The role of mitochondria in osteoarthritis

Francisco J. Blanco, Ignacio Rego and Cristina Ruiz-Romero

Abstract | Mitochondria are important regulators of cellular function and survival that may have a key role in aging-related diseases. Mitochondrial DNA (mtDNA) mutations and oxidative stresses are known to contribute to aging-related changes. Osteoarthritis (OA) is an aging-associated rheumatic disease characterized by articular cartilage degradation and elevated chondrocyte mortality. Articular cartilage chondrocytes survive and maintain tissue integrity in an avascular, low-oxygen environment. Recent *ex vivo* studies have reported mitochondrial dysfunction in human OA chondrocytes, and analyses of mitochondrial electron transport chain activity in these cells show decreased activity of Complexes I, II and III compared to normal chondrocytes. This mitochondrial dysfunction may affect several pathways that have been implicated in cartilage degradation, including oxidative stress, defective chondrocyte biosynthesis and growth responses, increased cytokine-induced chondrocyte inflammation and matrix catabolism, cartilage matrix calcification, and increased chondrocyte apoptosis. Mitochondrial dysfunction in OA chondrocytes may derive from somatic mutations in the mtDNA or from the direct effects of proinflammatory mediators such as cytokines, prostaglandins, reactive oxygen species and nitric oxide. Polymorphisms in mtDNA may become useful as biomarkers for the diagnosis and prognosis of OA, and modulation of serum biomarkers by mtDNA haplogroups supports the concept that mtDNA haplogroups may define specific OA phenotypes in the complex OA process.

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Introduction

Osteoarthritis (OA) is characterized by late-onset degeneration of articular cartilage, and is the most common joint disease associated with aging. Prevalence studies show that OA usually develops after the age of 45 years, and affects more than 10% of the population. However, its prevalence increases with age, and most people aged over 65 years exhibit this pathology.[1] OA is the leading cause of permanent work incapacitation and one of the most common reasons for visiting primary care physicians. As populations in the developed world age, it is estimated that the number of OA cases will double in the next three decades. Thus, this disease has experienced a large increase in research interest in the past decade.

As the chondrocyte is the only cell type present in mature cartilage, the OA disease process is characterized by changes in these cells. OA chondrocytes have been shown to produce extracellular-matrix-degrading proteins, such as the matrix metalloproteinases (MMPs), and proinflammatory cytokines, predominantly interleukin (IL)-1 and tumor necrosis factor (TNF).[2] Prostaglandins, nitric oxide (NO) and other reactive oxygen species (ROS), which are also produced by chondrocytes, have also been reported to be important mediators of OA.[3–5] Enhanced chondrocyte proliferation and apoptosis associated with the activation of chondrocytes are also important features of OA pathophysiology. While the progressive loss of articular cartilage is the most important characteristic of OA, other structural processes in the joint are also involved, such as synovial inflammation, osteophyte formation and remodeling of subchondral bone.

The mitochondrion, found in most eukaryotic cells, is a membrane-enclosed organelle that converts nutritional molecules into ATP via oxidative phosphorylation.[6] A eukaryotic cell typically contains about 2,000 mitochondria, which occupy approximately 20% of the total cell volume.[7] Each mitochondrion is formed of an inner and an outer membrane composed of phospholipid bilayers and proteins (Figure 1). The outer mitochondrial membrane encloses the organelle and has a protein-to-phospholipid ratio of about 1:1 by weight, which is similar to the plasma membrane of eukaryotic cells. The inner mitochondrial membrane contains several proteins that perform oxidation reactions in the respiratory chain, along with ATP synthase, which converts ADP to ATP in the matrix. Other proteins on this membrane function as specific transporters of metabolites and proteins into and out of the matrix.[8]

Morphologically, the inner mitochondrial membrane is compartmentalized into numerous cristae, which increase the surface area and maximize the ATP generation capability. NADH dehydrogenase (Complex I), succinate dehydrogenase (Complex II), cytochrome c reductase (Complex III) and cytochrome c oxidase (Complex IV) are protein complexes in the inner membrane that perform the transfer and incremental release of energy from the donated electrons, which is used to pump protons (H^+) into the intermembrane space

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Key points

- Mitochondrial functions, including mitochondrial respiratory chain (MRC) activity and ATP synthesis, are altered in osteoarthritis (OA) chondrocytes
- Mitochondrial dysfunction may influence several of the specific pathways involved in OA pathology, including oxidative stress, chondrocyte apoptosis, cytokine-induced chondrocyte inflammation and matrix catabolism, and calcification of the cartilage matrix
- OA chondrocyte mitochondrial dysfunction may originate from somatic mutations in the mitochondrial DNA (mtDNA) or from the direct effects of proinflammatory cytokines, prostaglandins, reactive oxygen species and nitric oxide on the MRC and ATP synthesis
- mtDNA haplogroups may serve as useful biomarkers for the diagnosis or prognosis of OA, and might define distinct, specific OA phenotypes with different levels of serum OA biomarkers

(Figure 1). The protons then pass through ATP synthase (sometimes referred to as Complex V) to provide energy for the creation of ATP. While this process is efficient, a small number of electrons may only partially reduce oxygen to form the toxic free radical superoxide, which can cause oxidative damage to the mitochondria that contributes to their impaired function.[9]

Over the past decade, the importance of mitochondria in cell biology has been emphasized by emerging evidence that mitochondrial protein alterations may cause some human diseases.[8,9] These protein modifications can lead to important organ and cellular dysfunctions that contribute to such neurologic conditions as Alzheimer disease, Parkinson disease and Huntington disease. Other diseases to which mitochondrial degeneration contribute include some types of cancer, type 2 diabetes mellitus, cardiovascular diseases, various neuromuscular syndromes and some aging processes.[10]

The role of mitochondria in OA is currently being studied in detail, and recent investigations have yielded new and exciting knowledge, which we aim to describe in this Review. Although multiple components of the joint are adversely affected by OA, here we will focus on the articular cartilage.

The mitochondrion in OA pathogenesis

As articular cartilage chondrocytes are traditionally classified as highly glycolytic cells,[11,12] mitochondrion-mediated OA pathogenesis has not, until relatively recently, been investigated in great detail. However, alterations in some mitochondrial functions, such as ATP production and modulation of calcium levels, and the redox state of the mitochondria could explain some mechanisms that induce cartilage degradation by chondrocytes during OA, such as increased oxidative stress, cytokine production and subsequent inflammation-mediated matrix breakdown, cartilage matrix calcification and increased cell death.

To understand OA pathogenesis and cartilage degradation, it should be noted that there are no blood vessels, lymphatic channels or neural elements entering or transiting adult articular cartilage. Numerous studies strongly indicate that the major source of nutrients for articular cartilage is synovial fluid,[13] and, because synovial fluid is an ultrafiltrate of plasma, chondrocytes receive their nutrition through a double diffusion system. First, nutrients cross the synovial barrier into the synovial fluid, then diffuse across the articular cartilage matrix to reach the cells. Because of the relative impermeability of the matrix, molecular size and electrical charge markedly affect the diffusion of nutrients.[14]

Oxygen is the terminal electron acceptor for mitochondrial cytochrome c oxidase in all human tissues, and is also a substrate for many other enzymes, including oxygenases and oxidases. Oxygen tension varies among tissues, and depends on several factors, including the density of cells in the tissue volume, the cellular metabolic rate, the diffusivity of oxygen through the extracellular matrix, and the quality of the vascular supply. As articular chondrocytes are oxygenated only by diffusion from the synovium (Figure 2), and because of the asymmetry of the oxygen supply, an oxygen gradient must exist across the tissue.[15,16] While cells at the surface are estimated to receive 5–7% oxygen (compared to 13% in arterial blood[17]), chondrocytes in the deepest regions of the cartilage receive only a very low oxygen tension (<1%).[11,12] These findings suggest that articular cartilage chondrocytes must survive and maintain tissue integrity in an avascular, low-oxygen environment, with asymmetric, decreasing oxygen and glucose concentration gradients from the superficial to the deep zones. It seems likely that chondrocytes in the deeper zones may require adaptively increased anaerobic glycolysis, while those in superficial areas use aerobic respiration to support ATP synthesis. In this sense, the number of mitochondria is higher in chondrocytes from superficial than deep zone (F. J. Blanco, unpublished data).

The results of a number of experiments indicate that cultured chondrocytes generate ATP through glycolysis and produce lactic acid as an end product.[14] However, the capability of these cells to utilize oxidative metabolism for the production of chemical energy is supported by *in vitro* observations that chondrocytes contain mitochondrial dehydrogenases, that isolated mitochondria can utilize tricarboxylic acid (TCA) cycle (also known as the citric acid cycle or Krebs cycle) substrates, and that these mitochondria contain the enzymes required for oxidative phosphorylation and electron transport.[18,19] In addition, cultured human articular chondrocytes were shown to possess similar levels of enzymatic activity in the mitochondrial respiratory chain (MRC) when compared to other mesenchymal cells.[20] Indeed, it has been reported that mitochondrial oxidative phosphorylation may account for as much as 25% of the ATP production in cartilage.[21,22] However, it is important to keep in mind that the majority of these results were obtained using *in vitro* models, which means that the chondrocytes are cultured in standard medium with high glucose concentrations (4.5 mM) and under aerobic conditions (high oxygen tension). Interestingly, recent *in vitro* findings showed that hypoxia differentially modulates the chondrocyte proteome in normal and OA human chondrocytes, including some mitochondrial proteins. OA cells showed less-extensive modulation than normal chondrocytes,

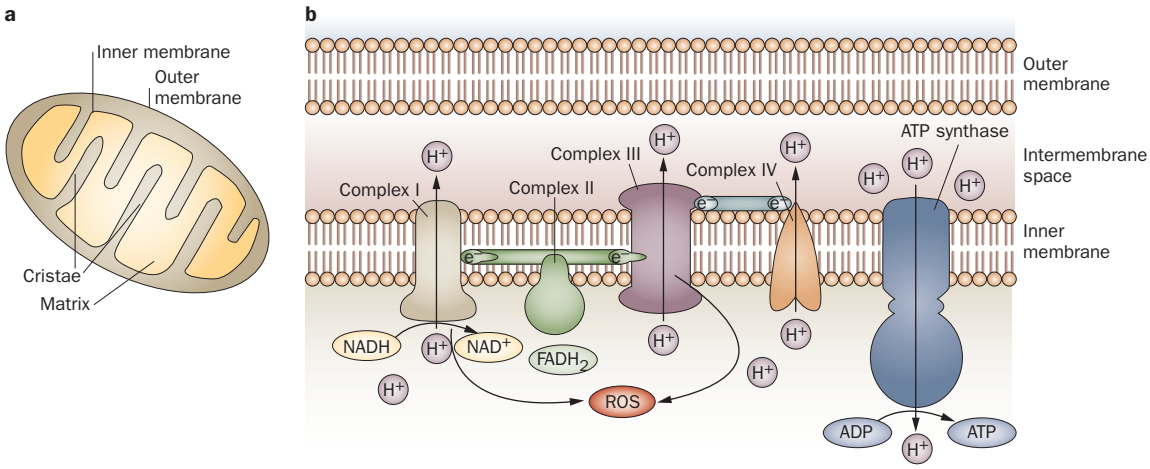

Figure 1 | Structure and function of mitochondria. **a** | Mitochondria contain inner and outer membranes composed of phospholipid bilayers and proteins. The outer mitochondrial membrane encloses the entire organelle, and contains porins that are involved in the transport of molecules ≤5 kDa. Larger molecules require active transport by mitochondrial membrane transport proteins. The inner mitochondrial membrane is compartmentalized into cristae, which expand the surface area of the inner mitochondrial membrane and enhance its capacity to generate ATP. It contains proteins that perform the oxidation reactions of the electron transport chain and ATP synthesis (oxidative phosphorylation). **b** | The tricarboxylic acid cycle produces reduced cofactors (three molecules of NADH and one molecule of $FADH_2$) that donate electrons to the electron transport chain. Protein complexes use energy released from electrons to pump protons into the intermembrane space, which creates a membrane potential that is used to convert ADP to ATP. Leakage of electrons from the electron transport chain can result in the production of ROS. Abbreviation: ROS, reactive oxygen species.

which indicates a lower capacity of diseased cells to react under a hypoxic milieu.[23]

Mitochondria and the inflammatory response

Ex vivo studies have revealed dysfunction of the mitochondria in human OA chondrocytes.[20] Analysis of the MRC in OA chondrocytes shows decreased activity of Complexes I, II and III compared to that seen in normal chondrocytes. On the other hand, the significantly increased activity of citrate synthase in OA chondrocytes suggests that mitochondrial mass is increased compared to that in normal cartilage cells.[20] The increase in OA chondrocyte mitochondrial mass might be a compensatory mechanism to offset the deficiency in electron transfer and resultant low ATP production (intracellular ATP was decreased by 50%) and reduced mitochondrial membrane potential ($\Delta\psi m$).[20,21,24]

OA is a rheumatic disease associated with increased production of ROS by chondrocytes in the cartilage. It is now established that the MRC is one of the most important sites of ROS production.[25] Interestingly, it has been reported that inhibition of Complex III activity with antimycin-A (a specific MRC inhibitor) induced ROS synthesis in human articular chondrocytes.[26] In addition, the inhibition of Complexes III or V in these cells induces the production of other proinflammatory stimuli, including cytokines IL-1, IL-6 and IL-18, prostaglandin E2 (PGE2), the chemokines IL-8 and monocyte chemotactic protein 1, and the proteases MMP1, MMP3 and MMP13.[27,28]

These experimental results help explain some of the specific pathogenic pathways implicated in OA. Several *in vitro* studies using human articular chondrocytes have indicated that the use of specific MRC inhibitors suppressed the synthesis of proteoglycans and collagen.[21,24,28] In addition, the inhibition of Complex I using rotenone *in vitro* reduced the proteoglycan content of superficial and middle-zone cartilage and increased the release of glycosaminoglycan from the cartilage to the supernatant.[28]

Calcium metabolism and chondrocyte survival

Mitochondria store calcium to help maintain cellular Ca^{2+} homeostasis. Studies have indicated that chondrocyte mitochondria are specialized for the transport of calcium, highlighting their importance in ECM calcification.[29,30] Mineralization has been demonstrated both in matrix vesicles and within the mitochondria themselves: indeed, calcium and phosphorus are present in single mitochondrial granules in chondrocytes from the growth plate and in certain extracellular particles distinct from matrix vesicles.[29] When mitochondrial respiration is directly suppressed, matrix-vesicle-mediated mineralization of chondrocytes is promoted.[21,22] Microcalcifications are present in OA cartilage, and the role of mitochondria in this process has been suggested.[22]

Histological studies of OA cartilage show a decreased number of chondrocytes compared with normal cartilage.[31] Several authors have suggested that increased apoptosis is responsible for this phenomenon.[32–34] Reported percentages of apoptotic chondrocytes in OA cartilage range from 0% to 6%.[35] The discrepancy in the percentages among these reports was probably the result of the different methodologies employed to detect apoptosis.[36] In addition, it is difficult to demonstrate typical apoptotic chondrocytes with accompanying apoptotic bodies even in advanced human OA articular cartilage; therefore, nonapoptotic programmed cell death due to chondroapoptosis or paraptosis mechanisms has been postulated.[37] An abundance of studies has investigated

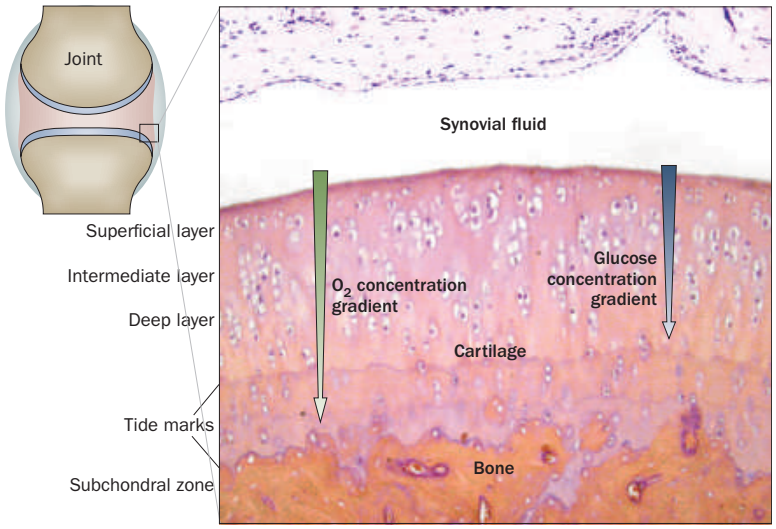

Figure 2 | Diffusion of glucose and oxygen into articular cartilage. Articular cartilage is avascular, which means that chondrocytes must obtain nutrients and oxygen via diffusion from the synovial fluid. Thus, these two compounds show a gradient of concentrations in the cartilage, being lower in the deeper layers than at the surface.

the mitochondrial pathways involved in chondrocyte apoptosis, which are reviewed elsewhere.[38]

Modifications to mitochondrial activity in OA

Keeping in mind that most mitochondrial proteins are encoded by the nuclear genome, mitochondria contain many copies of their own mitochondrial DNA (mtDNA). Human mtDNA is a 16,569 bp circular molecule containing 37 mitochondrial genes that encode 13 polypeptide MRC components, in addition to two ribosomal RNAs (rRNAs) and 22 transfer RNAs (tRNAs) that support protein synthesis within the mitochondrion using its own genetic code. Because mtDNA is only inherited maternally, many mtDNA single nucleotide polymorphisms (SNPs) have accumulated in sequence along maternal lineages.[39] In fact, a number of stable SNPs in mtDNA coding regions have been used to define related groups of mtDNA haplogroups.[39] Interestingly, 95% of the European population fall into one of nine mtDNA haplogroups: H, I, J, K, T, U, V, W and X (Figure 3).

The accumulation of mtDNA deletions and point mutations correlates with a decline in mitochondrial function, which is thought to contribute to pathologies such as Alzheimer disease and Parkinson disease, and aging.[40] Point mutations at specific sites in the mtDNA control region accumulate at high levels in certain tissues; for example, mutations occur at high frequency at T414G in cultured fibroblasts, A189G and T408A in muscle, and C150T in white blood cells.[41] Mutations at C150T in the mtDNA of chondrocytes have also been described (F. J. Blanco, unpublished data). Somatic mtDNA mutations induced by mutagens such as ROS or NO also contribute to the decline in mitochondrial activity that occurs in OA chondrocytes. Indeed, results from a 2009 study indicate that there is decreased mtDNA integrity and higher accumulation of mtDNA damage in chondrocytes from OA patients compared to those from normal individuals.[42] A reduced capacity of OA chondrocytes to repair mtDNA damage is reportedly associated with apoptosis.[42] In a study examining the accumulation of the mtDNA4977 deletion (which is associated with aging) in knee cartilage, its frequency was found to increase in aging cartilage, so this mutation may have a role in the development of OA.[43]

The accumulation of mtDNA mutations may result from increased production of ROS or defects in the mitochondrial antioxidant system.[25] An important scavenger of ROS is superoxide dismutase (SOD), a catalyst for the dismutation of superoxide to hydrogen peroxide and oxygen. Mitochondrial superoxide dismutase 2 (SOD2; also known as MnSOD) is an enzyme that functions to protect mitochondria from oxidative stress.[25] A localized SOD deficiency can result in the formation of oxidizing species, such as peroxynitrite. Two studies demonstrated that the expression of SOD2 at both gene and protein levels was significantly decreased in OA chondrocytes and OA cartilage.[44,45] Immunohistofluorescence studies of cartilage from age-matched normal individuals and OA patients revealed that SOD2 is largely missing in OA cartilage, but occurs regularly in the superficial layer of normal cartilage.[44]

The role of NO in chondrocyte death and apoptosis has become an important focus of research interest. Studies suggest that NO induces apoptosis in chondrocytes by reducing the activity of Complex IV and decreasing the $\Delta\psi m$.[3,21] Besides inhibiting respiration, NO has additional effects on mitochondria by inducing ROS and mtDNA damage, which have roles in cell death.[46] However, the precise role of NO in the induction of chondrocyte death is currently a subject of debate.[2,47,48] NO may not be the sole mediator of chondrocyte death, and a role for peroxynitrite, a reaction product of NO and superoxide anions, is postulated.[47] The suggestion that the type of chondrocyte death may be determined by the balance between intracellular NO and ROS is particularly interesting: a low concentration of ROS might promote apoptosis in the presence of NO, while a high concentration of ROS might promote necrosis.[2,49] An *in vitro* experiment using human cartilage chondrocytes found that cells from elderly donors (aged ≥50 years) were more susceptible to NO-induced cell death compared with cells from young donors (aged 18–50 years). This susceptibility correlated with a higher ratio of oxidized glutathione to reduced glutathione, providing evidence that increased oxidative stress with aging makes chondrocytes more susceptible to oxidant-mediated cell death.[50] Finally, a study of peroxynitrite-mediated chondrocyte apoptosis revealed that the predominant mode of cell death involves the calpains, a group of calcium-dependent cysteine proteases, and that peroxynitrite-induced mitochondrial dysfunction in cells leads to caspase-independent apoptosis.[51]

Other molecules with catabolic activity, such as PGE2 and some proinflammatory cytokines, including TNF and IL-1β, occur at high concentrations in the synovial fluid of OA-affected joints, and may act to modify

mitochondrial activity. In human chondrocytes, both TNF and IL-1β might decrease ATP production by inhibiting the activity of Complex I in the MRC, as well as by reducing $\Delta\psi m$ and inducing mtDNA damage.[28,52] In addition, stimulation of OA chondrocytes with PGE2 is known to decrease $\Delta\psi m$ and ATP generation.[53]

Interestingly, *in vivo* animal studies support some of these findings. Chondrocytes were examined for ATP depletion and changes in mitochondrial ultrastructure before and during the development of spontaneous knee OA in male Hartley guinea pigs.[54] The authors found that, as knee OA developed, spontaneous NO release from organ cultures of knee cartilage increased twofold between 2 months and 8 months of age. Knee chondrocyte intracellular ATP levels declined by approximately 50% during the same period, although no mitochondrial ultrastructure abnormalities were recorded. Together with aging-associated ATP depletion in chondrocytes, an increased lactate-to-pyruvate ratio was observed. This observation suggests that augmented glycolysis is an adaptation in response to mitochondrial dysfunction.

Role in OA diagnosis and prognosis

The conceptualization and development of early diagnostic strategies has been a major goal for OA research. During the past 5 years, new approaches for discovering and verifying OA biomarkers have emerged, among which are genomic, proteomic and metabolomic technologies. The genomic strategy is currently the most used, as it allows identification of candidate genes that are potentially involved in OA and in cartilage degradation. Unfortunately, most genomic studies with a focus on OA do not take the mitochondrial genome into account.

A number of studies have investigated the relationship between mtDNA haplogroups and specific diseases[55] and aging.[56] Our study revealed a correlation between mtDNA haplogroups and OA, demonstrating that mtDNA haplogroup J protects against knee and hip OA.[57,58] In contrast, an increase in radiologic severity of knee OA seems to be associated with mtDNA haplogroup U. Those patients who carry mtDNA haplogroup J and who have knee OA seem to have decreased radiographic disease severity.[57]

Associations have been found between serum levels of molecular markers of cartilage degradation, mtDNA haplogroups and the presence of knee OA, as well as between mtDNA haplogroups and radiologic Kellgren and Lawrence (K/L) grades. Overall, mtDNA haplogroup H carriers had higher serum molecular marker levels than did carriers of haplogroup J. This indicates that mtDNA haplogroups have a significant influence on the serum levels of some protein biomarkers, but not all, suggesting that both mtDNA-dependent and mtDNA-independent serum biomarkers exist (Box 1).[59]

The two primary functions of mitochondrial oxidative phosphorylation are to provide ATP for cellular energy and to generate heat for thermal regulation.[60] As mentioned above, an important biochemical factor in the OA process is ROS production by mitochondria.[4,5] Considering the hypothesis that climate influenced the selection of European mtDNA haplogroups, as

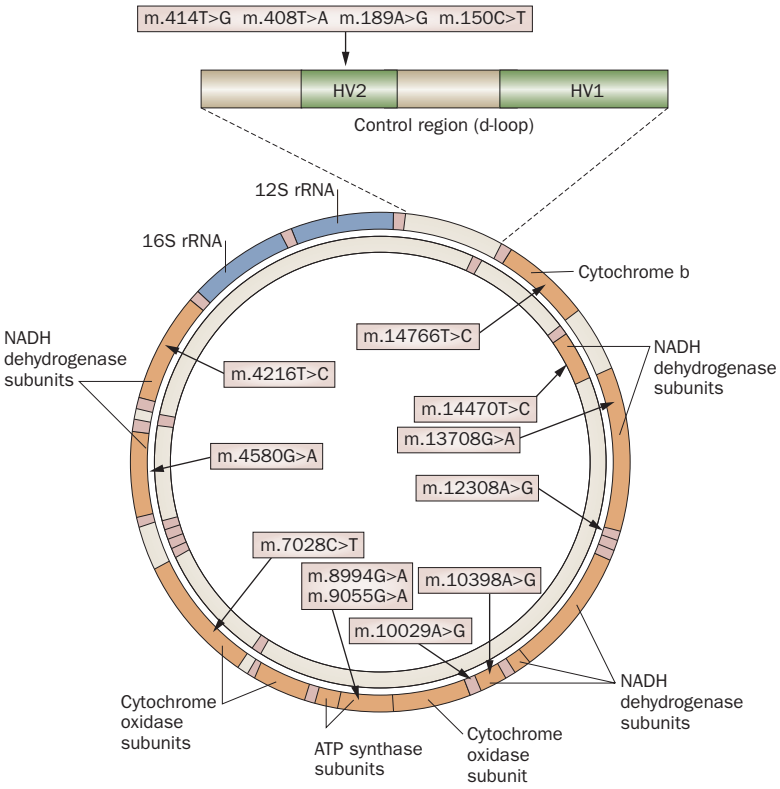

Figure 3 | SNPs giving rise to mtDNA haplogroups. Schematic of the mtDNA molecule showing the SNP sites in the hot-spot d-loop HV2 region and some of the characteristic SNPs that identify the nine most common European mtDNA haplogroups (H, I, J, K, T, U, V, W or X) resulting from world migration patterns that caused humans to adapt to colder climates. Abbreviations: HV, hypervariable region; mtDNA, mitochondrial DNA; rRNA, ribosomal RNA; SNP, single nucleotide polymorphism.

postulated by Mishmar *et al.*[61] and Wallace *et al.*,[62] some SNPs characteristic of haplogroup J may reduce the mitochondrial coupling efficiency. As a consequence, ATP production would be reduced, but the resulting increase in oxidation of the MRC would lead to decreased ROS production,[63] which would protect against diseases associated with oxidative stress, such as OA (Figure 4). If this is true, individuals carrying haplogroup J would have a lower risk of developing OA than those in the same age group who carry a non-J haplogroup.[57] Interestingly, in several European studies, carriers of haplogroup J were found to have increased longevity[56,64,65] and a decreased risk of developing Parkinson disease.[55]

On the basis of all these results, mtDNA haplogroups should be considered along with other potential biomarkers in the diagnosis and prognosis of knee and hip OA. The finding that serum biomarkers seem to be modulated by mtDNA haplogroups supports the role of mtDNA variations in the complex OA process. Also, it seems possible that mtDNA haplogroup typing could define specific OA phenotypes.

Mitochondria as therapeutic targets

Molecules that modulate or improve mitochondrial activity are not currently included in OA treatment regimens. As mitochondrial dysfunction explains several of

Box 1 | mtDNA haplogroups affect markers in OA

Various mtDNA haplogroups influence the markers of cartilage degradation in OA. Analyses were performed by comparing the serum levels of OA-related molecular markers in human participants carrying mtDNA haplogroups J, H or U.[59]

Markers at altered levels in haplogroup J carriers

Coll2-1. Lower levels in haplogroup J carriers than H carriers

Coll2-1NO_2. Lower levels in haplogroup J carriers than H carriers

C2C. Lower levels in haplogroup J carriers than H and U carriers

CPII. Lower levels in haplogroup J carriers than H carriers

C2C/CPII. Lower levels in haplogroup J carriers than H and U carriers

Matrix metalloproteinase 3. Higher levels in healthy haplogroup J carriers compared with OA-affected J and U carriers

Matrix metalloproteinase 13. Lower levels in haplogroup J carriers than H carriers

Haplogroup-independent markers

YKL-40

Hyaluronic acid

Matrix metalloproteinase 1

Myeloperoxidase

Cathepsin K

Abbreviations: mtDNA, mitochondrial DNA; OA, osteoarthritis.

the events occurring during the pathogenesis of OA, improving mitochondrial activity may prove to be a therapeutic alternative for patients with this disease. We now know that mitochondrial dysfunction participates in chondrocyte apoptosis, and numerous preclinical studies indicate that mitochondrial modulation reduces chondrocyte apoptosis.[38] Some promising approaches to preserving cell viability are focused on synthetic inhibitors of caspase 9, a key component of the mitochondrial apoptosis pathway.[66,67] When OA was induced in dogs by unilateral anterior cruciate ligament transection, OA knee cartilage incubated *in vitro* with the caspase 9 inhibitor Z-LEHD-FMK exhibited a marked decrease in the degree of chondrocyte apoptosis.[68] Following induced mechanical injury to human or horse cartilage explants, loaded explants cultured *ex vivo* in Z-VAD-FMK (a broad-spectrum caspase inhibitor) or a specific caspase 9 inhibitor showed a 40% reduction in the rate of apoptosis.[69,70] In addition, chondrocytes overexpressing Bcl-2 were resistant to apoptosis induced by either serum withdrawal or retinoic acid treatment.[71]

Therapies designed to reduce ROS synthesis or block ROS action have different effects. For example, two studies have demonstrated that vitamin C induces apoptosis in a cell culture of chondrocytes,[72,73] and ascorbic acid supplementation increased cartilage degradation and worsened the severity of spontaneous OA in an animal model.[74] On the other hand, a diet supplemented with vitamins E, C, A, B_6 and B_2 and selenium diminished the development of mechanically-induced OA in male STR/1N mice.[75] In the last few years it has been reported that resveratrol seems to be an effective *in vitro* anti-inflammatory agent that also has a chondroprotective capacity through suppression of ROS, tumor suppressor protein p53, PGE2 and IL-1 production.[53,76] Other interesting results suggest that the chondroprotective effects of hyaluronic acid on oxidatively stressed chondrocytes are due to preservation of mitochondrial function and amelioration of mitochondrion-driven apoptosis.[77]

Future research directions

The major goals for OA researchers are the definition of early diagnostic strategies and the development of new therapeutic targets. Existing therapies, which are intended only to treat symptoms and alleviate pain, do not effectively slow the rate of disease progression. Effective strategies with which to increase our understanding of OA pathogenesis are essential for the development and evaluation of new disease-modifying therapies. More research is needed to identify the early molecular players in OA pathogenesis.

As mitochondrial dysfunction has been associated with OA, mitochondrial proteins represent an attractive target for early-stage pharmacological treatments. A mitochondrial proteomic study of OA chondrocytes identified 23 proteins that showed significantly altered expression. Bioinformatic analysis identified some of the biochemical pathways that may be altered in OA mitochondria, which included the oxidative stress response, energy production, mitochondrial membrane organization and apoptosis.[44] Future, more-detailed studies to examine the role of these proteins in OA pathogenesis should lead to the discovery of new drugs that can reduce cartilage degradation.

The initial stages of OA are asymptomatic in most patients, so extensive cartilage deterioration is usually already present by the time a diagnosis can be made. Currently, a diagnosis of OA depends on the description of pain symptoms by the patient, the detection of joint stiffness, and joint destruction as measured by radiography. The limitations of the available diagnostic tests have triggered substantial interest in finding new, specific biological markers for cartilage degradation that will facilitate earlier diagnosis of joint destruction, more-accurate prognosis, and pathological evolution studies. The mtDNA haplogroups, other genetic mitochondrial markers and some mitochondrial proteins might represent new biomarkers, but they require validation in further independent studies utilizing a wider range of OA populations than those performed previously.

As chondrocytes exist under hypoxic conditions in their natural environment, future *in vitro* research and experiments must take this into account. The trans-mitochondrial cytoplasmic hybrid cell system (also known as 'cybrids'), which allows the study of mitochondrial metabolism in a cellular model with a common nuclear background, is a potentially useful methodology with

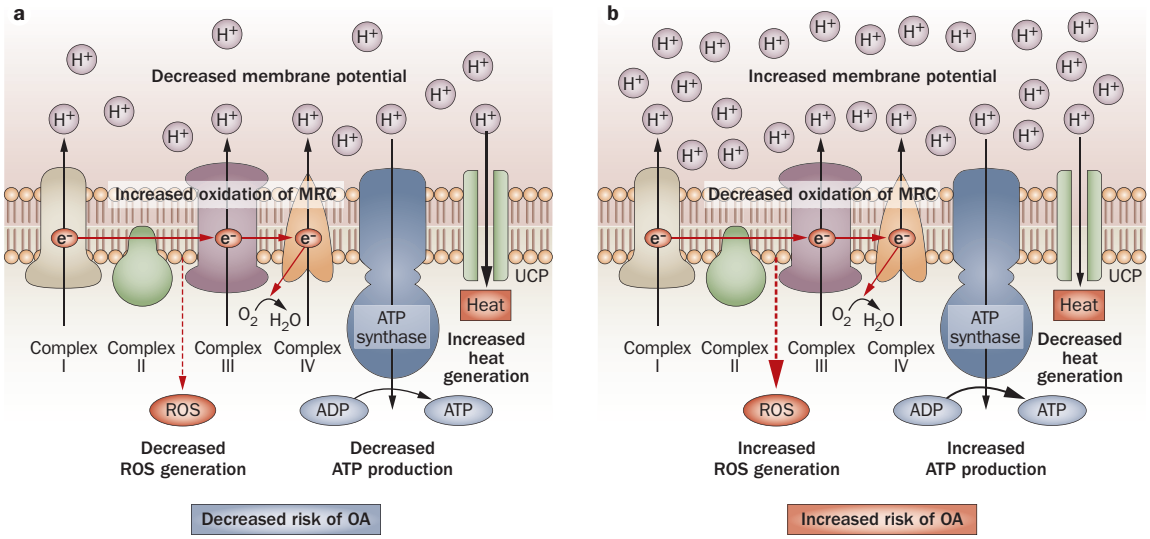

Figure 4 | Influence of mtDNA haplogroups in OA. **a** | Haplogroup J carriers have mtDNA mutations that partially uncouple mitochondrial oxidative phosphorylation. These uncoupling mutations lead to increased heat generation and decreased ATP production, but also decrease mitochondrial ROS production by increasing the oxidation of the MRC. Decreased ROS production reduces the oxidative damage caused to the cell, protects against apoptosis and decreases cartilage degradation, resulting in a decreased risk of knee OA. **b** | In non-J haplogroup carriers, mitochondrial oxidative phosphorylation is tightly coupled, leading to increased ATP production but also increased ROS generation, which causes increased cartilage degradation and a higher risk of knee OA. Abbreviations: MRS, mitochondrial respiratory chain; mtDNA, mitochondrial DNA; OA, osteoarthritis; ROS, reactive oxygen species; UCP, uncoupling protein.

which avoid the limitations of studying chondrocytes extracted from patients.[78]

As the majority of the currently published results were obtained from *in vitro* or *ex vivo* models, it will be necessary to replicate the results *in vivo* to confirm the role of mitochondria in OA. Animal models are important tools in experimental medical science, but classic methodological approaches to the development of animal models (such as the generation of transgenic or knockout mouse strains) in which to study mitochondrial dysfunction have failed because of their embryonic-lethal effects. For this reason, several groups have addressed this issue using an elegant approach that can generate *de novo* mtDNA mutations experimentally.[79] If the proofreading activity of DNA polymerase subunit γ (POLG) is eliminated while its polymerase activity is preserved, mtDNA mutations accumulate because of uncorrected errors during replication. In mice with such proofreading-deficient POLG (mtDNA-mutator mice), mtDNA mutations accumulate to high levels in all tissues. By 8 weeks of age, homozygous *Polg*$^{-/-}$ animals appeared normal, but by 25 weeks they began to exhibit pathology frequently seen in human aging, including weight loss, alopecia, osteoporosis, kyphosis, cardiomyopathy, anemia, gonadal atrophy and sarcopenia. The presence of OA has not been studied in these animals, but it could be a useful model in which to study the role of mitochondria in this disease.

Conclusions

Mitochondrial functions, including MRC activity and ATP synthesis, are altered in OA chondrocytes. Mitochondrial dysfunction may influence several of the specific pathways involved in OA pathology, including oxidative stress, chondrocyte biosynthetic responses, chondrocyte apoptosis, cytokine-induced chondrocyte inflammation with matrix catabolism, and calcification of the cartilage matrix. OA chondrocyte mitochondrial dysfunction may originate from somatic mutations in the mtDNA or from the direct effects of proinflammatory cytokines, prostaglandins, ROS and NO on the MRC and ATP synthesis. mtDNA haplogroups may serve as useful biomarkers for the diagnosis or prognosis of OA. Indeed, the modulation of serum biomarkers by mtDNA haplogroups supports their role in the complex OA process, and might define distinct, specific OA phenotypes.

The evidence presented in this Review strongly supports the hypothesis that chondrocyte mitochondrial impairment is a mediator for the onset and progression of cartilage degradation in OA. Promising therapies for OA include those that target the basic mitochondrial processes, particularly energy metabolism and free-radical generation. To confirm the role of mitochondria in OA, it will also be necessary to demonstrate these results in appropriate animal models and in large cohorts of OA patients, such as the OAI (Osteoarthritis Initiative), MOST (Multicenter Osteoarthritis Study) or the Framingham OA cohort.

Review criteria

Articles from the PubMed database were located using the following search terms: “mitochondria”, “mitochondrion”, “chondrocytes”, “cartilage” and “OA”. English language articles were selected for review based on their relevance to the role of the mitochondrion in OA pathogenesis and treatment.

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Author contributions

I. Rego and C. Ruiz-Romero researched data for the article. All authors made substantial contributions to discussion of the content. F. J. Blanco wrote the article. All authors performed review/editing of the manuscript before submission.