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The Relationship between mitochondrial energy metabolism changes and neurodegenerative disease

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ملخص

الخلايا العصبية هي خلايا متمايزة للغاية ، ومتخصصة في الاتصال بين الخلايا وتتطلب وظائفها الفسيولوجية كميات كبيرة من الطاقة. إنها تشكل هدفًا مميزًا في حالة الإجهاد الأيضي ، وتدعم أهمية الميتوكوندريا لعملها وجود نمط ظاهري عصبي ثابت تقريبًا في الأمراض المرتبطة بطفرات الحمض النووي للميتوكوندريا. تشارك الميتوكوندريا في عدد كبير من العمليات الفيزيولوجية المرضية مثل إنتاج الطاقة ، استقلاب الأحماض الأمينية والدهون ، استتباب الكالسيوم ، التوليد الحراري ، النقل المحوري ، موت الخلايا المبرمج. من المحتمل أن يساهم الخلل الوظيفي في كل من هذه العمليات في آليات التنكس العصبي ، وبالتالي تم إثبات تورط فشل الميتوكوندريا في الأمراض التنكسية العصبية الرئيسية مثل مرض الزهايمر ومرض باركنسون وهنتنغتون.

الكلمات المفتاحية: الأمراض العصبية التنكسية ، الخلل الوظيفي ، الميتوكوندريا.

Abstract

Neurons are highly differentiated cells, specialized in intercellular communication and whose physiological functions require large amounts of energy. They constitute a privileged target in the event of metabolic stress and the importance of mitochondria for their functioning is supported by the presence of an almost constant neurological phenotype in diseases linked to mitochondrial DNA mutations. Mitochondria are involved in a large number of pathophysiological processes such as energy production, amino acid and lipid metabolism, calcium homeostasis, thermogenesis, axonal transport, apoptosis. A dysfunction of each of these processes is likely to contribute to the mechanisms of neuro-degeneration and the involvement of mitochondrial failure has thus been established in the main neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington.

Keywords: Neuro-degenerative diseases, Dysfunction, Mitochondria.

Résumé

Les neurones sont des cellules hautement différenciées, spécialisées dans la communication intercellulaire et dont les fonctions physiologiques nécessitent de grandes quantités d‟énergies. Ils constituent une cible privilégiée en cas de stress métabolique et l‟importance des mitochondries pour leur fonctionnement est soutenue par la présence d‟un phénotype neurologique quasi constant dans les maladies liées aux mutations de l‟ADN mitochondrial. Les mitochondries sont impliquées dans grand nombre de processus physiopathologiques tels que la production énergétiques des, métabolisme des acides aminés et les lipides, homéostasie calcique, la thermogenèse, transport axonal, l‟apoptose. Un dysfonctionnement de chacun de ces processus est susceptible de contribuer aux mécanismes de neuro- dégénérescence et l‟implication d‟une défaillance mitochondriale a ainsi été établie dans les principales maladies neuro-dégénératives que sont la maladie d‟Alzheimer, de Parkinson, d‟Huntington.

Mots clés : Maladies Neuro-dégénératives, Dysfonctionnement, Mitochondrie

Dedication

To my dearest mother who is the spark of my life and who always supported and pushed me forward so that I could reach the end of the tunnel...

To the pure soul of my father whom I promise to be his pride and joy while praying to the Merciful to gather us in the highest paradise...

To my two brothers "Abderrahim ,Abdennour" and my sister "Maroua" who make my days full of joy...without forget my brother in law "Zidane "

To my beautiful nieces "Ranim ,Yousr , Rassil , and nephew "Anis"

To my best friend and soulmate "Soundes"

To my paternal family "Chergui" and maternal family "Belkhir"...

To all my relatives and friends...

I dedicate this modest work.

Safa Chergui

Dedication

There are a number of people without whom this thesis might not have been written. and to whom I am greatly indebted.

To my mother "fatma" , who continues to learn. grow and develop and who has been a source of encouragement and inspiration to me throughout my life. a very special thank you for providing a „writing space" and for nurturing me through the months of writing.

And also for m'y father , Allah yarhmek.

And my sister " Mariem " And all my friends.

Samra Hamza

Dedication

To the one who made heaven under her feet, to the one who overwhelmed me with the flood of her tenderness, to the one who burned to light my path, to my mother..

To the one who made the effort of the years in order to climb the ladders of success, to my dear father..

To my second mother, my beloved grandmother "Akila"..

To those who shared with me my joys and sorrows, To my two brothers " Alla, Imed " and my sister "sadjida"..

To my friend, my sister, and my soulmate "Ines"..

To the beauties "Awatef, Thouraya, Samiha" and To the most beautiful aunt "Saliha"

To my family "Rouabhia ", and To the friends with whom life brought me together.

I dedicate my joy and happiness on this beautiful day to all of you.

Narimene Rouabhia.

Thanks

We first thank our Creator, Allah the Omniscient.

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List of abbreviations

ATP …….. Adénosine Triphosphate
ADP ……..Adénosine Diphosphate
APP ……...Amyloid Precursor Protein
AIF ……Apoptosis inducing factor
ABAD ……Amyloid Beta-binding Alcohol Dehydrogenase
BH ……..Bcl-2 homology
CMA ……..Chaperone-Mediated Autophagy
CypD ……..cyclophilin D
CNS ……Central nervous system
DRPLA ……..Dentatorubral–Pallidoluysian Atrophy
EMRE ……... Essential for MCU Regulator
ER ……..Endoplasmic Reticulum
ERAD ……ER-associated Degradation
ETC ……The mitochondrial transport chain
HD ……Huntington's disease
IF ……..Intermediate Filament
IMM …….Inner Mitochondrial Membrane
IP_3Rs ……Inositol 1,4,5 trisphosphate Receptors
LRRK2 …….Leucine-rich Repeat kinase 2
LTP …….. Long-term Potentiation
LCD ……. Lysosomal cell death
LMP ……. Lysosomal membrane permeabilization
MCU …….Mitochondrial Calcium Uniporter
MAMs ……..Mitochondria-associated Membranes
MICU2 ……..Mitochondrial Calcium Uptake 2
MICU1 ……..Mitochondrial Calcium Uptake 1
MICU3 ……..Mitochondrial Calcium Uptake 3
MAPT ……Microtubule-Associated Protein Tau
MAP ……..Mitogen-activated protein
mPT ……...Mitochondrial permeability transition
mPTP …….Mitochondrial permeability transition pore
MOMP ……...Mitochondrial outer membrane permeabilization
MLK ……..Mixed Lineage Kinase Domain-like
NCLX …… Sodium–Calcium Exchanger
NIs ……..Nuclear inclusions
NFTs ……...Neurofibrillary tangles
NO ……..Nitric oxide
NOX ……NADPH oxidase
OMM ……..Outer Mitochondrial Membrane
OXPH……..Oxidative phosphorylation
PQC…….. ...Protein Quality Control
PolyQ ……..Polyglutamine
PSEN1 …….Presenilin 1
PSEN2 …….Presenilin 2
PD ……Parkinson's disease

PARP …… **…...**Poly(ADP-ribose) polymerases
PMD……..Primary Mitochondrial Diseases
RyRs……... …..Ryanodine Receptors
ROS ……..Reactive Oxygen Species
SERCA ……Smooth Endoplasmic Reticulum Ca2+ ATPase
SBMA …….spinal bulbar muscular atrophy
SCAs ……Spinocerebellar Ataxias
TDP-43 ……TAR DNA binding protein
TCA ……..Tricarboxylic Acid
TNF …… .Tumor necrosis factor
UPS ……..Ubiquitin–Proteasome System
VDACs …….Voltage-Dependent Anion Channels
WHO ……The World Health Organization

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Introduction

Neurons are highly differentiated cells specializing in intercellular communication and whose physiological functions require large amounts of energy. They are a prime target in cases of metabolic stress and the importance of mitochondria for their operation is supported by the presence of an almost constant neurological phenotype in disease-related mutations of mitochondrial DNA. Mitochondria are involved in many physiological processes such as ATP energy production, the metabolism of amino acids and lipids, calcium homeostasis, thermogenesis, the production of reactive oxygen species, transport and axonal apoptosis. A malfunction of each of these processes may contribute to neurodegeneration mechanisms. The involvement of mitochondrial failure has been established in the major neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's and amyotrophic lateral sclerosis. A better understanding of the pathophysiological mechanisms is needed to consider new therapeutic approaches.

Mitochondria are central to various cellular processes that include ATP production, intracellular Ca2+ signaling, and the generation of reactive oxygen species.

- We will first discuss the interactions of these parameters for experimental stimulation conditions that can be related to the physiological range.

- We will then describe how mitochondrial and metabolic dysfunction develops during pathological neuronal activity, focusing on neurodegenerative and its experimental models.

- The aim is to illustrate that the structure of the mitochondrial compartment is highly dynamic in neurons and that there is a fine-tuned coupling between neuronal activity and mitochondrial function.

- The mitochondria are of central importance for the complex behavior of neurons.

Our thesis is organized as follows:

- In General introduction, we start with a general introduction, followed by the objectives and the reading plan.
- In chapter 01, we present the mitochondrion. We will discuss its history and definition as well as its basic principles. We describe its implementation: structure, role, oxidative phosphorylation, and mitochondrial energy metabolism.
- In chapter 0 2, we provide a set of definitions and properties of neuro-death mechanisms and mechanisms of protein toxicity in neurodegenerative diseases, and characteristics of reactive oxygen species in oxidative stress. Finally, we present dysfunction of the ubiquitinproteasomal system.

- In chapter 03, we detail our approach. First we justify mitochondrial dysfunction in neurodegenerative diseases, then we expose the roles of calcium and mitochondrial in Alzheimer's disease. Finally, we discuss factors affecting mitochondrial imbalance, such as pesticide poisoning.

Chapter 1: Mitochondria

Introduction

Mitochondria are known as cellular powerhouses. Since more than 60% of cellular ATP is produced through mitochondrial oxidative phosphorylation, calling mitochondria the cellular powerhouse seems appropriate. However, due to an overemphasis on the ATP production of mitochondria, we might have blindly credited the majority of the mitochondria‟s pathophysiological involvement to their function as the cellular powerhouse. Such negligence became obvious during the last decade when the interlaced nature of biological events in the nucleus, cytosol, and mitochondria started being uncovered. Among the diverse aspects of mitochondria, mitochondrial redox biology seems to be emerging as the master modulator of mitochondrial, cytosolic, and nuclear biological events. **(FaucheuxMartin, and , *al* 2003)**

By no means, it is plausible to provide in-depth insight into specific subjects regarding mitochondria, unless the aforementioned subject is sufficiently narrow, considering the amount of knowledge we have gained during the last two decades. Readers will easily find many excellent recent reviews regarding various aspects of mitochondria. In this regard, this review aims to provide a broad historical and conceptual review of mitochondria and mitochondrial redox biology. In consequence, readers will befittingly find an introductory overview, albeit a bird‟s eye view, on the subject of mitochondria, mitochondrial free radical biology, and mitochondrial antioxidants and antioxidant enzymes. **(Betarbet; Sherer*al.* 2000)**

1.1. Origine and Evolution

1.1.1. Origine of mitochondrial proteomes

Mitochondrial proteomes are inherently chimeric and differ substantially in protein content amongst eukaryotic groups. Although they typically consist of ∼1,000 proteins, the number of proteins of endosymbiotic origin in mitochondrial proteomes is surprisingly low. Latest estimates suggest that only 10–20% of proteins in mitochondria show alphaproteobacterial affinity. An additional 20–30% of mitochondrial proteomes have phylogenetic affiliations more generally to proteobacteria Many of these could be proteins of true mitochondrial endosymbiotic origin that have lost their alphaproteobacterial signature, although some could easily be independent LGTs. A large number of mitochondrial proteins (∼40% of mitochondrial proteomes) have no known prokaryotic or viral homologs Many of the genes encoding these proteins originated in the protomitochondrial phase before the cenancestral mitochondrion and the diversification of modern eukaryotes, and their specific origins are unclear. However, a significant proportion of

mitochondrial proteins with no detectable prokaryotic homologs are „lineage-specific" and likely evolved in specific eukaryote groups after LECA. The remaining proteome fraction (~15%) has prokaryotic, non-proteobacterial affinities. **(Gabaldón, Szklarczykr, Gray 2007,2010,2015)**

Alphaproteobacterial rools

- Many alphaproteobacterial-derived proteins (encoded in both mitochondrial and nuclear genomes) serve direct or indirect roles in aerobic respiration. **(Gabaldónt, Wang 2007,2014)**
- These proteins take part in the ETC that conserves energy through chemiosmosis to make ATP, the mitochondrial ribosome that supports the translation of genes encoded in the mitochondrial genome, many of which encode ETC components, the Krebs cycle that feeds reduced cofactors (NADH and FADH2) to the respiratory chain, the oxidative decarboxylation of pyruvate that feeds acetyl-CoA into the Krebs cycle, the β-oxidation pathway for fatty acids that provides NADH for the respiratory chain and acetyl-CoA to the Krebs cycle, the biosynthesis of cofactors (Fe/S clusters, heme and biotin) that are required for the assembly of many proteins of the respiratory complexes and other mitochondrial enzymes. **(Gabaldónt, Wang 2007,2014)**
- The biosynthesis of cardiolipin and ubiquinone, which are essential for the proper function of the respiratory chain. Interestingly, for the foregoing systems involving multi-protein complexes, the central core subunits are of alphaproteobacterial descent (e.g., respiratory complexes, ribosomes, translocons, and the MICOS complex). **(Gabaldónt, Wang 2007,2014)**
- A large proportion of mitochondrial proteins of eukaryotic origin also function in the mitochondrial inner and outer membranes (e.g., protein import, metabolite transport, organelle division, etc.). **(Szklarczy, gabaldónt 2010,2007)**

1.1.2. Evolution of mitochondrial genomes

Mitochondrial genomes are vastly reduced in gene content and simplified compared to the genomes of their alphaproteobacterial relatives. The content of complete genomes of alphaproteobacteria sequenced so far range from 800 to 8000 genes with their common ancestor having ~3000. **(Boussau B and *al*., 2004)**

In sharp contrast, comparisons of diverse mitochondrial genomes suggest that 69 different conserved protein-coding genes and a full set of tRNA and ribosomal RNA genes were present in the genome of the mitochondrial cenancestor. **(Burger and *al*., 2013)**

Although the mitochondrial cenancestral genome likely encoded a few more proteins, it is still a miniscule fraction of modern mitochondrial proteomes**. (Burger and *al*., 2013)**

From the foregoing it should be clear that hundreds (if not thousands) of genes were lost from the endosymbiont genome during the „proto-mitochondrial" phase of evolution. The reductive evolutionary process likely started once the mitochondrial symbiont was no longer capable of replicating outside of the host cell. The confinement to host cells reduced the symbiont population size leading to the increased fixation of slightly deleterious mutations. **(Mmcutcheon and *al*., 2011)**

Inevitably, this resulted in an increase in rates of sequence evolution and increased A+T nucleotide composition and led to loss of non-essential genes. Similar reductive trajectories are well documented for genomes of insect endosymbionts obligate intracellular parasites and the cyanobactéries symbiont in Paulinella chromatophora. **(Nowack *al*… 2016)**

- Most eukaryotes possess several dozen genes on their mitochondrial genomes. The core genes on mitochondrial genomes conserved across many eukaryotes encode ETC omponents (e.g. subunits of complexes I, III, IV and V) and translation (tRNAs and rRNAs). Other genes, such as those encoding ribosomal proteins, complex II, heme maturation enzymes, cytochrome c oxidase assembly proteins and the translation elongation factor tufA, are much more patchily distributed.

The surprisingly large differences in mitochondrial gene contents across eukaryotic diversity are the result of multiple events of EGT in different lineages, which sometimes relocate the same genes, in parallel, to the nucleus. **(Maier *al*… 2013).**

1.2. Structure of mitochondria

Mitochondria may have a number of different shapes. A mitochondrion contains outer and inner membranes composed of phospholipid bilayers and proteins. The two membranes have different properties because of this double-membraned organization **(Albers and *al*., 2005)**.

There are five distinct parts to a mitochondrion:

- The outer mitochondrial membrane

- The inter-membrane space (the space between the outer and inner membranes)
- The inner mitochondrial membrane
- The cristae space (formed by infoldings of the inner membrane) The matrix (space within the inner membrane), which is a fluid.

Mitochondria fold to increase surface area, which in turn increases ATP (adenosine triphosphate) production. Mitochondria stripped of their outer membrane are called mitoplasts. **(British Society., 2013)**

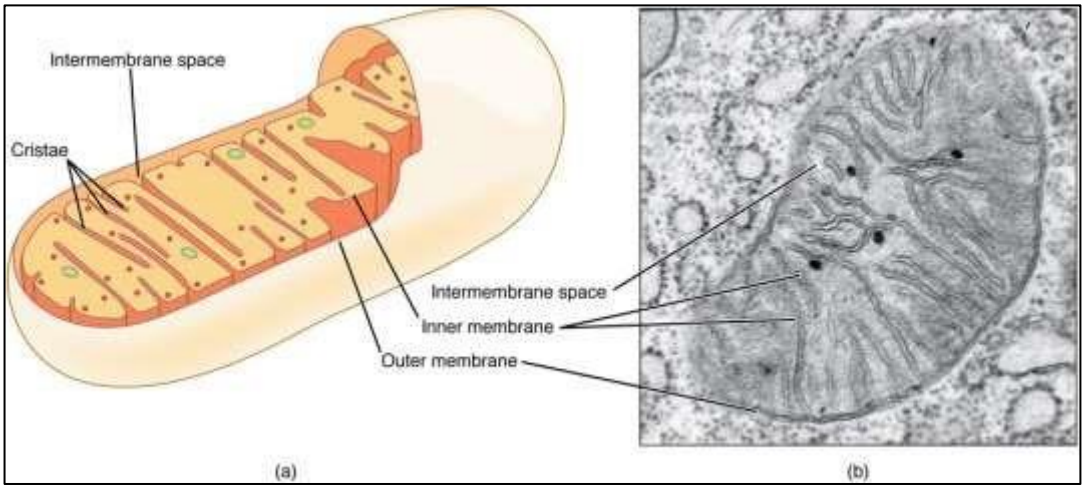

Figure 1.1: *Structure of the mitochondria* ***(British society., 2013)***

1.2.1. Outer membrane

- The outer membrane is 75 Å in thickness.
- The outer mitochondrial membrane resembles more of the plasma membrane in structure and chemical composition.
- It consists of phospholipids and proteins.
- It is permeable to NADH2.

- Pouring in the outer membrane allow small molecules to be exchanged between the cytoplasm and the inter-membrane space. **(Koolman *al*., 2005)**.

1.2.2. Inter membrane space

- The mitochondrial inter-membrane space is the space between the outer membrane and the inner membrane.

- The concentrations of small molecules, such as ions and sugars, in the inter-membrane space are the same as in the cytosol.

- Large proteins must have a specific signaling sequence to be transported across the outer membrane, so the protein composition of this space is different from the protein composition of the cytosol.

- One protein that is localized to the inter-membrane space in this way is cytochrome c. **(Chipuk *al*., 2006).**

1.2.3. Inner membrane

The inner mitochondrial membrane contains proteins with three types of functions:

- Those that perform the electron transport chain's redox reactions

- ATP synthase, which generates ATP in the matrix,

- Specific transport proteins that regulate metabolite passage into and out of the mitochondrial matrix. **(Albers , Johnson , 2005)**.

1.2.3.1. Cristæ

- The inner mitochondrial membrane is compartmentalized into numerous folds called cristae, which expand the surface area of the inner mitochondrial membrane, enhancing its ability to produce ATP.

- Mitochondria within the same cell can have substantially different crista-densities, with the ones that are required to produce more energy having much more crista-membrane surface.

These folds are studded with small round bodies known as F1 particles, or oxysomes. **(Mannella , 2006)**

1.2.4 . Mitochondrial matrix

- The matrix is the space enclosed by the inner membrane. It contains about 2/3 of the total proteins in a mitochondrion.
- The matrix is important in the production of ATP with the aid of the ATP synthase contained in the inner membrane.
- The matrix contains a highly concentrated mixture of hundreds of enzymes, special mitochondrial ribosomes, tRNA and several copies of the mitochondrial DNA genome.
- Of the enzymes, their major functions include oxidation of pyruvate and fatty acids and the citric acid cycle. **(Alberts Johnson and *al*., 2005)**

1.3. Role of the mitochondria

1.3.1. Production of ATP

Mitochondrial ATP production is the main energy source for intracellular metabolic pathways **(Schapira, 2006).** The human mitochondrial (mt) ATP synthase, or complex V is the 5th multi subunit oxidative phosphorylation (OXPHOS) complex. It synthesizes ATP from ADP in the mitochondrial matrix using the energy provided by the proton electrochemical gradient **(Capaldi and *al*., 1994; Nijtmans and *al*., 1995; Zeviani and Di Donato., 2004).**

1.3.2. Calcium Transport

The electrochemical gradient across the IMM is the driving force for calcium transported across the mitochondria inner membrane by the recently identified **(Baughman and *al*., 2011, De Stefani and *al*., 2011)** mitochondrial calcium uniporter (MCU). Uptake into the mitochondria of small physiological levels of calcium is thought to regulate mitochondrial metabolism and ATP production. **(Glancy and *al*., 2013; Lui and Orourke , 2009)** In the heart, an increase in contractility is mediated by an increase in the cytosolic calcium transient. The increase in cytosolic calcium is transmitted to the mitochondria via Ca uptake into mitochondria, which leads to activation of the calcium-sensitive mitochondrial dehydrogenases **(Denton and *al*., 1980)** and several complexes of electron transport

Calcium efflux from cardiac mitochondria occurs via the Na-Ca exchanger (NCXL) **(Boyman and *al*., 2013)** Calcium transits the outer mitochondrial membrane (OMM) via the voltage-dependent anion channel. Mitochondrial Na-Ca exchange has been shown to regulate **mitochondrial** calcium levels and to connect mitochondrial calcium to intracellular sodium, such that the rise in sodium that occurs during hypertrophy and heart failure is reported to lead to alterations in mitochondrial calcium that lead to altered redox and metabolis. **(Lui and *al*., 2014, Shimizu and *al*., 2015).**

1.3.3. Mitochondria and Cell Death

Mitochondria have been recognized as playing a central role in both apoptotic and necrotic cell death. The triggering event in mitochondria-mediated apoptosis is permeabilization of the OMM, which allows the release of apoptogens, including cytochrome c, SMAC/DIABLO, Omi/HtrA2, AIF, and EndoG. **(Whelan and *al*., 2010)** What these proteins share in common is that they perform healthy functions within the mitochondria but are toxic in the cytosolic compartment. For example, in healthy cells, cytochrome c participates in electron transport at the IMM as part of oxidative phosphorylation. **(Chipuk and *al*., 2010).**

1.4. Oxidative phosphorylation

Mitochondria play a number of vital roles in the eukaryotic cell, among which the most important one is the production of ATP during oxidative phosphorylation (OXPHOS). The heavily folded inner membranes of mitochondria called cristae accommodate many copies of the respiratory chain components or OXPHOS complexes (I–IV). Together with ATP synthase (complex V), they form the machinery for the production of ATP, the energy currency of the cell.

Complexes I–IV are multi-subunit enzymes that work in concert to create an electrochemical proton gradient across the mitochondrial inner membrane that is used by the F1Fo ATP synthase (complex V) to produce ATP via oxidative phosphorylation, although complex II is not directly able to pump protons. NADH or succinate generated during glycolysis, fatty acid oxidation, and in the citric acid cycle forms the fuel for the respiratory chain.

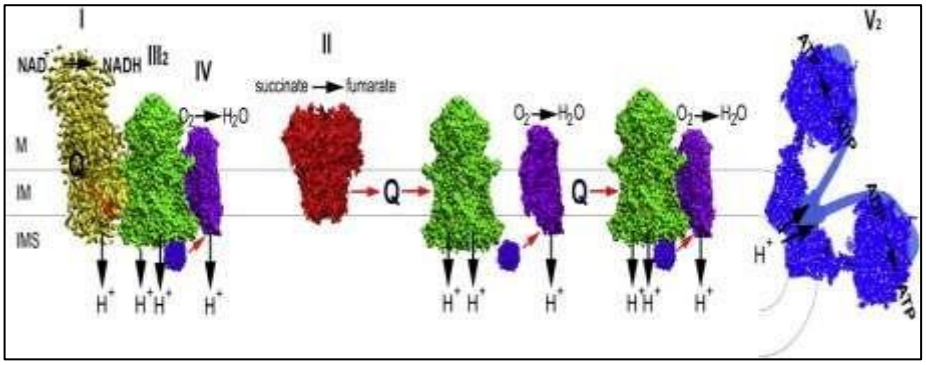

Figure 1.2: *Plasticity model of the mitochondrial electron transport chain* ***(Y. Chaban,, al 2005)***

1.4.1. Significance of the OXPHOS super-complexes

The structural study of the OXPHOS super-complexes is of special importance for understanding the function of the respiratory chain complexes. Most mitochondrial diseases and disorders, including Parkinson's and Alzheimer's diseases, are associated with genes that encode for OXPHOS proteins. **(Bender, *al*. 2006)**

Therefore, the OXPHOS system appears to be an attractive drug target in the treatment of cancer and mitochondria-associated diseases. Understanding the supra-molecular organization of the OXPHOS system and its role in the functioning of the respiratory chain is essential for the development of such a treatment.**(Diehnal., 2009)**

1.4.1.1. Stabilising factors for the respirasome

In various organisms, respirasomes appear to be very stable because they can be isolated as whole entities without significant degradation. In recent years, a lot of effort has been placed into the search for factors that are responsible for gluing together the respirasome components. Cardiolipin is an anionic phospholipid formed by two phosphatidyl groups bound by glycerol. It is exclusively found in the mitochondrial inner membrane where one of its major roles is to specifically interact with proteins and modulate their functions. **(Althoff *al*., 2011)**

1.4.1.2. Respirasome functions

In the respiratory chain, all enzymes are linked by electron exchange. Ubiquinone physiologically interconnects complexes I and III by accepting electrons from the first and delivering them to the latter. It is known that most of the harmful mitochondrial superoxide originates from complexes I and III. Complexes I and III are assembled within the respirasome such that the ubiquinone binding sites in the bovine respirasome are at a close distance of 13 nm. Short movements of ubiquinone make sense not only to speed up the electron transfer process as a whole,

but also to minimize loss of the electrons during transfer and the production of harmful oxygen radicals. Another crucial point in the OXPHOS electron pathway is the shuttling of cytochrome c between complexes III and IV. **(N.V. al. 2011)**

1.4.2. Dimeric ATP synthase

The first biochemical evidence for a dimeric organization of the ATP synthase complex in yeast came from the BN-PAGE work by Arnold and colleagues. Later, dimers were found in bovine Arabidopsis and several other organisms. Interestingly, dimerisation of ATP synthases seems to be a characteristic feature of mitochondria because no evidence has yet been obtained that supports the existence of dimers in bacterial membranes. **(Garcia Montes de Oca and *al.*, 2012)**

The structural information about the dimeric ATP synthases comes from either single particle EM of detergent-solubilised proteins or subtomogram averaging in situ. The latter method provides direct insight into the supra-molecular organization of macromolecules in membranes but at a lower resolution than single particle reconstructions.

1.4.2.1. Angular distribution in dimers of ATP synthases

The interacting monomers in ATP synthase dimers of different species form a variety of angles that must be discussed.Obviously, detergent treatment during isolation may affect the angle.

Several groups observed different angles between monomeric ATP synthases in S. cerevisiae using different amounts of detergent. A 2D averaging of the dimers extracted with digitonin at a ratio of 5 g per g of protein revealed mainly two types of super-complexes with angles of either 35° or 90°**.(Dudkina.,*al* 2006)**

1.4.2.2. Functions of the dimeric ATP synthase

The oligomerisation of ATP synthases in cristae must have a special reason. The angular association of ATP synthases in membranes points to the possible role of complex V in a local bending of the cristae membrane. Indeed, tomography studies of cristae membranes from various organisms revealed ATP synthases in highly curved areas of cristae. It is likely that the shape of the curved dimers causes the membrane curvature rather than that a curved membrane induces the kink in the ATP synthase dimers. **(Davies, *al*. 2011)**

1.5. Mitochondrial energy metabolism

The effects of aging on mitochondrial energy metabolism are tissue specific and are more prominent in tissues whose parenchyma contains mostly postmitotic cells such as brain, heart, and skeletal muscle. **(Grinblat and *al*.,1986)**

PDH activity in the brain was found to decrease with age **(Zhou and *al*., 2008)**. In addition, there is an age-dependent decrease in succinyl-CoA:3-oxoacid Co-A transferase (SCOT) activity, a key mitochondrial matrix enzyme that metabolizes ketone bodies to acetyl-CoA ; the decreased SCOT activity as a function of age was due to irreversible protein post-translational modifications**(Lam and *al*., 2009)**, ketone body metabolism is a temporary mechanism that prevents the further decline of brain mitochondrial bioenergetic capacity which is associated with decreased activities of PDH and cytochrome oxidase. 2-Deoxy-D-glucose treatment induced ketogenesis, and this resulted in increased ketone body metabolism in the brain and a significant reduction of both amyloid precursor protein and amyloid-β **(Yao and *al*., 2010).**

Mitochondrial function is also regulated by NO, largely on reversible binding to cytochrome oxidase **(Brown, 1965)**, and at higher concentrations, it inhibits electron transfer at the bc1 segment of the respiratory chain **(Poderoso and *al*., 1999)**

Mitochondrial metabolic states regulate the diffusion of both NO and H2O2 from mitochondria to cytosol **(Boveris and *al*., 2006)**. Interestingly, the tissue levels of mtNOS have been reported to decrease with age, particularly in the brain **(Boyd and Cadenas , 2002)**

1.5.1. Mitochondrial redox homeostasis

After the initial reports on intact heart and liver mitochondria as an active source of H2O2 by Chance and Boveris **(Boveris and Chance,1973)**, further work established that superoxide anion ($O_2^{\bullet-}$) was the stoichiometric precursor of mitochondrial H2O2 and that it was primarily generated during ubisemiquinone auto-oxidation **(Boveris and Cadenas , 1975)** and, secondarily, by reverse electron transfer at the NADH-dehydrogenase segment. **(Turrens JF and Boveris,1980)** Components of complex I and complex III were reported to generate $O_2^{\bullet-}$ **(Cadenas , 2004)**. Since the activities of complexes I, III, and IV decrease during aging

- Higher oxidant production is observed : The rates of $O_2^{\bullet-}$ and H2O2 formation increase with age and are higher in mitochondria from tissues of ad libitum-fed mice than in those on caloric-restricted diets $O_2^{\bullet-}$ **(Lass and *al*., 1998)**.

- Formed on oxidation of the outer UQ pool (UQO), can be vectorially released into the cytosol, in part, through a voltage-dependent anion channel **(Han and al., 2003)**. Thus, cytosolic levels of H2O2 reflect the mitochondrial energy status **(Boveris and *al*., 1999).**

1.5.2. GSH-based systems

GSH, synthesized in the cytosol from glycine, glutamate, and cysteine in a two-step process by the enzymes γ-glutamylcystéinesynthetase and GSH synthase **(Griffith , 1999)**, is imported into the mitochondria through the dicarboxylate and oxoglutarate carriers on the inner mitochondrial membrane **(Griffith and Meister , 1985)**.

- The mitochondrial GSH pool can apparently function autonomously from the cytosolic GSH pool in response to local changes in the production of mitochondrial oxidants. **(Hurd and *al*., 2005)**
- Mitochondrial GSH protects against oxidative stress largely as a cofactor for glutathione peroxidases (GPxs), glutathione-S-transferases, sulfiredoxins, and glutaredoxins (Grxs) **(Mari and *al*., 2009)**. GPx1 localizes mainly in the mitochondrial matrix, whereas GPx 4 (also referred to as phospholipid hydroperoxideGPx) **(Schuckelt and *al*., 1991)** occurs in the inner mitochondrial membrane ; the latter detoxifies mainly phospholipid hydroperoxides . **(Borchert and *al*., 2006)**

1.5.3. Trx-based systems

The reducing power for peroxiredoxins (Prx) is transmitted through thiols of the Trxsystem :NADPH→Thioredoxinreductase (TrxR)→TrxPrx) **(Zhang and *al*., 2007)**. A comprehensive study on immunohistochemical mapping of all six Prx subtypes in the mouse brain revealed that astrocytes and microglia were reactive to Prx6 and Prx1, respectively ; immunoreactivity for Prx1 and Prx4 in the nuclei of oligodendrocytes ; in neurons, Prx3 and Prx5 were found in the stratum lucidum of the hippocampus and Prx2 in the habenular nuclei **(Jin and *al*., 2005)**

Of these Prxs, Prx2 was critical for the maintenance of hippocampal synaptic plasticity against age-associated oxidative damage by a mechanism entailing the oxidant- and age-dependent mitochondrial decay of hippocampal neurons, in addition, the expression of Prx2 in hippocampal neurons increased as a function of age **(Kim and *al*., 2011)**

1.5.4. Mitochondrial dynamic remodeling

Mitochondria are highly dynamic organelles and undergo continuous fusion and fission throughout their life cycle. These processes regulate not only mitochondrial morphology, but also their biogenesis, transportation, cellular localization **(Chan , 2006).**

- Mitochondrial fusion is regulated by GTPases optic atrophy-1 (OPA1) and mitofusin-1/2 (Mfn1/Mfn2), which are responsible for the fusion of outer- and inner mitochondrial membranes respectively **(Song and *al*., 2009).**
- Mitochondrial fission is controlled by dynamin-related protein 1 (Drp1) and fission protein 1 homolog (Fis1) **(Ishihara and *al*., 2009).**

Mitochondrial dynamics are closely related to the energy-redox axis. A coordinated balance between fission and fusion is essential for cell function **(Cerveny and *al*., 2007).**

Similar effects on mitochondrial metabolism are shown when mitochondrial fission is inhibited

- The down-regulation of Drp1 leads to a loss of mitochondrial membrane potential and DNA content, a decrease of mitochondrial respiration and cellular ATP levels, as well as an oxidized cellular redox status and cytochrome c release **(Frank and *al*., 2001).**

1.5.5. The Energy-Redox Axis and Nuclear Transcriptional Pathways

1.5.5.1. Transcriptional control of mitochondrial biogenesis

The decline of mitochondrial function with age and in neurodegeneration is associated with reduced mitochondrial biogenesis and decreased activity of its major regulator, PGC-1α. PGC-1α levels were found to be decreased with age, and the decline was rescued by caloric restriction in skeletal muscle **(Hepple and al., 2006)** PGC-1α levels and mitochondrial function were positively linked to lifespan extension in several rodent genetic models **(Al-Regaiey and al., 2005)**. Overexpression of PGC-1α in a model of mitochondrial myopathy significantly prolonged lifespan **(Wenz and al., 2008).**

As a key regulator of mitochondrial biogenesis and function, PGC-1α is regulated at multiple levels, including its transcription, post-translational modification, localization, and degradation.

- The regulation of PGC-1α expression involves CREB **(Wu and al., 2006)** and mTORYY1 pathways, making them important modulators of mitochondrial metabolic function in aging **(Schieke and al., 2006).**

- Post-translationally, PGC-1α is activated by Sirt1 by deacetylation after the translocation of the former into the nucleus during stress conditions **(Anderson and al., 2008).**
- AMPK is another regulator of PGC-1α activity either by direct phosphorylation of PGC-1α **(Jager and al., 2007)** or by indirectly enhancing Sirt1 activity **(Canto and al., 2009)**

1.5.5.2. Mitochondrial regulation of transcriptional pathways

The signal communications between the nucleus and mitochondria are not unidirectional. Perturbations of mitochondrial energy and redox status can also be transmitted to the nucleus to induce cellular adaptive or compensatory responses. This process involves several mitochondriongenerated second messengers, such as ATP, H2O2, NO, and processes involved in the dysregulation of Ca2+ homeostasis and the maintenance of cytosolic NAD+/NADH ratios. As the primary indicator of mitochondrial metabolic status, altered ATP levels in the cells affect AMPK activity and positively modulate energy-consuming anabolic processes through mTOR either directly **(Dennis and al., 2001)** or indirectly, ATP signaling is also involved in the regulation of mitochondrial biogenesis through the AMPK-PGC1α pathway **(Canto and Auwerx , 2009).** Hence, the mitochondrial bioenergetic state is an important modulator of cell growth and proliferation **(Brew and leeuwenburg , 2003).**

As important intermediate metabolites, both NAD+ and NADH, and their ratio, affect mitochondrial function by modulating mitochondrial permeability transition pore and Ca2+ homeostasis **(Ying, 2006).** The age-dependent decline in intracellular NAD+levels and NAD+/NADH ratio were observed in parallel with decreased Sirt1 activity **(Braidy and al., 2011).** Although the inner mitochondrial membrane is impermeable to NAD+ and NADH, it was found that the malate-aspartate NADH shuttle could play a critical role in the activation of the downstream targets of caloric restriction, such as sir2 (Sirt1 homolog) **(Easlon and al., 2008).**

Transcription factors, such as HSF-1, p53, and NF-κB, can be directly activated on oxidative stress **(Finkel and Holbrook , 2000),** while some other factors, including NRF-1, NRF-2, and FoxO, can be activated/inhibited indirectly via redox-sensitive kinase signaling pathways such as JNK, ERK, p38, and PI3K/Akt **(Brigelius-Flohe and Flohe , 2011).**

The role of H2O2 in NF-κB activation has been critically reviewed **(Oliveira-Marque and al., 2009)**, and it was concluded that H2O2 does not function as an inducer of NF-κB but it is largely involved in the regulation of NF-κB-related pathways **(Dinkova-Kostova and al., 2002).**

Conclusion

From a toxicological point of view, mitochondria and mitochondrial redox biology are unmethodically connected to bioenergetics and oxidative stress/damage. Mitochondria are involved in pathophysiological cellular processes other than ATP production. In addition, mitochondrial redox biology influences cellular processes through redox modulation, which is now described as "eustress," in contrast to "oxidative stress."

Now, we should place mitochondria at the center of the cellular response to environmental stress. Pathophysiological responses may transpire through not only ATP production but also through catabolic/anabolic metabolism and retrograde-signaling, albeit the exact nature has not yet been clarified. The retrograde signal influences the mitochondrial morphology, biogenesis, and kinetics. In addition, experimental approaches and novel technologies must be devised to adequately evaluate the environmental stressor-induced toxicity manifested through the mitochondria.

Chapter 2: Neurodegenerative diseases

Introduction

Neurodegenerative diseases are a heterogeneous group of disorders that are characterized by the progressive degeneration of the structure and function of the central nervous system or peripheral nervous system. Common neurodegenerative diseases include Alzheimer‟s disease and Parkinson‟s disease.

2.1. Neuro-death mechanisms

Neuronal cell death used to be an enigmatic area of study. After decades of the molecular dissection of the mechanism, neuronal cell death is the outcome after a neuron activates wellorchestrated programs in order to terminate its existence, a process that can be triggered by internal or external signals throughout the cell‟s lifetime. During the development of the human central nervous system (CNS), neurogenesis is often accompanied by massive neuronal loss, a necessary part of constructing a functionally adequate command center. In spite of occasional or arranged death events, extensive neuronal loss rarely occurs in mature CNSs. However, in many neurodegenerative diseases, a significant increase in neuronal loss occurs compared to age-matched controls, which also correlates with longitudinal examinations of disease progression, cell death, and regional brain shrinkage, which will ideally facilitate the development of treatments to counteract the progression of diseases. In general, mature CNS neurons are very resistant to cell death compared to immature neurons. **(Kole; Annis.2013)**

2.1.1. Apoptosis

Apoptosis, also called programmed cell death, in biology, is a mechanism that allows cells to self-destruct when stimulated by the appropriate trigger. Apoptosis can be triggered by mild cellular injury and by various factors internal or external to the cell; the damaged cells are then disposed of in an orderly fashion. As a morphologically distinct form of programmed cell death, apoptosis is different from the other major process of cell death, known as necrosis. Apoptosis involves condensation of the nucleus and cytoplasm, followed by cellular partitioning into welldefined fragments for disposal.**(Paul . Schlesinger 2010)**

2.1.1.1. Apoptosis by the extrinsic / Death receptor pathway

Apoptosis is triggered by two principal pathways: the intrinsic (or mitochondrial) pathway and the extrinsic (or death receptor) pathway (Figure 2.1). The extrinsic apoptosis pathway is triggered by the ligation of tumor necrosis factor (TNF)-family death receptors at the cell surface.

Receptor ligation can result in recruitment of Fas-associated death domain protein (FADD), which in turn binds pro-caspase-8 molecules, allowing autoproteolytic processing and activation of caspase-8 to occur . Once activated, caspase-8 may in turn activate downstream effector caspases by direct proteolytic cleavage or indirectly by cleavage of the BH3-only protein Bid to produce tBid, which translocates to the mitochondria to induce Bax activation and mitochondrial outer membrane permeabilization (MOMP) as discussed below. TNF- and Fas ligands can cause apoptosis in some neurons during inflammation, and a Fas-dependent apoptotic pathway involving p38 nitric oxide (NO) and then classical caspase-dependent apoptosis has been described for motor neurons. **(Haase , al 2008)**

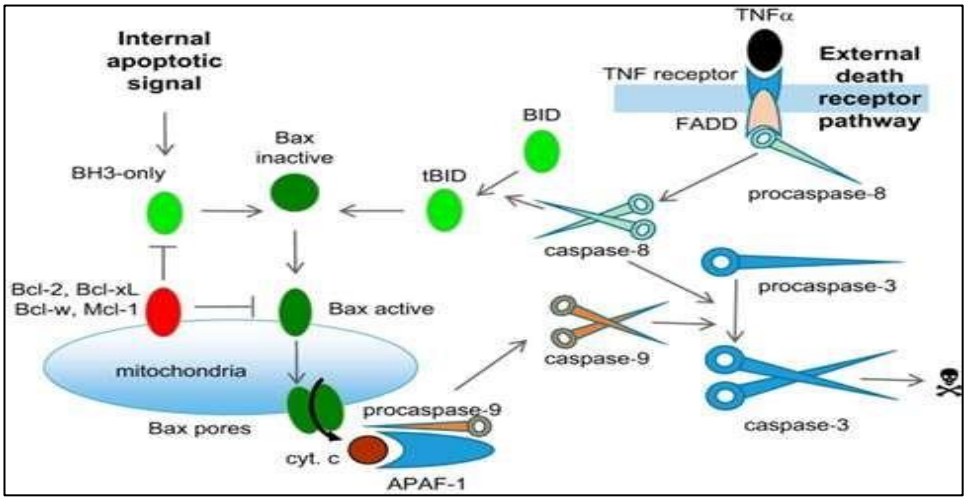

Figure 2.1: *Overview of apoptosis.* ***(Micheau O, Tschopp J. 2003)***

2.1.1.2. Apoptosis by the intrinsic/mitochondrial pathway

The intrinsic apoptosis pathway centers on the regulation of MOMP by the Bcl-2 family of proteins (Figure 2.2). Members of the Bcl-2 family share homology within at least one of up to four Bcl-2 homology (BH) domains, which are required for the homo-and heterotypic interactions that determine the decision to undergo MOMP. Bax and Bak, which contain BH1–3, are thought to be almost entirely required for the execution of apoptosis via the intrinsic pathway.**(Cheng EH, al. 2001).**

Thus, the induction of intrinsic apoptosis in neurons is entirely dependent on Bax expression and activation, and indeed, deletion and inhibition of Bax prevents aberrant neuronalcell death in a number of in vitro and in vivo models of neurodegeneration.

(D'Orsi B, Engel , al. 2016)

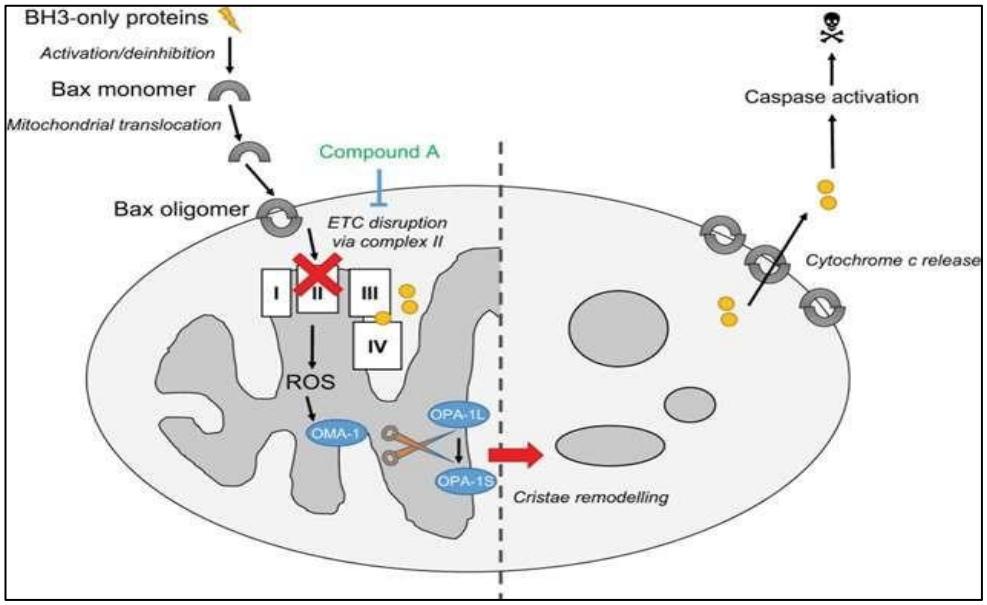

Figure2.2 :*Bax signaling at the mitochondria* ***.(Cheng EH, al. 2001)***

2.1.2. Necrosis

Necrosis is the death of a circumscribed area of plant or animal tissue as a result of disease or injury. Necrosis is a form of premature tissue death, as opposed to the spontaneous natural death or wearing out of tissue, which is known as necrobiosis. Necrosis is further distinguished from apoptosis, or programmed cell death, which is internally regulated by cells, plays a critical role in embryonic development, and serves as a protective mechanism against disease and other factors. **(GauravShukla 2007)**

2.1.2.1. Necroptosis

The best-characterized form of regulated necrosis is necroptosis. Necroptosis is defined as a necrotic cell death dependent on the kinase activity of Receptor Interacting Kinase 1 (RIP1) and kinase activity of RIP3, which in turn phosphorylates and activates MLKL. Activation of the necrosome results in the oligomerization of phosphorylated MLKL at the plasma membrane, causing cell rupture. This is due to the pore-forming activity of the MLKL oligomer or modulation of Na+ or Ca2+ channels. **(Dondelinger , al. 2014).**

2.1.2.2. Cell death by mitochondrial permeability transition

Cells can die as a result of mitochondrial permeability transition (mPT), and this is a distinct form of cell death. mPT defines a large increase in the permeability of the inner mitochondrial

membrane in response to elevated concentrations of calcium, usually resulting in uncoupling of oxidative phosphorylation, cellular energy depletion, and necrotic cell death. The permeability transition is caused by the opening of a so-called mitochondrial permeability transition pore (mPTP), a nonselective pore with a diameter of 1.4–2.3 nm, which makes the inner membrane freely permeable to protons, metal ions, and all small molecules (10,000 Da). Currently, the general consensus is that the mitochondrial matrix enzyme cyclophilin D (a peptidyl-prolyl cis-trans isomerase) is an essential component of mPTP. to form a pore in the mitochondrial inner membrane. **(, Lemasters JJ 2002)**

2.1.2.3. Lysosomal cell death (autolysis)

Lysosomal cell death (LCD) is defined as cell death resulting from lysosomal membrane permeabilization (LMP). The diagnosis of LCD as the cause of cell death is imprecise as it can be triggered by events that activate other cell death pathways and occur alongside, and cross-react with, other cell death mechanisms. **(Villalpando Rodriguez 2013)**

2.1.3. Autophagic Cell Death and Autosis

Autophagy normally functions to prevent cell death, but if excessive, can cause cell death (Figure 2.3). Autophagy is a process of cell self-eating whereby cell constituents are delivered to the lysosome for digestion and recycling.

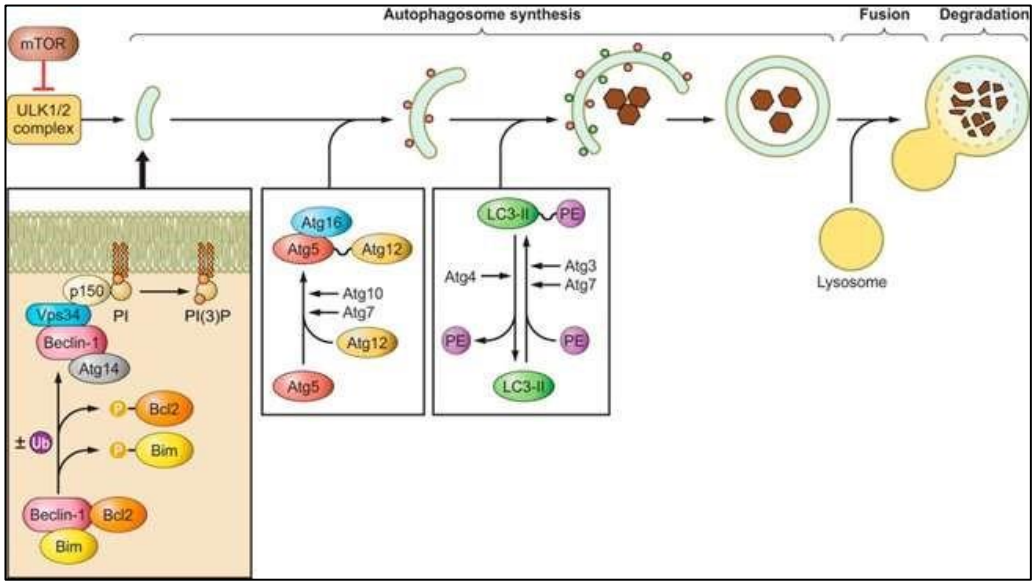

Figure 2.3: *Autophagy and autophagic cell death* ***(Deter RL, De Duve C 1997)***

2.1.4. Cell Death by Phagocytosis (Phagoptosis)

Cell death by phagocytosis refers to a cell dying as a result of being phagocytosed by another cell. The key distinguishing characteristic of this form of cell death is that inhibiting phagocytosis of the cell prevents the death of the cell. Dead or dying cells are rapidly phagocytosed (secondary phagocytosis) and inhibition of phagocytosis in this case will not prevent cell death but rather cause dead cells to accumulate. In this case, inhibition of phagocytosis will prevent cell death. **(Brown , Neher JJ 2014)**

2.2. Mechanism of proteins toxicity in neurodegenerative disease

Protein toxicity can be defined as all the pathological changes that ensue from accumulation, mis-localization, and/or multimerization of disease-specific proteins. Most neurodegenerative diseases manifest protein toxicity as one of their key pathogenic mechanisms, the details of which remain unclear. By systematically deconstructing the nature of toxic proteins, we aim to elucidate and illuminate some of the key mechanisms of protein toxicity from which therapeutic insights may be drawn, we focus specifically on protein toxicity from the point of view of various cellular compartments such as the nucleus and the mitochondria. We also discuss the cell-to-cell propagation of toxic disease proteins that complicates the mechanistic understanding of the disease progression as well as the spatiotemporal point at which to therapeutically intervene.

2.2.1. Protein toxicity in the nucleus

Nuclear inclusions (NIs) of toxic proteins in neurons are observed in approximately 20 different neurodegenerative diseases **(Woufle J, 2008)**. Although a growing body of evidence indicates nuclear dysfunction to be central to the pathogenesis of several neurodegenerative diseases, the precise role of neuronal intranuclear inclusion bodies in the disease pathogenesis is still a matter of debate. There is a view that microscopically visible NIs are not toxic, but are instead self-protective structures or incidental byproducts of the pathogenic process **(Arrasate M and al., 2004).**

PolyQ diseases may be representative neurodegenerative diseases associated with nuclear protein toxicity **(Havel LS, Li S, Li XJ, 2009).** There are at least nine polyQ diseases, including HD, dentatorubral–pallidoluysian atrophy (DRPLA), spinal bulbar muscular atrophy (SBMA), and the spinocerebellar ataxias (SCAs) **(Shao J, Diamond MI, 2007),** Each of these nine polyQ diseases is caused by CAG (Q) repeat expansion mutation in each of the disease-responsible genes [e.g., the huntingtin (htt) gene mutation for HD] **(Pouladi MA and al., 2013).**

Transcriptional and epigenetic alterations have been shown to contribute to the broad spectrum of neuronal phenotypes ranging from early neuropathic features to late-stage neuronal cell death in polyQ diseases **(Kwon MJ and al., 2017).** For instance, recent studies showed that polyQ proteins induced early changes to the dendrite morphology through the perturbation of RNA granule formation and transcriptional cascades regulating the ER-to-Golgi (COPII) pathway **(Chung CG, Kweon JH and al., 2017).**

2.2.2. Protein toxicity in the cytoplasm

Many of the disease proteins are prone to accumulate in the cytoplasm, in which the pool of potential target molecules differs significantly from that of the nucleus. For example, it is the cytoplasm in which the protein quality control (PQC) system mostly resides, not in the nucleus **(Ciechanover A, Kwon YT, 2015).** The cytoplasm also contains a more elaborate cytoskeleton compared to the nucleus. The accumulation of misfolded proteins in neurodegenerative diseases inevitably burdens the PQC system, which comprises the ubiquitin–proteasome system (UPS), chaperone-mediated autophagy (CMA), macro autophagy, and ER-associated degradation (ERAD) **(Ciechanover A, Kwon YT, 2015).** UPS impairment is considered to be one of the major contributing factors of neurodegenerative disease pathogenesis **(Deriziotis P and al., 2011).**

CMA is a selective protein degradation system that eliminates proteins harboring a pentapeptide KFERQ-like motif, which is found in approximately 30% of cytosolic proteins **(Ciechanover A, Kwon YT, 2015)**. When folded properly, the KFERQ motif is not exposed to the surface, Several disease-associated proteins such as LRRK2 and α-synuclein also harbor KFERQ-like motifs that are recognized by CMA for degradation. As for LRRK2, its binding to the lysosomal membrane is enhanced by certain mutations, thereby facilitating accumulation of α-synuclein among other CMA substrates **(Orenstein SJ and al., 2013),** macro autophagy activity is reduced due to the failure in cargo recognition by autophagic vacuoles **(Martinez-Vicente M and al., 2010).**

Pathological inclusions of cytoskeletal proteins, such as neuronal intermediate filament (IF) proteins or the microtubule-associated protein tau (MAPT), are neuropathological signatures in various neurodegenerative diseases **(Cairns NJ and al., 2004).** Specifically, tau-associated microtubule defects are linked to a range of neurodegenerative diseases known as “tauopathies” **(McMurray CT, 2000).** Furthermore, formation of ADF/coiling-actin filament bundles (rods) that can occlude neurites and block vesicle transport has been implicated in neurodegenerative diseases **(Bamburg JR, 2010).** In addition to these changes in cytoskeletal structures, accumulation of toxic

disease proteins can lead to defects in axonal transport **(Chevalier-Larsen E, Holzabaur EL, 2006).**

2.2.3. Protein toxicity in the mitochondria

The importance of the mitochondria to cell survival can easily be envisaged, as they are the organelles primarily responsible for ATP production in eukaryotic cells. Thus, mitochondrial dysfunction can be detrimental for cell survival, which can be catastrophic particularly to the brain, for the following reasons.

- First, most neurons cannot be replaced and thus need to be maintained due to their postmitotic nature. This will inevitably lead to the accumulation of mitochondrial toxicity, by which the irreplaceable neurons will eventually succumb to death.
- Second, the excitability of neurons allows for significant influx of calcium ions that are buffered by mitochondria, the dysfunction of which will lead to excitotoxicity.
- Third, the elongated morphology of neurites entails a local supply of ATP by the mitochondria, the dysfunction of which will perturb growth and maintenance of neurites **(Abramov AY and al., 2017).**

The following six toxic disease proteins that accumulate in mitochondria will be discussed: amyloid beta, amyloid precursor protein (APP), α-synuclein, mutant htt, TDP-43, and poly-GR DPRs.

Extracellular amyloid beta accumulation is one of the key pathological hallmarks of AD, in which mitochondrial dysfunction is often observed **(Spuch C, and al., 2012).** No direct mechanistic link between amyloid beta and mitochondrial dysfunction was identified until Lustbader et al. Showed in 2004 that amyloid beta can localize to the mitochondria and directly bind to amyloid beta-binding alcohol dehydrogenase (ABAD) to induce mitochondrial toxicity **(Lustbader JW and al., 2004).** Amyloid beta has also been shown to interact with cyclophilin D (CypD), an integral component of the mitochondrial permeability transition pore (mPTP) **(Du H, Guo L and al., 2008).**

Mitochondrial dysfunction is not unique to AD. In HD, an energy-deficit related to mitochondrial dysfunction **(Browne SE and al., 1997).** The mechanisms by which mutant htt proteins induce mitochondrial dysfunction have been shown to be as diverse as that in AD. Aside from the mutant htt perturbing transcription of genes related to mitochondrial biogenesis and function in the nucleus **(Cui L and al., 2006),** it could also directly interact with mitochondrial proteins **(Kim YE and al., 2016).** The N-terminal fragment of mutant htt localizes to the mitochondria **(Panov AVand al., 2002)** both in vivo and in vitro, and it interacts with the TIM23

complex, thereby clogging the mitochondrial import process **(Yano H and al., 2014).** These toxic interactions of mutant htt with mitochondrial proteins, depolarize mitochondrial membrane potential, and ultimately lead to neuronal demise **(Panov AV and al., 2002).**

2.2.4. Propagation of toxic disease proteins

Within 6–12 months after the inoculation of amyloid beta-containing brain extracts derived from either AD patients or aged APP transgenic mice into the hippocampus and neocortex of young APP transgenic mice, amyloid beta deposition and its associated pathology were widespread in the brain **(Kane MD, 2000).** Likewise, an intracerebral administration of brain or spinal cord homogenates prepared from symptomatic α-synuclein transgenic mice facilitated the appearance and spread of Lewy pathology in presymptomatic recipient transgenic mice **(Luk KC, 2012).** The spread of pathological changes was recapitulated by a local injection of synthetic α-synuclein fibrils or tau filaments in presymptomatic transgenic mice, suggesting that aggregates, but not other factors in the brain homogenates, are sufficient for the spreading of the pathological changes in the brain **(Iba M and al., 2015).** Finally, selective overexpression of transgenic tau, amyloid beta, or αsynuclein in a population of neurons could trigger the spread of misfolded proteins to the interconnected brain regions in transgenic mice **(Helwig M and al., 2016)**

2.3. Increase in oxidative stress

2.3.1. Characteristics of reactive oxygen species

2.3.1.1 Types of reactive oxygen species (ROS)

Oxygen is susceptible to radical formation due to two unpaired electrons present in the outer electron shell. Reactive oxygen species (ROS) are defined as a group of reactive molecules derived from oxygen, which are generally short-lived and highly reactive because of their unpaired valence electrons. ROS include, but are not limited to free radicals (superoxide, O2-), hydroxyl radical (·OH), or non-radicals (hydrogen peroxide, H2O2) (Figure 2.4) **(Gandhi S et al., 2012)**

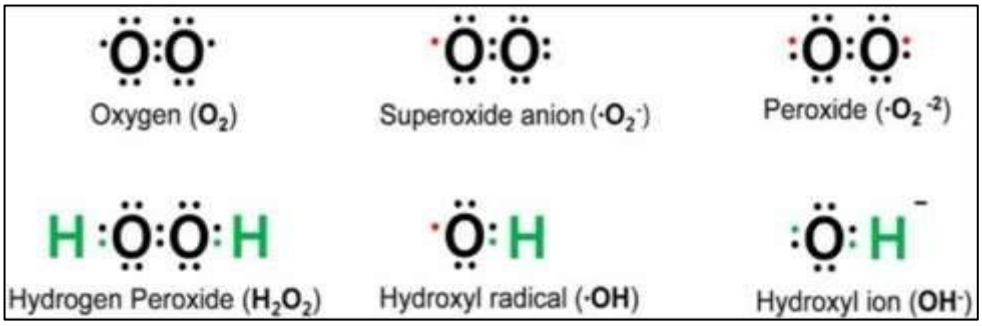

Figure 2.4: *Common reactive oxygen species (ROS).* ***(ExpNeurobiol ,2015)***

Vitamin E is a lipid-soluble antioxidant that can attenuate the effects of peroxide and protect against lipid peroxidation in cell membranes

O2- is suggested to play a gateway role in ROS production. O2- may be transformed into the more stable form of H2O2 by superoxide dismutases (SOD) (Figure 2.4). It may also be protonated to form HO2-. H2O2 may have potential to generate highly reactive hydroxyl radicals ·OH, while it can further be divided into H2O and O2 by catalase, glutathione peroxidase, and other peroxidases . ·OH is known to be one of the most reactive ROS that are mainly responsible for the cytotoxic effects of ROS ·OH can be generated from H2O2 and O2- and is catalyzed by iron ions through the Fenton reaction that refers to Fe2+-mediated decomposition of H2O2. **(Zorov DB et al..., 2014)**

2.3.1.2. Mitochondrial ROS production In brain

The mitochondrion is the primary source of ROS production in the majority of cells. Under normal physiological condition, up to 2% of the total cellular mitochondrial O2 consumption may be related to the generation of ROS including O2-. **(Orrenius S et al., 2007)**

Multiple ways of mitochondrial ROS productions have been proposed, which are mainly modulated by the mitochondrial respiratory chain complexes. **(Widlansky ME et al., 2011)**

The mitochondrial electron transport chain (ETC) consists of five multi-subunit complexes including NADH-coenzyme Q (CoQ) reductase (NADH dehydrogenase, Complex I), succinate dehydrogenase (Complex II), coenzyme Q-cytochrome c reductase (Complex III), cytochrome C oxidase (Complex IV), and ATP synthase (Complex V). **(Song P et al., 2015)**

Complex I is responsible for ROS production of O2- .and facilitates electron transfers from NADH to CoQ. During this step, protons are also translocated from the matrix to the intermembrane space**. (UBolisetty S et Jaimes EA, 2013)**

Complex II is involved in the reduction of CoQ and is known to be involved in producing low levels of O2-. **(McLennan HR et al., 2013)**

Complex III, on the other hand, is involved in the generation of O2- in the intermembrane space. The generation of O2- is especially enhanced when the electron transfer is reduced the increased membrane potential. **(UBolisetty S et Jaimes EA, 2013)**

Interestingly, the capacity of these enzymes to produce ROS may vary among the organs or during disease conditions**. (Turrens JF, 2003)**

For instance, Complex I appears to contribute to the production of most of O2- in the brain, while Complex III is considered as the primary source of O2- in the heart and lung**. (Turrens**

JFJF, 2003)

In addition, within mitochondria, ETC Complex I and III are regarded as the main producers of O2- . ROS productions from Complex I is approximately one-half of those from complex III in heathy state, while Complex I exerts the primary role in ROS productions under pathological conditions ranging from accelerated aging to neurodegenerative diseases **(Zorov DB et al..., 2014)**

A. Mitochondrial ROS production NADPH oxidases (NOX)

Nox, a transmembrane enzyme complex, is known to be another important endogenous source of O2- production as the result of the catalyzing the electron transfer from NADPH to oxygen. **(Infanger DW et al..., 2006)**

Nox is found highly in phagocytes (neutrophils, eosinophils, monocytes and macrophages, called as Phox or NOX2) as well as in the endothelium of cardiovascular tissue. **(Babior BM et Lambeth JD, 2004)**

Until now, seven Nox isoforms have been identified in mammalian cells including Nox1 to Nox5 and dual oxidases (Duox1 and Duox2). Each Nox isoform has unique cellular localization, regulation, and function. **(Song P, et al., 2015)**

For instance, Nox4 and Nox2 are abundant, whereas Nox1 is less in endothelial cells In contrast, Nox1 and Nox4 are the more highly expressed isoforms in vascular smooth muscle cell than Nox2.

Nox2 which is mainly expressed in phagocytes and produces large amounts of ROS, can help kill the foreign organisms as a part of the immune defense system. **(Patten DA et al., 2010)**

On the other hand, Nox produces relatively less ROS at a slow and sustained rate in cardiovascular tissue and exerts a role as intracellular signaling molecules. Previous studies have reported that Nox4 is one of the most common isoforms in vascular structures. **(Song P et al., 2012)** In addition, contrary to Nox1 and Nox2, Nox4 is fundamentally active in the cardiovascular systems and the primary source of H2O2 production rather than O2- production. **(Gordillo G et al., 2010)**

2.3.1.3. Antioxidant pathway

A. Superoxide dismutase (SOD)

SOD plays a significant role in catalyzing the breakdown of highly reactive O2- to less reactive H2O2 and oxygen. **(Dasuri K Et al.,2013)**

Cytosolic copper/zinc-SOD (SOD1), mitochondrial manganese SOD (SOD2), and extracellular SOD (SOD3) are three distinct isoforms of SOD that have been identified. SOD1 and SOD2 are mainly involved in the elimination of O2- in the cytosol and mitochondria, respectively.

(Dasuri K et al., 2013) B. Glutathione peroxidases

GPX contains a family of multiple isoenzymes which catalyze the reduction of H2O2 and lipid peroxides utilizing GSH as an electron donor. **(Gandhi S, Abramov AY, 2012)**

GPX is located in both cytosol and mitochondria. In mammals, there are five different isoforms of selenium-dependent glutathione peroxidases (GPX1-4 and 6) and three non-selenium congeners (GPX 5, 7 and 8) that have cysteine instead of selenocysteine. **(Brigelius-Flohé R et Maiorino M, 2013)**

Antioxidant function of GPXs depends on each isoform and location in the cells ; GPX1 exists universally in the cytosol and mitochondria, GPX2 does in the epithelium of intestine, and GPX3 does in the plasma. **(Brigelius-Flohé R et Maiorino M, 2013)**

It is noteworthy that GPX1 has been regarded as one of the major antioxidant enzymes in the brain, which is expressed predominantly in microglia but not in neurons, Studies have suggested that upregulation of GPX1 could be one of the protective responses against neuronal injury **(Power JH et Blumbergs PC, 2009)**

C. catalase

Catalase is responsible for the conversion of H2O2 to water and oxygen using either iron or manganese as a cofactor. **(Gandhi S et al., 2012)**

Catalase is located in peroxisomes and also found in the cytoplasm and mitochondria. **(Gandhi S et Abramov AY, 2012)**

The role of catalase is minor at low levels of H2O2, but becomes increasingly important at higher levels of H2O2. (**Gandhi S et Abramov AY, 2012)**

D. Peroxiredoxins

PRX are thiol-specific peroxidases that catalyze the reduction of H2O2 as well as other organic hydroperoxides and peroxynitrite. **(Dasuri K et al., 2013)**

Among the six PRX isoforms, PRX1, 2, and 4 are present in the cytoplasm as well as in the nuclei. In addition, PRX1 is also expressed in the mitochondria and peroxisomes, while PRX4 is found in the lysosomes. **(Espinosa-Diez C et al., 2015)**

PRX3 is exclusively localized in the mitochondria. **(Espinosa-Diez C et al., 2015)**

Whereas PRX5 is found in the mitochondria, cytoplasm, nuclei, and peroxisomes. **(Seo MS et al., 2000)**

All PRX utilize a conserved active-site cysteine residue in order to directly reduce peroxide. **(Hall A et al., 2011)**

Since PRX are abundant in eukaryotic cells, constitute approximately more than 1% of cellular proteins, and show high reactivity, PRX are responsible for the reduction of up to 90% of mitochondrial H2O2 and almost 100% of cytoplasmic H2O2. **(Hall A et al., 2011)**

E. Glutathione

GSH, a tripeptide synthesized from glutamate, cysteine, and glycine, exerts protective function of cell survival against oxidative stress. **(Gandhi S et al., 2012)**

In the brain, in vivo GSH is produced by the consecutive actions of two enzymes ; γ dipeptide of γglutamylcysteine is formed by -glutamylcysteine synthetase, using glutamate and cysteine as substrates. And this dipeptide is further combined with glycine by the catalyzing action of glutathionine synthetase to synthesize GSH. **(Lee M et al., 2010)**

Several studies have reported that GSH is involved in inhibiting apoptotic cell death and DNA damage in cells following oxidative stress. **(Presnell CE et al., 2013)**

F. Vitamin E / Vitamin C

- Vitamin E is a lipid-soluble antioxidant that can attenuate the effects of peroxide and protect against lipid peroxidation in cell membranes. **(Gandhi S et al., 2012)**
- Vitamin C is a water-soluble antioxidant, which is involved in the removal of free radicals by electron transfer and also acts as a cofactor for antioxidant enzymes. **(Song P et al., 2015)**

2.3.1.4. Oxidative stress : excessive accumulation of ROS

In a healthy condition, the production of ROS is balanced by various antioxidant systems. **(Gandhi S et al., 2012)**

Oxidative stress is a condition of imbalance between ROS production and antioxidant defenses, resulting in excessive accumulation of ROS. **(Dasuri K et al., 2013)**

Oxidative stress may be related to cell membrane damage from lipid peroxidation, changes in protein structure and function due to protein oxidation, and structural damage to DNA. **(Gandhi Set bramov AY, 2012)**

As the brain is one of the most metabolically active organs in the body, it is vulnerable to oxidative stress particularly because of the following reasons.

First, the brain has a high oxygen demand, which constitutes 20% of the body oxygen consumption.

Second, the redox-active metals such as iron or copper exist abundantly in the brain and they are actively involved to catalyze ROS formation.

Third, the high levels of polyunsaturated fatty acids are found in the brain cell membranes and react as substrates for lipid peroxidation. **(Wang X et Michaelis EK, 2012)**

Fourth, there are relatively low levels of GSH in the brain, which plays a role of endogenous antioxidant in the elimination of ROS. **(Ferreira ME et al., 2015)**

2.3.2. Evaluation of oxidative stress in neurodegenerative diseases

2.3.2.1. Measurement of oxidative stress in peripheral blood

Since oxidative stress may be a common pathophysiological mechanism underlying various neurodegenerative diseases, several surrogate markers for oxidative stress or antioxidant activity, including circulating lipid peroxides, GSH, and vitamins C and E, have been assessed in peripheral blood. **(Ferreira ME et al.,2015)**

A previous study has shown that AD patients demonstrated the decreased peripheral levels of vitamins A, C, and E along with lower activities of SOD and glutathione peroxidase. **(Lovell MA et al., 1995)**

The levels of GSH in plasma have been suggested as a significant predictor of cognitive functions in patients with AD, implying the relationship between lower plasma levels of GSH and more severe cognitive impairment. **(McCaddon A et al., 2003)**

Although the results have been inconsistent, the activity of SOD in erythrocyte would be altered in patients in PD**. (Kalra J et al., 1992)**

Increased SOD activity may contribute to the protection mechanism against enhanced production of O2- relating to neurodegenerative diseases. **(Younes-Mhenni et al., 2007)**

2.4. Dysfunction of the ubiquitin proteasomal system

The ubiquitin-proteasome system (UPS) is one of the major protein degradation pathways, where abnormal UPS function has been observed in cancer and neurological diseases. Many neurodegenerative diseases share a common pathological feature, namely intracellular ubiquitinpositive inclusions formed by aggregate-prone neurotoxic proteins. This suggests that dysfunction of the UPS in neurodegenerative diseases contributes to the accumulation of neurotoxic proteins and to instigate neurodegeneration.

2.4.1. Ubiquitin proteasomal system

Ubiquitin is an evolutionarily conserved 76-amino acid moiety covalently tandemly linked to target protein components for degradation, and is required for degradation of about 80% intracellular proteins in eukaryotes **(Pickart, 2001).** The presence of ubiquitin in intracellular inclusions has been found in various neurodegenerative diseases **(Todi and Paulson, 2011)** Protein ubiquitination occurs through the coordinated activity of several enzymes, including an ubiquitin activating enzyme (E1 ligase), conjugating enzyme (E2 ligase), and E3 ligase.

Protein ubiquitination occurs through the coordinated activity of several enzymes, including an ubiquitin activating enzyme (E1 ligase), conjugating enzyme (E2 ligase), and E3 ligase. Initially, a single ubiquitin moiety is attached to an active-site Cysteine residue within the E1 ligase through a thioester bond in an ATP dependent manner.

The activated ubiquitin is then transferred to an ubiquitin conjugating enzyme-E2 ligase. Finally, the E2 ligase will cooperatively transfer the ubiquitin chain with a specified E3 ligase to a particular substrate, whereby the 26S proteasome will target the polyubiquitinated protein for degradation

E3 ligases are grouped into three classes according to their unique domains, including Really Interesting New Gene (RING) or U-box domain-containing E3 ligases, Homologous to E6AP Cterminus (HECT) domain-containing E3 ligases, and RING-between-RING (RBR) domaincontaining E3 ligases **(Atkin and Paulson, 2014; Morreale and Walden, 2016).**

RING E3 ligases catalyze the transfer of ubiquitin directly from E2 ligases to the substrates by binding both of them. **(Ravid and Hochstrasser, 2008).**

The UPS is involved in protein quality control and removal of misfolded and aggregated proteins, and dysfunction of the UPS is implicated in the pathogenesis of neurodegenerative diseases **(Popovic et al., 2014).**

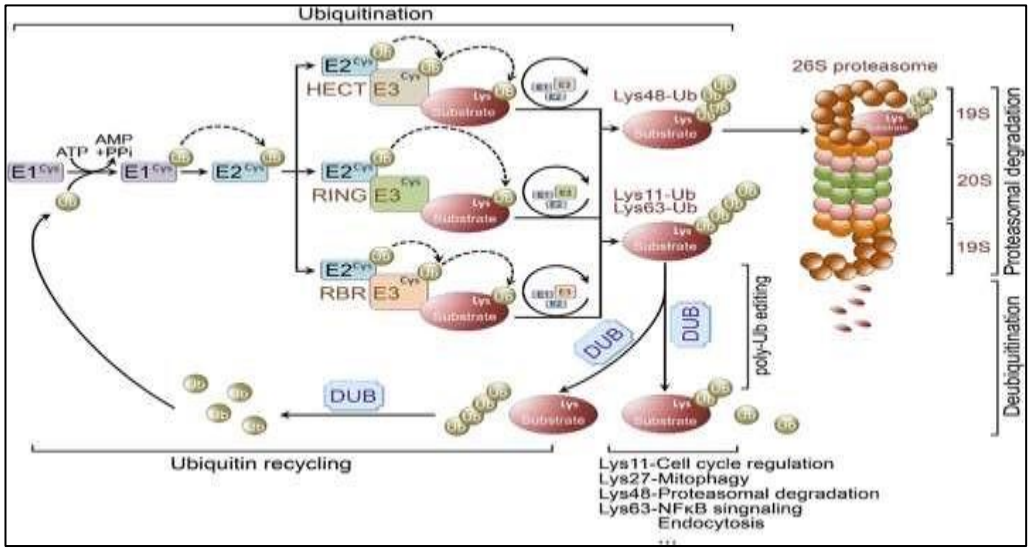

Figure 2.5 : *Ubiquitination and ubiquitin-proteasome system.(https://doi.org/10.3389/fnagi.2016.00303)*

2.4.2. Dysregulation of the UPS in Alzheimer's disease

The presence of ubiquitin in NFTs and amyloid plaques in AD brain has been described as early as 1987 **(Cole and Timiras, 1987; Mori et al., 1987; Perry et al., 1987).** Ubiquitin-targeted proteasomal activity declines with age Tg2576 AD mouse model brain, and the presence of dystrophic neurites and clustering of tubular endoplasmic reticulum has been observed in RTN3 transgenic mouse brain **(Hu et al., 2007; Sharoar et al., 2016).** Interestingly, intracellular Aβ accumulation and impaired proteasome function can be reversed by the ubiquitin E3 ligase parkin **(Rosen et al., 2010).** Parkin expression is downregulated in AD brains, and reduced parkin expression may contribute to the accumulation of intracellular Aβ. Moreover, parkin expression results in Aβ reduction which can be dampened by proteasome inhibitors **(Rosen et al., 2010).**

A direct interaction between APP and ubiquitin ligases also contributes to amyloid pathology. HRD1, an endoplasmic reticulum-associated degradation (ERAD) ubiquitin E3 ligase, is reduced in AD brains **(Kaneko et al., 2010).** HRD1 interacts with APP through its Proline-rich region, and this interaction reduces Aβ generation through promoting APP ubiquitination and degradation **(Kaneko et al., 2010).** In addition, APP can bind to ubiquitin E3 ligases Stub1 and CRL4CRBN through the APP cytosolic region **(ACR Del Prete et al., 2016).** ACR promotes the ubiquitination of several presynaptic proteins and regulates neurotransmitter release **(Del Prete et al., 2016).**

Polyubiquitinated tau has also been found in the brains of AD patients **(Perry et al., 1987),** where ubiquitin conjugation usually occurs at Lys254, Lys311, and Lys353 within the tau microtubulebinding domain **(Cripps et al., 2006).** In AD brains, accumulation of

hyperphosphorylated, ubiquitinated tau protein has been found at both presynaptic and postsynaptic terminals, which is associated with dysfunction of the UPS **(Tai et al., 2012).** Recently, Myeku et al. Reported that tau can induce dysfunction of the 26S proteasome which can be prevented by activating cAMP-PKA signaling. Protective effects derived from cAMP-PKA signaling are mediated through phosphorylation of the 26S proteasomal subunit **(Myeku et al., 2016).**

Conclusion

People all over the world are suffering from neurodegenerative diseases, which are illnesses that lead to cell death in the brain.

The recognition that many neurodegenerative diseases are associated with some sort of intra- or extracellular proteinaceous aggregates has sparked major interest in the idea that these amorphous deposits may play a pathogenic role in the demise of specific subsets of neurons in various brain diseases.

Chapter 3: Mitochondrial dysfunction in neurodegenerative diseases

Introduction

Mitochondria affect cellular functions via their roles in ATP production, lipid synthesis, reactive oxygen species (ROS) generation, and Ca2+ regulation. Recent studies of mitochondriadependent Ca2+ handling have revealed the molecular identities of Ca2+-control components, including the mitochondrial calcium importer (MCU) **(Mammucari et al., 2017).**

In aged animals and humans, mitochondrial functional impairment is a key hallmark of brain aging **(Grimm and Eckert, 2017, Mattson and Arumugam, 2018, Muller et al., 2018).** Alzheimer‟s disease (AD), Parkinson‟s disease (PD), Huntington‟s disease (HD), and other agingrelated neurodegenerative diseases also show mitochondrial defects.

3.1. Calcium Deregulation in Alzheimer's disease

During neurotransmission, a rise in intracellular calcium following membrane depolarization transmits the signal to synapses. Calcium signaling in neurons is, therefore, crucial for neurotransmission and for maintaining synaptic plasticity and generating long-term potentiation (LTP), which forms the basis of learning and memory through the progressive strengthening of synapses **(Morris R G, 2003, Khawamoto E m and al., 2012).**

Unsurprisingly, disruptions to calcium signaling have debilitating consequences on neuronal function. Evidence for calcium deregulation in AD was initially found over 25 years ago in fibroblast cells isolated from AD patients, which showed enhanced endoplasmic reticulum (ER) calcium uptake and ER calcium release **(Peterson C and al.,1985, Ito E and al., 1994).**

Many familial AD PSEN mutations are also linked to dysregulated calcium signaling **(Supnet C, 2010).** In addition to PSEN1/2′s role as the catalytic subunit of gamma-secretase, PSEN1/2 regulates ER calcium stores, a function that is notably gamma-secretase independent, demonstrating that PSEN1 and PSEN2′s impact on neuronal function extends beyond Abeta generation **(Wang Y and al., 2009, Tu H and al., 2006).**

PSEN1/2 are transmembrane proteins present on most endomembranes but predominate on the ER membrane, where they have been found to physically interact with ER calcium channels, including the two main ER calcium release channels, inositol 1,4,5-trisphosphate receptors (IP3Rs) and ryanodine receptors (RyRs) **(Smith I F and al., 2005, Cheung K H and al., 2010, Cheung K H and al., 2008).**

Loss of PSEN1 function has also been shown in Xenopus oocytes to increase the activity of smooth endoplasmic reticulum Ca2+ ATPase (SERCA), which is responsible for pumping calcium into the ER to maintain cytosolic calcium levels. Elevated SERCA activity in turn leads to overloading of ER calcium stores and a compensatory release of ER calcium **(Green K and al., 2008)**. PSEN itself may act as a passive ER calcium leak channel, which similarly results in ER calcium overfilling and exaggerated ER calcium release **(Tu H and al., 2006).**

3.1.1. Mitochondria and ER Crosstalk in Presenilin Mutants, and in Sporadic AD

The mitochondria-associated membranes (MAMs) are regions where the ER closely associates with the outer mitochondrial membrane (OMM). These MAMs contain a specialized distribution of phospholipids and proteins to enable ER–mitochondria communication. MAMs regulate a variety of processes including mitochondrial metabolism and energy production, the ER stress response, lipid synthesis **(Pinton P, 2018).**

Calcium homeostasis is also highly dependent on MAM function. IP3R and RyR localization is concentrated at the MAMs to promote the rapid uptake of calcium into the mitochondria **(Rizutto R and al., 1998).** MAMs are also enriched in voltage-dependent anion channels (VDACs), an ion channel on the OMM that regulates the transportation of a variety of ions and metabolites into and out of the mitochondria, and is primarily responsible for calcium uptake into the mitochondria **(Hedskog L. and al., 2013, Fujimoto M, Hayashi T, 2011)**

There is growing evidence that ER–mitochondrial communication is perturbed in AD. It has been shown in neurons of both sporadic and familial AD patients and an AD mouse model that there are increased ER–mitochondria contact points and expression of MAM-associated proteins, including IP3Rs, RyRs, and VDACs. Abeta exposure to hippocampal neurons was also shown to increase ER–mitochondrial contact and promote transfer of calcium from the ER into the mitochondria **(Hedskog L. and al., 2013).**

Further suggesting disruption of MAM function is a common characteristic observed in AD. PSEN1/2 localization is also concentrated at the **MAMs (Area-Gomez, E and al., 2009).** Accordingly, PSEN1 and PSEN2 FAD mutations have been shown to alter lipid and phospholipid synthesis, lipid exchange, and calcium transfer between the ER and mitochondria **(Area-Gomez E and al., 2012; Zampese, E and al.,2011).**

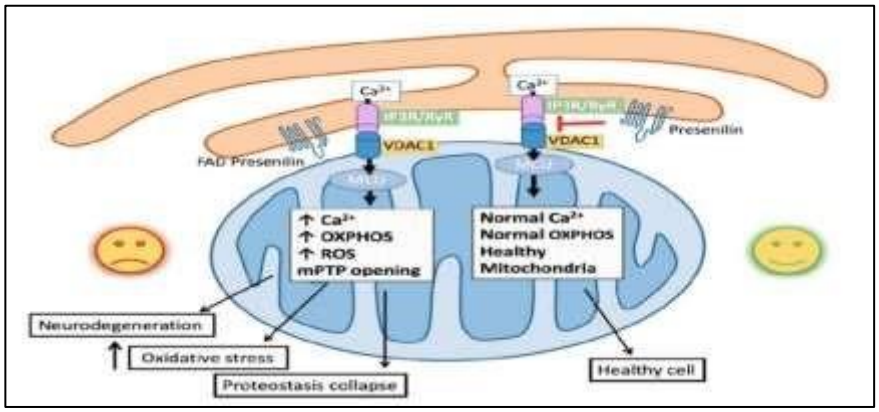

Figure 3.1: Schematic of familial Alzheimer‟s Disease *(FAD) mutations facilitating endoplasmic reticulum (ER)–mitochondrial calcium transfer. (https://doi.org/10.3390/ijms21239153)*

3.1.2. Mitochondrial Calcium and AD

Intracellular calcium greatly impacts mitochondrial function. Indeed, calcium plays a direct role in stimulating enzymes of the tricarboxylic acid (TCA) cycle and electron transport chain leading to increased oxidative phosphorylation **(Balaban, R.S, 2009,Denton, R.M, 2009,Carafoli E, 2010, Ivannikov M V, Macleod, G.T, 2013).** Mitochondria, in turn, regulate cellular calcium signaling by sequestering and buffering cytosolic calcium. The positioning of the ER calcium channels at the MAMs facilitates calcium transfer into the mitochondria. Selective transport of calcium into the matrix across the inner mitochondrial membrane (IMM) is accomplished by the highly calcium selective mitochondrial calcium uniporter (MCU) protein complex **(Mishra, J and al., 2017, Baughman J M and al., 2011, De Stefani D and al., 2011).**

The MCU complex is composed of four core components: the pore forming MCU protein, an auxiliary subunit EMRE **(essential for MCU regulator)** and the MICU gatekeepers, MICU1 and MICU2/3 **(Kamer K J, Mootha V K, 2015, Pallafacchina G and al., 2018,Patron M and al., 2014).**

The MCU complex regulates calcium uptake into the matrix primarily through the MICU1 and MICU2/3 proteins that sense calcium through their conserved calcium-binding EF hand domains **(Perocchi F and al.,2018).** Recently, MICU2 has been shown bind to MICU1 and together these proteins allow for gatekeeper activity. Specifically, elevation in cytosolic calcium promotes

calcium binding to the EF hands of the MICU1-MICU2 heterodimer, enabling MICU1 to facilitate MCU activity allowing calcium entry into the mitochondria **(Wang C and al.,2020, Fan M, 2020).**

The mitochondria at synapses are important not only for ATP delivery but also for tightly regulating calcium concentration at the synapses for effective neurotransmission **(Pivovarova N B, Andrews S B, 2010).** Considering the abundant evidence implicating intracellular calcium dysregulation in AD, it is likely that regulation of intracellular calcium through mitochondrial calcium buffering is a factor in this process **(Jouaville L and al., 1999).**

Due to MICU1 and MICU2/3 calcium-sensing and gating properties, increased cytosolic calcium can promote mitochondrial calcium uptake **(Mishra, J and al., 2017).** Multiple studies have also reported that expression of cytosolic calcium binding proteins calmodulin, calbindin D28K, and parvalbumin is reduced in AD patients and AD models, which would presumably free up calcium to bind MICU1 and MICU2/3 and activate MCU **(Riascos D and al., 2011,McLachlan D R and al., 1987,Ahmadian S.S and al., 2015,Ali F and al., 2019).** Therefore, it is unsurprising that mitochondrial calcium is elevated in AD models **(Hedskog L and al., 2013).**

Although mitochondrial calcium homeostasis can be restored through calcium efflux pathways, which are regulated primarily through the sodium–calcium exchanger NCLX **(Boyman L and al., 2013),** excessive calcium uptake or impairments to calcium efflux can overwhelm mitochondrial calcium capacity. Which when combined with other stressors such as oxidative damage, results in the formation and opening of the mitochondrial permeability transition pore (mPTP) **(Kim, G.H and al., 2015).**

Prolonged opening of the mPTP leaves the mitochondria open to the osmotic influx of cytosolic solutes and water, which causes the matrix to swell and rupture. Cytochrome c is also released from the mitochondria from prolonged mPTP opening, leading to the initiation of apoptosis. This process has been observed in several AD mouse models **(Sanz-Blasco S and al., 2008).**

3.1.3. Calcium-Induced Changes to Mitochondrial Activity Promote ROS Production

Oxidative stress plays a significant role in the pathogenesis of AD **(Birnbaum J H and al., 2018, Pohanka M, 2014).** In vivo and in vitro studies show a direct relationship between oxidative stress and AD **(Pohanka M, 2014,Harrison F E and al., 2009,Tönnies E, Trushina E, 2017).**

In a variety of AD animal models, high ROS levels have been shown to cause extensive neuronal death and cognitive decline **(LaFerla F M, Green K N, 2012).** Neurons are especially vulnerable to oxidative damage induced by mitochondrial respiration due to their especially high energy and oxygen demand, which in turn produces greater relative levels of ROS **(Kim G H and**

al., 2015). It has also been proposed that the regions of the brain that first undergo neurodegeneration in AD occur due to their increased susceptibility to oxidative damage, which can result from greater energy demands or higher basal levels of ROS required for signaling **(Wang X, Michaelis E, 2010).** Elevated mitochondrial calcium levels have been associated with increased oxidative stress in AD models. Impairment to NCLX function that prevents mitochondrial calcium efflux in a mouse AD model resulted in excessive mitochondrial calcium and oxidative stress **(Jadiya P and al., 2019).** Mitochondrial calcium influx has also been shown to induce mitochondrial damage by stimulating oxidative phosphorylation, increasing the amount of ROS generated as a byproduct **(Tamagno E and al., 2006, Butterfield DA and al., 2002).**

3.2. Role of mitochondrial dysfunction in the Alzheimer's disease

3.2.1. Inpaired energy metabolism implicates mitochondrial dysfunction in AD

Brain constitutes on average 2% of the total body weight, but utilizes 25% of total body glucose and 20% of body oxygen consumption in resting awake state. As one of the high-energy consuming organs, brain is vulnerable to impaired energy metabolism such that even mild changes in energy metabolism in human brain closely associates with the disturbance in nervous function. In fact, impaired energy metabolism is one of the earliest and most consistent features in AD. **(Kapogiannis D et Mattson MP, 2011)**

Glucose is the predominant substrate for the human adult brain under physiological conditions, and its utilization is widely used as one primary measure to assess energy metabolism in the brain**. (Kapogiannis D et Mattson MP, 2011)**

Using fluoro-2-deoxyglucose positron-emission tomography (FDG-PET), greater decline in glucose utilization was consistently found in the hippocampus and cortex in AD brain as compared to individuals without dementia. Among many brain regions, the posterior cingulate cortex is metabolically affected in the earliest clinical stages of AD**.(Kapogiannis D et Mattson MP, 2011)**

Glucose hypometabolism, to a lesser extent in terms of magnitude or spatial distribution, was also observed in patients with mild cognitive impairment (MCI), a prodromal stage of AD, suggesting an early role in the course of disease. **(Croteau E et al., 2018)**

84-months longitudinal study clearly demonstrated an ApoE4-associated brain-region specific longitudinally declined glucose metabolism pattern in the context of MCI. **(Paranjpe MD et al., 2019)**

Such an early role is further supported by the finding of abnormally low rates of glucose metabolism in the vulnerable brain regions in young adults carrying apoE4 allele in their 20s, several decades before the possible onset of dementia. **(Reiman EM et al., 2004)**

Accepted as a hallmark of the disease, cerebral glucose hypometabolism assessed with FDG-PET is now used as a common biomarker for early detection of AD and can predict the conversion from MCI to AD with reasonable sensitivity and accuracy , which underscores the critical role of impaired energy metabolism in the course of AD**.(Arbizu J et al., 2018)**

Glucose hypometabolism in AD brain was generally interpreted as impaired energy metabolism through oxidative phosphorylation, which thus strongly implicates the involvement of mitochondrial dysfunction early in the course of AD.

Oxygen metabolism measured by positron-emission tomography (PET) detection of Oxygen-15 is another primary measure for brain energy metabolism, which provides direct evidence for mitochondrial function through electron transport chain (ETC) in the brain. Cerebral metabolic rate of oxygen was significantly decreased in the frontal, parietal and temporal cortex in AD, which showed significant correlation with severity of dementia . **(Ishii K et al., 1996)**

3.2.2. Mitochondrial deficits in Alzheimer's disease

3.2.2.1. Disrupted mitochondrial bioenergetics in AD

Consistent with impaired energy metabolism in AD, gene expression studies repeatedly identified defects in mitochondrial related metabolic pathways in AD, which provided direct evidence for impaired bioenergetic machinery in mitochondria of AD. **(Liang WS et al., 2008)**

For example, a genome-wide transcriptome study in laser-capture micro-dissected neurons found significantly greater proportion of under expressed nuclear genes encoding mitochondrial ETC subunits in the posterior cingulate cortex than those in the primary visual cortex, a region that is relatively spared metabolically in AD vs. Control. **(Liang WS et al., 2008)**

A microarray analysis and quantitative RT-PCR studies found 15 out of 51 members of the glycolytic, TCA cycle, oxidative phosphorylation, and associated pathways were significantly downregulated in AD. **(Brooks WM et al., 2007)**

More recent microarray data confirmed significant downregulation in nuclear-encoded but not mitochondria-encoded OXPHOS genes in the hippocampus of AD patients, which however was puzzlingly increased in the hippocampus from MCI patients. **(Mastroeni D et al., 2017)**

Complex I of OXPHOS was downregulated while complexes III and IV showed increased mRNA expressions in both early and definite AD brain specimens . **(Manczak M et al., 2004)** A bioinformatics analysis of four transcriptome datasets for the hippocampus of AD patients identified OXPHOS pathway as one of most significant pathways involved in AD . **(Zhang L et al., 2015)**

Gene set enrichment analysis demonstrated that mitochondrial oxidative phosphorylation (OXPHOS) downregulation and mitochondrial import pathways disruption were hallmarks of AD . **(Sorrentino V et al., 2017)**

3.2.2.2. Increased oxidative stress in Alzheimer's disease

Reactive oxygen species (ROS) are unavoidable byproducts during electron transport of aerobic respiration in the mitochondria due to electron leaks at complex I and complex III and it is estimated that mitochondria contribute approximately 90% of the cellular ROS **.(Balaban RS et al., 2005)**

While ROS serve important signaling roles, when in excess, they lead to oxidative stress with extensive damage. Mitochondria are susceptible to oxidative damage despite the presence of an antioxidant system and damaged mitochondria are less efficient producers of ATP and more efficient producers of ROS. Therefore, increased oxidative stress could be both the cause and consequence of mitochondrial dysfunction**.(Balaban RS et al., 2005)**

A large body of evidence demonstrated increased oxidative damage to almost all types of macromolecules in the brain of AD patients including proteins, sugar, lipid and nucleic acids. **(Butterfield DA etHalliwell B, 2019)**

For example, significant increase in protein carbonyls and 3-nitrotyrosine modification as protein oxidation markers and elevated glycation and glycooxidation marking oxidative modifications to sugars were widely reported in the brains of patients with AD and MCI . **(Wang X et al. ,2014)**

Lipid peroxidation products such as reactive aldehydes including 4-hydroxynonal, malondialdehyde (MDA), and acrolein were increased in multiple brain regions affected in AD and MCI . **(Zhu X et al., 2012)**

AD brains demonstrated significant increase of 8-hydroxydeoxyguanosine (8-OHdG) and 8-hydroxyguanosine (8-OHG) respectively in DNA (including mtDNA, which will be discussed in more detail in the next session) and RNA . **(Nunomura A et al., 2012)**

3.2.2.3. Disturbed mitochondrial genomic homeostasis in Alzheimer's disease

Mitochondria maintain their own DNA called mtDNA, which is a multicopy (1–10 copies per mitochondrion), extrachromosomal genome that codes for 13 mitochondrial core proteins of the ETC complexes and 2 rRNA and 22 tRNAs necessary for mitochondrial protein synthesis . **(Taanman JW, 1999)**

While mtDNA is critical to the proper function of mitochondria, it is prone to oxidative damage due to its proximity to the site of ROS generation and relative lack of DNA-protective

histones and efficient DNA repair mechanisms, which gives rise to mutations . **(Yan MH et al., 2013)**

Mutations in mtDNA, whether through inheritance or gradual somatic accumulation, propagate through clonal expansion, which may eventually gain momentous deleterious effects after exceeding critical threshold, and compromise mitochondrial function and result in cell death and disease. **(Swerdlow RH, 2018)**

Many patients with primary mtDNA mutations demonstrated pronounced cognitive deficits quite similar to those commonly seen in AD. **(Inczedy-Farkas G et al., 2014)**

Which supports a critical role of mtDNA in proper cognitive function. Interestingly, it is reported that in families with a history of dementia, a consistently identified risk factor for AD, maternal transmission is significantly more frequent than paternal transmission. Along this line, maternal family history of AD is associated with increased atrophy in AD-vulnerable brain regions **.(Honea RA et al., 2011)** a pattern of progressive reduction of brain glucose metabolism. **(Mosconi L et al., 2010)** and higher white matter hyperintensity load in temporal and occipital lobes in cognitively normal individuals, pointing towards family of origin effects. Given the maternal inheritance of mtDNA, this implicated a potential role of inherited mtDNA variability in AD. Indeed, while no primary mtDNA mutations were associated with AD, multiple studies have found that mtDNA SNPs and germ line variants (i.e., haplogroups) likely play a role in AD . **(Salvado G et al, 2019)**

3.2.3. Mechanisms underlying mitochondrial dysfunction in Alzheimer's disease

3.2.3.1. Abnormal mitochondrial fusion and fission in AD

Mitochondria are highly dynamic organelles undergoing continuous fusion and fission in the cytoplasm, a process that is essential for maintaining a healthy pool of mitochondria with proper distribution**.(Zhu X et al., 2013)**

The molecular mechanisms of the fusion and fission are still under intensive exploration but accumulating evidence suggest that a group of large GTPase domain-containing proteins play critical roles, which either enhance mitochondrial fission such as DLP1(also referred to as Drp1), or promote mitochondrial fusion such as Mfn1, Mfn2 and OPA1 (Figure 3.2). **(Mishra P et Chan DC,2014)**

Deficits in either fission or fusion cause human neurological disorders, which underscores the importance of balance of mitochondrial fission and fusion in neuronal function and brain health. **(Mishra P et Chan DD, 2014)**

Early studies demonstrated ultrastructural damage to the susceptible pyramidal neurons in the biopsied brain tissues of AD. **(Hirai K et al., 2001)**

More detailed analysis revealed altered size and number and reduced aspect ratio of mitochondria in these neurons, suggestive of a potential fragmented mitochondrial network in AD brain . **(Wang X et al., 2008)**

Fragmented mitochondria could cause mitochondrial bioenergetics deficits either through negative impact to the proper complex assembly critical for ETC function. (Liu W et al., 2011) Moreover, it also caused reduced exchange of mitochondrial contents exacerbating mtDNA deficits , all prominent features found in AD brain (Figue 3.1**).(Liu W et al., 2011)**

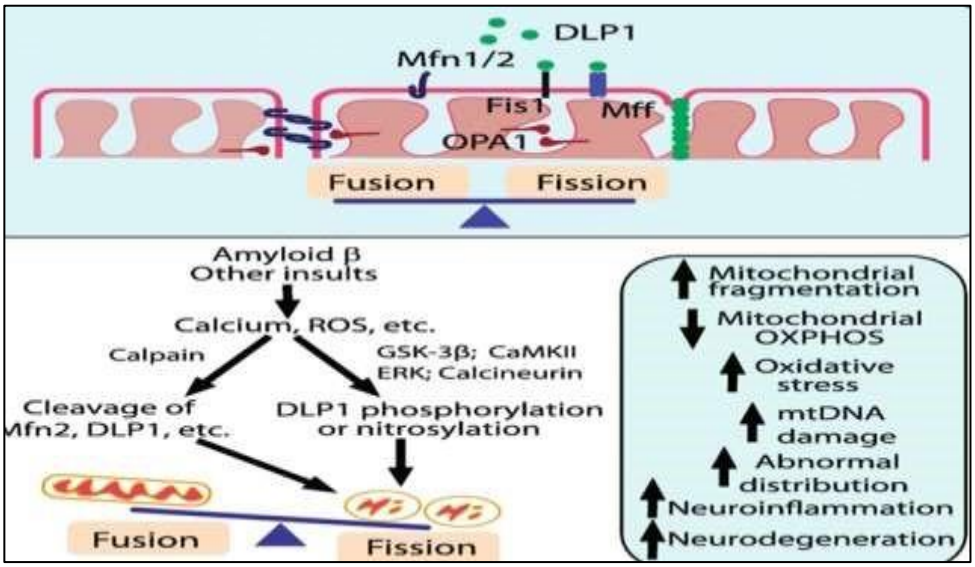

Figure 3.2: Abnormal mitochondrial fusion and fission in AD. (W***ang W et al., 2020)***

3.3. Factors affecting mitochondrial imbalance

Mitochondria are critical for the provision of ATP for cellular energy requirements. Tissue and organ functions are dependent on adequate ATP production, especially when energy demand is high. Mitochondria also play a role in a vast array of important biochemical pathways, including apoptosis, generation and detoxification of reactive oxygen species, intracellular calcium regulation, steroid hormone and heme synthesis, and lipid metabolism. The complexity of mitochondrial structure and function facilitates its diverse roles but also enhances its vulnerability. Primary disorders of mitochondrial bioenergetics, or Primary Mitochondrial Diseases (PMD), are due to inherited genetic defects in the nuclear or mitochondrial genomes that result in defective oxidative phosphorylation capacity and cellular energy production. Secondary mitochondrial dysfunction is observed in a wide range of diseases such as Alzheimer‟s and Parkinson‟s disease.

3.3.1. Environmental toxins and deleterious effect on mitochondria

The environment plays a significant role in human health and disease. A systematic study conducted by the World Health Organization (WHO) estimated that 22% of the global disease burden, including mental, behavioral, and neurological disorders, Environmental factors were defined as the physical, chemical, and biologic environment of the human host and related behavior. Environmental toxins are pervasive in all aspects of patients' lives, including those found in the air, water, soil, food, and consumer products. **(Chen et al., 2017)**

3.3.1.1. Mitochondrial vulnerability to toxins

In recent years, the deleterious effect of environmental toxins on mitochondrial function has been extensively studied in humans and model organisms such as rodents, fish, zebrafish, and cellular models. (**Zieminska et al., 2016)**

A large number of environmental factors, including pesticides such as rotenone and paraquat, are now widely recognized as mitochondrial toxins, specifically neurotoxins.**(Youngster et al., 1987)**

3.3.2. Environmental toxins and neurodegenerative disorders with mitochondrial dysfunction

Defects in mitochondrial function cause diverse and complex human diseases. The contribution of mitochondrial dysfunction has been reported in major environment-related multifactorial diseases, such as respiratory diseases, viral infections, neurological disorders, cardiovascular diseases , and cancer . Although harmful exposure to environmental pollutants is ubiquitous among all populations, disease manifestation occurs in only a subset of the population. Interaction between environmental factors and genetic predisposition, e.g., ecogenetic variants, is therefore likely to be a necessary prerequisite to disease manifestation.**(Saneto et al., 2013)**

3.3.2.1. Alzheimer’s disease

AD is the most common cause of dementia, with more than 30 million people projected to be affected in the next 20 years . AD is now the third leading cause of death in the United States, after cardiovascular disease and cancer. Familial, autosomal dominant AD accounts for 5–10% of all AD cases, while late-onset, sporadic AD cases account for 90–95% of all AD cases. No genetic etiology other than conferred risk associated with susceptibility alleles has been identified for sporadic AD.

This suggests that environmental and/or epigenetic factors play an important role in initiating and influencing the cascade of events that contribute to the emergence of AD. A link to

environmental exposure has been described for ingested, absorbed, and inhaled toxins. Key neuropathologic features include neurofibrillary tangles.**(Bredesen, 2016)**

Conclusion

Mitochondria not only supply the energy for cell function but also take part in cell signaling. This review describes the dysfunction of mitochondria in aging and neurodegenerative diseases.

Article analysis

cells
Review

The Role of Mitochondria in Reactive Oxygen Species Generation and Its Implications for Neurodegenerative Diseases

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Abstract: Mitochondria are dynamic cellular organelles that consistently migrate, fuse, and divide to modulate their number, size, and shape. In addition, they produce ATP, reactive oxygen species, and also have a biological role in antioxidant activities and Ca^{2+} buffering. Mitochondria are thought to play a crucial biological role in most neurodegenerative disorders. Neurons, being high-energy-demanding cells, are closely related to the maintenance, dynamics, and functions of mitochondria. Thus, impairment of mitochondrial activities is associated with neurodegenerative diseases, pointing to the significance of mitochondrial functions in normal cell physiology. In recent years, considerable progress has been made in our knowledge of mitochondrial functions, which has raised interest in defining the involvement of mitochondrial dysfunction in neurodegenerative diseases. Here, we summarize the existing knowledge of the mitochondrial function in reactive oxygen species generation and its involvement in the development of neurodegenerative diseases. **Keywords:** electron transport chain; mitochondria; neurodegenerative diseases; oxidative damage

1. Introduction

Mitochondria are important cellular organelles that control various vital physiological processes in the bodies of organisms. Being a major consumer of oxygen, mitochondria utilize approximately 98% of the total amount of inhaled oxygen. Mitochondria efficiently generate energy that is required for almost all types of cellular activities; this energy is also used to contract both voluntary and involuntary muscles [1,2]. Additionally, the produced

energy is used to sustain ionic gradients across the plasma membrane, which are essential for the excitability of excitable cells, permitting the accumulation of secreted material into vesicles, and allowing for the vesicle fusion and cycling essential for the secretion of neurotransmitters [3]. Thus, a mitochondrial functional disorder causes different disorders, such as alterations in tissue functions that may manifest as disease, as well as major defects in tissue function that may lead to handicap or death. In fact, mitochondria are necessary in the bodies of animals not just to provide energy, but also to maintain other physiological activities of cells, such as cellular calcium signaling, and any defect in this process might lead to dysfunction and disease [4,5].

The respiratory chain in mitochondria is a greatly efficient system. It catalyzes alternating one-electron oxidation reduction reactions that predisposes each carrier of an electron to side reactions with oxygen. It has been shown that mitochondria are the major intracellular source of reactive oxygen species under normal physiological conditions [6,7]. According to estimates, 1–2% of total daily oxygen

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consumption goes to reactive oxygen species production, and a woman with an average weight of 60 kg generates 160–320 mmol of free radicals each day from cellular respiration, while a man of 80 kg produces almost 215–430 mmol per day [8].

Neurodegenerative diseases are a group of heterogeneous disorders with discrete clinical symptoms and genetic etiologies. The diseases are characterized by the progressive loss of physiologically or anatomically associated neuronal systems. Parkinson's disease and Huntington's disease are typical examples of neurodegenerative diseases [9]. In spite of this heterogeneity, mitochondrial dysfunction is considered to be a unifying basic mechanism involved in different types of neuronal degeneration. Mitochondrial dysfunction has widespread detrimental consequences for cellular functions; for instance, it leads to impaired energy generation, impaired cellular calcium buffering, the activation of phospholipases and proteases, nitric oxide synthase, and the production of reactive oxygen species [10]. Thus, they play a crucial role in ageing, and can directly interact with variety of specific proteins that are thought to be involved in genetic forms of neurodegenerative disorders [11,12]. Furthermore, there are several lines of evidence that suggest that mitochondrial dysfunction is linked with neurodegenerative disorders [10]. However, our understanding is limited regarding the different mechanisms by which mitochondrial dysfunction influences physiological functions. Here, our main focus is to review recent data on the role of mitochondria in reactive oxygen species and its deleterious impact on the progression of neurodegenerative diseases.

2. The Structure and Functions of Mitochondria

A mitochondrion is a double-membraned, semi-autonomous cellular organelle that is separatedfrom the cytoplasm of a cell by the mitochondrial membranes. The outer mitochondrial membrane is spongy in nature, which allows for free cross-movement of small, uncharged molecules and ions through the porin [13,14]. However, larger molecules, particularly proteins, have to be transported inside the mitochondrion via special translocases [15]. The inner membrane is highly impermeant and forms the main barrier between the mitochondrial matrix and the cytosol. The space between the outer and inner mitochondrial membranes is called

the intermembrane space and harbors a variety of different proteins, which seem to be involved in different physiological functions of the cell. The inner membrane has at least three morphologically discrete subregions, including cristae, cristae junctions, and boundary membranes. These structures alter their shape in response to the cell's metabolic needs and stress [16,17].

The structure of cristae appears to differ greatly between different tissues, and the functional importance of these variations in the structure of cristae remains largely mysterious [4]. Different groups of researchers have attempted to generate detailed three-dimensional reconstructions of cristae and to model the bioenergetic consequences of altering the shape of their structure [18–20]. Some researchers have correlated the complex structure of cristae with their energy demands; for instance, Perkins et al. [18] compared the structure of cristae in the mitochondrion of rod and cone cells, and observed greater cristae connectivity, narrower crista junctions, and almost 3-fold more surface area for cristae membranes in cone cells compared to that in rods. Further, they suggested that, as cones and rods utilize a different bioenergetic signature, the aerobic ATP demand and production is higher in cones than in rods. Hence, cones use two different strategies to enhance their aerobic ATP production: increasing the number of mitochondria and increasing the surface area of cristae membranes. However, it must be noted that we still have little exact information regarding the functional importance of the complex features of, and structural variations in, mitochondria.

Mitochondria regulate various biological processes in a cell for its survival and precise functioning. They functionally control the production of energy, the electron transport chain, cell signaling, apoptosis, and programmed cell death, while their functional disorder has serious health consequences. Considering the involvement of mitochondria in the key processes of cells as well as their health impacts, researchers all over the world are attempting to explore the role of mitochondrial dysfunction in the pathogenesis of different diseases [3,21,22].

3. Mitochondrial Sites for the Generation of Free Radicals

Reactive oxygen species are produced in various cellular compartments. However, mitochondria are a major contributor to reactive oxygen species, as they generate almost 90% of the total number of cellular reactive oxygen species [23]. Hence, mitochondria seem to represent one of the key sources of reactive oxygen species production in the majority of cell types. However, mitochondria in different cells/tissues may clearly differ in their capacity to generate free radicals using various substrates, and this capacity of the mitochondria may depend on the composition of a membrane, the age of an organism, and the species of animal. In a mitochondrion, at least eight sites are known to be involved in the production of reactive oxygen species. However, as shown in Figure 1, mitochondrial complexes I, II, and III mainly lie within the respiratory chain, and are thought to make a major contribution to the generation of reactive oxygen species [24,25]. Oxidative phosphorylation is the main process that produces unpaired electrons. These unpaired electrons interact with O_2, resulting in the production of greatly reactive free radicals (superoxide ions). The superoxide ions are converted into other reactive oxygen species, such as H_2O_2 and hydroxyl ions (-OH) [4].

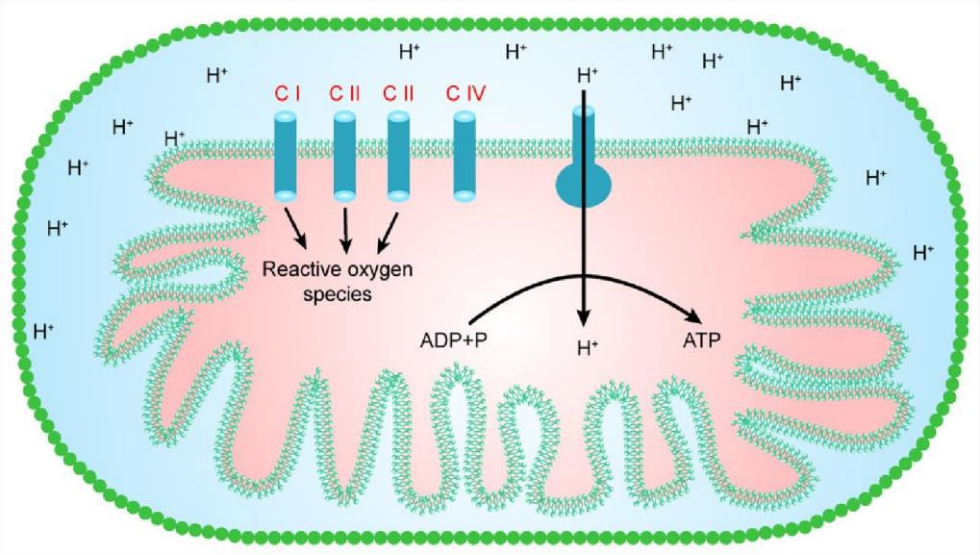

Figure 1. The major sites for the production of reactive oxygen species in a mitochondrion.

4. Complex I and the Generation of Free Radicals

Mitochondrial complex I, also known as NADH CoQ reductase, catalyzes the electron transfer from NADH to ubiquinone, which is accompanied by the movement of protons from the mitochondrial matrix to the intermembrane space [26,27]. During the 20th century, it was shown that mitochondrial complex I is involved in the generation of reactive oxygen species [24]. Additionally, it is believed that mitochondrial complex I is a key source of reactive oxygen species and is a major contributor to cellular oxidative stress. Hence, since then, an enormous literature has been developed on the mechanism by which reactive oxygen species are produced from complex I. For instance, the transfer of an electron at complex I may cause the production of reactive oxygen species, whereas rotenone, a complex I inhibitor, seems to suppress the generation of reactive oxygen species, presumably by preventing the electron's passage further into the distal end where reactive oxygen species are produced [4,28]. Cadenas et al. [8] suggested that water-soluble co-enzyme Q homologs are utilized as electron acceptors in isolated complex-I-induced H_2O_2 formation; the production rate of H_2O_2
was partially prevented by rotenone, suggesting that water-soluble quinones react with oxygen when reduced at sites both downstream and upstream of the rotenone block. The one electron donor to oxygen in complex I is a non-physiological hydrophilic site [29,30] that reduces many quinones to their corresponding semiquinone forms, which are unstable and reduce oxygen to a superoxide. This mechanism is shared by several quinones, including anthracyclines [31], and the CoQ analog idebenone [32]. The hydrophilic, rotenone-insensitive site can apparently reduce oxygen to reactive oxygen species in the absence of intermediate acceptors [33]. Furthermore, many studies have reported that mitochondrial complex I is a major source of reactive oxygen species production in mitochondria [34,35] and localizes the oxygen-reducing site between the ferricyanide and the quinone reduction sites [36].

A recent study proposed two sites in a complex I reactive oxygen production model using themitochondria of rat skeletal muscle. One site is in equilibrium with the NAD pool, seemingly the flavin of the FMN moiety, and the other site is dependent not only on the NAD redox state, but also on the proton motive force Δp and the reduction state of the Q pool, seemingly a semiquinone in the Q-binding site

[37]. Concurrently, Pryde and Hirst [5] devised a single mechanism of reactive oxygen species production by complex I under all conditions (during both NADH oxidation and reverse electron transfer) using bovine heart sub-mitochondrial particles. Generally, NADH-induced reactive oxygen species generation is prevented by complex I flavin-site inhibitors, but not by inhibitors of ubiquinone reduction and it is independent of the proton motive force (Δp). A reverse electron transfer by complex I in sub-mitochondrial particles, driven by the oxidation of succinate and the Δp generated by ATP hydrolysis, reduces the flavin, leading to NAD^+ and O_2 reduction. However, similar to forward electron transport, reverse electron-transfer-stimulated reactive oxygen species generation is prevented by ubiquinone reduction and flavin site inhibitors. The potential dependence of NADH-stimulated reactive oxygen species generation (set by the NAD^+ potential) matches with that of reverse electron-transfer-stimulated reactive oxygen species generation (set by the succinate potential and Δp), and they both match the potential dependence of the flavin. Hence, the study suggested that NADH- and reverse electron-transfer-stimulated reactive oxygen species are generated by the flavin.

While mitochondrial complex I has been shown to be a key source of reactive oxygen species, and also a remarkable contributor to cellular oxidative stress in mitochondria, its ability to produce reactive oxygen species may vary under different oxidation–reduction pressure conditions [38]. In particular, a study on bovine heart mitochondria showed that the concentration and ratio of NAD^+ and NADH regulate the production rate of reactive oxygen species [39]. For instance, at a lower NADH level, rotenone seemed to prevent the generation of reactive oxygen species in coupled sub-mitochondrial particles from the heart of beef. So, this phenomenon excludes a crucial function of complex I in reactive oxygen species under a lower reduction state of the iron–sulfur clusters of complex I. However, at a higher NADH level, rotenone induced reactive oxygen species generation. Recently, it was discovered that, with the exception of the concentration and ratio of NAD^+ and NADH, many other factors also control the production of reactive oxygen species. For example, in reverse electron transport, the generation of reactive oxygen species is influenced by changes in the concentration of O_2, the magnitude of the proton motive force, and the redox states of the co-enzyme Q (CoQ) and NADH pools [35]. Such a condition simulates the state of reduction of complex I when the respiratory chain is impaired.

Collectively, mitochondrial complex I generates large amounts of free radicals using two biological mechanisms: (1) a high ratio of NAD^+/NADH leads to a reduction of the FMN site on complex I; and (2) electron transport to the CoQ pool coupled with a high protonmotive force Δp leads to reverse electron transport. Although the site at which free radicals are generated during reverse electron transport has not been identified, the rate at which free radicals are generated under reverse electron transport appears to be the highest that can occur in mitochondria [37,39]. Furthermore, the mechanisms underlying the generation of free radicals that have been developed by the sub-mitochondrial particles, the isolated enzyme, and the coupled membrane vesicles need to be tested on mitochondrial complex I.

5. Complex II and the Generation of Free Radicals

The question of whether complex II, which is also known as succinate dehydrogenase, is a major source of reactive oxygen species remained controversial for many decades. Previous studies on isolated mitochondria and cells showed that complex II was not

recognized as a remarkable contributor to reactive oxygen species until it experienced mutation [6,40]. Recently, the generation of reactive oxygen species from complex II of mitochondria has gained extensive scientific interest. Since then, many studies executed on sub-mitochondrial particles and intact isolated mitochondria have demonstrated that complex II, under specific conditions, and particularly when it is supplied with a high succinate concentration (almost 5 mM), produces a considerable amount of reactive oxygen species [41,42]. Interestingly, a recent study on skeletal muscle mitochondria of rats demonstrated that complex II produces reactive oxygen species in both the forward and reverse reactions as well as with a low and a high concentration of succinate [43,44]. Additionally, the authors suggested that complex II generates reactive oxygen species in the reverse reaction, for instance when electrons are provided from the reduced ubiquinone pool, and in the forward reaction, for instance when the electrons are supplied from succinate. Furthermore, they proposed a mechanism for the production of reactive oxygen species through complex II that entirely depends upon its possession of the carboxylate binding site and the enzyme reduction state; e.g., reactive oxygen species are produced when the binding site for carboxylate is not occupied and the flavin is reduced. Finally, it has been demonstrated that the complex-II-associated rates approach or exceed the maximum rates of complexes I and III, indicating that complex II may be an important contributor to the physiological and pathological generation of reactive oxygen species [24].

It has been shown that a functional loss of mitochondrial complex II can lead to succinate accumulation and enhanced reactive oxygen species production in cells [45]. This appears to particularly be the case for mutation in the subunits of complex II, and it is believed that it is associated with certain diseases. Mitochondrial complex II contains four subunits: SDHA, SDHB, SDHC, and SDHD. Mutation is mostly associated with SDHB, SDHC, and SDHC, while mutation in SDHA rarely occurs. A loss of SDHB, SDHC, and SDHD would allow for the acceptance of an electron, but not progression along the respiratory chain, and consequently may enhance the production of reactive oxygen species [46]. Ishii et al. [47] suggested that a mutation in the SDHC subunit of complex II in fibroblasts of a transgenic mouse and conditional transgenic mice stimulates dysfunction of the respiratory chain in mitochondria and enhances reactive oxygen species' generation. Likewise, in a study on hamster fibroblasts, the authors observed that SDHD is also responsible for the production of reactive oxygen species [48]. Further, a mutation in SDHC of complex II triggered hypersensitivity that enhanced the concentration of oxygen in Caenorhabditis elegans, and stimulated the production of reactive oxygen species that was found in succinate sub-mitochondrial particles and fueled mitochondria from the mutant Caenorhabditis elegans [49,50].

To summarize, a growing amount of evidence suggests that mitochondrial complex II is an important modulator of reactive oxygen species generation under different physiological conditions. Hence, it can adapt different functions as a generator or regulator of reactive oxygen species depending on the supply of substrate, etc. [45,46]. Complex II's biological functions need a rigorous analysis to identify the source of reactive oxygen species. This will be useful to provide a clear understanding of complex II's functions in various pathophysiological conditions. Furthermore, the basic mechanisms by which mitochondrial complex II generates reactive oxygen species should be further studied in various model organisms.

6. Complex III and the Generation of Free Radicals

Complex III is an important multi-subunit, membrane-bound enzyme that is essential to respiratory energy transduction pathways in various organisms. Apart from energy production, it is known to contribute a considerable number of reactive oxygen species [51,52]. A remarkable advancement has been made in our understanding of complex III's biological role in the production of reactive oxygen species. Various studies have described the mechanism by which complex III generates reactive oxygen species. According to these studies, it can produce reactive oxygen species by both a forward and a reverse electron transfer.

The first report regarding the involvement of complex III in reactive oxygen species generation surfaced in 1970s. The study explored the role of complex III in reactive oxygen species production using mitochondria from a rat's heart. It showed that antimycin A, an oxidative phosphorylation inhibitor, cannot entirely prevent electron transport from ubiquinol to cytochrome c: the antimycin-A-insensitive reduction of cytochrome c is mediated by reactive oxygen species [53]. Another study proposed a mechanism for electron transport and reactive oxygen species generation from complex III, and suggested that two electrons from ubiquinol oxidation would be transported to the cytochrome c, but by using two pathways. One of the electrons would be transferred to cytochrome c through the chain reactions such as from quinol oxidation center to the iron–sulfur protein and finally to cytochrome c1, while the second one would be delivered to O_2 for the production of reactive oxygen species. Additionally, preventing the quinone reduction at the quinone reduction (Qi) center using inhibitor molecules (e.g., antimycin A) remarkably enhanced quinol oxidation (Qo) center mediated reactive oxygen species generation [54]. Further, loss of iron–sulfur protein abolishes reactive oxygen species generation, whereas the loss of cytochrome b retains production of reactive oxygen species [55,56]. Many studies investigated the impact of particular Qi and Qo center inhibitors e.g., antimycin A, myxothiazol, on reactive oxygen species generation described complementary reactions in detail [57]. For example, declining electron transfer rate between the hemes b H and b L of cytochrome b, or eradicating the following oxidation of both the hemes by the Qi center inhibitor antimycin A, caused accumulation of electrons on cytochrome b [58]. This resulted in the accumulation of semiquinone radical at the Qo center and to the escape of electrons to O_2 to produce reactive oxygen species [57]. If a semiquinone radical is formed at the Qo center during the normal turnover of a complex III, reactive oxygen species generation is likely to be relatively low to reduce electron leakage and energy wastage. However, reactive oxygen species generation at the Qo center possibly become remarkable under specific conditions, such as a highly reduced quinone pool and presence of inhibitor molecules (e.g., antimycin A). These abnormal conditions may occur only in extreme physiological conditions and in situation of complex III damage [59,60]. A recent study proposed a mechanism for generation of reactive oxygen species, when a complex III undergoes damage during disease resulted in the opening of permeability transition pores, consequently malate/succinate-fueled mediated generation of reactive oxygen species from complex III due to activation of malic enzyme through increases in matrix [Mg^{2+}], [ADP] and [NAD^+]. Further generation of reactive oxygen species in these physiological conditions is related to Mg^{2+} dependent NADH production by malic enzyme. For maximal production of reactive oxygen species, the production rate of NADH has to be almost equal

or below that of NADH oxidation, as further increase in NADH elevate ubiquinol-related complex III reduction beyond the optimal range for reactive oxygen species generation [61].

The production of reactive oxygen species by reverse electron transfer pathway has gained attention in the current decade. Consequently, many studies were conducted to explore the mechanism and rate of reactive oxygen species generation by this pathway. Most of these studies suggested that partial oxidation of the quinone pool in a physiologically relevant condition remarkably enhances the rate of reactive oxygen species generation by antimycin A inhibited complex III [62,63]. Another study on sub-mitochondrial particles showed that complex III mediated reactive oxygen species generation is higher, when complex II activity is inhibited by its inhibitors (i.e., oxaloacetate or malonate), linking quinone pool redox state to reactive oxygen species generation by the Qo center of complex III. They inferred that produced reactive oxygen species was generated at the Qo center via reverse electron transfer from reduced heme b L of cytochrome b to O_2 by semiquinol [64]. Afterwards, Quinlan et al. [42] argued that this effect is perhaps due to the redox state of hemes b H and b L of cytochrome b, which are highly sensitive to the membrane potential and redox state of quinone pool. Similarly, studies using bacterial model provided evidences that reactive oxygen species generation at the Qo center also used reverse electron transfer from reduced heme b L of cytochrome b, later on Sarewicz et al. [7] proposed a mechanism that shows transport of the Fe/S protein cluster from the Qo center enhanced reactive oxygen species production, while its stagnation reduced the production at Qo center.

Taken together, the exact mechanism for production of reactive oxygen species at the Qo center has still remained to elucidate and is currently a matter of debate. Furthermore, remarkable amount of reactive oxygen species from the Qo center have been measured under non-native conditions, for example, in the presence of antimycin A (inhibitor of the Qi center) [53,57]. Therefore, the basic question that remains to be explored is which physiological processes or factors promote production of reactive oxygen species from the Qo center in vivo.

7. Other Cellular Components and Free Radicals

There is increasing evidence that besides mitochondria, cellular components and other factors are also involved in the production of reactive oxygen species. Peroxisome, endoplasmic reticulum phagocytic cells, neuro-inflammation, dopamine, genetic mutation, anti-oxidant depletion etc. may induce the reactive oxygen species in cells. Thus, contributing to degeneration of neurons by lipid peroxidation, DNA and protein oxidation [65,66].

8. Environmental Sources of Oxidative Stress and Neurodegenerative Disorder

Besides the endogenous factors, exogenous sources of reactive oxygen species also contribute in the pathogenesis of neurodegenerative diseases including Parkinson's and Huntington's disease etc. Multiple lines of evidence suggest the etiology of neurodegenerative disorders is multifactorial and comprised of an interaction between environmental factors and genetic predisposition. Migliorea and his coworkers [67] reviewed that the environmental exposure to air pollution, metals, herbicides, insecticides and diet are the major risk factors in pathogenesis of neurodegenerative diseases by stimulating oxidative stress. Hence, to understand the pathogenesis of neurodegenerative

disorders exogenous factors are also equally important, however, they are beyond the scope of the present review.

9. Physiological Functions of Mitochondria in Neurodegenerative Disease

In recent years, important advancements have been made in the field of medical science to understand initiating factors and cure of neurodegenerative diseases, however still it is a major cause of concern in the health profession. Neurodegenerative diseases can be divided into Parkinson's disease, Alzheimer's disease, multiple sclerosis, injury to the central nervous system by chronic low-grade hypoxia, motor neuron diseases, Wilson's disease, Huntington's disease and Freidreich's ataxia [9]. Exact knowledge on the pathophysiological mechanisms is still insufficient and unclear in all of these neurodegenerative diseases. However, functional disorders of mitochondria have been reported to be a major cause in pathogenic process. A key challenge of modern neuroscience is to investigate and understand the level to which these variations in the functions of mitochondria depict the level (primary or secondary components) of the pathophysiological process, further to understand the fundamental biological pathways which lead to the initiation and development of neurodegenerative disease. A recent study suggested that mitochondria play critical functions in most neurodegenerative disorders. The mitochondria are mainly involved in ATP generation, reactive oxygen species generation, antioxidant activity and Ca^{2+} buffering. Neurons being high energy demanding cells are closely related to maintenance, dynamic activity, and functions of mitochondria. In most neurodegenerative diseases, mitochondrial dynamics and activities are impaired, causing low ATP production, high levels of reactive oxygen species, and apoptosis [68]. In this article our main focus is to review recent data regarding Huntington and Parkinson's disease and the role of mitochondria in progression of these neurodegenerative diseases, particularly through generation of reactive oxygen species (Figure 2).

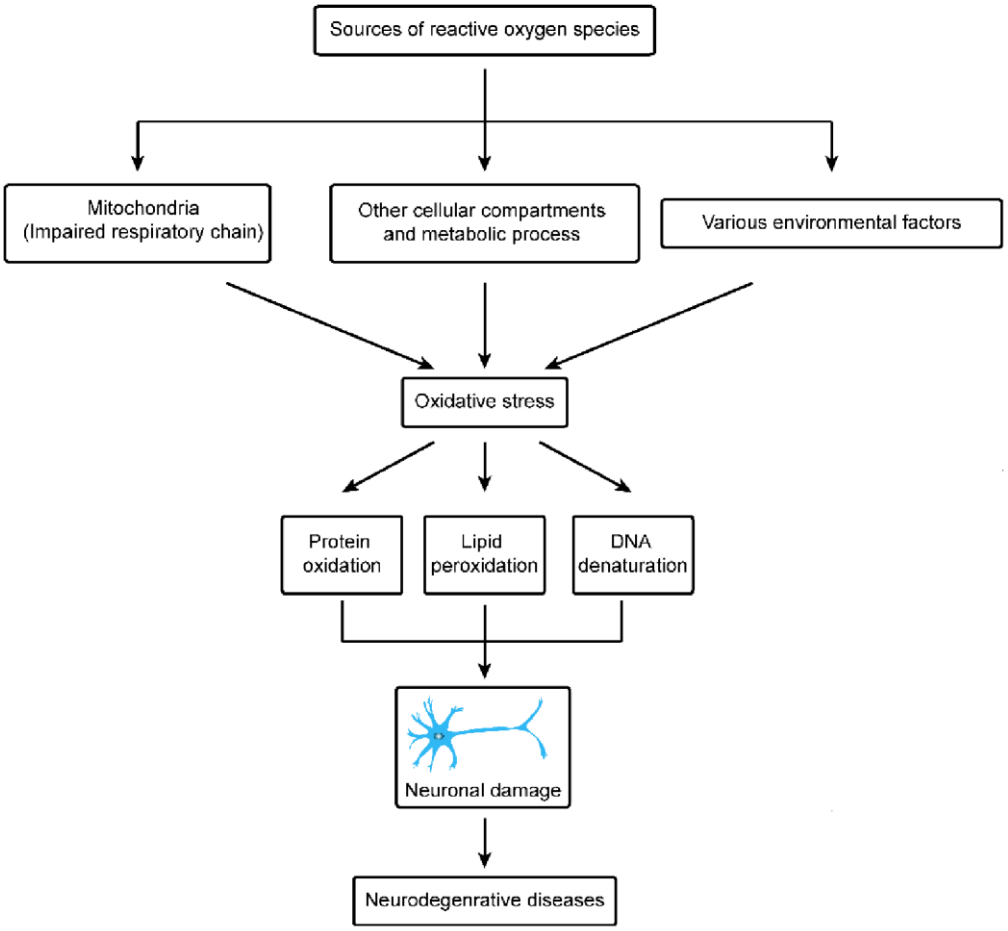

Figure 2. The causes of oxidative stress in neurodegenerative diseases.

10. Mitochondria and Huntington's Disease

Huntington's disease is a detrimental neurodegenerative disorder, and people from the United States of America and Australia are highly affected by this disease, while a lower number of cases are also reported from China, Africa, Japan and other countries [69,70]. This disease is characterized by the irregular expansion of CAG trinucleotide repeats in exon 1 of the gene, which resides on the chromosome 4 and translates into an abnormal huntingtin protein. Healthy individuals usually contain 6 to 35 repeats of CAG, while this number is observed more than 36 repeats in patients [71]. Medically, Huntington's disease is caused by dysfunction of motor neurons, psychiatric disturbances, and cognitive decay, consequently causing the patient to experience progressive muscle loss and brain dysfunction [72,73]. This disorder is commonly fatal within 15 to 20 years after disease onset.

Emerging evidence suggests that mitochondrial dysfunction is associated with the pathogenesis of Huntington's disease. Panov et al. [74] observed mitochondria extracted from lymphoblasts of Huntington's disease patients contain lower mitochondrial membrane potential compared with the control. Likewise, they found similar characteristics in mitochondria isolated from the brains of transgenic mice that transcribe mutant huntingtin protein. This physiological defect led the initiation of behavioral and pathological abnormalities. Based on these observations, they concluded that calcium abnormalities of the mitochondria arises at the onset of Huntington's disease pathogenesis and probably is the direct influence of the huntingtin protein mutation on the tissues and organelle;

however, it remains undiscovered exactly how the huntingtin mutant protein elicits its harmful effects. Mattson et al. [75] reviewed that mitochondrial-mediated oxidative stress, perturbed homeostasis of Ca^{2+}, and apoptosis contribute to the onset of Huntington's disease.

A recent study further highlighted this mechanism and demonstrated that the functional impairment of mitochondria and dysregulation of transcription are involved in Huntington's disease pathogenesis. For example, Cui et al. [76] suggested that the mutant huntingtin protein causes perturbation of mitochondrial functions by preventing expression of PGC-1α, which controls different metabolic processes, including respiration and mitochondrial biogenesis. Crossbreeding of PGC-1α knockout mice with Huntington's disease knock in mice lead to enhanced neurodegeneration of striatal neurons and motor abnormalities in mice with Huntington's disease. Whereas, expression of PGC-1α partially reverses the toxic effects of mutant huntingtin in cultured striatal neurons.

11. Mitochondria and Parkinson's Disease

Parkinson's disease is one of the most predominant neurogenerative diseases. Clinically, it is characterized by apoptotic loss of dopaminergic neurons, resulting in subsequent and gradual loss of muscle control. This disease is diagnosed by resting tremors, rigidity of muscle, change in gait and speech, postural instability, depression, fatigue, anxiety, sleep disturbances, and decline in dementia and cognition. Premature death of patients occurs due to complications e.g., pneumonia, injuries etc. [77]. Parkinson's disease is prevalent in the adult population, usually in people over 65 years. The male population is approximately 1.5–2 times more susceptible to this disease than the female population [78]. There are multiple lines of evidence that link functional disorders of mitochondrial complex I and dopaminergic neurons in Parkinson's disease [79]. A most appealing model of this disease was discovered in the 20th century, which described how MPTP (1-methyl-phenyl 4-phenyl-1,2,3, 6-tetrahydropyridine) of the mitochondria is responsible for Parkinson's disease [80,81]. Later on, several researchers independently discovered that sporadic Parkinson's disease patients have reduced complex I in different brain areas, peripheral cells and neural and extra neural tissues and in cells (cytoplasmic hybrid), which are derived from Parkinson's disease patients [70,82,83]. Another study on hybrid cell lines showed that complex I deficiency is correlated with the increased reactive oxygen species generation [84]. Further, many studies based on model organs and human neuroblastoma cells showed that in some patients, the disorder may reflect exposure to the plant extracted insecticide rotenone (complex I inhibitor) [85,86]. Exposure to low doses of rotenone induce apoptosis in human neuroblastoma cells. While, rotenone inhibition stimulates aggregation and accumulation of ubiquitin, α-synuclein, gradual oxidative damage and caspase-dependent cell death, which is the central mechanism in Parkinson's disease pathogenesis. The binding site for rotenone appears irrefutably defined as mitochondrial complex I. It seems injury of cells is not a simple role of metabolic machinery inefficiency, as similar levels of ATP suppression stimulated by other poisonous chemicals such as glycolysis inhibition by 2-deoxyglucose completely failed to cause approximately the same cell injury. Rather, cells protect themselves using their antioxidant protection system, suggesting that oxidative stress is the primary mechanism for cell injury. Mitochondrial complex I is particularly susceptible to modification caused by oxidative stress, and thereby is a potential producer of reactive oxygen species [85].

A recent study on cell lines, human brain tissues and mice suggest that mutations in α-synuclein is associated with the pathogenesis and progression of Parkinson's disease. Further, point mutations in α-synuclein are responsible for its decreased association with mitochondria associated membranes, coincident with a lower degree of apposition of endoplasmic reticulum with mitochondria, a reduce in mitochondria associated membranes function and increase in fragmentation of mitochondria [86]. Mutations in Parkin and PINK1 also contribute in the progression of different types of Parkinson's diseases. The Parkin gene is highly expressed in brain tissues including the substantia nigra, and contains 12 exons, of which five (exons 3–7) are generally deleted and cause the pathogenesis and progression of disease [87]. Likewise, PINK1 (PTEN-induced kinase 1) is a mitochondrially-located molecule and has a protective impact on a cell. A mutation in its kinase domain can make cells susceptible to oxidative stress, and is thereby involved in the progression of disease [88].

Besides the above-mentioned factors, many others have been reported to be involved in dysfunction of mitochondria associated membranes. However, these factors were identified in other brain associated disorders, which is beyond the scope of our review. In short, c-secretase activity itself is highly enriched in a sub-compartment of the endoplasmic reticulum that is biochemically and physically connected to mitochondria. Mutation in the catalytic components of c-secretase (Presenilin-1 and -2) increase mitochondria associated membranes functions and communication between endoplasmic reticulum and mitochondria, which is the prominent characteristic of the familial and sporadic forms of Alzheimer disease. These results will help to understand calcium deregulation, mitochondrial dysfunction and oxidative stress in this disease and will also help to explore the contribution of this mutant in other brain associated diseases [89]. Similarly, Sigma 1 receptor plays a crucial biological role in the protection of motor neurons and its mutation lead to degeneration of motor neurons and caused Amyotrophic lateral sclerosis. A recent study reported that mutation occurs in highly conserved amino acid reside in the sigma receptor of *SIGMAR1* gene. The neuronal cells
which express this mutant protein are less resistant to apoptosis stimulated by cellular stress [90]. Here, we garnered different contributing factors which are responsible for neurodegenerative diseases e.g., Alzheimer disease and that should be the focus of future studies to determine the biological role of these factors in brain associated diseases.

On the whole, cellular viability depends on the biological functions of mitochondria, and alterations in its biological functions can lead to cell functional abnormality and even cell death. Neuronal cells are especially susceptible to mitochondrial dysfunction due to their dependence for energy requirement on the mitochondrial metabolism. Dysfunction of mitochondrial respiration has been demonstrated to be involved in the pathogenesis of neurodegenerative diseases. However, currently the biological functions of mitochondria in neurodegenerative diseases appear to extend well beyond abnormalities in respiration. Although it remains to be explored whether alterations of mitochondria in neurodegenerative diseases constitute a primary or a secondary event, or are just part of a larger multifactorial pathogenic process.

12. Oxidative Stress and Parkinson's Disease

The brain is the central organ in living organisms and is a major consumer of oxygen to provide uninterrupted supply of energy to approximately 86 billion neurons for their daily

activities, it has been estimated it uses almost 20% of the basal oxygen (O_2) from the total oxygen supplied to the human body [91,92]. This uptake of oxygen is utilized by mitochondria in respiratory chain to generate energy/ATP and reactive oxygen species are produced as by-product other than energy production, which is a major cause of DNA damage and one of the eminent characteristics of Parkinson's disease [93,94].

Growing evidence suggests that oxidative metabolism has extensive functions in the Parkinson's disease, as defective respiratory chain and mutation of mitochondrial DNA in the dopaminergic neurons are the most common features of Parkinson's disease patients [95,96]. In the neurons of patients, metabolism of dopamine is greatly linked to oxidative stress and its biochemical degradation produces reactive oxygen species including hydrogen peroxide (H_2O_2) and other metabolic products. As the H_2O_2 is highly membrane permeable, these products enter into adjacent neurons where it has ability to interact with Fe^{2+} to hydroxyl radicals [97,98]. The remarkable elevation of iron in the redox active form in the dopaminergic neurons plays a key function in pathogenesis of Parkinson's disease [99]. A recent study proposed a mechanism for iron accumulation in the brain neurons; functional disorder of mitochondria not only enhance reactive oxygen species generation but also decrease synthesis of iron–sulfur cluster and unorthodox activation of Iron Regulatory protein 1, a key regulator of iron homeostasis in a cell. This protein stimulates the accumulation of iron and hydroxyl radicals in a cell [100]. Iron ions can produce reactive oxygen species since ferrous ions (Fe^{2+}) and ferric ions (Fe^{3+}) can react with hydrogen peroxide and superoxide, respectively, in a chain reaction producing the hydroxyl free radical which together with oxidation of dopamine can induce neurotoxicity [101,102]. The iron accumulation in different areas of brain particularly in the dopaminergic neurons have been reported in the Parkinson's disease, which suggest that iron mediated lipid peroxidation plays a major biological role in the development and progression of this disease [103,104]. For instance, the accumulation of byproducts of lipid peroxidation have been observed in the cerebral spinal fluid and serum of the patients [105]. A recent study suggested that the neurodegenerative process is increased, when neuro-melanin containing organelles accumulate high load of toxins and iron during aging. Additionally, the release of neuromelanin from degenerating neurons stimulates microglia and latter cause neurons death with further release of neuromelanin initiates a self-propelling mechanism of neuro-inflammation and neurodegeneration [106].

It has been shown with the onset of Parkinson's disease metabolism of lipid peroxidation and proteins oxidation is increased in the dopaminergic neurons, and antioxidant level is also decreased. Additionally, thiobarbituric acid, malondialdehyde, and 4-hydroxy-2,3-nonenal level is also elevated in the substantia nigra of the patients [107,108]. Later, two-fold increase in protein oxidation in the substantia nigra was observed [109,110]. Furthermore, decrease in glutathione contents in human brain enhances the level of hydroxyl radicals in the patients [111]. Likewise, several experimental studies on human and model organisms suggest that glutathione peroxidase activities and glutathione contents are significantly decreased in the brain [112,113]. Multiple evidences, suggest that reduced activities of enzymatic and non-enzymatic antioxidants particularly in the dopaminergic neurons play crucial role in the progression and development of this disease [114–116]. Another study demonstrated that decrease in glutathione level and increase in oxidized glutathione contents is a common feature in the patients, whereas glutathione level is also decreased in the substantia nigra, due to loss of neurons [117].

13. Oxidative Stress and Huntington's Disease

Besides major advancements in the field of molecular biology and medical sciences, the precise mechanism of neuronal death in Huntington's disease remained mysterious. However, numerous reports suggest that oxidative stress play a major role in the development and progression of the disease [118,119]. In fact, susceptible brain neurons in the disease may not be able to cope the conditions of increasing reactive oxygen species. The higher level of reactive oxygen species may trigger intracellular cascades of oxidative stress through oxidizing DNA and protein, and inducing peroxidation of lipids in plasma membrane [120,121]. Mutant huntingtin proteins affect the mitochondrial activities by stimulating the opening of the mitochondrial permeability transition pore with the concomitant release of cytochrome c and the stimulation of the apoptotic mitochondrial pathway. This abnormal interaction also changes calcium buffering of mitochondria, further deteriorating mitochondrial disorder and enhancing reactive oxygen species production [122,123]. This mutant huntingtin protein interacts with Drp1, elevates GTPase Drp1 enzymatic activity, enhances abnormal dynamics of mitochondria and consequently, defective synaptic deficiencies and anterograde mitochondrial movement [124].

Although the oxidative damage has not been detected in the early stages of disease, yet it is assumed to have a crucial role in the progression of Huntington's disease [125]. Many researchers observed a changed expression pattern and activity of nitric oxide synthase, antioxidants, and ascorbate in R6/2 HD and R6/1 transgenic mouse [126,127]. Reactive oxygen species generation is also enhanced in the striatum of transgenic mice [128], whereas, a recent study observed increase in lipid peroxidation in different mouse models [129]. Furthermore, decreased expression of antioxidants has also been detected in the model species, while increase of these antioxidant enzymes reduce the toxic effects of mutant huntingtin proteins in cultured neurons [130].

In the late stages of disease, level of oxidative stress plays a crucial role in the progression of Huntington's disease. Mitochondrial dysfunction and impaired respiratory chain have been thought to involve in the reactive oxygen species mediated Huntington's disease pathogenesis [131,132]. Brain tissues of the patients show a higher level of dysfunction in the components of oxidative phosphorylation [128]. Elevated oxidative stress markers and depletion of antioxidants in brain and peripheral tissues have also been observed in the patients [133]. Increase in reactive oxygen species in the patients causes the denaturation of other biomolecules, for instance, it has been proposed that reactive oxygen species mediated mitochondrial dysfunction, links with defective glucose metabolism in Huntington's disease patients [134,135]. Another study suggested that the increase in reactive oxygen species level enhance lipid peroxidation and damage the cellular membranes, further, the higher lipid peroxidation is associated with lower glutathione content in Huntington's diseases patients compared with the healthy subject [132,136]. A recent study on mice model organism proposed that oxidative stress greatly denature the DNA, and this denaturation accumulates at CAG repeats in a length dependent manner. Similarly, the rate of DNA damage is increased under the relevant physiological condition, for example reactive oxygen species mediated decrease in protein level and depletion of major base excision repair enzymes [137]. Later on, comparative study of striatal cells derived from Huntington's diseases knock-in mice expressing mutant hunting versus wild cells indicated that higher level of reactive oxygen species greatly damages non-enzymatic antioxidants along with the other proteins [138]. Additionally, reactive oxygen species

mediated abnormal metabolism of tryptophan further worsen the ongoing brain functional disorder [139–141].

Altogether, the mechanisms implicated in the pathogenesis and progression of Huntington's and Parkinson's disease have not been fully understood. However, there is increasing evidence that oxidative stress is one of the key events in the pathogenesis of these diseases. Apart from oxidative stress, failure of antioxidant enzymes in neurodegenerative disease patients plays a crucial role in neuro-degeneration. In the human brain, reactive oxygen species are mainly produced by mitochondrial dysfunction, dopamine metabolism and inflammation of neurons. Thus, the different protective mechanisms implicated in the modulation of these biological processes are an interesting area of research focus in current years. Moreover, the study of Huntington's and Parkinson's disease related proteins in combination with experimental research using model organism has yielded substantial insights into the molecular pathways of neuro-degeneration and highlighted previously unidentified mechanisms by which oxidative stress contributes to these diseases.

14. Conclusions

Neurodegenerative diseases are life-threatening disorders, rapidly spreading in the aged population, with the number of cases rapidly increasing worldwide. These diseases impose a substantial health burden both on the patients, their families and society. The mechanisms implicated in the pathogenesis and development of neurodegenerative diseases have not yet been completely understood. However, some biological mechanisms have been proposed for the development and pathogenesis of these diseases, of which functional disorders of mitochondria and oxidative stress are key mechanisms considered to be involved in the progression of these diseases. In brain tissues, reactive oxygen species are generated from mitochondrial disorders, dopamine metabolism and inflammatory neurons. Therefore, protective biological mechanisms, which contribute to the modulation of these biological processes, are an important area for future studies. Based on the results of previous studies, various therapeutic approaches such as restoring functions of mitochondria and reducing oxidative damage have been developed. However, despite encouraging outcomes in model organisms, many clinical trials have failed to describe an influence on the development of disease. Failures from these approaches analyzed so far should guide future, newer strategies.

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Article analysis

The Role of Mitochondria in Reactive Oxygen Species Generation and Its Implications for Neurodegenerative Diseases Abstract

Mitochondria are dynamic cellular organelles that consistently migrate, fuse, and divide To modulate their number, size, and shape. In addition, they produce ATP, reactive oxygen species, and also have a biological role in antioxidant activities and Ca2+ buffering. Mitochondria are thought to play a crucial biological role in most neurodegenerative disorders. Impairment of mitochondrial activities is associated with neurodegenerative diseases, we summarize the existing knowledge of the mitochondrial function in reactive oxygen species generation and its involvement in the development of neurodegenerative diseases.

Introduction

A mitochondrion is a double-membraned, semi-autonomous cellular organelle that is separated, from the cytoplasm of a cell by the mitochondrial membranes. The outer mitochondrial membrane, is spongy in nature, which allows for free cross-movement of small, uncharged molecules and ions through the porin **(Bayrhuber and *al.*,2008 ; Dai and *al* 2018)**, They functionally control the production of energy, the electron transport chain, cell signaling, apoptosis, and programmed cell death **(Picard and *al.*, 2011 ; Dai and *al.*, 2018 ; Zheng and *al.*, 2018)**.

Reactive oxygen species are produced in various cellular compartments. However, mitochondria are a major contributor to reactive oxygen species (Balaban R S and *al.*, 2005), mitochondria seem to represent one of the key sources of reactive oxygen species production in the majority of cell types, to generate free radicals using various substrates, and this capacity of the mitochondria may depend on the composition of a **(Lenaz , 2001 ; Brand , 2010).**

Mitochondrial complex I, also known as NADH CoQ reductase, catalyzes the electron transfer from NADH to ubiquinone, which is accompanied by the movement of protons from the mitochondrial matrix to the intermembrane space **(Bolisetty , Jaimes, 2013 ; Warnaua, 2018)**. During the 20th century, it was shown that mitochondrial complex I is involved in the generation of reactive oxygen species **(Lenaz , 2001).** Mitochondrial complex I generates large amounts of free radicals using two biological mechanisms (1) a high ratio of NAD+/NADH leads to a reduction of the FMN site on complex I ; and (2) electron transport

to the CoQ pool coupled with a high protonmotive force Dp leads to reverse electron transport**. (Treberg and *al* 2011; Kussmaul , Hirst , 2006).** Many studies executed on sub-mitochondrial particles and intact isolated mitochondria have demonstrated that complex II, under specific conditions, and particularly when it is supplied with a high succinate concentration (almost 5 mM), produces a considerable amount of reactive oxygen species. **(Votyakova, Reynolds , 2001 ; Quinlan and *al*., 2011).**

Cellular components and other factors are also involved in the production of reactive oxygen species. Peroxisome, endoplasmic reticulum phagocytic cells, neuro-inflammation, dopamine, genetic mutation, anti-oxidant depletion etc. **(Phaniendra and *al*., 2015 ; Weng and *al*., 2018).**

Multiple lines of evidence suggest the etiology of neurodegenerative disorders is multifactorial and comprised of an interaction between environmental factors and genetic predisposition. Migliorea and his coworkers. **(Halliwell , Cross , 1994).**

In recent years, important advancements have been made in the field of medical science to understand initiating factors and cure of neurodegenerative diseases, however still it is a major cause of concern in the health profession. Neurodegenerative diseases can be divided into Parkinson's disease, Alzheimer's disease (Migliorea L, Coppedè F., 2009).

In the neurons of patients, metabolism of dopamine is greatly linked to oxidative stress and its biochemical degradation produces reactive oxygen species including hydrogen peroxide (H2O2). **(Perier, Vila , 2012 ; Nagatsu , Sawada , 2006).**

Material

- Mitochondria
- Mice
- Cell lines
- Human brain tissues
- Heart sub-mitochondrial
- Skeletal muscle
- Rate demonstrated
- A rat's heart
- Inhibitor molecules (antimycin A)

Results

There is increasing evidence that oxidative stress is one of the key events in the pathogenesis of these diseases. Failure of antioxidant enzymes in neurodegenerative disease patients plays a crucial role in neuro-degeneration.

Conclusion

Neurodegenerative diseases are life-threatening disorders, rapidly spreading in the aged population, with the number of cases rapidly increasing worldwide, some biological mechanisms have been proposed for the development and pathogenesis of these diseases, of which functional disorders of mitochondria and oxidative stress are key mechanisms considered to be involved in the progression of these diseases despite encouraging outcomes in model organisms, many clinical trials have failed to describe an influence on the development of disease. Failures from these approaches analyzed so far should guide future, newer strategies.

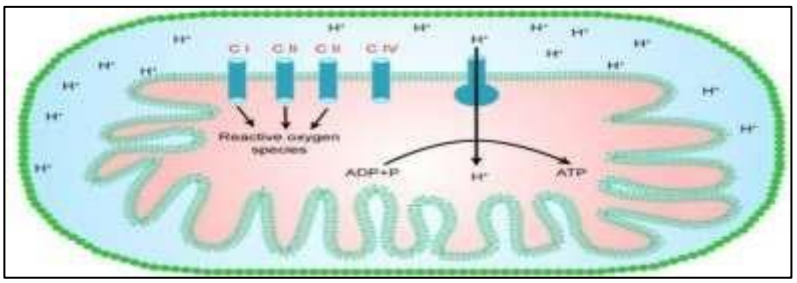

Figure 4.1: The major sites for the production of reactive oxygen species in a mitochondrion **(Lenaz, 2001; Brand, 2010)**

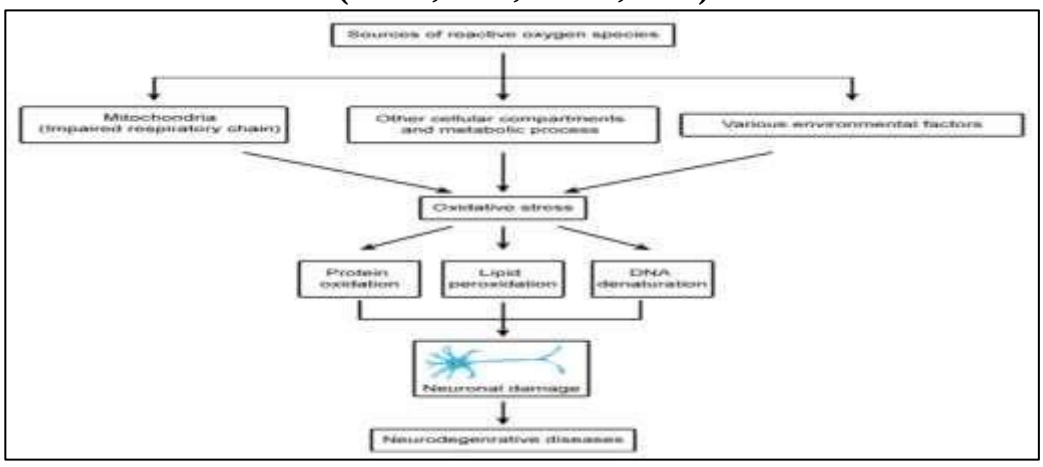

Figure 4.2: The causes of oxidative stress in neurodegenerative diseases **(Jodeiri and Ghaedi, 2017)**

Conclusion

Throughout this thesis, we have been interested to mitochondria in neurons form a compartment that is highly dynamic in structure and function. This involves mitochondrial transport in neuronal segments and strategic positioning of mitochondria at sites where ATP supply and Ca2+ handling is required. Moreover, a fine-tuned coupling between neuronal activity and mitochondrial function exists that is mediated by ions and substrates. Thus, mitochondria are critically involved in neuronal survival, neurotransmission. New highresolution imaging techniques will help to extend our knowledge on the physiological interactions between neuronal and mitochondrial functions in different types of neurons, brain regions, and developmental stages of the CNS. Under pathophysiological conditions, like Alzheimer disease, the tight coupling between neuronal activity and mitochondria has devastating effects on mitochondrial function, implying mitochondrial Ca2+ overload, synchronous depolarization of the mitochondrial compartment, and enhanced ROS production. This might cause energy failure, release of pro-apoptotic factors, and oxidative damage of lipid membranes and DNA, even in the acute phase of disease activity.

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