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Exosome Release Is Modulated by the Mitochondrial-Lysosomal Crosstalk in Parkinson's Disease Stress Conditions

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Abstract

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra (SN) pars compacta region of the brain. The main pathological hallmark involves cytoplasmic inclusions of α-synuclein and mitochondrial dysfunction, which is observed in other part of the central nervous system other than SN suggesting the spread of pathogenesis to bystander neurons. The inter-neuronal communication through exosomes may play an important role in the spread of the disease; however, the mechanisms are not well elucidated. Mitochondria and its role in inter-organellar crosstalk with multivesicular body (MVB) and lysosome and its role in modulation of exosome release in PD is not well understood. In the current study, we investigated the mitochondria-lysosome crosstalk modulating the exosome release in neuronal and glial cells. We observed that PD stress showed enhanced release of exosomes in dopaminergic neurons and glial cells. The PD stress condition in these cells showed fragmented network and mitochondrial dysfunction which further leads to functional deficit of lysosomes and hence inhibition of autophagy flux. Neuronal and glial cells treated with rapamycin showed enhanced autophagy and inhibited the exosomal release. The results here suggest that maintenance of mitochondrial function is important for the lysosomal function and hence exosomal release which is important for the pathogenesis of PD.

Keywords Mitochondrial dysfunctions · Mitochondria-lysosome crosstalk · Exosome release · Parkinson's disease

Introduction

Parkinson's disease (PD) is a chronic, progressive, neurodegenerative movement disorder including motor as well as non-motor symptoms. It affects 1% of the population of over 60 years of age and 3% of people over 80 years of age, and an estimated seven to ten million people are affected worldwide [1, 2]. Neuronal loss in the substantia nigra leads to a decrease in the dopamine levels in the corpus striatum, which leads to the motor symptoms, namely, tremor, rigidity and bradykinesia. The cellular hallmark of PD is the presence of the intracytoplasmic Lewy bodies and Lewy neurites, composed of protein aggregates, fats and polysaccharides. The protein aggregates contain α-synuclein, neurofilaments, ubiquitin, Parkin and Synphilin [3]. There are emerging evidences which show that misfolded proteins spread through the brain along anatomically connected networks to other neuronal regions thereby promoting progressive decline [4]. PD occurs sporadically as well as in a familial form. Mutations in genes like SNCA, LRRK2 and VPS35 are related to autosomal dominant forms of PD, while PARKIN, PINK1 and DJ1 are associated with autosomal recessive form of PD [5]. The key molecular pathways regulated by these genes involved in PD are emerging; however, their role in progression to different brain regions is not well understood.

The dopaminergic neurons are specifically vulnerable in PD; however, α-synuclein aggregation and neuronal degeneration are observed in non-dopaminergic parts of the brain as well, like neocortex, brain stem and olfactory bulb [6], suggesting that the pathology spreads to other types of brain cells including microglia and astrocytes and other parts of the brain. Emerging studies suggest that exosomes play a major role in inter-neuronal and neuron-glia communication in the brain [7]. Exosomes are a class of extracellular vesicles ranging in the size approximately 30–150 nm, which are released from almost all cell types including neuron, glial and astrocytes [8].

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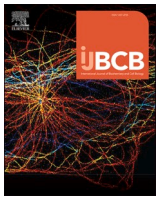

hsa-miR-320a mediated exosome release under PD stress conditions rescue mitochondrial ROS and cell death in the recipient neuronal and glial cells

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ABSTRACT

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by dopaminergic neuronal cell death. Emerging evidence suggest exosomes as a crucial player in the progression and pathogenesis of PD via intercellular communication between different cell types in brain. Exosome release is enhanced from dysfunctional neurons/glia (source cells) under PD stress and mediates the transfer of biomolecules between different cell types (recipient) in brain leading to unique functional outcomes. Exosome release is modulated by alterations in the autophagy and lysosomal pathways; however, the molecular factors regulating these pathways remain elusive. Micro-RNAs (miRNAs) are class of non-coding RNAs that regulate gene expression post-transcriptionally by binding target mRNA and modulate its turnover and translation; however their role in modulating exosome release is not understood. Here, we analyzed the miRNAs-mRNAs network which target cellular processes regulating exosome release. hsa-miR-320a showed the maximum mRNA targets of autophagy, lysosome, mitochondria and exosome release pathways. hsa-miR-320a regulate ATG5 levels and modulate exosome release under PD stress conditions in neuronal SH-SY5Y and glial U-87 MG cells. hsa-miR-320a modulates autophagic flux, lysosomal functions, and mitochondrial ROS in neuronal SH-SY5Y and glial U-87 MG cells. Exosomes derived from hsa-miR-320a expressing source cells under PD stress conditions were actively internalized in the recipient cells and rescued cell death and mitochondrial ROS. These results suggest that hsa-miR-320a regulates autophagy and lysosomal pathways and modulates exosome release in the source cells and derived exosomes under PD stress conditions rescue cell death and mitochondrial ROS in the recipient neuronal and glial cells.

1. Introduction

Parkinson's disease is one of the most prevalent neurodegenerative disorder affecting approximately ten million people worldwide (Maserejian et al., 2020). The accumulation of misfolded α-synuclein aggregates also known as Lewy bodies in the substantia nigra region lead to neuronal loss results in both motor and non-motor defects in PD patients (Mehra et al., 2019). Non-motor symptoms appear prior to the onset of motor symptoms including pain, depression, sleep trouble, olfactory dysfunctions and gastrointestinal (GIT) alterations (Schapira et al., 2017). Evidences now suggest that substantia nigra is not the sole area with alpha synuclein aggregation and neuronal loss, instead it spreads to different brain regions (Dagher and Zeighami, 2018; Henrich et al., 2020; Port, 2018; Ruppert et al., 2020), however the molecular mechanisms are still not well understood. Emerging reports suggest the role of nanovesicles termed as exosome in intercellular communication and its implication in the progression of PD pathogenesis (Yu et al., 2020). Brain cells including neurons, astrocytes and microglia actively communicate with each other via exosomes (Bavisotto et al., 2019). Moreover, exosomes mediated α-synuclein transfer to different cell types lead to cell death in specific regions of the brain (Guo et al., 2020b; Jacquet et al., 2021; Xia et al., 2019). Recent reports from our lab and other groups have shown enhanced exosome release and progression under PD stress conditions (Currim et al., 2021; Zhu et al., 2021); however the release mechanism and its effect after uptake in the bystander cells leading to neuronal dysfunction is not well understood. Inter-organellar crosstalk between different membrane bound organelles like mitochondria and ER with endo-lysosomal system is important for exchange of different biomolecules, maintaining the equilibrium either for degradation or secretion via exosome (Currim et al., 2021;

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DNA damage induces STING mediated IL-6-STAT3 survival pathway in triple-negative breast cancer cells and decreased survival of breast cancer patients

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Abstract

Triple-negative breast cancer is aggressive and metastatic breast cancer type and shows immune evasion, drug resistance, relapse and poor survival. Anti-cancer therapy like ionizing radiation and chemotherapeutic drug majorly induces DNA damage hence, alteration in DNA damage repair and downstream pathways may contribute to tumor cell survival. DNA damage during chemotherapy is sensed by cyclic GMP-AMP synthase(cGAS)-stimulator of interferon genes (STING), which determines the anti-tumor immune response by modulating the expression of programmed cell death ligand-1 (PD-L1), immune suppressor, in the tumor microenvironment. Triple-negative breast cancer cells are cGAS-STING positive and modulation of this pathway during DNA damage response for survival and immune escape mechanism is not well understood. Here we demonstrate that doxorubicin-mediated DNA damage induces STING mediated NF-κB activation in triple-negative as compared to ER/PR positive breast cancer cells. STING-mediated NF-κB induces the expression of IL-6 in triple-negative breast cancer cells and activates pSTAT3, which enhances cell survival and PD-L1 expression. Doxorubicin and STAT3 inhibitor act synergistically and inhibit cell survival and clonogenicity in triple-negative breast cancer cells. Knockdown of STING in triple-negative breast cancer cells enhances CD8 mediated immune cell death of breast cancer cells. The combinatorial treatment of triple-negative breast cells with doxorubicin and STAT3 inhibitor reduces PD-L1 expression and activates immune cell-mediated cancer cell death. Further STING and IL-6 levels show a positive correlation in breast cancer patients and poor survival outcomes. The study here strongly suggests that STING mediated activation of NF-κB enhances IL-6 mediated STAT3 in triple-negative breast cancer cells which induces cell survival and immune-suppressive mechanism.

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TNF-α-induced E3 ligase, TRIM15 inhibits TNF-α-regulated NF-κB pathway by promoting turnover of K63 linked ubiquitination of TAK1

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ABSTRACT

Ubiquitin E3-ligases are recruited at different steps of TNF-α-induced NF-κB activation; however, their role in temporal regulation of the pathway remains elusive. The study systematically identified TRIMs as potential feedback regulators of the TNF-α-induced NF-κB pathway. We further observed that TRIM15 is "late" response TNF-α-induced gene and inhibits the TNF-α-induced NF-κB pathway in several human cell lines. TRIM15 promotes turnover of K63-linked ubiquitin chains in a PRY/SPRY domain-dependent manner. TRIM15 interacts with TAK1 and inhibits its K63-linked ubiquitination, thus NF-κB activity. Further, TRIM15 interacts with TRIM8 and inhibits cytosolic translocation to antagonize TRIM8 modualted NF-κB. TRIM8 and TRIM15 also show functionally inverse correlation in psoriasis condition. In conclusion, TRIM15 is TNF-α-induced late response gene and inhibits TNF-α induced NF-κB pathway hence a feedback modulator to keep the proinflammatory NF-κB pathway under control.

1. Introduction

The NF-κB family of transcription factors are activated by a range of pathophysiological stimuli including viral and bacterial factors (LPS, dsRNA), antigen receptors, DNA damage, reactive oxygen species (ROS), and cytokines (TNF-α, IL-1), [1–3]. Activated NF-κB promotes transcription of a several target genes that include growth factors, chemokines, cytokines, immune modulators, regulators of apoptosis, acute response genes, cell adhesion molecules [1,3]. NF-κB pathway is tightly regulated by post-transcriptional and post-translational regulatory mechanisms, however, its dysregulation leads to prolonged activation of NF-κB and chronic inflammatory conditions hence critical for organismal survival and fitness [4].

Pleiotropic cytokine TNF-α is an activator of the NF-κB pathway and inflammation [1,5]. The increased level of TNF-α and persistent activation of proinflammatory NF-κB is observed in many pathological conditions including cancer [6–9]. TNF-α activated NF-κB target genes have been classified in 'early' 'mid' and 'late' response genes based on their temporal expression pattern [10]. Some of the early response genes like NFKBIA (IκBα) and TNFIP3 (A20) act as negative feedback regulators of the pathway and dynamics of gene expression by controlling NF-κB oscillations [10–12]. The modulation of the NF-κB pathway by late responsive genes and its implication in feedback regulation to restrict inflammation had not yet been explored.

Ubiquitination plays a critical role in the regulation of TNF-α mediated NF-κB pathway. cIAP1/2 mediated Lys-63-linked polyubiquitination of RIP1, recruits TAK1 [5,13]. After recruiting TAK1 to the complex, TAK1 is K(Lysine)-63-linked polyubiquitinated and activated to recruit IκB kinase (IKK) complex. Dual phosphorylation of IκBα by IKK complex leads its K48-linked polyubiquitination by $SCF^{\beta TrCP}$ ubiquitin ligase complex [5,13] which is degraded through ubiquitin proteasome system (UPS). Removal of K63-linked ubiquitin chains by deubiquitinase (DUB) like A20 and CYLD negatively regulates NF-κBactivation and plays a pivotal role in modulating NF-κB pathway [4,5,13]. TNF-α stimulation also promotes the expression of several E3 ligases including various TRAFs, cIAPs, and XIAP and most of these modifiers positively regulate the pathway [1]. Surprisingly to date, no TNF-α-induced E3 ligase had been shown regulating the pathway in a

Abbreviations: TNF-α, Tumor necrosis facotr-alpha; TRIMs, Tripartite motif containing proteins; CCLE, Cancer Cell Line Encyclopedia; TRIM8, Tripartite motif containing protein 8; TRIM15, Tripartite motif containing protein 15; Baf-A1, Bafilomycin-A1; UPS, Ubiquitin proteasome system; GEO, Gene Expression Omnibus.

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The analog of cGAMP, c-di-AMP, activates STING mediated cell death pathway in estrogen-receptor negative breast cancer cells

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Abstract

Immune adaptor protein like STING/MITA regulate innate immune response and plays a critical role in inflammation in the tumor microenvironment and regulation of metastasis including breast cancer. Chromosomal instability in highly metastatic cells releases fragmented chromosomal parts in the cytoplasm, hence the activation of STING via an increased level of cyclic dinucleotides (cDNs) synthesized by cGMP-AMP synthase (cGAS). Cyclic dinucleotides 2' 3'-cGAMP and it's analog can potentially activate STING mediated pathways leading to nuclear translocation of p65 and IRF-3 and transcription of inflammatory genes. The differential modulation of STING pathway via 2' 3'-cGAMP and its analog and its implication in breast tumorigenesis is still not well explored. In the current study, we demonstrated that c-di-AMP can activate type-1 IFN response in ER negative breast cancer cell lines which correlate with STING expression. c-di-AMP binds to STING and activates downstream IFN pathways in STING positive metastatic MDA-MB-231/MX-1 cells. Prolonged treatment of c-di-AMP induces cell death in STING positive metastatic MDA-MB-231/MX-1 cells mediated by IRF-3. c-di-AMP induces IRF-3 translocation to mitochondria and initiates Caspase-9 mediated cell death and inhibits clonogenicity of triple-negative breast cancer cells. This study suggests that c-di-AMP can activate and modulates STING pathway to induce mitochondrial mediated apoptosis in estrogen-receptor negative breast cancer cells.

Keywords Stimulator of interferon gene (STING) · Cyclic GMP AMP synthase (cGAS) · Interferon regulatory factor3 (IRF-3) · Apoptosis · Cyclic dinucleotides (cDNs)

Introduction

The crosstalk between tumor cells, infiltrating immune cells and stroma in breast cancer tumor microenvironment (TME) provides an optimal niche for the growth and proliferation of cancer cells [1]. Hypoxic TME of solid tumors promotes the clonal evolution of the cancer cells which leads to the progression of the tumor [2]. Hypoxic TME can also induce necrotic cell death leading to the release of intrinsic danger-associated molecular patterns (DAMPs), which can activate innate immune response [3]. The activation of the innate immune system and its regulation during tumorigenesis is emerging [4] however, its role in the acquisition of tumorigenic phenotype, its physiological and chemical modifiers are not well understood.

Our previous reports demonstrated that innate immune regulators are uniquely positioned at mitochondria which in turn links the inflammatory pathways and metabolism, hence playing an important role in the metabolic adaption of tumor cells [5]. STING (Stimulator of interferon gene) is also known as MITA, MPYS, TMEM 173 is localized at the ER/mitochondria contact site and is a major regulator of the type I immune response. Interestingly, STING is differentially expressed in ER/PR positive and negative breast cancer patients, therefore can differentially regulate inflammatory cell death [6]. The implication of increased level of STING in triple-negative breast cancer cells and association with metastasis and resistance to cell death is not well understood.

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RESEARCH Open Access

TNF-α differentially modulates subunit levels of respiratory electron transport complexes of ER/PR +ve/−ve breast cancer cells to regulate mitochondrial complex activity and tumorigenic potential

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Abstract

Background: Tumor necrosis factor-α (TNF-α) is an immunostimulatory cytokine that is consistently high in the breast tumor microenvironment (TME); however, its differential role in mitochondrial functions and cell survival in ER/PR +ve and ER/PR −ve breast cancer cells is not well understood.

Methods: In the current study, we investigated TNF-α modulated mitochondrial proteome using high-resolution mass spectrometry and identified the differentially expressed proteins in two different breast cancer cell lines, ER/PR positive cell line; luminal, MCF-7 and ER/PR negative cell line; basal-like, MDA-MB-231 and explored its implication in regulating the tumorigenic potential of breast cancer cells. We also compared the activity of mitochondrial complexes, ATP, and ROS levels between MCF-7 and MDA-MB-231 in the presence of TNF-α. We used Tumor Immune Estimation Resource (TIMER) webserver to analyze the correlation between TNF-α and mitochondrial proteins in basal and luminal breast cancer patients. Kaplan-Meier method was used to analyze the correlation between mitochondrial protein expression and survival of breast cancer patients.

(Continued on next page)

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Expression of expanded *FMR1*-CGG repeats alters mitochondrial miRNAs and modulates mitochondrial functions and cell death in cellular model of FXTAS

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ABSTRACT

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a progressive neurodegenerative disorder caused by an expansion of 55 to 200 CGG repeats located within 5′UTR of *FMR1*.These CGG repeats are transcribed into RNAs, which sequester several RNA binding proteins and alter the processing of miRNAs. CGG repeats are also translated into a toxic polyglycine-containing protein, FMRpolyG, that affects mitochondrial and nuclear functions reported in cell and animal models and patient studies. Nuclear-encoded small non-coding RNAs, including miRNAs, are transported to mitochondria; however, the role of mitochondrial miRNAs in FXTAS pathogenesis is not understood. Here, we analyzed mitochondrial miRNAs from HEK293 cells expressing expanded CGG repeats and their implication in the regulation of mitochondrial functions. The analysis of next generation sequencing (NGS) data of small RNAs from HEK293 cells expressing CGG premutation showed decreased level of cellular miRNAs and an altered pattern of association of miRNAs with mitochondria (mito-miRs). Among such mito-miRs, miR-320a was highly enriched in mitoplast and RNA immunoprecipitation of Ago2 (Argonaute-2) followed by Droplet digital PCR (ddPCR)suggested that miR-320a may form a complex with Ago2 and mitotranscripts. Finally, transfection of miR-320a mimic in cells expressing CGG permutation recovers mitochondrial functions and rescues cell death. Overall, this work reveals an altered translocation of miRNAs to mitochondria and the role of miR-320a in FXTAS pathology.

1. Introduction

FXTAS is a late onset inherited neurodegenerative disorder characterized by progressive intention tremor, gait ataxia and cognitive decline [1,2]. Nearly,1 in ~3000 male and 1 in ~5000 female can be affected by FXTAS and disease symptoms get more pronounced with the age [3]. FXTAS is caused by an expansion of 55 to 200 CGG repeats (known as premutation) at the 5′UTR of the *FMR1* gene located on the long arm of X chromosome [4]. The expanded CGG repeats are transcribed into RNAs that titrate specific RNA binding proteins such as the DROSHA/DGCR8 complex involved in regulation of the processing of microRNAs (miRNAs) [5]. Consequently, expression of various miRNAs are altered in FXTAS [6,7]. CGG repeats embedded in the 5′UTR of *FMR1* are translated into a toxic polyglycine-containing protein, FMRpolyG, through initiation via a non-canonical ACG start codon located upstream of the repeats [8–10]. However, it is still not understood if CGG RNA and/or FMRpolyG protein contribute to mitochondrial alterations leading neuronal cell dysfunctions and death [11].Importantly, recent findings suggest that mitochondrial dysfunctions including loss of mitochondrial membrane potential, ATP and mitochondrial transcripts and proteins are associated with FXTAS pathogenesis [11–14]. We have recently shown decreased expression levels of mitochondrial transcripts

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Biology of the Cell

REVIEW

Do different exosome biogenesis pathways and selective cargo enrichment contribute to exosomal heterogeneity?

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Abstract

Exosomes are emerging intercellular communicators essential for cellular homeostasis during development and differentiation. The dysregulation in exosome-mediated communication alters cellular networking leads to developmental defects and chronic diseases. Exosomes are heterogeneous in nature depending on differences in size, membrane protein abundance, and differential cargo load. In this review, we have highlighted the latest developments in exosome biogenesis pathways, heterogeneity, and selective enrichment of various exosomal cargoes including proteins, nucleic acids, and mitochondrial DNA. Furthermore, the recent developments in the isolation techniques of exosome subpopulations have also been discussed. The comprehensive knowledge of extracellular vesicle (EV) heterogeneity and selective cargo enrichment during specific pathology may provide a clue for disease severity and early prognosis possibilities. The release of specific exosome subtypes is associated with the progression of specific disease type and hence a probable tool for therapeutics and biomarker development.

KEYWORDS
biogenesis, cargo, exosomes, heterogeneity, miRNA, mitochondrial DNA

INTRODUCTION

Intercellular communication is essential for development and adult body homeostasis. Cytokines and chemokines, neurotransmitters, and hormones are well-known mediators for intercellular crosstalk (Thurley et al., 2018). The discovery in the early 80s in reticulocyte cells revealed a novel way of cellular communication via extracellularly released nanovesicles termed exosomes (Harding et al., 1983; Pan & Johnstone, 1983). Exosomes, initially considered as cellular waste, is important for intercellular communication and signaling outcomes in the recipient cells as suggested by several studies in the last decade (Pitt et al., 2016). Extracellular vesicles (EVs) include three main categories, microvesicles, exosomes, and apoptotic bodies. Exosomes (30–150 nm) belongs to endosome origin, microvesicles (100 nm–1 μm) originate through direct outward pinching of the plasma membrane (PM) and apoptotic bodies (50–5000 nm) are released from the dying cells into the extracellular space (Nicolas & Goodwin, 2019). Exomeres, non-membranous vesicles with a size less than 50 nm have been recently added to the EV category (Q. Zhang et al., 2019). The terms exosomes, EVs, and microvesicles are sometimes used interchangeably but recent reports have confirmed the functional differences. Although the isolation of specific subtypes is challenging; the recent advancements in isolation techniques made possible to isolate specific sub population of EVs. Subtypes of EVs are categorized based on their biophysical as well as biochemical properties like size [small (sEVs) and large (lEVs)], membrane tetraspanin enrichment [CD63/CD9/CD81], and density [high (HD) and low density (LD)] EVs (Lässer et al., 2018).

Different cellular pathways regulate exosome biogenesis including endosome-originated—Endosome Sorting Complex Required for Transport (ESCRT) pathway, membrane lipid-linked ceramide pathway and alternative pathways which include molecular regulators apart from ESCRT and ceramide pathways. However; the pathway(s) regulating the biogenesis and release of