for monitoring and treatment of mania episodes in bipolar disorder.[57] In addition, miR-29c expression is increased in extracellular vesicles extracted from prefrontal cortex samples of patients with bipolar disorder. miR-29c is linked with neural development and signaling, and a target for lithium treatment.[38] The analysis of EVs from anterior cingulate cortex of bipolar disorder patients showed increased miR-149 and miR-29c expressions. The increased miR-149 expression originates from the glial cells and inhibits glial cell proliferation.[40] Exosomes originating from astrocytes within niche of neurogenesis might transfer the stress signals from peripheral blood (e.g. corticosteroids, cytokines) and disturb neurogenesis in depression and bipolar disorder.[58] Exosome characteristics could be a marker for different stages of bipolar disease and prognosis.

Schizophrenia and extracellular vesicles

Schizophrenia is characterized by reoccurring or chronic psychosis along with other positive and negative (involving mood and cognition deficits) symptoms.[59] Pathophysiology of schizophrenia is associated with abnormal neurotransmission (including dopamine, serotonin, glutamate and many other neurotransmitters), genetic predisposition (including synaptogenesis, neurotransmitters, and immune system), intrauterine and early childhood exposures, autoimmune disorders and substance use. Schizophrenia is linked excess dopaminergic activity in mesolimbic tract, decreased dopamine in prefrontal cortex, decrease in NMDA glutamate receptor (mesocortical dopamine neurons) and GABAergic inhibitory activities.[60-62]

According to the RDoC positive valance system matrix, schizophrenia is linked with altered reward valuation/prediction linked with prefrontal/striatal activation, reduced reward learning and action selection. The impairment in explicit positive reinforced learning can be explained with increased dopamine in basal ganglia and reduced dorsolateral prefrontal cortex activity, which also explains the impairment the action towards valuated outcomes.[63] The auditory hallucinations in schizophrenia are linked with cognition, social processes and negative valance constructs (including sustained and acute threat).[64]

The postmortem prefrontal cortex samples of schizophrenia patients have increased amounts of exosomal miR-497, when it's compared to control patients.[38,65] In addition to miRNAs, EVs can transport misfolded proteins or neurotransmitters, which might play a part in the pathogenesis in schizophrenia.[41] Since the exosomes are capable to carry large molecules like brain-derived neurotrophic factor or platelet-derived growth factor receptor, it can hypothesized that extracellular vesicles might also carry neurotransmitter receptors on their membranes.[66,67] Glutamatergic and dopaminergic receptors on extracellular vesicles might provide valuable information regarding the neural receptor expression status and help to fine-tune the treatment.

Alzheimer's disease and extracellular vesicles

Alzheimer's disease (AD) is characterized with increasing decline of cognitive functions as a result of neural degeneration, especially in older individuals. The most noticeable feature of AD is the progressive memory loss but it's commonly accompanied by other cognitive deficits e.g. behavioral, and psychological signs.[68]

The pathophysiology of AD is characterized by accumulations of Tau and β-amyloid proteins in intra- and extracellular compartments, respectively.[69] The etiology of AD can be explained with genetic (e.g. amyloid precursor protein APP, presenillin, and some ApoE alleles), metabolic (e.g. diabetes mellitus, dyslipidemia, obesity), vascular (e.g. cerebral atherosclerosis, hypertension), neuro-inflammatory factors (e.g. trauma, free radicals).[70-72] The neuropsychiatric symptoms in AD, like agitation, depression or delusions, can be linked with RDoC cognitive systems domain, however there is significant overlapping between different RDoC constructs by explaining these signs and symptoms.[73]

The previous studies revealed that some fraction of β-amyloid protein secretion to extracellular compartment occurs through exosomes. Amyloid precursor protein (APP) is cleaved into β-amyloid proteins and fragments of β-carboxyl-terminal (CTFs) via secretase enzymes (β and γ, which are involved in AD pathogenesis) within exosomes. This cleavage process begins in endosomes and multivesicular bodies within the intracellular compartment, and continues in the exosomes in extracellular domain. The β and γ cleavage products of APP leads the formation of β-amyloid plaques in extracellular compartment of CNS.[74,75] The β-amyloid formation also increases the neuro-inflammation within the CNS.[76]

Additionally, exosomes are also responsible for spreading of the hyper-phosphorylated Tau proteins in AD. Thus, these neurofibrillary tangles may contaminate the healthy neurons via EVs and interfere with the metabolism and intracellular neurotransmitter transport system of these cells.[77]

Nevertheless, it is also demonstrated that the neural exosomes are responsible for clearance of β-amyloid plaques via microglia within CNS.[78] This shows the both detrimental and beneficial effects of exosomes in the pathogenesis of AD.

Along with the proteins, miRNAs are also released within exosomes and play significant roles in AD pathogenesis. The alterations in exosomal miRNA expressions in AD can be detected in CSF and/or peripheral blood samples. The role of miRNAs in neurodegeneration is multifaceted, while some miRNA molecules (like miRNA-219, miR-124a) favor myelination and signaling, others (like miR-193b, let-7) accelerate neural degeneration through increasing neuro-inflammation and amyloid plaque formation.[79]

CONCLUSION

The RDoC constructs provide valuable information for choosing candidates for biomarker molecules for both diagnostic and therapeutic purposes in psychiatric disorders. By using methods of bioinformatics, these potential biomarkers can be extracted from RDoC domain matrices. Extracellular vesicles provide a chance for measuring different classes of biomarkers (e.g. miRNA, proteins and lipids) simultaneously, which might increase the predictive values of testing and help to overcome the difficulties of working with a diagnostic modality relying on a single class of molecule. Using multiple markers on extracellular vesicles carry a huge potential for improving of understanding and managing of psychiatric disorders.

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