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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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Mitohormesis; Potential implications in neurodegenerative diseases

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ABSTRACT

Mitochondrial dysfunction is known to be associated with neurodegenerative diseases (NDDs), which is a major burden on the society. Therefore, understanding the regulation of mitochondrial dysfunctions and its implication in neurodegeneration has been major goal for exploiting these mechanisms to rescue neuronal death. The crosstalk between mitochondria and nucleus is important for different neuronal functions including axonal branching, energy homeostasis, neuroinflammation and neuronal survival. The decreased mitochondria capacity during progressive neurodegeneration leads to the altered OXPHOS activity and generation of ROS. The ROS levels in narrow physiological range can reprogram nuclear gene expression to enhance the cellular survival by phenomenon called mitohormesis. Here, we have systematically reviewed the existing reports of mitochondrial dysfunctions causing altered ROS levels in NDDs. We further discussed the role of ROS in regulating mitohormesis and emphasized the importance of mitohormesis in neuronal homeostasis. The emerging role of mitohormesis highlights its importance in future studies on intracellular ROS mediated rescue of mitochondrial dysfunction along with other prevailing mechanisms to alleviate neurodegeneration.

1. Mitochondrial dysfunction and neurodegeneration

Neurodegenerative diseases (NDDs) includes spectrum of disorders characterized by progressive loss of neurons in central and peripheral nervous system (Ellis and Fell, 2017; Jellinger, 2010; Hoogendam, 2017). These include range of neuronal pathologies where PD and AD are most prevalent affecting 8% population worldwide increasing socioeconomic burden of society (Association, 2019). Other NDDs such as multiple sclerosis (MS), Huntingtońs disease (HD), amyotrophic lateral sclerosis (ALS), Fragile X-associated tremor ataxia syndrome (FXTAS) and mostly all types of dementias cause vast impact on overall health and economy of the countries worldwide (Chitnis and Weiner, 2017; Patten et al., 2010; Schapira et al., 2014; Hagerman and Hagerman, 2015; Tzvetkov and Atanasov, 2018). Though, NDDs are caused by various mechanisms, they do share some common features at cellular and physiological levels which majorly involve oxidative stress, neuroinflammation, excitotoxicity (abnormal protein dynamics due to protein misfolding), proteolytic stress, and mitochondrial dysfunction (Jellinger, 2010; Chitnis and Weiner, 2017; Hung, 2018; Bond, 2012) .

Mitochondria apart from its traditional role as being powerhouse of the cell, have emerged as a versatile organelle involved in calcium regulation, antioxidant response, inflammation and cell survival (Spinelli and Haigis, 2018; Romero-Garcia and Prado-Garcia, 2019; Prizes, 2011). It also acts as signaling hub to regulate translation of candidate mitochondrial protein in response to the intra- and extracellular stimulus (Yi et al., 2018). In neuronal cells, mitochondria occupy ~ 40% volume of cytosol and is known to play crucial role in maintaining neuronal physiology (Fieni et al., 2012). Mitochondria play essential roles in axonal maintenance, destinies and branching and higher densities of mitochondria are observed in distal axons which is important for the axon extension (Matsuo et al., 2013). In neuronal cells, mitochondrial shape, dynamics and functions are different at different sub neuronal sites and mitochondria localized at the growth cone are more hyperpolarized than along the axon shaft (Jacobs and Coyne, 2013; Verburg and Hollenbeck, 2008; Ketschek and Gallo, 2010). Similarly, it is observed that mitochondrial position and stalling in axons

Abbreviations: OXPHOS, Oxidative phosphorylation; ETC, Electron transport chain; ROS, Reactive oxygen species; PD, Parkinson's disease; AD, Alzheimer's disease; MPTP, Mitochondrial permeability transition pore; PUFA, Polyunsaturated fatty acids; PINK1, PTEN-induced kinase 1; FOXO, Fork head box transcription factors; RISC, RNA-induced silencing complex; NRF-1,2, Nuclear respiratory factor 1,2; HIF-1α, Hypoxia-inducible factor 1-alpha; CCO-1, Cytochrome *c* oxidase subunit 1; PGC-1α, Peroxisome proliferator-activated receptor-gamma coactivator-1alpha; ATFS-1, Activating transcription factor associated with stress-1; UBL-5, Ubiquitin like-5.

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Review

The emerging molecular mechanisms for mitochondrial dysfunctions in FXTAS

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ABSTRACT

Fragile X-associated tremor/ataxia syndrome (FXTAS) is an inherited neurodegenerative disorder caused by an expansion of 55-200 CGG repeats at 5UTR of FMR1 gene, known as premutation. The main clinical and neuropathological features of FXTAS include progressive intention tremor, gait ataxia, neuronal cell loss and presence of ubiquitin-positive intranuclear inclusions in neurons and astrocytes. Various mitochondrial dysfunctions are reported in *in vitro/vivo* models of FXTAS; however, the molecular mechanisms underlying such mitochondrial dysfunctions are unclear. CGG expansions are pathogenic through distinct mechanisms involving RNA gain of function, impaired DNA damage repair and FMRpolyG toxicity. Here, we have systematically reviewed the reports of mitochondrial dysfunctions under premutation condition. We have also focused on potential emerging mechanisms to understand mitochondrial associated pathology in FXTAS. This review highlights the important role of mitochondria in FXTAS and other related disorders; and suggests focus of future studies on mitochondrial dysfunction along with other prevailing mechanisms to alleviate neurodegeneration.

1. FXTAS: overview of pathogenicity

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late onset neurodegenerative disorder usually observed after 50–55 years of age [1,2]. The main clinical features of FXTAS are progressive intention tremor, parkinsonism, cerebellar gait ataxia and cognitive decline [3]. FXTAS is also often found to be associated with neuropathy, thyroid dysfunctions, hypertension, immune dysfunction and cardiac arrhythmias [4]. At the histopathological level, FXTAS is characterized by neuronal cell loss and by the presence of large ubiquitin-positive intranuclear inclusions in both neurons and astrocytes [5]. Rare ubiquitin positive inclusions are also observed in non-CNS organs like kidney and thyroid [6], At the genetic level, FXTAS is caused by the presence of 55 to 200 CGG repeats embedded within the 5′ untranslated region(UTR) of the Fragile X mental retardation(*FMR1*) gene (OMIM *309550), located on the q-arm of the X chromosome [7,8]. FXTAS, Fragile X-associated primary ovarian insufficiency (FXPOI) and Fragile X-associated Neuropsychiatric Disorders (FXAND) are caused by expansions of 55 to 200 CGG repeats (premutation), while Fragile X Syndrome (FXS) is caused by expansions greater than 200 CGG repeats (full mutation) [9,10]. The prevalence of the CGG premutation is estimated to range between 1 in 110 to 250 in females and 1 in 260 to 800 in males [11,12]. However, due to incomplete penetrance it is estimated that 1 in ~3000 male and 1 in ~5000 female are at risk of developing FXTAS, with disease symptoms getting more pronounced with age [13].

At the molecular level, the CGG premutation promotes 2 to 8 folds higher levels of *FMR1* mRNA in premutation carriers compared to control individuals [14,15]. Importantly, constitutive/inducible CGG expression of *FMR1* mRNA containing the CGG premutation manifest acute cellular toxicity in human neuronal cell (SK-N-MC) and similar results were also obtained in premutation model of Drosophila and mouse [7,16–20]. Evidences in the last decade have proposed several mechanisms of CGG repeats mediated pathology including RNA gain of function (RNA mediated titration of specific RNA binding proteins), altered DNA damage response and Repeat Associated Non-AUG (RAN) translation of the CGG repeats into toxic proteins, notably FMRpolyG [21–27]. When expressed in human cells and transgenic animals, FMRpolyG is prone to aggregate and localizes within the nucleus forming ubiquitin-positive inclusions. Importantly, FMRpolyG expression appears necessary to mediate the toxicity of the CGG premutation in human neuronal cells (SK-N-SH cells, expressing 95 CGG repeats), *Drosophila* and mouse models of FXTAS [28,29]. The role of these mechanisms causing neuronal dysfunction in FXTAS and FXS is not well understood.

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FMRpolyG alters mitochondrial transcripts level and respiratory chain complex assembly in Fragile X associated tremor/ataxia syndrome [FXTAS]

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ABSTRACT

Fragile X-associated tremor/ataxia syndrome (FXTAS) is an inherited neurodegenerative disorder caused by an expansion of 55 to 200 CGG repeats (premutation) in *FMR1*. These CGG repeats are Repeat Associated non-ATG (RAN) translated into a small and pathogenic protein, FMRpolyG. The cellular and molecular mechanisms of FMRpolyG toxicity are unclear. Various mitochondrial dysfunctions have been observed in FXTAS patients and animal models. However, the causes of these mitochondrial alterations are not well understood. In the current study, we investigated interaction of FMRpolyG with mitochondria and its role in modulating mitochondrial functions. Beside nuclear inclusions, FMRpolyG also formed small cytosolic aggregates that interact with mitochondria both in cell and mouse model of FXTAS. Importantly, expression of FMRpolyG reduces ATP levels, mitochondrial transmembrane potential, mitochondrial supercomplexes assemblies and activities and expression of mitochondrial DNA encoded transcripts in cell and animal model of FXTAS, as well as in FXTAS patient brain tissues. Overall, these results suggest that FMRpolyG alters mitochondrial functions, bioenergetics and initiates cell death. The further study in this direction will help to establish the role of mitochondria in FXTAS conditions.

1. Introduction

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a neurodegenerative disorder characterized by progressive intention tremor (parkinsonism), gait ataxia and cognitive decline [1]. In addition, neuropathy, thyroid dysfunctions, hypertension, immune dysfunction and cardiac arrhythmias are also observed in some patients of FXTAS [2]. FXTAS affects 1 in ~3000 male and 1 in ~5000 female and disease symptoms get more pronounced with the age [3]. The Fragile X mental retardation (*FMR1)* gene, located on the q-arm of the chromosome X, encodes for the FMRP-RNA binding protein, which is involved in regulation of transport and local translation of mRNAs in brain and is essential to synaptic plasticity and neuronal development [4–6]. An expansion of CGG trinucleotide repeats within the 5′-UTR of *FMR1* causes different neuropathological conditions based on the number of CGG repeats.

Firstly, expansions exceeding 200 CGG repeats, which are called full mutations, are the main cause of Fragile X syndrome (FXS), a neurodevelopmental disease characterized by intellectual disability and autism. Expansions over 200 CGG repeats lead to hypermethylation and silencing of the *FMR1* promoter. Hence, the FMRP protein encoded by *FMR1* is absent, which ultimately results in alterations of the brain synaptic plasticity [7]. Secondly, Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) is caused by presence of 55 to 200 CGG repeats, which is named premutation [8]. The prevalence of the CGG premutation carrier varies among populations but is estimated to range between 1 in 110 to 250 in females and 1 in 260 to 800 in males [9,10]. At the histopathological level, FXTAS is characterized by neuronal cell loss and presence of large ubiquitin-positive intranuclear inclusions in both neurons and astrocytes [11]. Rare ubiquitin positive inclusions are also observed in non-CNS organs like kidney and thyroid [12]. At the molecular level and in strict contrast to Fragile-X, the CGG premutation expansion does not inhibit but promotes *FMR1* expression in FXTAS, resulting in 2 to 8 folds higher levels of *FMR1* mRNA in FXTAS patients compared to control individuals [13,14]. Importantly, expression of mutant RNA containing the CGG premutation is pathogenic both in cell and animal models [15–20]. Studies in the last decade have identified two main mechanisms of how CGG repeats expression can be toxic for

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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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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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TRIM32 regulates mitochondrial mediated ROS levels and sensitizes the oxidative stress induced cell death

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ABSTRACT

Emerging evidence suggests that ubiquitin mediated post translational modification is a critical regulatory process involved in diverse cellular pathways including cell death. During ubiquitination, E3 ligases recognize target proteins and determine the topology of ubiquitin chains. Recruitment of E3 ligases to targets proteins under stress conditions including oxidative stress and their implication in cell death have not been systemically explored. In the present study, we characterized the role of TRIM32 as an E3 ligase in regulation of oxidative stress induced cell death. TRIM32 is ubiquitously expressed in cell lines of different origin and form cytoplasmic speckle like structures that transiently interact with mitochondria under oxidative stress conditions. The ectopic expression of TRIM32 sensitizes cell death induced by oxidative stress whereas TRIM32 knockdown shows a protective effect. The turnover of TRIM32 is enhanced during oxidative stress and its expression induces ROS generation, loss of mitochondrial transmembrane potential and decrease in complex-I activity. The pro-apoptotic effect was rescued by pan-caspase inhibitor or antioxidant treatment. E3 ligase activity of TRIM32 is essential for oxidative stress induced apoptotic cell death. Furthermore, TRIM32 decreases X-linked inhibitor of apoptosis (XIAP) level and overexpression of XIAP rescued cells from TRIM32 mediated oxidative stress and cell death. Overall, the results of this study provide the first evidence supporting the role of TRIM32 in regulating oxidative stress induced cell death, which has implications in numerous pathological conditions including cancer and neurodegeneration.

1. Introduction

Mitochondria are dynamic organelles and are implicated in various cellular functions including metabolism, cell death, inflammation, and immunity apart from its role in bioenergetics to maintain cellular homeostasis [1–3]. The equilibrium of the healthy mitochondrial network is maintained in the cells through the dynamic process of fusion and fission. The stressed or damaged mitochondria are labeled with ubiquitin (Ub) and selectively degraded through the process known as mitophagy in order to remove them from the healthy network [4]. Any defect in mitophagy or in the fusion and fission process leads to accumulation of defective mitochondria, resulting in the production of excessive ROS and initiates cell death [5]. This phenomenon has been observed in many pathological conditions including neurodegeneration and autoimmune diseases [6,7].

Ub is versatile molecule that can form different types of Ub chains of different topology on the target proteins through seven conserved lysine residues [8,9]. The presence of atypical Ub chains on the target proteins regulate their stability and impart unique functional outcomes. The reported evidence suggests that ubiquitination during oxidative stress is initiated on the mitochondria which regulates mitophagy and cell death [10]. It was observed that K63 linked ubiquitination is initiated through Bre1 ubiquitin ligase during oxidative stress that determines cell survival [11]. The process of ubiquitination is achieved by the sequential action of three enzymes: E1 (Ub activating enzyme), E2 (Ub conjugating enzyme), E3 (Ub ligases). The E3 ligases are terminal proteins in ubiquitination process and provide pathway specificity as they recognize the substrate and initiate the transfer of Ub. Interestingly, the human genome contains more than 1000 E3 Ligases and their functions are not well understood. Moreover, the role of specific E3 Ligases, their recruitment to mitochondria and regulation of cell death pathways during oxidative environment have not been well studied [12].

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Enforced lysosomal biogenesis rescues erythromycin- and clindamycin-induced mitochondria-mediated cell death in human cells

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Abstract

Antibiotics are the front-line treatment against many bacterial infectious diseases in human. The excessive and long-term use of antibiotics in human cause several side effects. It is important to understand the underlying molecular mechanisms of action of antibiotics in the host cell to avoid the side effects due to the prevalent uses. In the current study, we investigated the crosstalk between mitochondria and lysosomes in the presence of widely used antibiotics: erythromycin (ERM) and clindamycin (CLDM), which target the 50S subunit of bacterial ribosomes. We report here that both ERM and CLDM induced caspase activation and cell death in several different human cell lines. The activity of the mitochondrial respiratory chain was compromised in the presence of ERM and CLDM leading to bioenergetic crisis and generation of reactive oxygen species. Antibiotics treatment impaired autophagy flux and lysosome numbers, resulting in decreased removal of damaged mitochondria through mitophagy, hence accumulation of defective mitochondria. We further show that over-expression of transcription factor EB (TFEB) increased the lysosome number, restored mitochondrial function and rescued ERM- and CLDM-induced cell death. These studies indicate that antibiotics alter mitochondria and lysosome interactions leading to apoptotsis and may develop a novel approach for targeting inter-organelle crosstalk to limit deleterious antibiotic-induced side effects.

Keywords Antibiotics · Side effects · Mitochondria · Lysosome · Autophagy

Introduction

Antibiotics are considered as one of the more important discoveries of modern medicine by preventing large-scale morbidity and mortality associated with microbial infectious diseases. However, it has been observed that certain populations are more prone to antibiotic's side effects [1]. In particular, severe side effects in infants, children, and aging

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NLRX1 regulates TNF-α-induced mitochondria-lysosomal crosstalk to maintain the invasive and metastatic potential of breast cancer cells

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ABSTRACT

An increased level of proinflammatory cytokines, including TNF-α in tumor microenvironment regulates the bioenergetic capacity, immune evasion and survival of cancer cells. Emerging evidences suggest that mitochondrial immune signaling proteins modulates mitochondrial bioenergetic capacity, in addition to the regulation of innate immune response. The optimal oxidative phosphorylation (OxPhos) capacity is required for the maintenance of functional lysosomes and autophagy flux. NLRX1, a mitochondrial NOD family receptor protein, regulates mitochondrial function during apoptosis and tissue injury. However, its role in regulation of mitochondrial and lysosomal function to modulate autophagy flux during inflammatory conditions is not understood. In the current study, we investigated the role of NLRX1 in modulating TNF-α induced autophagy flux and mitochondrial turnover and its implication in regulating the invasive and metastatic capability of breast cancer cells. Expression analyses of clinical breast cancer samples and meta-analysis of multiple public databases revealed that NLRX1 expression is significantly increased in basal-like and metastatic breast carcinoma as compared to non-basal-like and primary breast cancer. Depletion of NLRX1 expression in triple-negative breast cancer cells, altered the organization and activity of OxPhos complexes in presence of TNF-α. NLRX1 depletion further impaired lysosomal function and hence the turnover of damaged mitochondria through mitophagy in presence of TNF-α. Importantly, loss of NLRX1 decreased OxPhos-dependent cell proliferation and migration ability of triple-negative breast cancer cells in presence of TNF-α. These evidences suggest an essential role of NLRX1 in maintaining the crosstalk of mitochondrial metabolism and lysosomal function to regulate invasion and metastasis capability of breast cancer cells.

1. Introduction

Breast cancer is the second most common malignancy diagnosed among women worldwide and a leading cause of mortality in women population [1]. The heterogeneity of breast cancer makes them a challenging solid tumor to diagnose and treat. A primary or early breast tumor, characterized by the luminal-like subtype and presence of estrogen receptor (ER), progesterone receptor (PR) and/or HER2 status, is potentially curable. The surgical removal of the tumor combined with hormonal and systemic therapy is considered to be the best treatment option [2]. In contrast, basal-like subtype and triple-negative (ER, PR and HER2, negative) metastatic breast cancer is currently considered as incurable with a long-term survival rate of < 5% [3]. The study of the cellular pathways involved in differential regulation of the invasive phenotype of triple negative compared to ER/PR positive tumors would help to understand the intratumoral heterogeneity and clonal evolution of the metastatic breast cancer cells.

Chronic inflammation, a hallmark of solid tumor, is intricately associated with initiation and progression of many cancer types, including breast cancer [4]. The specific role of inflammation in

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NLRX1 resides in mitochondrial RNA granules and regulates mitochondrial RNA processing and bioenergetic adaptation

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ABSTRACT

The role of mitochondria is emerging in regulation of innate immunity, inflammation and cell death beyond its primary role in energy metabolism. Mitochondria act as molecular platform for immune adaptor protein complexes, which participate in innate immune signaling. The mitochondrial localized immune adaptors are widely expressed in non-immune cells, however their role in regulation of mitochondrial function and metabolic adaption is not well understood. NLRX1, a member of NOD family receptor proteins, localizes to mitochondria and is a negative regulator of anti-viral signaling. However, the submitochondrial localization of NLRX1 and its implication in regulation of mitochondrial functions remains elusive. Here, we confirm that NLRX1 translocates to mitochondrial matrix and associates with mitochondrial FASTKD5 (Fas-activated serine-threonine kinase family protein-5), a bonafide component of mitochondrial RNA granules (MRGs). The association of NLRX1 with FASTKD5 negatively regulates the processing of mitochondrial genome encoded transcripts for key components of complex-I and complex-IV, to modulate its activity and supercomplexes formation. The evidences, here, suggest an important role of NLRX1 in regulating the post-transcriptional processing of mitochondrial RNA, which may have an important implication in bioenergetic adaptation during metabolic stress, oncogenic transformation and innate immunity.

1. Introduction

ATP generation through oxidative phosphorylation (OxPhos) is one of the major function of mitochondria, besides its additional important roles in numerous biosynthetic reactions, maintaining intracellular ion homeostasis, apoptosis and innate immune signaling [1]. Mitochondrial outer membrane and mitochondrial contact sites serve as molecular platform for the assembly of dynamic signaling complexes that forms during viral infection [2]. Mitochondrial outer membrane proteins namely, MAVS and STING act as adaptors for the downstream activation of anti-viral signaling [3, 4]. NLRX1 (Nod-Like Receptor (NLR) protein family member) negatively regulates innate immune responses during viral infections. Recent reports from our lab and others have shown that mitochondrial immune signaling proteins are widely expressed in non-immune cells and their levels are altered during tumor progression [5, 6]. Similarly, the role of NLRX1 in controlling mitochondrial metabolic functions and apoptosis during inflammatory condition and tissue injury is emerging [7, 8]. However, its sub-mitochondrial localization and molecular mechanism(s) of regulating mitochondrial function is not well understood.

The OxPhos system, embedded in inner mitochondrial membrane, is composed of five multiprotein complexes forming the mitochondrial respiratory chain (MRC). The MRC complexes are assembled from nearly 100 protein subunits, of which approximately 90 subunits are encoded by nuclear genes and imported into mitochondria. The remaining 13 subunits are encoded by mitochondrial DNA (mtDNA) [9]. In addition, mitochondrial genome encodes 22 tRNAs and 12S and 16S mt-rRNAs that are essential for the synthesis of mitochondria-encoded proteins. As all protein factors involved in the expression of mitochondrial genome are nuclear encoded, a strict coordination between nuclear and mitochondrial gene expression programs is necessary, especially during physiological responses requiring changes in energy demands, such as conditions of altered carbon sources [10]. The regulatory mechanisms of mitochondrial gene expression and its

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TRIM8 regulated autophagy modulates the level of cleaved Caspase-3 subunit to inhibit genotoxic stress induced cell death

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ABSTRACT

In cancer patients, treatment modalities like chemotherapy and radiation exert their anticancer effects by inducing DNA damage. The cancer cells can survive under genotoxic stress by inducing DNA damage response (DDR) or can undergo cell death. The process of autophagy is emerging as crucial regulator of cell survival during different stress conditions. Post translational modification through ubiquitin plays an essential role in DDR during genotoxic stress conditions. Ubiquitin ligases regulate autophagy and cell death pathways however their role during genotoxic stress conditions is not understood. In the current study we identified TRIM8, RING E3 Ligase, as a novel regulator of autophagy during DDR. TRIM8 regulates lysosomal biogenesis and autophagy flux. The turnover of TRIM8 is high and is stabilized during genotoxic stress conditions. TRIM8 regulated autophagy is essential for its cytoprotective role during genotoxic stress induced cell death. TRIM8 stabilizes the turnover of XIAP during genotoxic stress and forms complex with XIAP and caspase-3 to inhibit its activation in presence of etoposide. TRIM8 mediated autophagy promotes degradation of cleaved caspase-3 subunits. This study described TRIM8, as a novel regulator of DDR-autophagy crosstalk, which may play role in survival of cancer cells in presence of genotoxic agents.

1. Introduction

The cell has evolved DNA damage response (DDR) to ensure genomic integrity and cell survival. DDR includes sensing of the DNA damage, recruitment of DNA repair proteins and repair. During DNA damage response (DDR), cell cycle check points are activated leading to either repair or cell death in irreversible DNA damage conditions. DNA double stranded breaks (DSBs) detection by ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and Rad3 related) promotes activation of apoptosis by inducing p53 [1] however many other regulators still needs to be identified. The DNA repair process and cell survival/death pathway are intricately linked to maintain cellular homeostasis [2,3]. Any defect in this process can lead to genomic aberrations including chromosomal translocations, mutations deletions/additions leading to malignant transformation of the cells. Cancer cells often have compromised DDR which help them in avoiding cellular checkpoints and proliferate. During radiation and chemotherapy of cancer patients, the genomic DNA of malignant cells is the target and DDR induced apoptosis is initiated to eliminate cancer cells [4]. The mechanism of cross talk of different cell survival and death pathways and their regulators during genotoxic stress in cancer cells needs to be identified and investigated.

Genotoxic agent induced autophagy have been implicated both in initiation of cell death as well as cell survival, hence its role further needs more investigation for targeted therapeutic intervention [5,6]. The crosstalk between autophagy and apoptosis is crucial as apoptosis acts as the last resort for cells in severe or persistent genotoxic stress [7]. Autophagy maintains cellular fitness by elimination of defective organelles, dysfunctional proteins and aggregates [8–10]. Interestingly, autophagy has also been shown essential for removal of DNA damage induced extranuclear DNA that can lead to activation of inflammatory pathways. It is observed that damaged DNA is exported outside the nucleus and its autophagy mediated degradation in lysosomes is essential for cell survival. Failure in clearance of extranuclear DNA leads to activation of inflammatory pathways by intracellular DNA sensors like cGAS/STING [11]. It had been observed that knockout of p62/

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Review Article

Recent Advances in Immunotherapy for the Treatment of Cancer

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Abstract | Immunotherapy based approaches to modulate the body's own immune defense mechanism to fight cancer is gaining momentum due to its successful application for treating different types of cancers recently. There are four major types of immunotherapy approaches including nonspecific immunotherapy using immune-modulatory molecules, monoclonal antibody therapy, vaccines, and cellular immunotherapy. The use of antibodies for treating cancers has been well established there are about dozen antibodies currently available in clinical practice for the treatment of various cancers. Approval of Dendritic cell based therapy by regulatory authorities has led to the recognition of immune cells as drug for treatment of clinical disease. Other modes of immune cell based therapies including Natural killer cells and T cells are also under various stages of development. This review is aimed to provide an overview of different modes of immunotherapy and its application in human and animals.

Keywords | Immunotherapy, Dendritic cell, NK cells, Monoclonal antibodies, Cancer

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INTRODUCTION

Immunotherapy for cancer is a rapidly advancing field of translational science having enormous therapeutic application potential for cancer. Cancer immunotherapy has been recognized as 'breakthrough of the year 2013' by the journal Science and it was also recognized by release of a special issue on cancer immunotherapy by the journal Nature recently.

There are four major areas of cancer immunotherapy- nonspecific immunotherapy, monoclonal antibody therapy, vaccines, and cellular immunotherapy as depicted in the Figure 1. Nonspecific immunotherapy includes use of cytokines and other chemicals that stimulate a general immune response or remove the blockades of immune activation against cancers. Monoclonal antibodies are used for tagging the cancer cells for immune mediated killing or altering the cell signalling pathways to inhibit proliferation or inducing apoptosis. Cancer vaccines include dendritic cells based vaccines where these antigen presenting cells were loaded with tumour associated antigens *ex vivo* and then injected back in to the patients. The cellular immunotherapy approach utilizes *ex vivo* expanded specific population of immune cells (T-cells, NK cells, gamma delta T cells etc.) for the treatment of cancer. Some of these approaches are approved by US FDA (Food and Drug Administration) and some are still in the experimental stages. Several clinical trials are undergoing worldwide by several academic & research institutions and companies to bring novel therapies to cancer patients based on cancer immunotherapy approaches.