A Possible Link between Dysregulated Mitochondrial Calcium Homeostasis and Citrullination in Rheumatoid Arthritis

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Rec date: April 19, 2018; Acc date: May 16, 2018; Pub date: May 21, 2018  
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Abstract  
Rheumatoid arthritis (RA) is characterized by citrullination of peptides and proteins mediated by calcium-dependent peptidyl arginine deaminase enzymes (PADs). Various mechanisms of intracellular and extracellular protein citrullination have been elucidated so far. Here, we highlight one more important possible mechanism that could lead to intracellular citrullination due to dysregulated mitochondrial Ca\(^{2+}\) homeostasis i.e., inability of mitochondrial uptake of Ca\(^{2+}\) during physiological signaling and triggering the generation of autoimmune disease. Spontaneous secretion of intracellular PAD may be the result of high cytosolic Ca\(^{2+}\) due to a disturbance in mitochondrial calcium homeostasis secondary to oxidative damage without any compromise to the cell membrane. Various environmental triggers like air pollutants also induce oxidative stress (OS) which may compromise the mitochondrial integrity and disturb Ca\(^{2+}\) homeostasis. Adoption of simple lifestyle modification like yoga and meditation optimizes reactive oxygen species (ROS) levels and maintains mitochondrial integrity by increasing COX activity, thus may curb citrullination process and its sequelae.

Keywords: Calcium homeostasis; Citrullination; Mitochondrial integrity; Peptidyl Arginine Deaminases (PAD); Rheumatoid arthritis; Yoga and meditation

Introduction

Citrullination is an essential contributor to the pathogenesis of rheumatoid arthritis (RA). This post-translational conversion of arginine to citrulline residues requires peptidyl arginine deiminase enzymes (PADs) which are calcium-dependent. Under physiological conditions, calcium stimulation is required to activate the mostly inactive PADs resulting in citrullination of a number of structural proteins e.g., vimentin, fibrinogen, filaggrin, and keratin and proteins involved in the regulation of gene transcription e.g., histones [1]. Hence, the enzymatic activity of PADs may impact gene expression by directly citrullinating transcription factors or by regulating histones and thus impact the epigenome. Recently, it has been reported that hypoxia promotes citrullination and PAD production in human fibroblast-like synoviocytes [2]. Although, the mechanisms promoting citrullination in RA are not fully elucidated but heightened citrullination propagates inflammation in an NF-xB-dependent expression of IL-1\(\beta\) and TNF\(\alpha\) [3]. Zhou Y et al. highlights novel pathways of extracellular protein citrullination, cellular localization of calcium (Ca\(^{2+}\)) dependent peptidyl arginine deiminases (PAD) and their activity in physiological and pathological conditions. This finding also sheds light upon mechanisms responsible for intracellular protein citrullination which are distinct from the ones mediated by PAD activation via Ca\(^{2+}\) influx due to the compromised cell membrane [4]. Various other pathologies have also been associated with the abnormal protein citrullination like multiple sclerosis, Alzheimer’s disease, prion diseases, psoriasis as well as cancer [5-8].

Millimolar Ca\(^{2+}\) levels are required by intracellular PAD activity, but how citrullination takes place even in the presence of an intact cell membrane remains uncertain. One possibility could be dysregulation of mitochondrial Ca\(^{2+}\) homeostasis i.e., inability of mitochondrial uptake of Ca\(^{2+}\) during physiological signaling [9]. Mitochondria play a crucial role in different intracellular pathways of signal transduction, ranging from energy production to cell death. The fine modulation of mitochondrial Ca\(^{2+}\) homeostasis plays a fundamental role in various pathophysiological processes. It regulates different cell processes controlled by buffering intracellular cytosolic Ca\(^{2+}\) as well as exerts a positive role on oxidative metabolism within mitochondria by regulating Ca\(^{2+}\) dependent Krebs’ cycle enzymes [10]. However, excessive mitochondrial Ca\(^{2+}\) entry consequent to stress stimuli causes opening of the mitochondrial permeability transition pore (mPTP) and release of proapoptotic factors which eventually lead to cell death [11]. The mPTP is voltage-gated inner membrane channel which is activated by matrix calcium overloading and reactive oxygen species (ROS) [12]. Full opening of the mPTP results in increased production of mitochondrial reactive oxygen species (mROS) and release of most matrix metabolites including mROS, calcium, NAD\(^{+}\), and glutathione. As a result, the mitochondrial membrane potential collapses, oxidative phosphorylation and mitochondrial metabolism are inhibited, the matrix swells, and on prolonged opening the outer membrane ruptures, releasing intermembrane space proteins [13]. Prolonged pore opening in a large number of mitochondria in the cell can lead to cell death by necrosis or similar pathways.

Discussion

Activation of mPTP by mitochondrial calcium overloading and mROS is seen enhanced during aging and in aging-associated degenerative diseases like depression, osteoarthritis, RA [14]. The oxidative damage to mitochondria is more severe and persistent than damage to nuclear DNA. The process of immunosenescence is accelerated and occurs prematurely in RA [15]. There is accelerated telomere length attrition in antigen-unprimed naïve T cells of RA patients due to inefficient telomere maintenance resulting in loss of telomeres, unraveling of heterochromatin, low expressions of DNA
repair nucleases like MRE11A, changed the expression of cell-cycle regulators which leads to accumulation of T cell aging [16]. Oxidative stress (OS) is responsible for the fragmentation of nuclear/ mitochondrial DNA, increased oxidative DNA damage, inefficient DNA mismatch repair system and accumulation of DNA adducts like 8-oxo-hydroxy-7,8-dihydro-2’-deoxyguanosine, 1,N(6)-etheno-2’-deoxyadenosine, and heptanone-etheno-2’-deoxyctydine which results in progression of autoimmune disease like RA [17]. Excessive physiological buffering capacity due to ROS generation during respiratory bursts results in OS. There is perturbation in mitochondrial homeostasis leading to excessive ROS production, impaired mitochondrial dynamics, electron transport chain defects, bioenergetics imbalance and increased AMPK activity, decreased mitochondrial NAD+ and altered metabolism, and mitochondrial calcium accumulation. Such mitochondrial signals activate p53/p21 and/or p16/p5b pathways resulting in cellular senescence [18]. Oxidative damage to calcium transporters leads to calcium overload and hence inducing more frequent opening of mPTP. Intracellular Ca2+ levels in normal cells are much lower than the optimal Ca2+ levels required for PAD activation. Hence, it has been proposed that PAD activation occurs only during cell death or necrosis when PAD enzymes leak out into the extracellular matrix from dying cells, or vice versa, with the high Ca2+ concentration activating PADS [19].

Hence, spontaneous secretion of intracellular PAD may be the result of high cytosolic Ca2+ due to a disturbance in mitochondrial calcium homeostasis secondary to oxidative damage to mitochondria and flares of RA is seen with increased environmental exposure especially air pollution. Air pollutants also induce OS which may compromise the mitochondrial integrity and disturb Ca2+ homeostasis. Mitochondria are the powerhouse organelle, a place where oxidative metabolism takes place, which plays a pivotal role in health and disease. Most of the mitochondrial activities are driven in a Ca2+ dependent manner, in turn affecting the activities of various Ca2+ dependent enzymes like PADS. Development of specific drugs that act on mitochondrial Ca2+ homeostasis is being developed which may open the way to new biochemically designed therapeutic approaches in the treatment of several disabling disorders like RA. Studies in our laboratory have shown yoga-based lifestyle intervention (YBLLI) improves mitochondrial integrity as is evident by increasing COX activity, reducing OS, upregulating the total anti-oxidant capacity and telomerase enzymes, which both aid in maintenance of telomere length and genomic stability, thus reducing and delaying onset of age-related chronic diseases and complex lifestyle disorders [20-23]. Also, YBLLI reduces the levels of acute phase reactants like CRP, pro-inflammatory cytokines, OS levels in RA [24].

Conclusion

The ongoing studies in our laboratory on the impact of yoga and meditation have shown significant upregulation in the expression of antioxidant, anti-inflammatory genes, genes of cell cycle control, DNA repair genes and elevation of tolerogenic molecules which both aid in immune-modulation and reversal of autoimmunity. Improvement in mitochondrial integrity with normal Ca2+ levels by maintaining homeostasis, thus prevent activation of PAD and citrullination of tissue fibrinogen. This may prevent numerous sequelae of excessive citrullination and dysregulated gene expression. Thus, we hypothesize that activation of PAD may be due to loss of mitochondrial integrity secondary to OS. OS can be induced due to various exogenous and endogenous factors. Majority of factors that induce oxidative stress are modifiable like quitting smoking, decreasing intake of fast nutritionally depleted processed food, minimizing alcohol consumption, minimize exposure to xenobiotics and insecticides and pesticides and adopt simple mind body stress reduction techniques to delay accelerated aging and improve mitochondrial integrity. Thus, simple lifestyle intervention may maintain optimal ROS levels, mitochondrial integrity, Ca2+ homeostasis and prevent activation of PADS and thus reduce the incidence/severity of RA.

Author Contributions

RD and SG conceived the idea behind this commentary; SG wrote the manuscript; SG, AG and PC generated experimental data and; RD finalized the article.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

This study was funded by Science and Technology of Yoga and Meditation (SATYAM) (SR/SATYAM/SS/2016), Government of India, Ministry of Science and Technology, Department of Science and Technology (DST).

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