

# Cell Responses to Oxidative Stressors

Amelia Cataldi\*

*Dipartimento di Biomorfologia, Cattedra di Anatomia Umana, Facoltà di Farmacia, Università G. d'Annunzio, Chieti-Pescara, Via dei Vestini, 6, 66100, Chieti, Italy*

**Abstract:** Stress is a stimulus or a succession of stimuli tending to disrupt the homeostasis of an organism. An organism is consisting of a multitude of cells that singly undergo the effects of external factors that disturb or upset their homeostatic regulation. Stimuli acting as potential stressors are numerous, and include physical agents (ionizing radiation), non-physiological oxygen levels (hypoxia, hyperoxia) and chemotherapeutics. Lastly, also senescence, a physiological process occurring in all organisms, can be considered as a potential stressor.

The cell response to multiple oxidative stresses involves mitochondria, since these organelles represent the major source of Reactive Oxygen Species (ROS) that drive the occurrence of pathological conditions and ageing by activating specific signalling pathways. Nevertheless, under physiological conditions the cells are able to exert an antioxidant response which, controlling ROS/Reactive Nitrogen Species (RNS) homeostasis, is involved in mediating cell differentiation, proliferation and migration. Thus, this review focuses the attention to the role played by mitochondria in the physiological and non-physiological signalling responses of eukaryotic cells to some oxidative stresses, in order to identify potential therapeutic targets to counteract oxidative stress effects and mitochondrial-related pathologies.

**Keywords:** Oxidative stressors, ROS, mitochondria, signalling pathways, eukaryotic cells.

## INTRODUCTION

Stress is a stimulus or a succession of stimuli tending to disrupt the homeostasis of an organism. An organism is constituted by a multitude of cells that singly undergo the effects of internal and external factors that disturb or upset their homeostatic regulation. In fact, at the cellular level stress can be regarded as a disturbance to normal development, which may affect cell structure and function, stability, growth and survival. Stimuli acting as potential stressors are numerous, and include physical agents (ionizing radiation), non-physiological oxygen levels (hypoxia, hyperoxia) and chemotherapeutics. Lastly, also senescence, a physiological process occurring in all organisms, can be considered as a potential stressor [1]. Each one of these stressors activates a Reactive Oxygen Species (ROS) response at the cellular level that switches on the antioxidant machinery and physiological signalling pathways. When the threshold of stress is exceeded, this response is converted into a death signal, leading to apoptosis, necrosis or to neoplastic transformation following genomic instability Fig. (1). The cell response capability depends on the proteome expressed and therefore is species- and cell type-dependent. Cell stressors target a number of cellular functions such as cell cycle control, protein chaperoning and repair, chromatin stabilization and repair, removal of damaged proteins and certain aspects of metabolism [2-3].

## OXIDATIVE STRESSORS

### Ionizing Radiation

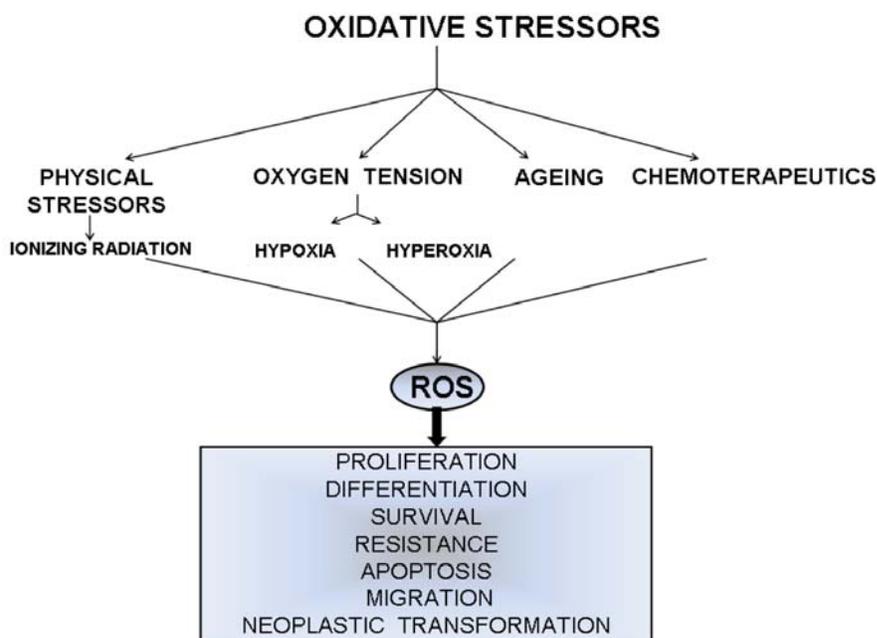
The effective target of ionizing radiation is DNA, but damage at DNA level is preceded by damage at the plasma membrane which, in turn, activates intracellular signalling processes, such as ROS production, finally leading to cell cycle arrest, apoptosis, stress response and DNA repair processes, depending on cell type and on dose of ionizing radiation [4-8]. At the cell membrane level ionizing radiation is able to initiate sphingomyelin hydrolysis by inducing acidic or neutral sphingomyelinase without the involve-

ment of any nuclear component [9-10]. Sphingomyelin cleavage results in the formation of phosphocholine and ceramide, a lipid second messenger that plays a role in the regulation of a number of cellular processes, among which proliferation, differentiation and apoptosis. Moreover, numerous nuclear signalling events, namely JNK/SAPK phosphorylating pathway of several transcription factors such as ATF2 and p53 [11-13], PKC $\delta$ /NF- $\kappa$ B pathway [14] and ERK/MAPK-mediated metalloproteinases activating pathway [15], are involved in radiation-induced response which leads to apoptosis or radioresistance [16-17].

### OXYGEN SENSING

Eukaryotic cells display diverse responses to varying oxygen levels that influence cell growth. Normal oxygen levels (21%; P<sub>O<sub>2</sub></sub>≈100 Torr) keep the enzymatic machinery in a physiological state. When hypoxic (5-10% oxygen; P<sub>O<sub>2</sub></sub>≈35-50 Torr) or hyperoxic (60-95% oxygen; 0.5% CO<sub>2</sub>) stresses are established, the mammalian body reacts with several changes in ventilation and at the cardio-circulatory system as well as with other specific tissue reactions such as angiogenesis, erythropoiesis and glycolysis [18-19]. Under hypoxia conditions at the cellular level increased anaerobic glycolysis, loss of contractility, changes in lipid and fatty acid metabolism, and possible irreversible membrane damage result in conditions incompatible with life [20]. Chronic hypoxia not only determines changes in cell metabolism but also is able to induce changes in DNA-protein interactions, leading to gene expression alteration [21-22]. Also 85% oxygen supplied to Sprague-Dawley rats for seven days in a polystyrene chamber induces tissue damage and functional alterations by influencing the production of free radicals, not only through the mitochondrial chain transport but also through NADPH oxidase family members [23]. Moreover, severe hyperoxia acts as a killer for cells with a very high metabolism or oxygen consumption [24], whereas a milder hyperoxia (70% oxygen) retards growth and induces apoptosis changes in vascular density and gene expression in transplanted gliomas in nude rats [25]. In addition, in cells exposed to high oxygen levels metabolism decreases with respect to cells maintained at normoxia, due to a lower rate of oxygen consumption, which leads to reduction in ATP generation. Instead, in cells exposed to hypoxia is activated HIF-1, which induces the production of rescue proteins as an adaptive response, and ATP levels do not diminish, due to a reduction in the

\*Address correspondence to this author at the Dipartimento di Biomorfologia, Cattedra di Anatomia Umana, Facoltà di Farmacia, Università G. d'Annunzio, Chieti-Pescara, Via dei Vestini, 6, 66100, Chieti, Italy; Tel: 39-0871-3554508; Fax #: 39-0871-3554568; E-mail: cataldi@unich.it



**Fig. (1).** Physiological and pathological cellular responses to oxidative stressors.

rate of utilization [26]. In any case, in spite of the diverse cell responses to hypoxia or hyperoxia, in both systems the signalling events are triggered by the generation of mitochondrial-derived oxygen species. Whatever the mechanisms, too high or too low oxygen levels can modify the metabolic homeostasis [27]. In spite of various negative effects, both hyperoxia and hypoxia exert beneficial effects in particular pathological conditions. In fact, hyperoxia is largely used in the therapy of hemorrhagic shock [28] and in nerve regeneration in early diabetes [29], and has been shown to be a useful adjunct in several models of ischemia reperfusion injury (IRI) including myocardial infarction [30]. Interestingly, in light of recent studies observing appetite suppression and body weight loss at high altitude, it has been suggested to apply hypoxia for the treatment of obesity and related disorders [31].

### CELLULAR SENEESCENCE

The ageing process is due to a large extent to the damaging consequence of free radical accumulation and action (lipid peroxidation, DNA damage, protein oxidation). Cellular senescence can occur following a period of proliferation, triggered by a cell-intrinsic mechanism (replicative senescence) or in a rapid manner in response to an acute stress (stress-induced premature senescence) [32]. Once cells have entered senescence, they undergo a dramatic change in morphology (volume increase, flattened cytoplasm etc.) that leads to the decline of their physiological functions. Studies performed on human fibroblasts have evidenced that the cell proliferation capability is limited by an intrinsic mechanism, related to the number of divisions through which cell lineages have gone, and triggers senescence when the predetermined limit for division is reached or changed under different oxygen or stress levels [33-34]. Interestingly, internal ROS can determine changes in nuclear structure, gene expression, protein processing and metabolism through the activation of specific signalling pathways, finally influencing cell mitotic ability [35]. Although repair systems are able to correct much damage, oxidative DNA lesions accumulate with age and represent fundamental contributors to the ageing process [36]. In spite of this, it is still not known whether ROS generation is only a secondary or rather a primary event in the occurrence of senescence, likewise it is suggested also for

Alzheimer's disease, in which it has been shown that free radicals are capable of mediating neuron degeneration and death [37].

### CHEMOTHERAPEUTIC AGENTS

Anticancer drugs can themselves act as stressors primarily because they induce DNA damage and interfere with important cellular functions, such as DNA replication, and secondarily because they are capable of inducing adaptive signals such as genotoxic stress response that could limit their clinical value. This particular stress involves ROS production and may activate different responses at the cellular level depending on its nature, duration and severity [3, 38]. In this respect, it has been shown that membrane lipids, proteins and DNA damage as well alterations in the redox status, energy metabolism, cell cycle and proliferation are key functional aspects of the cellular stress response. Among chemotherapeutic agents etoposide and TRAIL (Tumour Necrosis Factor-Related Apoptosis-Inducing Ligand) represent two antineoplastic drugs that have been investigated in our laboratory. Etoposide, useful for the treatment of a wide range of cancers, is an analogue of 4-demethylepipodophyllin benzylidene glucoside (DEPBG) and inhibits the DNA unwinding Topoisomerase II, which makes double-stranded cuts in DNA. It does not kill cells by blocking Topoisomerase catalytic function, but poisons this enzyme by increasing the steady state concentration of covalent DNA cleavage complexes. For this reason Topoisomerase II becomes a physiological toxin that introduces high levels of transient protein-associated breaks in the genome of treated cells. When these breaks become permanent and are present at sufficient concentration, they trigger a series of events that ultimately culminate in cell death by apoptosis, as evidenced by the high level of Bax protein and caspase-3 activation in etoposide-treated Jurkat T cells [6-7, 13, 39]. TRAIL is a member of the structurally related TNF family of cytokines that induces apoptosis in various neoplastic cell lines, including several of haematopoietic origin, displaying minimal or no toxicity on normal cells and tissues. It binds to several members of the TNF-receptor family, i.e. death receptors (DRs) 4 and 5, and anti-apoptotic decoy receptors (DcRs) 1 and 2. DR4 and DR5 contain a cytoplasmic region consisting of a stretch of 80 aminoacids, designated the death domain (DD) responsible for

transducing the death signal [39]. In human leukaemia Jurkat T cells TRAIL induces Topoisomerase I (Top I) synthesis at the onset of apoptosis. Topoisomerase I is known to relax supercoiled DNA generated by transcription, replication and chromatin remodelling and to be trapped as cleavage complexes (Top I cc) at sites of oxidative DNA lesions. In this specific experimental model, Top I cleavage is mediated by caspase-3 which is a mediator of the apoptotic response induced by TRAIL [40]. Moreover, other agents exist such as resveratrol, a natural polyphenol, whose effect has been tested on mtROS (mitochondrial ROS) production in cultured human coronary arterial endothelial cells. This agent, by attenuating mitochondrial oxygen species production, could confer vasoprotection, improve endothelial function and prevent complications of diabetes, and thus has been proposed as a potential candidate for treatment of metabolic diseases [41]. Lastly, combined chemotherapy of gastric cancer with 5-fluorouracil and mitomycin exerts synergistic cytotoxic effects via ROS formation and p53-dependent apoptotic pathway induction, leading to mitochondrial dysfunction and caspase activation [42].

Moreover, the stress response to chemotherapy may be circumvented or modulated by co-existing disorders that do not allow the optimal evaluation of pharmacological interventions effects [43]. In fact, especially in the case of cancer, it is not known whether interfering with components of the molecular response would increase physiological defence mechanisms against the malignant phenotype or whether it would enable tumour cells to adapt to chemotherapy or to undergo apoptosis [44]. Landriscina *et al.* [45] report that drug resistance can be considered as a lack of response or a partial response to therapy, or a tumour re-growth after an initial response, suggesting that adaptive responses to oxidative stress may share common mechanisms with drug resistance. Tumour cells produce a large amount of ROS that are counteracted by an altered antioxidant machinery that lead to the dysregulation of cellular signalling and to the activation of pro-survival mechanisms [45]. In any case, the imbalance between cell survival and cell death due to pharmacological interventions is decisive for sensitivity or resistance to DNA-damaging chemotherapeutic agents and lastly for the outcome [6, 39]. Indeed, the response to chemotherapy in solid tumours leads to the induction of resistance to drugs that act primarily on rapidly dividing cancer stem cells, such as breast, prostate, brain and pancreas cells, which continue to proliferate to form a tumour mass, whereas current cancer therapeutics based on tumour regression may target and kill differentiated tumour cells, which compose the bulk of the tumour, and spare the rare cancer stem cell population [46].

## CELL RESPONSES

### Role of Mitochondria

All the above-mentioned oxidative stressors produce a cell response involving mitochondria. Mitochondria are cytoplasmic organelles containing the oxidative phosphorylation machinery, which allows the generation of 90% of the cellular energy in the form of adenosin triphosphate (ATP) molecules. Moreover, mitochondria control intracellular  $\text{Ca}^{++}$  metabolism and signalling, regulate thermogenesis and, when dysfunctional, determine accumulation of oxidative damage to DNA, proteins and lipids by producing high levels of ROS and RNS [47-49]. ATP production derives by the enzymatic cleavage of a number of metabolic substrates like glucose. At the mitochondrial level, molecules like glucose provided by the external blood environment are transformed into pyruvic acid that, in turn, is degraded by pyruvate dehydrogenase into NADH (Nicotinamide Adenine Dinucleotide reduced) and Acetyl CoA. Then, three classes of enzymes work in cascade: i) oxidant enzymes (Krebs cycle enzymes), localized at the mitochondrial matrix, which degrade carbohydrates and fatty acids producing hydrogen atoms, that link to FAD and NAD determining FADH<sub>2</sub> and NADH<sub>2</sub> formation; ii) respiratory chain enzymes,

localized at the inner mitochondrial membrane, which transport electrons and protons to the final acceptor molecular oxygen ( $\text{O}_2$ ) to form  $\text{H}_2\text{O}$ ; iii) ATP synthetase enzymes, localized at the inner mitochondrial membrane, which synthesize ATP from ADP and Pi by using the energy produced by the respiratory chain enzymes and stored as electrochemical gradient. The energy derived from ATP cleavage is used for all the cellular physiological activities such as protein and lipid synthesis, muscular contraction, cell membrane active transport, nerve impulse transportation.

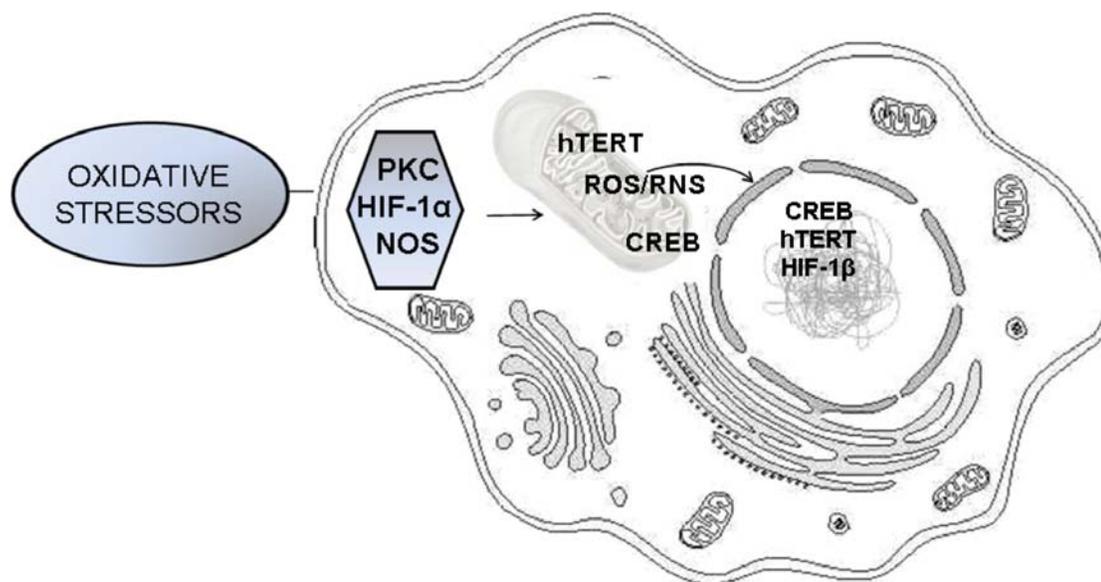
All aerobic organisms produce ROS that are partially reduced metabolites of molecular oxygen, but with higher activity relative to molecular oxygen. These include superoxide anion ( $\text{O}_2^-$ ) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), formed through one- and two-electron reductions of  $\text{O}_2$ , respectively, as well as the hydroxyl radical ( $\cdot\text{OH}$ ). The primary ROS generated by mitochondria is  $\text{O}_2^-$  also known as superoxide anion. When superoxide anion reacts with water more stable  $\text{H}_2\text{O}_2$  and molecular oxygen can be generated through a dismutation reaction, which occurs in mitochondria through the activity of matrix Mn-SOD (Superoxide Dismutase), as well as through Cu/Zn-SOD not only at mitochondrial intermembrane space but also at cytoplasmic level [26].

In addition to mitochondria also peroxisomes contribute to ROS production [50]. Peroxisomes are cytoplasmic organelles and major sites of oxygen consumption in several metabolic reactions. Oxygen consumption in the peroxisomes leads to  $\text{H}_2\text{O}_2$  production, which is used to oxidize a variety of molecules by anti-oxidant enzymes such as catalase, peroxidases and urate oxidases. In particular catalase, by decomposing  $\text{H}_2\text{O}_2$  into  $\text{H}_2\text{O}$  and  $\text{O}_2$ , is able to prevent the accumulation of this toxic compound. Thus, peroxisomes maintain a delicate balance with respect to the relative concentration or activities of these enzymes to ensure no net production of ROS. Actually, when peroxisomes are damaged by a stress like ionizing radiations, and their  $\text{H}_2\text{O}_2$  consuming enzymes are downregulated,  $\text{H}_2\text{O}_2$  is released into the cytosol and significantly contributes to oxidative stress [50].

Oxidative stress is viewed as an imbalance of normal "ROS/RNS homeostasis" leading to the accumulation of oxidative damage in cell constituents. Reactive Nitrogen Species (RNS) derive from the reaction of Nitric Oxide ( $\text{NO}^\circ$ ), generated in biological tissues by specific nitric oxide synthases (NOSs), with oxygen and water to form nitrate and nitrite anions. Overproduction of RNS is called nitrosative stress and occurs when RNS production in a system exceeds the system's ability to neutralize them. In healthy cells excessive ROS/RNS are decomposed by protective antioxidant machinery, represented by cytosolic glutathione S transferase and the thioredoxin system, including peroxiredoxin, especially concentrated in the endoplasmic reticulum [48]. Therefore only excessive ROS or RNS production, due to exogenous stresses and/or decrease in detoxification mechanisms [50], lead to oxidative stress and pathological conditions, such as diabetes mellitus, cardiovascular disease, rheumatoid arthritis, Alzheimer's and Parkinson's diseases and cancer [48]. On a time scale of years, modest but ongoing oxidative stress may play a significant role in the occurrence of aging and cancer. Lastly, while for many years ROS have been considered harmful, recently a role has been assigned to such products as mediators of physiological responses, such as cell differentiation, proliferation and migration, as evidenced in haemin-treated endothelial progenitor cells (EPC) [5, 51]. Thus, the interaction of oxidative stressors with mitochondria determines both physiological and stress responses that can activate nuclear and mitochondrial signalling pathways Fig. (2).

### ROS-MEDIATED INTRANUCLEAR SIGNALLING PATHWAYS ACTIVATION

Cellular signal transduction networks commonly include three steps: i) sensors that perceive the signals; ii) transducers that can amplify and integrate the signals; iii) effectors that adjust cell



**Fig. (2).** Mitochondria as key components of the intracellular signalling response to oxidative stressors.

function to signals. The cell membrane represents the barrier to the external environment and, containing signalling molecules, can activate a complex signalling network from outside inside the cell up to the nucleus, mediating not only the switching on of cell proliferation and differentiation but also the impairment of growth and proliferation and, in some cases, the occurrence of cell death (apoptosis) and of neoplastic transformation.

#### ROLE OF NITRIC OXIDE (NO) IN OXIDATIVE STRESS RESPONSES

The cell response to oxidative stressors involves the production of NO, deriving from an imbalance of normal ROS/RNS homeostasis [48], as a mediator which affects the mitochondrial permeability transition pore and cytochrome c release in a concentration-dependent manner [52]. Nitric oxide (NO) is a small, diffusible, multifunctional, highly reactive molecule, intracellular messenger with dichotomous regulatory roles under physiological and pathological conditions. Apart from its physiological functions, exerted by constitutively generated NO (neuronal signalling [53-54], blood pressure, clotting regulation, haematopoietic stem cell activity regulator [55]), excessive synthesis of NO could be toxic to the cells. In fact, depending on concentration, local environmental conditions (superoxide radical concentration, pH) and cell types, NO can act as a pro- or anti-oxidant or as a pro- or anti-apoptotic factor and can trigger both a pro- and an anti-inflammatory response [56]. Pro-apoptotic and pro-inflammatory functions seem to be associated with the cellular redox state, as a consequence of the rate of NO production, and with the formation of complexes with biological molecules such as iron, thiols, proteins, nucleic acids, lipids, sugars and reactive oxygen species [57-60] and expression of survival genes [61]. NO can directly induce cytochrome c release through a mitochondrial membrane potential loss, eventually activating the caspase-dependent apoptotic signal cascade. It binds to cytochrome c oxidase in the mitochondrial electron transfer chain. Under stress conditions, superoxide generated from mitochondria interacts with NO to form peroxynitrite, which induces mitochondrial dysfunction at the membrane level, cytochrome c release and apoptosis. Endogenous nitric oxide is synthesized from L-arginine by a family of NO synthases (NOS). These enzymes are homodimers whose monomers are themselves two fused enzymes: a cytochrome reductase and a cytochrome that requires three co-substrates (L-arginine, NADPH and O<sub>2</sub>) and five

cofactors or prosthetic groups (FAD, FMN, calmodulin, tetrahydrobiopterin and heme). Several distinct NOS isoforms are produced from three distinct genes [62]. These include two constitutive Ca<sup>++</sup>/CaM-dependent forms of NOS: nNOS (NOS1), whose activity was first identified in neurons and which maps at 12q24.2, and eNOS (NOS3) first identified in endothelial cells and mapping at 7q35-36. Endothelial nitric oxide synthase (eNOS) is an important enzyme in the cardiovascular system [63]. eNOS catalyzes the production of nitric oxide that in the cardiovascular system is a key regulator of blood pressure, vascular remodelling and angiogenesis [64-65] which can be regulated, in turn, by phosphorylation mechanisms at multiple sites [66].

The inducible form of NOS, iNOS (NOS2) is Ca<sup>++</sup>-independent and is expressed in a broad range of cell types, including macrophages and hepatocytes, and in response to a variety of stresses, such as inflammatory reactions [60]. NOS2 maps to 17cen-q12. In certain tissues, such as liver, brain, thymus and heart, a specific form of mitochondrial mtNOS has been detected [67]. Under physiological conditions mtNOS can produce NO, which reversibly (and competitively with O<sub>2</sub>) inhibits the mitochondrial cytochrome c oxidase, what seems to be its primary regulatory role in mitochondria. In this way O<sub>2</sub> production in the respiratory chain is enhanced by the modified redox state of cytochrome c oxidase. The hydroxyl radical °OH can also oxidize nitrite to nitrogen dioxide (°NO<sub>2</sub>), which can then combine with NO giving rise to N<sub>2</sub>O<sub>3</sub>. Another member of RNS family namely peroxynitrite (OONO<sup>-</sup>) can be obtained through the reaction of °NO with molecular oxygen and can further react with CO<sub>2</sub>, lipids etc. to yield hydroxyl radical °OH. Under stress conditions of increased cellular or mitochondrial °NO formation and increased O<sub>2</sub> release, accumulation of peroxynitrite alters mitochondrial oxidative phosphorylation and Ca<sup>++</sup> homeostasis, phenomena associated with diseases such as stroke, myocardial infarction, chronic heart failure, diabetes, circulatory shock, chronic inflammatory diseases, cancer, neurodegenerative disorders and ageing [48].

#### ROLE OF PROTEIN KINASES C (PKC) IN OXIDATIVE STRESS RESPONSES

At the cell membrane level the interaction of oxidative stressors activates specific signalling proteins among which Protein Kinases C are included.

Protein kinase C isozymes belong to a serine/threonine kinase family, ubiquitously expressed, involved in the cell transduction of exogenous signals regulating cell growth [68], differentiation [69], apoptosis induction [70], neoplastic transformation [71], ageing [72] and stress responsiveness [73-74]. Three main groups of PKC have been characterized: conventional PKC ( $\alpha$ 1,  $\beta$ 1,  $\beta$ 2,  $\gamma$ )  $\text{Ca}^{++}$ -, PS- and DAG-dependent, novel PKC ( $\delta$ ,  $\epsilon$ ,  $\eta$ ,  $\theta$ ,  $\mu$ ) PS- and DAG-dependent and atypical PKC ( $\zeta$ ,  $\iota$ ,  $\lambda$ ) PS- or PI3,4,5P3-dependent [75]. Although most cells express more than one type of PKC, differences among the isozymes patterns suggest that individual PKC are cell type- and stimulus-dependent and able to mediate distinct biological processes [76]. Indeed, in Friend cells exposed to ionizing radiation PKC zeta nuclear translocation is involved in mediating radioresistance [77-78]. In Jurkat lymphoblastoid cells PKC delta nuclear specific activity mediates caspase-3 activation, inducing apoptosis and, thus, an increased sensitivity to ionizing radiation [79]. By contrast, in K562 erythroleukaemia cells PKC delta nuclear translocation is involved in the activation of NF- $\kappa$ B-mediated survival pathway, possibly leading to the radioresistance displayed by these cells [14]. Interestingly, PKC isoforms are involved in the activation of splicing factors during postnatal rat heart development [80] and play a role in the response to hypoxic stimuli both at cellular [81] and tissue level [72] and during ageing [82-83].

#### ROLE OF HYPOXIA INDUCIBLE FACTOR-1 (HIF-1) IN OXIDATIVE STRESS RESPONSES

Changes in  $\text{O}_2$  concentration (hypoxia and hyperoxia), affecting mitochondrial function, determine the production of ROS, the activation of HIF and of downstream targets which regulate glycolysis, mitochondrial oxygen consumption, erythropoiesis, angiogenesis, cell survival or cell death and senescence [31, 84-87]. The different responses to changes in  $\text{O}_2$  concentration can be quantitative: a relative increase in ROS generation during hyperoxia, compared to hypoxia, might result from an increase in the rate of mitochondrial ROS generation due to a stronger inactivation of mitochondrial antioxidants or a longer duration of ROS generation. ROS release during hyperoxia can trigger cell death or senescence, whereas the relatively lower levels of ROS generated during hypoxia might signal an adaptive response such as, for example, HIF-dependent gene transcription [31]. HIF-1, composed of two subunits, HIF-1 $\alpha$  and HIF-1 $\beta$ , is identified as a transcriptional factor that regulates the cellular response to hypoxia through the binding to HRE (Hypoxia Responsive Element) gene at nuclear level [88-91]. Under normoxia, the intracellular level of HIF-1 $\alpha$  is kept low by rapid ubiquitination and subsequent proteasomal degradation, which depends on the hydroxylation of proline residues by PHD2, whereas under hypoxia the intracellular level and transcriptional activity of HIF-1 $\alpha$  increase as a result of suppressed PHD2 and HIF activities [92]. HIF-1 $\alpha$  is required for the up-regulation of Vascular Endothelial Growth Factor (VEGF) gene, which, by producing the specific protein, provides a mechanism to stimulate angiogenesis thus preventing depletion of local oxygen [86]. Thus HIF-1 $\alpha$  is considered a master regulator of  $\text{O}_2$  variations by governing adaptive patterns of gene expression [93]. Whereas HIF-1 $\alpha$  in normoxic conditions is continuously expressed and degraded by the ubiquitin proteasome system [84], under hypoxia it is not degraded, migrates into the nucleus where it forms a heterodimer with HIF-1 $\beta$ , recruits co-activators, such as p300/CBP, ref-1, TIF-2 etc, and trans-activates the expression of a multitude of target genes which produce rescue proteins such as PI-3-kinase and Akt [24, 81].

#### ROLE OF C-AMP RESPONSE ELEMENT BINDING PROTEIN (CREB) TRANSCRIPTION FACTOR IN NUCLEAR STRESS RESPONSES

The malfunction and changes in the biogenesis of mitochondria seem to exert some of the most pronounced effects on the organism.

If biogenesis is affected, it is reasonable to expect that mitochondrial turnover must be slower and the accumulation of modified lipids, proteins and DNA must also increase, further aggravating the situation resulting from the deficient activity of stressed mitochondria. Moreover, several nuclear receptors and other transcriptional factors such as NF- $\kappa$ B, AP-1, CREB (c-AMP Response Element Binding Protein) and p53, involved in growth, metabolic, developmental and apoptotic processes, have been detected at mitochondria level Fig. (2). These factors seem to play a key role in mitochondrial transcription and energy metabolism intervening in mediating apoptosis [94-97]. Among these factors CREB has been detected at the inner membrane of rat brain mitochondria [98].

The CREB/ATF (activating transcription factor) multigenic family is composed by several nuclear transcription factors with an identical cAMP-responsive element (CRE) consensus binding site. The prototype of this family is CREB (also known as CREB/CREM or CREB1), a 43 kDa-basic region-leucine zipper (bZIP) transcription factor, whose phosphorylation at Ser<sup>133</sup> [99] and association with the co-activator CREB binding protein (CBP) [100] or related molecules (p300) are necessary for gene trans-activation [101]. CREB can bind to DNA as a homodimer [102] or as a heterodimer with ATF1 (also known as TREB36), that is a bZIP protein highly related to CREB, widely expressed in different tissue types and implicated in cAMP- and calcium-induced transcriptional activation [103]. Due to the high number of CREB putative target genes [104], CREB elicits responses to a variety of signals, such as growth factors and stress [105] and is involved in several cellular processes such as proliferation, ageing and differentiation, survival and apoptosis [7, 72, 106-108]. In recent years deregulation or aberrant expression of CREB1 and ATF1 has been associated with cancer progression of solid tumours like breast cancer [109] and melanoma [110] or in the bone metastases development of prostate primary carcinoma [111].

CREB, once imported from the cytoplasm, where it is synthesized, into mitochondria, where transcription/translation of mtDNA encoded proteins takes place, can be phosphorylated by c-AMP-dependent-Protein Kinase (PKA) upon stress stimuli interaction [112] and its binding to mtDNA can affect the expression of mitochondrial genes and proteins [113-114]. However, the precise mechanisms of action of these transcription factors on mitochondrial gene expression need to be further investigated.

#### ROLE OF TELOMERES AND TELOMERASE IN CELLULAR STRESS RESPONSES

Telomeres are very sensitive indicators of cumulative oxidative stress. Telomeres are specialized DNA-protein complexes which prevent linear chromosome ends from fusion (end to end joining) and from being sensed as a DNA strand break which triggers growth arrest and other responses [115]. Telomere shortening is largely caused by the so called "end replication problem" since conventional DNA polymerases are not able to synthesize the ends of chromosomes completely due to a gap left by the last RNA primer. In addition, oxidative stress contributes largely to a cell type-dependent telomere shortening due to a telomere-specific DNA single-strand break repair inefficiency [116]. The enzyme involved in replication of the ends of eukaryotic chromosomes (capping) is the ribonucleoprotein Telomerase, composed by a catalytic protein subunit, human telomerase reverse transcriptase (hTERT), required for telomerase maintenance activity, and an RNA moiety (TERC) [117-118]. Telomerase balances terminal DNA losses by lengthening the ends of eukaryotic telomeric DNA through a RNA-template-mediated addition of tandemly repeated telomeric sequences. If telomere capping is lost due to shortening or damage, the telomere is recognized as DNA damage which can eventually results in cell cycle exit or in certain mammalian cells in apoptosis. Thus telomere shortening is involved in the response to some oxidative stresses such as different oxygen concentrations and

appears to be a biomarker of ageing [33, 116, 119-121]. In fact von Zglincki *et al.* [122] have demonstrated that in human fibroblasts exposed *in vitro* to mild hyperoxia telomeres shortening is faster under these conditions, whereas a lower oxidative stress decreases telomere shortening and increases the lifespan of cells in culture [122-123]. Since it has been also demonstrated that mitochondrial dysfunction via induction of telomere damage is an important component in the occurrence of senescence within dividing cellular populations [124], it might be suggested that mitochondrial DNA can be considered as a sensitive indicator of intracellular oxidative stress. Moreover, telomerase has been shown to be involved in the regulation of chromatin status and DNA damage responses [125]. Interestingly, significant changes in nuclear gene expression, such as glutamine synthase, adenylate cyclase 3 (ADCY3), growth hormone receptor (GHR) etc. in human cells seem to be induced by mitochondrial dysfunction [124] and improved by telomerase expression. Thus, a better telomere capping could contribute to an improved cellular and mitochondrial function, due to decrease of oxidative stress and thereby to preservation of the organelle function and lastly to the occurrence of stress and apoptosis resistance [126]. In light of such evidences a shuttling of telomerase from the nucleus to the mitochondria upon oxidative challenge has been proposed [127-130]. This translocation seems to occur in a time- and dose-dependent manner [131] and to represent an important physiological mechanism of mitochondrial protection, even though it is not clear through which mechanism TERT protects mitochondrial DNA. Some proposed mechanisms for this protection are the lowering in mitochondrial ROS generation, direct binding to and protection of mtDNA, improved DNA repair or accelerated degradation of mitochondria harbouring damaged DNA [130].

## CONCLUSION

The cell responses to different oxidative stresses represent a defence reaction to the damage that environmental forces inflict on macromolecules. Even though for many years ROS have been viewed as an inevitable and unwanted cell product, recently they have been assigned novel roles in the activation of specific signalling pathways mediating survival, differentiation and proliferation of healthy cells, or leading to pathological conditions and ageing when stress tolerance limits are exceeded [130]. Thus, the choice of the use of antioxidants, which can buffer the effects of oxidative stressors, in association with selective inhibitors of key signalling proteins depends on cell types and conditions, and allows to identify potential therapeutics targets for a number of stresses, mitochondrial-related pathologies and ageing. Again, it is crucial to pay attention to the dual role of ROS, since on one hand they are necessary for the production of cytotoxic effects and selective killing of tumour cells by a number of chemotherapeutic drugs, on the other hand they might play a role in inducing cellular survival mechanisms, therefore counteracting the intended effects of the drugs in terms of cancer cells eradication.

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## ABBREVIATIONS

AMP	=	Adenosine monophosphate
ATP	=	Adenosine triphosphate
ATF2	=	Activating Transcription Factor 2
CREB	=	c.AMP Responsive Element Binding Protein

DAG	=	Diacylglycerol
HIF-1	=	: Hypoxia Inducible Factor 1
HRE	=	Hypoxia Responsive Element
NO	=	Nitric Oxide
NOS	=	Nitric Oxide synthase
PKC	=	Protein Kinase C
PS	=	Phosphatidylserine
ROS	=	Reactive Oxygen Species
SAPK	=	Stress Activated Protein Kinase
-RNS	=	Reactive Nitrogen Species
TERT	=	Telomerase Reverse Transcriptase
Top I	=	Topoisomerase I
VEGF	=	Vascular Endothelial Growth Factor

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