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Mitochondrial Stress Response and Cancer

Jordan O'Malley[1,3], **Rahul Kumar**[1,3], **Joseph Inigo**[1], **Nagendra Yadava**[2], **Dhyan Chandra**[1,*]

[1]Department of Pharmacology and Therapeutics, Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA

[2]Department of Anesthesiology and Center for Shock, Trauma, and Anesthesiology Research, University of Maryland School of Medicine, Baltimore, MD, USA

[3]These two authors contributed equally to this work.

Abstract

Cancer cells survive and adapt to many types of stress including hypoxia, nutrient deprivation, metabolic and oxidative stress. These stresses are sensed by diverse cellular signaling processes leading to either degradation of mitochondria or alleviation of mitochondrial stress. This review will discuss signaling during sensing and mitigation of stress involving mitochondrial communication with the endoplasmic reticulum, and how retrograde signaling upregulates the mitochondrial stress response to maintain mitochondrial integrity. The importance of the mitochondrial unfolded protein response, an emerging pathway that alleviates cellular stress, will be elaborated with respect to cancer. Detailed understanding of cellular pathways will establish mitochondrial stress response as a key mechanism for cancer cell survival leading to cancer progression and resistance, and provide a potential therapeutic target in cancer.

Keywords

cancer cell survival; heat shock protein 60 (HSP60); mitochondrial unfolded protein response; mitochondrial stress response; cancer progression and therapeutic resistance

Cancer Cell Mitochondria and Stress Response Pathways

Cancer cells rely on the mitochondrial oxidative phosphorylation (OXPHOS) system to sustain high proliferative capacities, metastatic spread, chemotherapy resistance, and stemness [1–4]. Mitochondria generate other macromolecules besides ATP that are essential for cellular function. Mitochondria synthesize amino acids, fatty acids, cholesterol, heme, Fe-S clusters, nucleotides and a myriad of metabolic intermediates that can act as signaling molecules (reviewed extensively in [5]). The mitochondrion is home to other major metabolic pathways such as the tricarboxylic acid (TCA) cycle and fatty acid oxidation

[*]**Correspondence:** dhyan.chandra@roswellpark.org (D. Chandra). .

(FAO), which occur in the mitochondrial matrix. The TCA intermediates fuel OXPHOS Complexes and are used for biosynthesis of amino acids as well as redox balance while FAO is used as an alternate energy source for ATP [6–9]. Fatty acid and cholesterol synthesis can be upregulated in cancer cell mitochondria to be used as building blocks of the lipid bilayer membrane, an essential requirement for dividing cells. Mitochondrial cholesterol is the sole precursor for steroids including testosterone and estrogen, which drive prostate and breast cancer progression, respectively [10–12]. Therefore, cancer cells often induce mitochondrial biogenesis and metabolic reprogramming to keep up with the energy demands and to produce essential macromolecular building blocks required for constant cell division and oncogenic signaling. Since evasion of apoptosis is one of the hallmarks of cancer [13], cancer cells may increase the apoptotic threshold of mitochondria by activating mitochondrial maintenance programs.

Mitochondria play important roles in cellular physiology by affecting cell viability and function. Therefore, cellular stresses that impact mitochondria need to be managed to avoid any deleterious effects to cells. Cancer cells employ multiple stress response pathways to counteract exogenous or endogenous stressors and to enhance their survival and proliferation. These pathways include the integrated stress response (ISR), the cytosolic heat shock response (HSR), and the unfolded protein response (UPR) mediated by organelles such as the endoplasmic reticulum (ER) and mitochondrion [14–16]. Although the cytoprotective ISR pathway, mediated by the phosphorylation of eukaryotic translation initiation factor 2 alpha, is interlinked with other stress response pathways [14, 15], the activation of the endoplasmic reticulum UPR (UPR^{ER}) and the mitochondrial UPR (UPR^{mt}) maintains protein homeostasis specifically in these two organelles in response to exogenous or endogenous stressors [16, 17]. Mitochondria have evolved several maintenance and preservation pathways to attenuate various stresses, such as UPR^{mt}, mitochondrial fission and fusion, and mitophagy [15, 18–21]. In cancer cells, these mechanisms are deregulated due to altered signaling leading to long-term proliferative and survival advantages, which are intrinsically linked to tumor progression and therapeutic resistance [22–24]. This review describes current updates on mitigation of persistent cellular stresses mediated by the mitochondrial stress response (Figure 1) and highlights some outstanding questions that require immediate attention to efficiently target stress signaling to block tumor growth and to prevent development of therapeutic resistance in cancer.

Cellular Stress and Mitochondrial Dysfunction in Cancer

Mitochondria are interconnected with other cellular organelles and perform various functions including bioenergetics, macromolecular synthesis, cellular life and death decisions. These mitochondrial functions are deregulated in cancer rendering long-term survival and proliferative advantages to cancer cells leading to aggressive disease and therapeutic resistance [13, 25]. Key reasons for impaired mitochondrial function (i.e., dysfunction) in cancer cells include endogenous stresses such as hypoxia, and metabolic and proliferative stresses [26]. Mitochondrial dysfunction in cancer is adapted in a way that help cancer cells survive, proliferate, migrate, and develop plasticity [26, 27]. The adaptation of cancer cells under persistent stress is mediated by robust stress sensing and mitigation systems in mitochondria and other cellular compartments [28].

Stress sensing and mitigation signaling may require inter-organellar communications, mitochondrial biogenesis, unfolded protein response, mitochondrial dynamics and downstream signaling [15, 18–20, 29]. Mitochondrial biogenesis is controlled by both nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). MtDNA encodes 13 structural proteins of the OXPHOS system, 22 tRNAs, and 2 rRNAs [30]. Thus, mitochondrial biogenesis and function relies on import of the nDNA-encoded proteins, which are synthesized in the cytosol [31] and synthesis of the mtDNA-encoded proteins within mitochondria. Mutations of both nDNA and mtDNA compromise mitochondrial function and create mitochondrial stress leading to deregulation of cellular signaling and tumorigenesis. Warburg's original hypothesis suggests a causative role of the respiratory chain injury and enhanced aerobic glycolysis in cancer cells [32, 33]. However, current evidences also support that tumorigenesis requires both glycolysis and OXPHOS for energy production and the synthesis of macromolecules [13, 25, 34, 35]. Therefore, it is essential to understand the role of glycolysis and OXPHOS in tumor growth and progression in the broader context of mitochondrial stress response.

Mitochondrial Stress and Inter-organellar Networking

Mitochondria experience stresses in many forms including generation of mitochondrial reactive oxygen species (mtROS) due to hypoxia, metabolic stress, nutrient depletion, defects in protein folding within mitochondria, and defects in protein import [26, 31, 36]. Sensing and mitigation of these stresses require instant communication between various cellular compartments such as mitochondria, ER, lysosomes, and nucleus [15, 18–20, 28, 36]. In response to stress, mitochondria undergo dynamic changes and regulate their biogenesis as well as biogenesis of other cellular organelles such as lysosomes and ER. For example, in response to stress, a decline in ATP generation leads to the upregulation of AMP-activated protein kinase (AMPK), which enhances lysosomal biogenesis to degrade dysfunctional mitochondria via mitophagy and maintain mitochondrial homeostasis [37]. Inter-organellar crosstalk between ER and mitochondria via mitochondria-associated ER membranes (MAMs) ensures that mitochondrial stresses are shared between organelles [38–40].

Under normal condition, the ER stress transducer inositol-requiring enzyme 1α (IRE1α) maintains the composition and functions of MAMs, and mediates ER-to-mitochondria communication in order to fine-tune mitochondrial respiration via facilitating local Ca^{2+} transfer from the ER to the mitochondrial matrix [41]. Increased mitochondrial respiration and expression of the mitochondrial protease LONP1 upon ER stress, including increased ER stress induced by generation of mtROS, suggest a unique coordination and stress sensing mechanism between these two organelles [42–45]. However, persistent accumulation of Ca^{2+} in the mitochondrial matrix via MAMs induces the mitochondrial permeability transition pore (mPTP), which triggers apoptotic cell death [46, 47]. Additionally, MAMs regulate other tightly controlled cellular processes such as autophagy and mitochondrial dynamics. Given that MAMs are critical for various cellular functions, their deregulation may impact cancer progression and therapeutic resistance. Upregulation of mitochondrial fusion components such as mitochondrial GTPases mitofusin 1 (MFN-1) and mitofusin 2 (MFN-2) promote prostate cancer progression [48]. It is also interesting to note that MFN-1

and MFN-2 are important for physical tethering between mitochondria and ER at the MAMs [49]. By contrast, increased mitochondrial division and expression of GTPase dynamin related protein 1 (Drp1), an essential component of the mitochondrial division (mitochondrial fission) machinery, as well as decreased mitochondrial fusion promote tumorigenesis [50, 51]. Therefore, there is a context-dependent role of mitochondrial dynamics, ER, and MAMs to alleviate cellular stress and promote cancer progression. Notably, mitochondrial networking with other organelles are important to maintain cellular functioning, for example, crosstalk between mitochondria and nucleus is important for both normal and cancer cells.

The UPR^{mt} Alleviates Stress and Promotes Cancer Cell Survival

Cancer cells employ multiple stress response pathways to counteract endogenous and exogenous or environmental stresses. The cytosolic heat shock response (HSR) is regulated by the transcription factor heat shock factor 1 (HSF1) [52]. The HSR is induced by unfolded protein accumulation within the cytosol, which results in HSF1 trafficking to the nucleus to induce cytosolic chaperones, such as heat shock protein 27 (HSP27) and heat shock protein 90 (HSP90), which are involved in protein folding [53–55]. Stressors such as temperature, oxidative stress, and calcium depletion can cause protein denaturation and misfolding resulting in the accumulation of aggregates. The cellular response to accumulation of unfolded proteins is referred to as UPR, which is activated in different cellular compartments.

Mitochondrial-specific stress signaling was first reported in 1996 by Martinus et al. [56]. They observed that depletion of the mtDNA led to an increase in the expression of nuclear-encoded mitochondrial chaperones, HSP10 and HSP60 [56]. However, no change in the expression of HSP70, an ER chaperone, and HSP72, a cytosolic chaperone, were noted. Thus, it was concluded that mtDNA depletion resulted in mitochondria-specific stress response marked by the induction of mitochondrial chaperones [56]. Apart from mtDNA depletion, the accumulation of unfolded protein within the mitochondrial matrix can also result in the induction of mitochondria-specific stress proteins, such as HSP10 and HSP60 [57]. The knockdown of paraplegin (or SPG7), a mitochondrially localized protease, also induces mitochondrial stress response by impairing the assemblies of multiprotein complexes [58]. Therefore, in analogy to UPR^{ER}, the transcriptional induction of mitochondrial stress proteins in response to the accumulation of unfolded proteins within mitochondria was termed as the UPR^{mt}. Together, these findings support the notion that mitochondrial protein misfolding and accumulation of insoluble mitochondrial proteins activates UPR^{mt} [57, 59–61].

Destabilizing genetic mutations and oxidizing conditions can cause accumulation of insoluble aggregates of unfolded proteins in cells, which are not cleared efficiently by mitochondrial proteases [60, 61]. Both aging and hypoxia induce the formation of insoluble mitochondrial proteins (aggregates) that can be resolved after enhanced activation of UPR^{mt} [60]. Aging results in accumulation of mutations in both the mitochondrial and nuclear genomes. However, compared to nDNA, the mtDNA is more susceptible to mutations due to the lack of protective histones and fewer DNA repair mechanisms within mitochondria [62–

64]. The oxidative environment and lack of protective histones within mitochondria renders mtDNA more susceptible to damage by ROS. ROS can also directly oxidize mitochondrial proteins leading to their misfolding [65–68]. Thus, both mtDNA mutations and mitochondrial protein oxidation can induce UPR^{mt}.

The mitochondrial proteome is comprised of 1200–1500 proteins of mitochondrial and nuclear origins [69]. Only 13 of these, which are components of the OXPHOS system, are encoded by mtDNA [30, 70]. The remaining mitochondrial proteins including OXPHOS proteins are encoded by nDNA. The 13 mtDNA-encoded proteins must exist in a state of balanced stoichiometry with the rest of the nuclear-encoded OXPHOS proteins. Therefore, mtDNA depletion and mutations result in a "mitochondrial-nuclear imbalance" and trigger activation of UPR^{mt} [17, 71]. The status of UPR^{mt} activation ultimately determines the cell fate. The cancer cells may utilize UPR^{mt} to evade apoptosis and ensure their survival similar to cardiomyocytes [72].

The key proteins involved in UPR^{mt} are chaperones HSP10, HSP60, and mtHSP70 and proteases ClpP and LONP1. Among these, HSP60 is a key component of UPR^{mt} activation process across different species (Box 1). HSP10, HSP60, and mtHSP70 work together to properly fold denatured and nascent polypeptides [73]. The ClpP and LONP1 proteases cleave the irreversibly damaged proteins and mark them for degradation [74, 75]. Collectively, the chaperones and proteases restore proteostasis within mitochondria, and thus promote cell survival. Apart from the chaperones and proteases that resolve misfolded mitochondrial proteins, UPR^{mt} activation also induces expression of gene sets including those involved in metabolic adaptation such as glycolytic genes, OXPHOS recovery, and antioxidants such as MnSOD2 [76–78]. When the level of proteotoxic stress is insurmountable, then mitochondria release cytochrome c into the cytosol and initiate the intrinsic apoptotic pathway [79].

Retrograde Signaling and Mitochondrial Stress Relieving Response Pathways

To sense and mitigate mitochondrial stress, mitochondria-to-nuclear (retrograde) signaling is activated. The signals from mitochondria are relayed to the cytosol and nucleus by mitochondrial metabolites, such as TCA cycle intermediates, ATP, and ROS, which initiate key protein modifications (e.g. histone acetylation) and activate transcriptional regulatory elements in nuclear genes [80–82]. Several transcription factors are involved in UPR^{mt} activation. For example, the transcription factor CCAAT-enhancer-binding protein homologous protein (CHOP) is known to activate UPR^{mt}. AKT and AMPK are proposed to serve as sensors, which detect proteotoxic stress and elevations of ROS in mitochondria, leading to activation of CCAAT-enhancer-binding protein beta (C/EBPβ), followed by induction of CHOP [57, 83, 84]. Stress in the inter-membrane space of mitochondria, and not in the mitochondrial matrix, induces elevated levels of CHOP [85]. However, C/EBPβ and CHOP are better known to activate UPR^{ER} and are not specific to the activation of UPR^{mt} [86]. Unlike CHOP, a UPR^{mt}-specific transcription factor, ATFS-1 has been discovered in *C. elegans* [87]. Under normal conditions, ATFS-1 accumulates in the

mitochondria where it is constantly degraded by the LONP1 protease [88]. Under stress conditions, mitochondrial protein import becomes impaired and results in cytosolic accumulation of ATFS-1 [88]. Because ATFS-1 has both mitochondrial and nuclear localization signals, its cytosolic accumulation results in nuclear translocation where it facilitates the transcription of genes involved in UPR^{mt} and OXPHOS [77, 88]. ATFS-1 targets include HSP60, mtHSP70 and nuclear-encoded OXPHOS subunits. Thereby, ATFS-1 ultimately promotes OXPHOS recovery during mitochondrial stress [77]. It is interesting to note that UPR^{mt} activation limits pathogen infection as ATFS-1 induces innate immune genes [89, 90], which may modulate host cell signaling to promote cellular proliferation and cancer [91].

The mammalian homolog of ATFS-1 is activating transcription factor 5 (ATF5), which can be activated by heat shock [92], amino acid depletion [93], inhibition of proteasome function [94], increases in ROS [67, 94], and ER stress [94, 95]. ATF5 complements the genetic ATFS-1 deficiency in *C. elegans* by rescuing UPR^{mt} [67]. ATF5 may induce UPR^{mt} using a similar mechanism to ATFS-1 because both localize to mitochondria and the nucleus. ATF5 is required for transcriptional induction of HSP60, mtHSP70, and LONP1 in response to paraquat, a mitotoxic agent [67]. Like ATFS-1, the knockdown of ATF5 reduces mitochondrial respiration. ATF5 promotes OXPHOS recovery after mitochondrial stress [67]. In addition to transactivating UPR^{mt} components, ATF-5 is also reported to regulate expression levels of Egr-1, BCL-2, and MCL1 to mediate proliferation and survival in cancer [96–98]. ATF5 has been upregulated in many types of cancer such as colorectal, breast and pancreatic cancer, and contribute to tumor cell cells survival and growth [82, 99, 100]. Furthermore, high ATF5 levels in lung cancer and malignant glioma are correlated with reduced survival in patients [101, 102]. Therefore, pharmacological inhibition of ATF5 may provide therapeutic benefits to patients with cancer.

The activation of retrograde signaling ensures that mitochondrial stress is relieved. However, the presence of persistent stress may lead to dysfunctional mitochondria, which either leads to mitophagy or apoptosis. Mitophagy is initiated by identification of severely damaged mitochondria, which are tagged by PTEN-induced putative kinase 1 (PINK1)-dependent phosphorylation of ubiquitin ligase Parkin. The activated Parkin leads to poly-ubiquitination of multiple outer mitochondrial proteins, which target mitochondria for degradation upon their fusion with lysosomes [103, 104].

In the presence of a persistent internal stress, the mitochondrial outer membrane is permeabilized to release cytochrome c into the cytosol. Cytochrome c binds with the apoptotic protease-activating factor 1 (Apaf-1), which recruits caspase 9 to form the apoptosome, a large protein complex. The apoptosome activates caspase 9, which subsequently leads to the activation of executioner caspases such as caspase-3 and −7. This cascade of activating caspases initiates the process of cellular degradation *via* apoptosis [105–110]. Apoptosis can be inhibited by the prevention of cytochrome c release from mitochondria into the cytosol or prevention of cytochrome-c interaction with Apaf-1 by many factors including intracellular nucleotide pool [111–114]. Thus, mitochondrial dysfunction in the form of cytochrome c release plays a key role in initiating apoptosis. Each cell type has an apoptotic threshold, which is determined by mitochondrial homeostasis in

the presence of stress. When a stress disturbs mitochondrial homeostasis beyond the threshold, then cells initiate apoptosis. Compared to normal cells, cancer cells raise their apoptotic threshold by overexpressing prosurvival factors as well as by the activation of UPR^{mt} (Box 2) [79, 115].

Regulation of UPR^{mt} by Mitochondrial Bioenergetics

The key role of UPR^{mt} is to minimize the impact of mitochondrial stress and protect cells. Cells may experience mitochondrial stress by different ways, e.g. by OXPHOS defects and ROS production. A typical strategy used to induce UPR^{mt} in cell culture is the treatment of cells with paraquat. Paraquat causes superoxide production ($O_2^{\cdot -}$) by receiving electrons from the respiratory chain Complex III [67, 116]. The electrons from Complex III are trapped by paraquat before they reach Complex IV. Thus, paraquat disrupts electron flow along the respiratory chain and impairs OXPHOS, resulting in UPR^{mt} activation [116]. The knockdown of proteases affecting OXPHOS biogenesis, such as *spg-7* also induces UPR^{mt} in *C. elegans* [77]. Partial loss-of-function mutation in NDUFS7, a subunit of the respiratory chain Complex I, also induces mitochondrial stress response [117]. Thus, OXPHOS defects may play a regulatory role in UPR^{mt} activation. However, whether specific defects of the OXPHOS Complexes, e.g. isolated deficiencies of Complexes I-V, can induce UPR^{mt} needs further confirmation.

The sirtuins (Sirt 1–7), a family of NAD-dependent enzymes, detect cellular energy perturbations and alter the metabolic state of cells [118]. NAD^+ is used as a substrate by sirtuins such as Sirt1 and Sirt3 [119]. Sirt1 deacetylates PGC-1α, a master transcription factor controlling mitochondrial biogenesis [120]. The administration of NAD^+ and overexpression of Sirt1 increases transcription and translation of HSP60, ClpP and superoxide dismutase (SOD2) genes, which are involved in UPR^{mt} [121]. Sirt3 has been implicated in maintaining mitochondrial stability by activating antioxidant machinery during periods of mitochondrial proteotoxic stress [78]. The members of poly ADP-ribose polymerase (PARP) family also uses NAD^+ as a substrate, and compete with sirtuins for the use of NAD^+ [122]. An acute activation of PARP can deplete NAD^+ pool and initiate cell death. In *C. elegans*, the inhibition of PARP increases lifespan by boosting mtDNA content and ATP production. In mammalian cells, PARP inhibition also elevates HSP60, ClpP and SOD2 protein levels in a Sirt1-dependent manner [121]. Sirt1 is a key nuclear transcription factor, whose activity is highly responsive to NAD^+ levels. Therefore, PARP inhibition promotes UPR^{mt} by making NAD^+ available for Sirt1 [121].

The mitochondrial maintenance transcription factor nuclear respiratory factor 1 (NRF1) recruits Sirt7 to the promoters of mitochondrial ribosomal proteins and translational factors. In doing so, NRF1 represses mitochondrial metabolic activity and reduces protein-folding stress to promote stem cell quiescence [123]. Loss of Sirt7 increases transcription of HSP10, HSP60, ClpP, mtDNA content, and cell proliferation. These phenotypes are reversible when Sirt7 was reintroduced to the system [123].

Cancer cells rely on functional mitochondria to generate macromolecules required for unchecked proliferative capacity. To meet the increased demand for proliferation, cancer

cells reprogram their metabolism to enhance macromolecule biosynthesis, readjust bioenergetics, and redox status [124]. They also take up macromolecules from their microenvironment. Because of the increased demand for amino acids and protein synthesis, some of the non-essential amino acids may become essential for cancer growth. The TCA cycle is the hub of anabolic and catabolic metabolism. It is dynamically linked with the OXPHOS. The function of the respiratory chain is dependent on an adequate supply of NADH and $FADH_2$. Severe deficiency of the respiratory chain causes accumulation of NADH resulting in reductive stress, particularly inside mitochondria. The build-up of NADH blocks the TCA cycle by the feedback inhibition of the NAD^+-dependent dehydrogenases. This results in oxaloacetate deficiency, which is required to generate the amino acids such as aspartate. Aspartate is a nonessential amino acid synthesized inside mitochondria. Severe respiratory chain deficiency makes aspartate essential for cell proliferation [125]. The role of the respiratory chain in cellular physiology has been rediscovered after about 40 years. In late 1970s, the Scheffler group at University of California San Diego demonstrated that genetic respiratory chain deficiency could block the TCA cycle. The respiration-deficient lung fibroblasts were unable to grow in a bicarbonate-free medium. In addition, the respiration-deficient mutants also became dependent on aspartate and asparagine for their growth [126–128]. Aspartate is used in the urea cycle and released as fumarate, which links the urea cycle with the TCA cycle. The most important role of urea cycle is to clear toxic ammonia generated from the protein metabolism. The overexpression of a mutant ornithine decarboxylase (OTC), a urea cycle enzyme, in mitochondria led to the discovery of mitochondria-specific stress response or the UPR^{mt} [57]. Therefore, mitochondrial metabolism controls multiple aspects of cellular physiology. The major ones being the TCA and urea cycles, and Fe-S cluster biosynthesis. Alterations in the TCA cycle and urea cycles are implicated in cancer development [129, 130]. Without the UPR^{mt} activation, the mitochondria in cancer cells may not meet the demand for macromolecules biosynthesis required for cell growth and proliferation. Thus, UPR^{mt} is essential to maintain proper mitochondrial function to sustain unchecked proliferation during tumorigenesis (Box 2).

Concluding Remarks

The UPR^{mt} is a mitochondrial stress specific response. Like UPR^{ER} [131], the UPR^{mt} can play a pivotal role in evading apoptosis by alleviating mitochondrial stress in cancer cells. Cancer cells experience mitochondrial stress as they undergo unchecked cellular proliferation and generate ROS, which damage mtDNA and crucial mitochondrial proteins, such as components of the OXPHOS family causing mitochondrial dysfunction. Cancer cells rely on functional mitochondria to generate macromolecules such as amino acids, nucleotides and cholesterol to maintain their high proliferative capacity. Thus, cancer cells activate the mitochondrial stress response to alleviate mitochondrial dysfunction and protein aggregation, which subsequently promotes tumor growth and progression (Figure 2). These notions have been validated by recent findings indicating that persistent activation of UPR^{mt} provides survival advantage to cancer cells leading to tumor progression [22], suggesting that a mediator of UPR^{mt} such as superoxide dismutase 1 (SOD1) may represent a therapeutic target in cancer [132]. Mitochondrial stress relieving response requires inter-

organellar communication, retrograde signaling, mitophagy, mitochondrial bioenergetics, mitochondrial dynamics, and apoptosis. These cellular and biochemical signaling pathways provide multiple targets to develop efficacious therapeutics in various types of cancer (Figure 3). HSP60, a key player of the UPR^{mt}, is overexpressed in many cancer types, and its expression has been correlated with the metastatic potential of cancers and overall survival of cancer patients [133–137]. HSP60 upregulation within mitochondria raises cancer cells threshold for surviving stressful conditions. The extramitochondrial HSP60 may also promote tumor growth, apoptosis resistance, and metastatic spread (Box 3). An upstream regulator of UPR^{mt}, ATF5 functions as a pro-survival protein and promotes tumor progression and therapeutic resistance [98, 99, 138]. Increased mitochondrial fission mediated by Drp1 promotes tumor growth [50, 51] and pharmacological inhibition of mitochondrial fission improve survival in mice [139]. Importantly, targeting the components of mitochondrial stress response, such as mitochondrial ClpP protease and other mitochondrial peptidase, have shown selective therapeutic efficacy in cancer cells [140–143]. Although inhibiting UPR^{mt} offers a unique therapeutic strategy for targeting cancer cells, important challenges need to be addressed experimentally to exploit mitochondrial stress response pathways in cancer (see Outstanding Questions).

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Box 1.

HSP60 oligomerization, protein folding, proteotoxic stress in mitochondria

HSP60 is a 60-kDa protein encoded by the nuclear *HSPD1* gene [144] and translated in the cytosol. The newly translated peptide contains a mitochondrial import signal (MIS) [145] at the N-terminus [146]. The MIS is a sequence of 26 amino acids, the majority of which are hydroxylated, and contributes to mitochondrial import in cells [146]. The MIS is cleaved upon mitochondrial import and HSP60 reaches its final conformation [146, 147]. Each HSP60 monomer has three domains: the apical domain, intermediate domain, and equatorial domain. The equatorial domain contains the ATP-binding site, the apical domain binds unfolded proteins, and the intermediate domain acts as a hinge by connecting the apical and the equatorial domain. Once in the mitochondria, HSP60 monomers self-assemble into ring-shaped heptameric oligomers, two of which associate to form a barrel-shaped tetradecamer [73]. In the inactive state (ATP unbound), the exposed residues of the apical domain in the core of the barrel-like structure are mostly hydrophobic and bind unfolded, non-native proteins. In the active state, ATP binds the equatorial domain and the co-chaperonin HSP10 covers the activated complex. The culmination of these two events induce a conformational shift and causes hydrophilic residues of the apical domain to become exposed in the core of the barrel. This charge turnover from hydrophobic to hydrophilic drives the folding of the encapsulated substrate proteins and their subsequent release from the HSP60-HSP10 complex [73, 148–150]. Reduced expression of mitochondrial chaperones causes a buildup of misfolded protein aggregates resulting in a proteotoxic stress [78, 151]. The proteotoxic stress triggers ATF5 to translocate to the nucleus and activate UPR^{mt}.

Box 2.

Constitutive activation of the UPR^{mt} enhances apoptosis threshold in cancer cells

Elevated HSP60 expression has been observed in many cancers such as acute myeloid leukemia, breast ductal invasive carcinoma, pancreatic ductal adenocarcinoma, ovarian carcinoma, prostate adenocarcinoma, and others [135, 152]. Protective function of HSP60 and UPR^{mt} is based on hypothesis that it is a "chaperonopathy by mistake" [153]. Thus, instead of protecting the host, HSP60 facilitates cancer cell growth and proliferation by increasing the apoptotic threshold of the cell *via* folding and refolding of oncoproteins and denatured/partially misfolded proteins within mitochondria. An increase in the ratio of mutated and misfolded proteins to chaperones can trigger UPR^{mt}. The UPR^{mt} enhances the protein-folding capacity of the mitochondria by activating the transcription factor ATF5 and CHOP, which promotes transcription of genes involved in mitochondria quality control such as the protease LONP1, and chaperonins HSP10 and HSP60 [67, 154]. Therefore, the elevated HSP60 expression within cancer cells may be a sign of constitutively activated UPR^{mt}, which would increase the apoptotic threshold. Thus, increased HSP60 works against the host by aiding tumor progression *via* constitutive activation of the UPR^{mt} [153].

Box 3.

The extramitochondrial roles of HSP60 in cancer

In addition to its established localization in mitochondria, HSP60 also localizes to other cellular compartments such as cytosol and may contribute to malignant phenotypes (Figure I). Cytosolic HSP60 promotes cancer cell survival and contributes to malignant phenotypes function [155]. Cytosolic HSP60 interacts with the IKK complex and enhances the activation of IKK [155]. This interaction directly increases the expression of NF-κB targets such as Bfl-1/A-1 and MnSOD, and promotes cancer cell survival under stressful conditions [155]. Cytosolic HSP60 enhances IKK activation independent of its chaperone activity, suggesting alternative functions of HSP60 beyond protein folding (Figure I) [155].

Cytosolic HSP60 also interacts with proapoptotic proteins Bax and Bak but not with antiapoptotic Bcl-2 in adult cardiac myocytes [156]. HSP60 interacts with the tumor suppressor p53 and restrain its transcriptional function, which reduces Bax expression [134]. HSP60 upregulation also sequesters Bax in the cytosol, and thus inhibits onset of apoptosis in cancer cells (Figure I).

Overexpression of HSP60 significantly increases cellular migration and invasion *in vitro* as well as increased tumor volume and metastasis *in vivo* [157]. HSP60 directly interacts with β-catenin *via* its apical domain. β-catenin expression is critical for HSP60-mediated *in vitro* and *in vivo* metastatic activity [157] implying that HSP60 overexpression represents an alternate route for β-catenin activation. Intriguingly, the reverse process occurs in neuronal tissue, whereby canonical Wnt signaling (i.e., β-catenin -dependent) induces UPR^{mt} [158]. HSP60 is highly expressed on the surface of pancreatic metastatic cells. In these cancer cells, HSP60 seems to play a critical role in metastasis [159]. Surface HSP60 interacts with proteins involved in metastasis e.g. integrin α3β1 [160]. Activated integrin α3β1 induces motility and cell adhesion property in breast cancer cells [161] via preferential association with HSP60 located on the cell surface. Exogenous addition of HSP60 also activates integrin α3β1 and increases cell motility in breast cancer cells [160]. Conversely, mizoribine, the HSP60-binding drug, inhibits HSP60 association with integrin α3β1 [160]. Thus, activation of integrin α3β1 by HSP60 is independent of its chaperone activity (Figure I).

Outstanding questions

- Cellular stresses are sensed by various organelles with diverse mechanisms of action.

  How is the specificity of mitochondrial stress response determined?

- How does ATF5 translocate from mitochondria to the nucleus during stress response?

  This mechanism was elucidated in the homolog ATFS-1 in *C.elegans* but has yet to be determined in cancer cells.

- How does the mitochondria relay stress signal to other cellular organelles such as endoplasmic reticulum and nucleus in cancer cells?

- What are the underlying mechanisms of activation of ATF5 and retrograde signaling during mitochondrial stress response in cancer?

- HSP60, a key component of mitochondrial stress response, contains mitochondrial localization sequence and is traditionally located in the mitochondrial matrix. How does HSP60 regulate tumor promoting factors localized in the cytosol and nucleus?

Highlights

- Cancer cell mitochondria are susceptible to oxidative stress due to inherent production of reactive oxygen species and a lack of robust protective mechanisms causing persistent mitochondrial stress.
- Cancer cells activate mitochondrial stress response to alleviate endogenous stresses in order to prolong survival, enhance proliferation and metastatic potential.
- Mitochondrial unfolded protein response (UPR^{mt}) comprising chaperones and proteases
- remove proteotoxic stress to maintain cellular homoeostasis.
- Elevated expression of HSP60 and its upstream regulator ATF5 enhances apoptotic threshold in cancer cells leading to therapeutic resistance in various types of cancer.
- Targeting of mitochondrial stress response by pharmacologic inhibition of its components provides novel therapeutic targets in cancer.

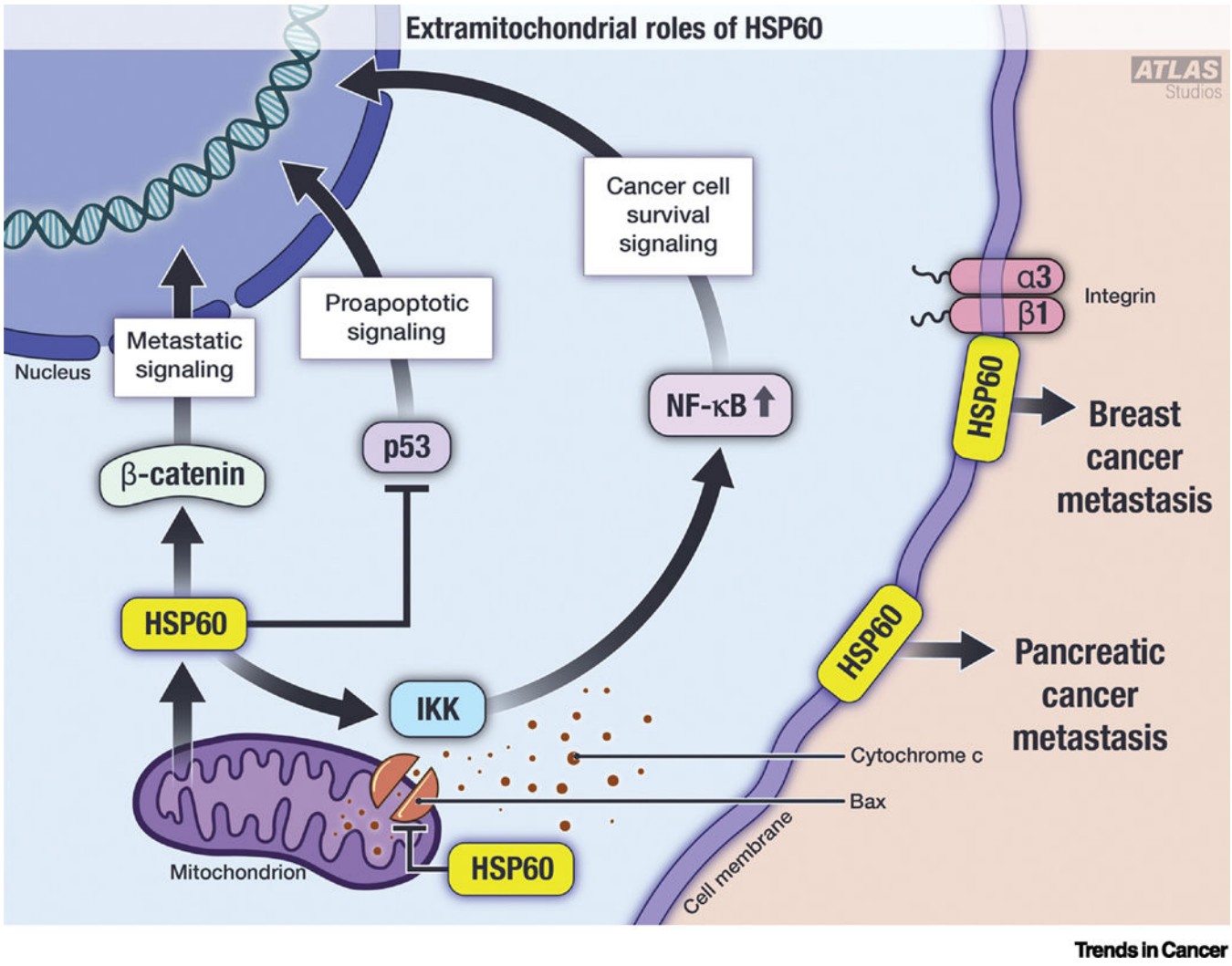

Figure I. Extramitochondrial roles of HSP60.

Graphical representation of nontraditional roles of HSP60. Cytosolic HSP60 independently interacts with β-catenin, IKK and p53 to induce metastatic signaling, cell survival, and inhibit pro-apoptotic signaling, respectively. Cytosolic HSP60 also binds with Bax at the mitochondrial membrane to inhibit cytochrome c release and inhibit intrinsic apoptosis. On the cell surface, HSP60 interacts with integrin α3β1 to promote breast cancer metastasis. Cell surface HSP60 is also implicated in pancreatic cancer metastasis.

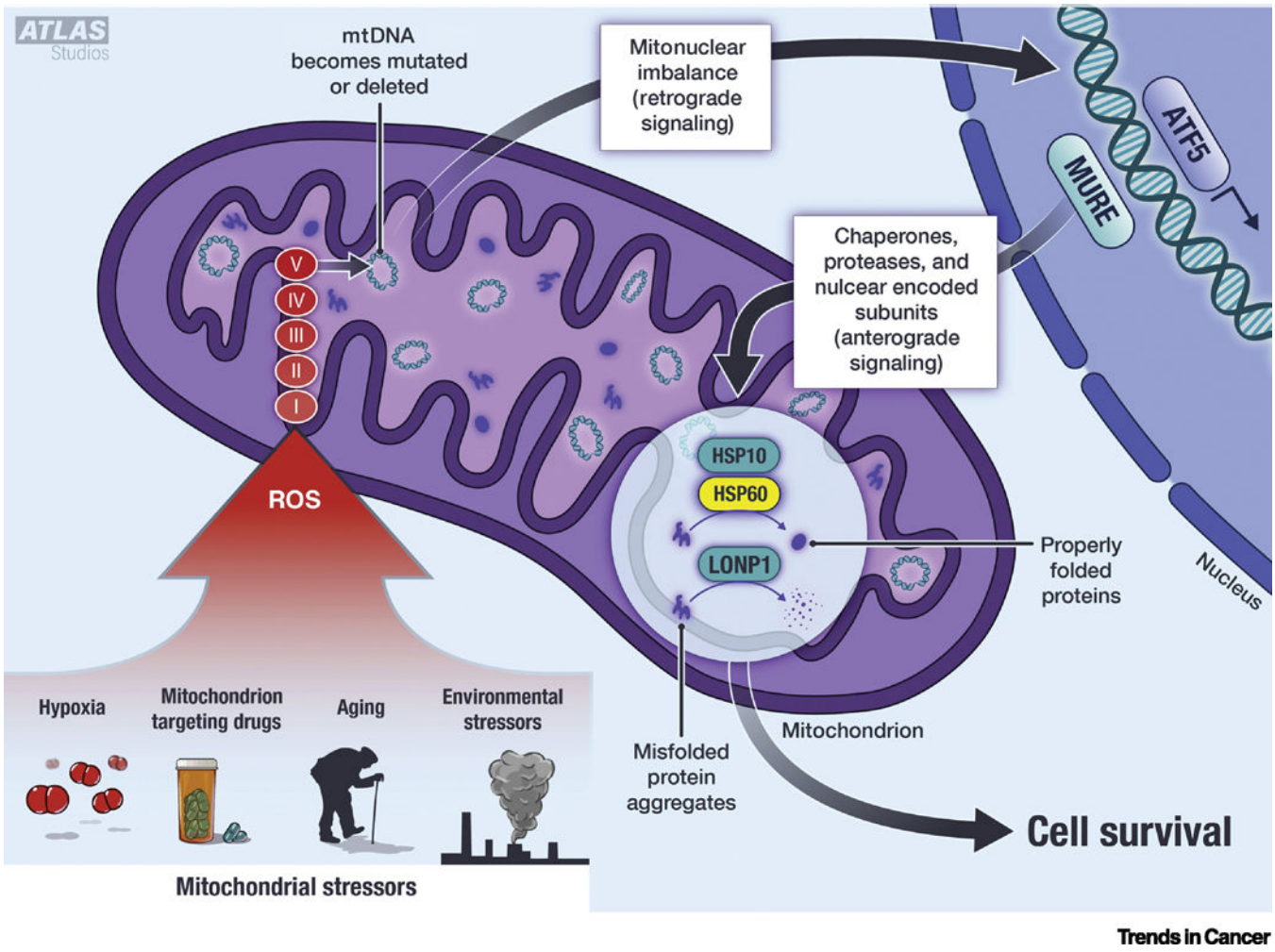

Figure 1. Key Figure. The activation of mitochondrial stress response and cellular signaling.

Mitochondrial stresses associated with aging, environmental toxins, hypoxia and mitotoxic drugs promote ROS production. ROS damages mitochondrial proteins including the OXPHOS complexes, which may further enhance ROS production. The continued ROS production damages mtDNA. The damaged mtDNA and OXPHOS complexes create an imbalance of the mitochondria-nuclear proteins and induce retrograde signaling. This includes nuclear translocation of the UPR^{mt} transcription factor ATF5. ATF5 binds to **M**itochondrial **U**nfolded Protein **R**esponse **E**lement (MURE) to initiate transcription of UPR^{mt} genes including HSP60, HSP10 and LONP1. These transcripts are translated and imported into the mitochondria (anterograde signaling). Once within the mitochondria, HSP60 and HSP10 work together to properly fold damaged proteins, and LONP1 cleaves and degrades those proteins that are damaged beyond repair. The UPR^{mt} proteins promote cell survival by maintaining mitochondrial integrity, thereby preventing cytochrome c release and inhibiting the initiation of intrinsic apoptosis. Abbreviations: HSP = heat shock protein, LONP1 = Lon Protease, ROS = reactive oxygen species, mtDNA = mitochondrial DNA, OXPHOS = oxidative phosphorylation, ATF5 = activating transcription factor 5, MURE = mitochondrial unfolded protein response elements.

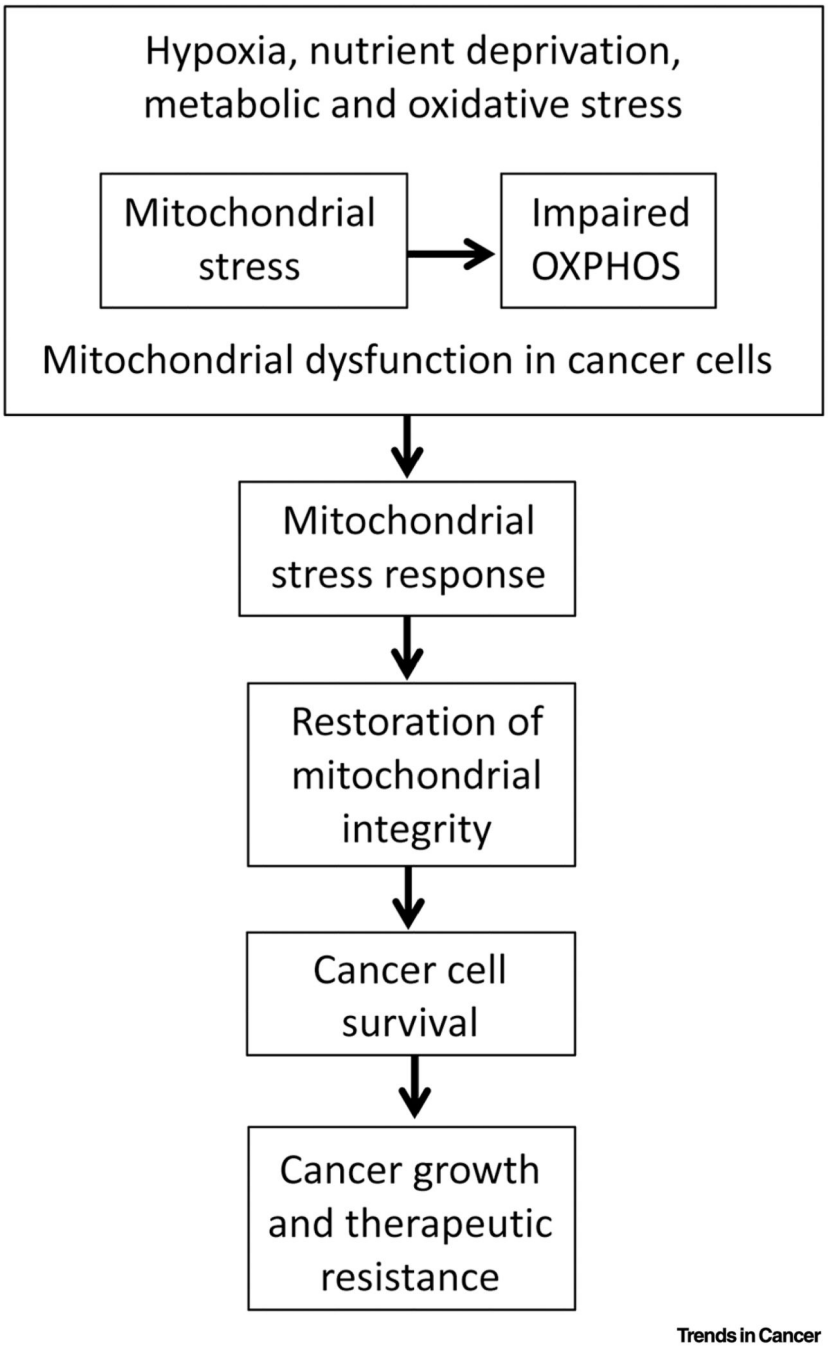

Figure 2. Mitochondrial stress response promotes cancer growth, progression and therapeutic resistance in cancer.

A schematic describing how different stresses induce mitochondrial dysfunction leading to the activation of the mitochondrial stress response, restoration of mitochondrial integrity, cancer cell survival, which subsequently promotes tumor growth and progression.

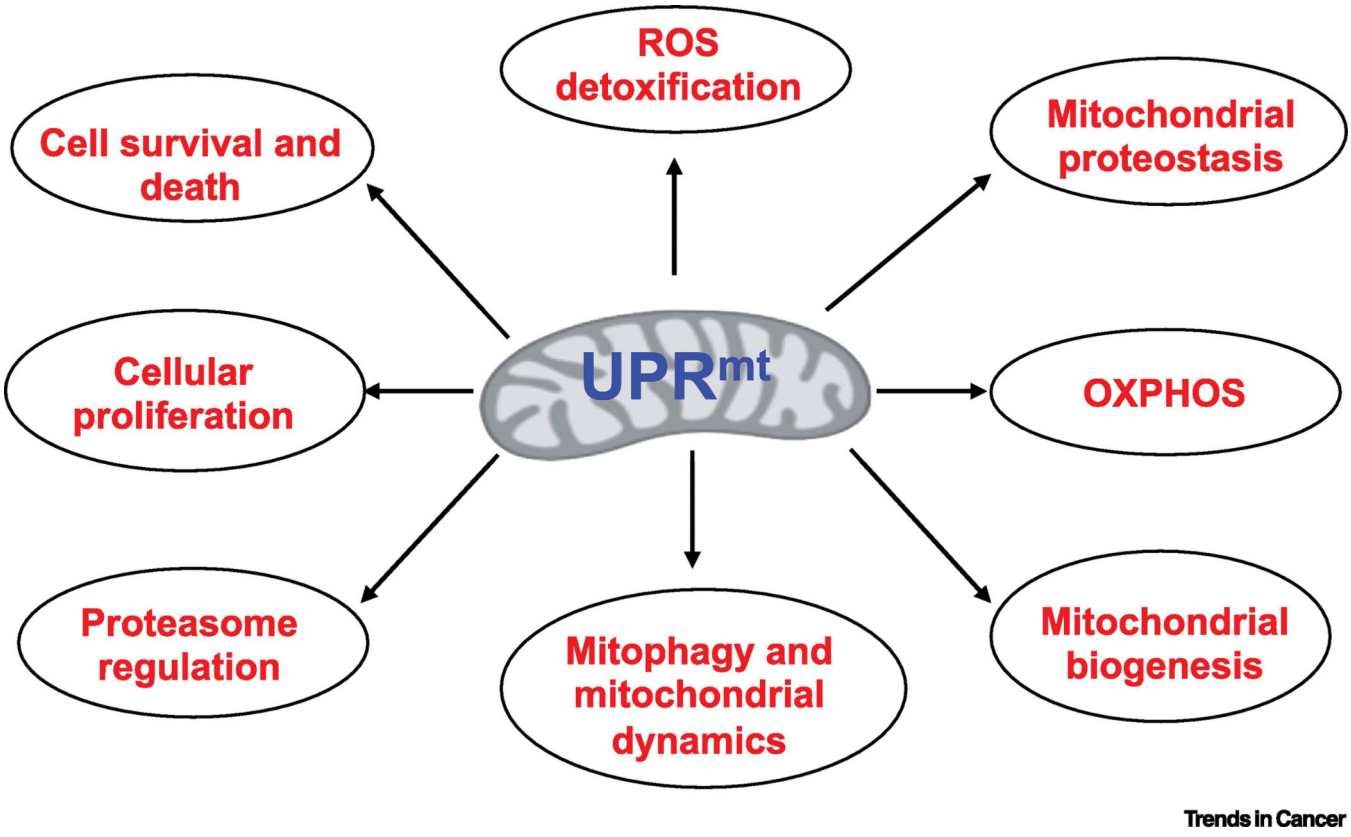

Figure 3. Mitochondrial stress response (UPR^{mt}) regulates various cellular signaling and functions.

A graphical representation depicting the overarching reach of UPR^{mt} signaling in various cellular functions including bioenergetics, mitochondrial homeostasis, ROS detoxification, cell survival and proliferation.