Molecular Medicine: Current Aspects

Scient Open Access
Exploring the World of Science

Open Access Full Text Article

Research Article

Mitochondrial Regulation in the Pathogenic Process of Inflammatory Arthritis by Microalgal *Mucidosphaerium* Species

This article was published in the following Scient Open Access Journal:
Molecular Medicine: Current Aspects
Received September 08, 2017; **Accepted** September 14, 2017; **Published** November 13, 2017

Chie K. Mifude[1], Hiroyuki Ishitobi[2,3], Shigeru Miyaki[2] and Kuniyoshi Kaseda[1]*

[1]Saravio Central Institute, Saravio Cosmetics Ltd., Oita, Japan
[2]Medical Center for Translational and Clinical Research, Hiroshima University Hospital, Hiroshima, Japan
[3]Faculty of Sociology Department of Sports Sociology and Health Sciences, Kyoto Sangyo University, Japan

Abstract

The excess generation of reactive oxygen species is involved in the pathogenesis of arthritis such as Rheumatoid Arthritis (RA) and Osteoarthritis (OA). The oxidative products are induced by inflammatory cytokines via mitochondria in synovium and articular cartilages. We found that IL-1β, one of the critical mediators of arthritis, impaired the balance of mitochondrial configuration in human Fibroblast-Like Synoviocytes (FLSs): the stimulus factor reduced the number of filamentous mitochondria and increased that of rounded mitochondria. In addition, the cellular ATP level was reduced but instead the ROS level was increased in the IL-1β-stimulated FLSs. Significant relationship was observed between the mitochondrial morphology and the functions.

The effects of the cytokine on mitochondrial morphology and functions were cancelled by the extract of a novel strain of *Mucidosphaerium* species RG92. The current findings of the mitochondrial roles in the process of arthritis along with the preventative function of the algal RG92 shall provide new methodology for the treatment of inflammatory arthritis.

Introduction

Chronic disease is a multi-factorial and polygenic disease, therefore, its pathogenesis is influenced by several genetic and environmental factors under diverse molecular pathways. In RA, the progressive destruction of articular cartilages and bones are observed [1]. Typically, the level of pro-inflammatory cytokines such as IL-1β and TNF-α becomes highly elevated in synovium at the initial stage of the RA pathology [2,3]. Although the level of pro-inflammatory cytokines in OA are generally lower than that in RA, joint inflammation still plays a role and thereby contributes to pain in OA, the main subjective symptom in the patients [4,5]. The homeostatic balance of joint tissues such as cartilage and synovium is regulated by extracellular stimuli including cytokines and mechanical stress [6,7]. The impaired balance causes the growth or the death of joint cells, correspondingly resulting in hyperproliferation of Fibroblast-Like Synoviocytes (FLSs) or apoptotic cell death of chondrocytes in RA and OA. In addition, up-regulated proteolytic enzymes degrade the extracellular matrix of cartilage and bone [8].

A mitochondrion, an indispensable organelle in eukaryote cells, not only supplies chemical energy in the form of ATP but also buffers calcium gradients and regulates programmed cell death [9]. In addition, Reactive Oxygen Species (ROS) is produced mainly during the oxidative phosphorylation in mitochondria. Excessive ROS generation damages cellular functions by oxidizing lipids, proteins and nucleic acids, along with enhancing inflammatory response as a second messenger in the pathogenesis of chronic diseases. In response to cellular demands and/or environmental stresses, mitochondria undergo morphological transitions that is regulated by dynamic collaboration of membrane fusion/fission proteins. The dysfunction of the organelle has been shown to be a mechanism underlying various inflammatory, autoimmune and age-related diseases [6,10].

In fact, mitochondrial dysfunction promotes and aggravates inflammatory response in human FLSs and chondrocytes [11,12]: IL-1β, in conjunction with the inhibitors of mitochondrial respiratory chain, synergetically induces the inflammatory cytokines/mediators such as IL-8, COX-2 and PGE_2, though the ROS-mediated NF-κB pathway. In addition, the stimulative cytokine reduces the mitochondrial biomass and membrane potential, resulting in the reduction of cellular ATP level and the induction of apoptotic

***Corresponding author:** Kuniyoshi Kaseda, Saravio Central Institute, Saravio Cosmetics Ltd., 1356-6 Oaza Tsurumi, Beppu, Oita, Japan, 874-0842, Tel: +81-977-75-8575, Fax: +81-977-75-8112, Email: kaseda@saravio.jp

cell death [13]. According to these reports, an antioxidant resveratrol attenuates the inhibitory effects of the inflammatory stimuli in both chondrocytes and FLSs [14,15]. These anti-inflammatory substances may represent a future strategy in controlling the inflammatory response of joint tissues.

The relationship between mitochondrial dysfunction and articular inflammation has been increasingly built up. For instance, the mitochondrial mutations are highly associated with the inflammation level of synovial tissue in inflammatory arthritis [16]. In addition, the excessive oxidative stress induced by defective mitochondria is closely correlated with synovial inflammatory progressions [17-19]. Interestingly, RA patients show higher incidence of mitochondrial mutations in FLSs compared with OA patients [20].

While energy metabolism dominantly depends on glycolysis in chondrocytes, mitochondrial oxidative phosphorylation plays a significant role in the cells [21,22]. Indeed, when human chondrocytes were exposed to either IL-1β or TNF-α, the energy producing organelle was fragmented and the respiratory system became inefficient, concomitantly with a decrease in the activity of ATP production and the elevation of superoxide level [23]. However, the relationship between mitochondrial morphology and function has not been quantitatively described in line with inflammatory response in joint cells. In the current work, we firstly investigated mitochondrial morphology and function in IL-1β-stimulated FLSs.

A better understanding of inflammation-related mitochondrial regulation shall be beneficial in developing a novel therapeutic approach for the prevention of arthropathies. In fact, TNF blocking therapy effectively suppresses oxidative stress and hypoxia-induced mitochondrial mutagenesis in inflammatory arthritis [17]. We have recently discovered a novel strain of microalga, *Mucidosphaerium* sp. RG92, and its extract inhibited the over expression of the pro-inflammatory cytokines in different types of human primary cells [24]. In addition, the algal extract suppressed the excess ROS production in FLSs, inhibited the abnormal proliferation of the cells and attenuated the gene expression of Matrix Metalloproteinases (MMPs), when the cells were stimulated by IL-1β. While those results imply that the microalgal extract could prevent the progresses of arthritis, mitochondrial involvement in the effects has yet to be revealed. Here, we describe that the algal RG92 dramatically attenuates the influence of IL-1β upon mitochondrial morphology and function in FLSs.

Materials and Methods

Materials

Primary FLSs were isolated from synovial autopsy of donors. Studies were approved human subjects/ethics protocols by Scripps Research Institute Human Subjects Institutional Review Boards. IL-1β was purchased from PeproTech (Rocky Hill, NJ). Unless otherwise stated, all other chemicals of analytical grade were obtained from either Sigma-Aldrich (St. Louis, MO) or Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The microalgal extract was prepared as described elsewhere [24].

Cell culture

FLSs (passage 5) were sub-cultured as described elsewhere [24]. In brief, the cells were grown in DMEM supplemented with 10% foetal calf serum and 50 Uml^{-1} penicillin/50 μgmL^{-1} streptomycin (Invitrogen, Carlsbad, CA, USA) in a 5% CO_2 atmosphere at 37°C.

Functional assays

FLSs were stimulated by 10 ng/ml recombinant human IL-1β for 24 hours. In order to test the effect of the microalge-derived ingredient, the cells were incubated with the algal extract for 24 hours in advance of the IL-1β stimulation. For the investigation of mitochondrial morphology, the fluorescent images were observed after the organelle were specifically stained with 100 nM Mito Tracker Red CMXRos (Invitrogen, Carlsbad, CA) [25]. For the detection of the level of cellular ATP and ROS, ''Cell'' ATP Assay reagent (TOYO INK CO., LTD., Tokyo) and DCFDA Cellular ROS Detection Assay Kit (Abcam, Cambridge) were used, respectively. Data are represented as the mean +/- SD of three to six independent experiments. Statistical analysis was performed using Student's t-test.

Results and Discussion

Inflammatory stimulus uncouples ATP synthetic reaction through mitochondrial morphological change

The mitochondrial morphology of primary synovial cells were observed under a fluorescent microscope. Similar to the previous report with a cutaneous dermal papilla cells [25], the energy producing organelle showed mainly two different configurations, i.e., filamentous and rounded types (Figure 1). 37% of the cellular population showed only filamentous mitochondria while 6% population showed only rounded mitochondria. The rest of the cells showed mixture of the two types (Table 1).

		Cellular population (%)		
IL-1β		**Filamentous mitochondria**	**Rounded mitochondria**	**Mixture**
-	Control (n = 500)	36.7±5.2	6.4±2.0	56.9±5.3
-	RG92 (n = 500)	37.0±4.5	5.1±1.7	57.9±3.4
-	NAC (n = 300)	34.0±2.9	12.3±2.9	54.0±0.0
+	Control (n = 600)	9.0±2.1	25.9±6.0	65.0±5.2
+	RG92 (n = 600)	36.0±5.0	8.4±4.0	55.7±7.0
+	NAC (n = 300)	29.0±6.2	15.0±3.6	55.7±3.4

Mitochondrial configuration was examined in the IL-1β-challenged FLSs. The microalgal extract (RG92) cancels the effect of the inflammatory cytokine, similar to an antioxidant, NAC. Data are represented as the mean +/- SD of three to six independent experiments. Total numbers of tested cells are shown in parentheses.

Table 1: Inflammatory and anti-inflammatory effects on mitochondrial morphology.

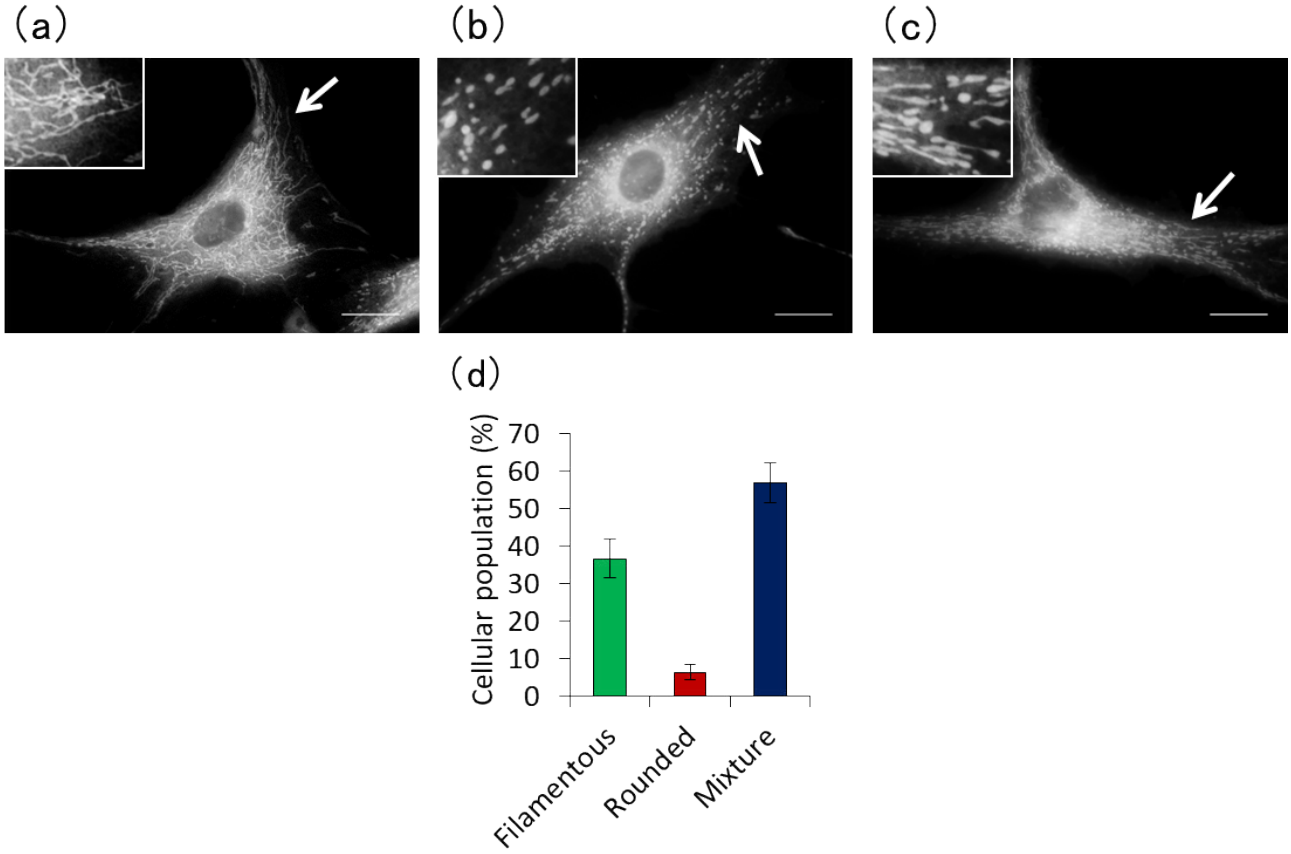

Figure 1: Filamentous and rounded mitochondria in human FLSs
Mitochondria of primary FLSs were visualized with Mito Tracker Red CMXRos. 37 % of the cell population shows filamentous mitochondria (a) and 6 % of the population shows rounded mitochondria (b). The rest of the cells show the mixture of the two types (c). Enlarged views of the arrowed areas are shown in the white boxes. Scale bars = 20μm. (d) The cellular population with each type of mitochondria. Data are represented as the mean +/- SD of five independent experiments.

As shown in Figure 2 and Table 1, when the cells were treated with IL-1β, the balance between the two morphologies was considerably affected, with the decreased level of filamentous mitochondria (9%) and the increased level of rounded mitochondria (26%). We previously demonstrated that the filamentous mitochondria produce more chemical energy ATP than the rounded mitochondria in other fibroblast-like cells, which is beneficial in cellular migration [25,26]. Therefore, we investigated mitochondrial functions in the presence or the absence of IL-1β. As shown in Figure 3, the level of cellular ATP and ROS were significantly decreased and increased, respectively, in the presence of the pro-inflammatory cytokine. Therefore, it is likely that IL-1β induces the uncoupling of the chemical process of ATP synthesis in the articular cells, in parallel with the morphological transition of mitochondria. In fact, as shown in Figure 4a, a linear relationships were observed in both ATP and ROS levels over the population of filamentous mitochondria. The opposite relationship was shown over the population of rounded mitochondria (Figure 4b). These results confirm that the mitochondrial configuration plays an important role in the coupled functions of ATP synthesis of the organelle [25,26]. It was previously reported that the surface of cristae increases through the collaboration of fusion proteins when energy is highly required [27,28]. It is intriguing to investigate fine structure of IL-1β-stimulated mitochondria in the future.

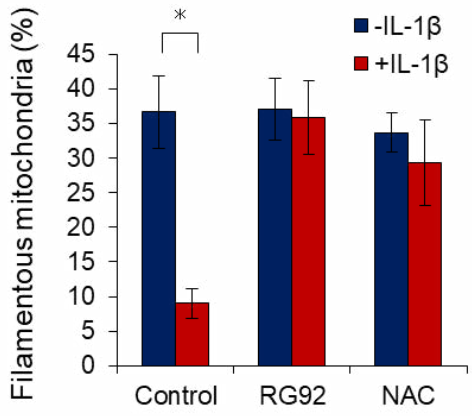

Figure 2: Anti-inflammatory RG92 negates IL-1β-induced morphological change of mitochondria
Cellular population of FLSs with filamentous mitochondria was measured in the different conditions. The incubation of the cells with 10 ng/ml IL-1β for 24 hours dramatically reduces the number of the elongated form. Both anti-inflammatory algal extract (RG92) and an antioxidant (NAC) negate the effect of the inflammatory challenge, while they do not change the morphological population in the absence of IL-1β. Data are represented as the mean +/- SD of three to five independent experiments. * indicates *P*-value <0.001 compared with control.

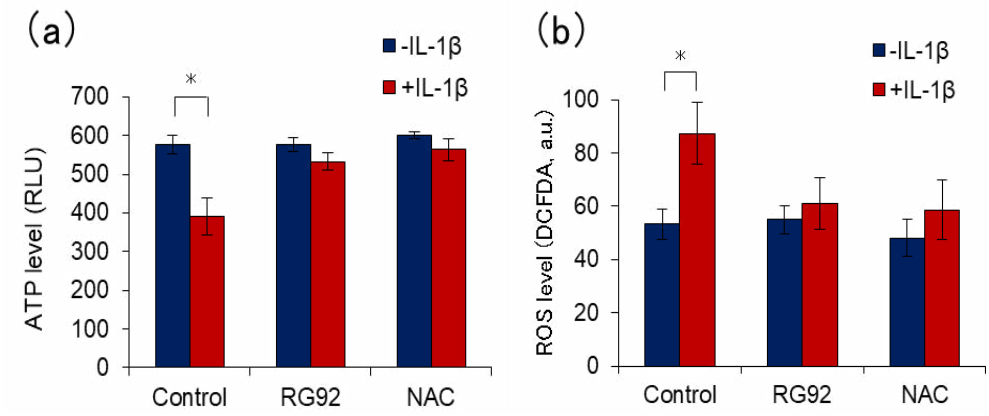

Figure 3: Anti-inflammatory RG92 negates IL-1β-induced uncoupling of mitochondrial function
(a) The level of intracellular ATP is significantly reduced by IL-1β. Neither the anti-inflammatory algal extract (RG92) nor a typical antioxidant (NAC) affects the ATP level in the absence of IL-1β, however, the both effectively suppress the IL-1β-induced inhibition of ATP synthesis.
(b) The level of ROS production is significantly elevated by the inflammatory stimulus. The algal extract suppresses the IL-1β-induced ROS elevation, similar to NAC (1mM). Neither the algal extract nor NAC affects the ROS level in the absence of IL-1β. Data are represented as the mean +/- SD of three independent experiments. * indicates *P*-value <0.05 compared with control.

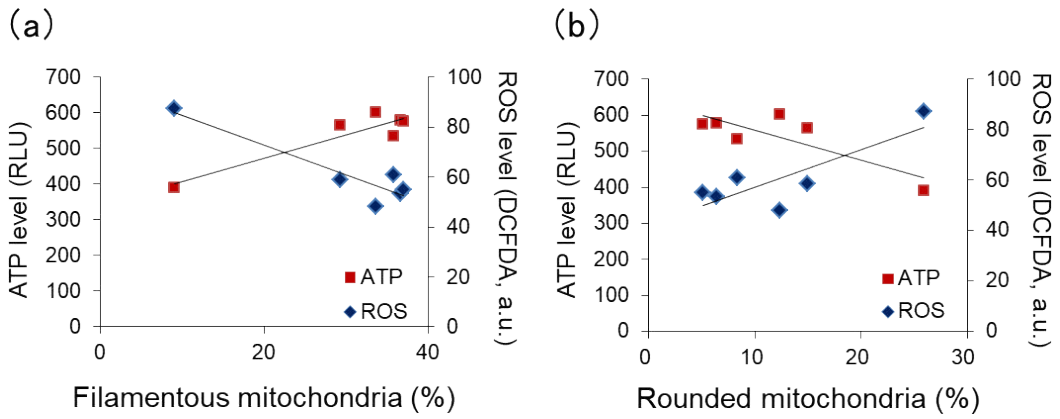

Figure 4: Relationship between mitochondrial morphology and functions
The determined levels of ATP and ROS were re-plotted against the corresponding number of filamentous mitochondria (a) or rounded mitochondria (b). The linear least-squares fittings show the correlation between the morphology of the organelle and its functions.

Microalgal RG92 prevents mitochondria from undergoing inflammatory damage

We have recently discovered a novel strain of microalga, *Mucidosphaerium* sp. (RG92), from a famous hot spring spa in Beppu in Japan. The algal extract shows anti-inflammatory effects in different types of human primary cells including FLSs: when the cells were stimulated by IL-1β, the gene expression of TNF-α, IL-1β, IL-6 and MMP-1, 3, 9 were up-regulated. The over expression of the risk factors of RA were effectively suppressed by the algal extract, which suggests that the novel microalga is an useful tool for the preventative application of the articular disease [24]. Here we examined the effect of the algal extract on mitochondrial activities in FLSs. As shown in Figure 2 and Table 1, the percentage of the cells that show filamentous and rounded mitochondria became back to the normal levels by the algal extract in the presence of IL-1β, while the numbers were unaffected by the bio-product in the absence of the pro-inflammatory cytokine. Importantly, the algal extract suppressed both the inhibition of ATP production and the enhancement of ROS generation that were induced by IL-1β (Figure 3). The effects of the extract were similar to a typical ROS scavenger NAC. Therefore, the algal extract restores the inhibitory effect of IL-1β in the process of the mitochondrial oxidative phosphorylation.

Conclusions

In the current work, we investigated mitochondrial configuration and functions in FLSs and found that IL-1β reduces the number of filamentous form of the organelle, concomitantly with the reduction of ATP level and the excess generation of ROS in the cells. The microalgal RG92 attenuated the effect of IL-1β on mitochondrial morphology and functions (Figure 5). The effects of the extract might be closely correlated to its anti-inflammatory functions and the detailed molecular mechanism is to be examined [24]. Even so, the discovery of the mitochondrial involvement in the cellular process of RA and OA along with that of the novel function of the algal extract will provide new methodology in the therapeutic approach of the diseases.

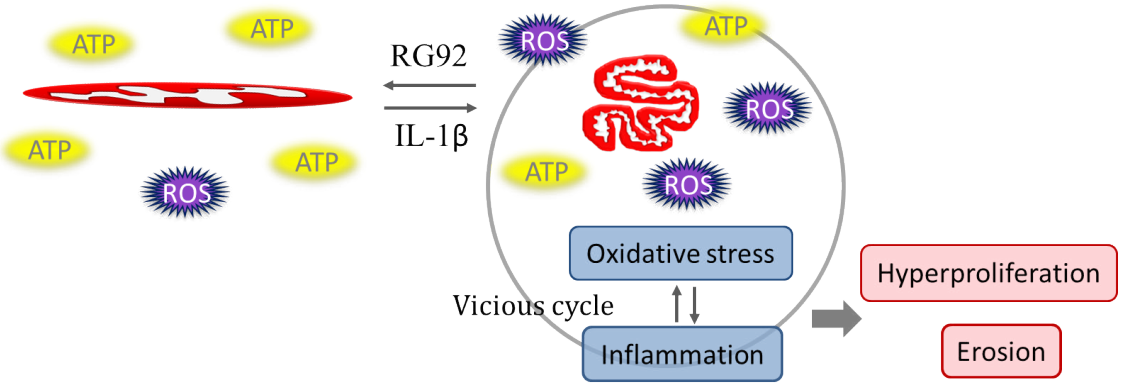

Figure 5: Mitochondrial damage in FLSs in inflammatory arthritis
At the early stage of chronic arthritis, one of the major pro-inflammatory cytokines, IL-1β, is produced. The pathogenic factor modifies mitochondrial morphology and functions in FLSs: rounded mitochondria become dominant with decreased ATP production and increased ROS generation in the presence of IL-1β. These mitochondrial modification is involved in the following processes of the articular disease, i.e., abnormal synoviocyte proliferation and cartilage degradation, though the vicious cycle of oxidative stress and inflammatory reactions. The microalgal RG92 cancels the effect of IL-1β on mitochondrial morphology and functions. Note that a rounded mitochondrion is shown as a tangled form, based on the previous report [26].

Abbreviations

IL: Interleukin; MMP: Matrix Metalloproteinase; NAC: N-Acetyl-L-Cysteine; ROS: Reactive Oxygen Species; TNF: Tumor Necrosis Factor

Conflicts on Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgement

We would like to thank Dr. Martin Lotz for providing the FLSs.

References

1. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med.* 2011;365(23): 2205-2219.
2. Kapoor M, Martel-Pelletier J, Lajeunesse D, et al. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol.* 2011;7(1):33-42.
3. Brennan FM, Maini RN, Feldmann M. Role of pro-inflammatory cytokines in rheumatoid arthritis. *Seminars in Immunopathology.*1998;20(1-2):133-147.
4. Liu-Bryan R, Terkeltaub R. Emerging regulators of the inflammatory process in osteoarthritis. *Nat Rev Rheumatol.* 2015;11(1):35-44.
5. Mathiessen A, Conaghan PG. Synovitis in osteoarthritis: current understanding with therapeutic implications. *Arthritis Research & Therapy.* 2017;19(1):18.
6. Lotz MK, Caramés B. Autophagy and cartilage homeostasis mechanisms in joint health, aging and OA. *Nat Rev Rheumatol.* 2011;7(10):579-587.
7. Miyaki S, Asahara H. Macro view of microRNA function in osteoarthritis. *Nat Rev Rheumatol.* 2012;8(9):543-552.
8. Bartok B, Firestein GS. Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. *Immunol Rev.* 2010;233(1):233-255.
9. Picard M, Shirihai OS, Gentil BJ, et al. Mitochondrial morphology transitions and functions: implications for retrograde signaling?. *Am J Physiol Regul Integr Comp Physiol.* 2013;304(6):R393-406.
10. Hernandez-Aguilera A, Rull A, Rodriguez-Gallego E, et al. Mitochondrial dysfunction: a basic mechanism in inflammation-related non-communicable diseases and therapeutic opportunities. *Mediators of Inflammation. 2013.*
11. Valcarcel-Ares MN, Riveiro-Naveira RR, Vaamonde-García C, et al. Mitochondrial dysfunction promotes and aggravates the inflammatory response in normal human synoviocytes. *Rheumatology (Oxford).* 2014;53(7):1332-1343.
12. Vaamonde-García C, Riveiro-Naveira RR, Valcárcel-Areset MN, et al. Mitochondrial dysfunction increases inflammatory responsiveness to cytokines in normal human chondrocytes. *Arthritis Rheum.* 2012;64(9):2927-2936.
13. Dave M, Attur M, Palmer B, et al. The antioxidant resveratrol protects against chondrocyte apoptosis via effects on mitochondrial polarization and ATP production. *Arthritis Rheum.* 2008;58(9):2786-2797.
14. Dvir-Ginzberg M, Mobasheri A, Kumar A. The Role of Sirtuins in Cartilage Homeostasis and Osteoarthritis. *Curr Rheumatol Rep.* 2016;18(7):43.
15. Nguyen C, Savouret JF, Widerak M, et al. Resveratrol, Potential Therapeutic Interest in Joint Disorders: A Critical Narrative Review. *Nutrients.* 2017;9(1):E45.
16. Harty LC, Biniecka M, O'Sullivan J, et al. Mitochondrial mutagenesis correlates with the local inflammatory environment in arthritis. *Ann Rheum Dis.* 2012;71(4):582-588.
17. Biniecka M, Kennedy A, Ng CT, et al. Successful tumour necrosis factor (TNF) blocking therapy suppresses oxidative stress and hypoxia-induced mitochondrial mutagenesis in inflammatory arthritis. *Arthritis Res Ther.* 2011;13(4):R121.
18. Filippin LI, Vercelino R, Marroni NP, et al. Redox signalling and the inflammatory response in rheumatoid arthritis. *Clin Exp Immunol.* 2008;152(3):415-422.
19. Lepetsos P, Papavassiliou AG. ROS/oxidative stress signaling in osteoarthritis. *Biochimica et Biophysica Acta.* 2016;1862(4):576-591.
20. Da Sylva TR, Connor A, Mburu Y, et al. Somatic mutations in the mitochondria of rheumatoid arthritis synoviocytes. *Arthritis Res Ther.* 2005;7(4):R844-851.
21. Cao Y, Zhang X, Shang W, et al. Proinflammatory Cytokines Stimulate Mitochondrial Superoxide Flashes in Articular Chondrocytes In Vitro and In Situ. *PLOS ONE.* 2013;8(6):e66444.
22. Martin JA, Martini A, Molinari A, et al. Mitochondrial electron transport and glycolysis are coupled in articular cartilage. *Osteoarthritis Cartilage.* 2012;20(4):323-329.
23. López-Armada MJ, Caramés B, Martín MA, et al. Mitochondrial activity is modulated by TNFalpha and IL-1beta in normal human chondrocyte cells. *Osteoarthritis Cartilage.* 2006;14(10):1011-1022.
24. Miyata M, Iwata S, Mifude KC, et al., (peer review process)
25. Mifude C, Kaseda K. PDGF-AA-induced filamentous mitochondria benefit dermal papilla cells in cellular migration. *Int J Cosmet Sci.* 2015;37(3):266-271.
26. Mifude C, Kaseda K. Shaping up mitochondrion in motion. *DERMATOLOGY Open Journal. 2016;*1(2):38-41.

27. Jakovcic S, Swift HH, Gross NJ, et al. Biochemical and stereological analysis of rat liver mitochondria in different thyroid states. *J Cell Biol*. 1978;77(3):887-901.

28. Wu PC, Lai CL, Liddel RH. Quantitative morphology of mitochondria in hepatocellular carcinoma and chronic liver disease. *Arch Pathol Lab Med*. 1984;108(11):914-916.

Copyright: © 2017 Kuniyoshi Kaseda, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.