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Interaction Between Mitochondria and Caspases: Apoptotic and Non-Apoptotic Roles

Meenakshi Tiwari[1], Lokendra Kumar Sharma[2], Ajit K. Saxena[1], Madan M. Godbole[3]

[1]Department of Pathology/ Laboratory Medicine, All India Institute of Medical Science-Patna, Bihar, India
[2]Biotechnology Program, Centre for Biological Sciences, Central University of Bihar, Patna Bihar, India
[3]Department of Molecular Medicine, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow- (U.P.) India

Email address:

meenakshimani79@yahoo.co.in (M. Tiwari), lokendra@cub.ac.in (L. K. Sharma)

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Abstract: Mitochondria, play an important role in a variety of processes including energy production, apoptosis, autophagy and inflammation. Caspases, are proteases that play an essential role in mediating apoptotic process of programmed cell death. An association between mitochondria and caspases is very well defined during apoptosis. Besides apoptosis, emerging data is strongly suggesting a direct association between mitochondria and caspases in regulation of a variety of non-apoptotic processes such as mitochondrial dynamics, autophagy, mitochondrial membrane permeabilization, immune responses and differentiation. On one hand mitochondria regulate activation and localization of caspases, caspases also regulate mitochondrial function, thus a feedback loop exist between mitochondria and caspases to regulate a variety of processes. In this review, we have focused on interaction between caspases and mitochondria in the regulation of each other for mediating a variety of apoptotic and non-apoptotic functions. Since both mitochondria and caspases play an important role in development of a variety of diseases like cancer, neurodegeneration, immune disorders, accelerated aging; understanding association between the two will pave the way for better understanding and designing the therapeutics modalities for a variety of diseases. This review is a part of special issue on mitochondria: implication in human health and diseases.

Keywords: Apoptosis, Autophagy, Caspases, Differentiation, Inflammation, Mitochondria, Mitochondrial Dynamics

1. Introduction

Mitochondria are double membrane cellular organelles that contain circular genome, mitochondrial DNA (mtDNA). Mitochondria are involved in several crucial functions of a cell that include generation of cellular energy source-ATP, metabolism, intracellular Ca^{2+} homeostasis, cell signaling, cell death and others. Any dysregulation in these mitochondrial functions are associated with a variety of human diseases and inherited disorders that includes neurodegenerative disorders, cardiomyopathies, metabolic syndrome, cancer, and obesity [1]. Mitochondria associates with a variety of proteins and signaling molecules to regulate the processes required for the maintenance of the normal functions or pathological conditions [2]. One such association is between mitochondria and caspases, cystine rich, aspartate specific proteases [3, 4]. Caspases play a canonical role in apoptosis, a programmed cell death pathway. Caspases are evolutionarily conserved family of proteins with at least 14 different mammalian members [5]. These caspases have been classified as initiators that includes caspase-8, caspase-9 and caspase-2, and executioner caspases that includes caspase-3, caspase-6 and caspase-7. Mainly intrinsic and extrinsic pathways are known to be involved in activation of initiator as well as executioner caspases. Evidence suggests that caspases are activated in cascades in which upstream (activator) caspases lead to activation of downstream (effectors) caspases [6]. Cells are exposed to extrinsic and intrinsic stresses, and caspases are activated as one of multiple stress responses to perform functions like apoptosis, autophagy and differentiation. Thus, caspases actively regulate animal development through both apoptotic and non-apoptotic functions [7]. The best known association between mitochondrion and caspases has been observed during apoptosis where mitochondrion and caspases coordinate to induce cell death in a programmed manner [3]. Emerging data as reviewed here further indicates that besides induction of apoptosis, mitochondria and caspases interact to

regulate crucial processes like autophagy, differentiation, inflammatory responses e.t.c. Furthermore, a role of caspases is also emerging in regulating mitochondrial functions like fusion, fission, mitochondrial membrane permeabilization (MMP) and reactive oxygen species (ROS) production. In this mini review, we have discussed a regulatory association between caspases and mitochondria to modulate a variety of processes that includes apoptotic as well as non-apoptotic functions. Since an important role for mitochondria and caspases has been identified in a variety of diseases and inherited disorders and are potential therapeutic targets; it becomes important to understand the association between the two to improve the understanding of the disease process and to determine effective therapeutic modalities.

2. Association of Caspases and Mitochondria During Apoptosis

Apoptosis, a programmed cell death pathway is the physiologically preferred pathway of cell death [8]**.** Apoptosis is involved in a variety of normal function of an organism that includes maintenance of growth, development, differentiation, immune responses and prevention of cancer. Also, under various pathological conditions, apoptosis is triggered depending upon the stressor and a variety of conditions. Any dysregulation of the apoptotic pathway may lead to the development of diseases [9, 10]**.** Apoptosis which is a tightly regulated process, involves sequential activation of variety of gene products in a well controlled manner. Caspases are the proteases that are the central executioners of apoptosis and are mainly responsible for the typical morphological features observed during apoptosis and results in non-inflammatory removal of the cell. With a few exceptions, all pathways of apoptosis leads to activation of caspases [5, 11]. Multiple cellular pathways trigger apoptosis and two main pathways leading to caspase activation have been well characterized: i) the extrinsic pathway initiated by activation of cell surface receptor thereby leading to direct activation of the initiator caspase-8, and ii) the intrinsic pathway that is regulated by mitochondria by release of a variety of factors that regulate downstream caspase activation including caspase-9 and caspase-3 [6].

For long, it is well established that mitochondria plays a central role in mediating the intrinsic pathway of apoptosis. Identification of Bcl-2, an anti-apoptotic protein that localizes to the outer membrane of mitochondria by Nguyen et. al., highlighted the role of MMP as a central event in induction of apoptosis [12]. Using cell free system, Newmeyer et. al. demonstrated the requirement of mitochondrial-rich fractions for the induction of the apoptotic changes in a cell-free system [13]. Furthermore, a loss of mitochondrial transmembrane potential ($\Delta\Psi m$) during apoptosis was identified as an upstream event in mediating caspase activation, and the event is mainly controlled by the Bcl-2 family of proteins. It has been well established that in response to a variety of signals, Bcl-2 family of proteins alter the integrity of the mitochondrial membrane, thereby regulate the release of proteins from mitochondria inner and outer membrane, and mitochondrial intermembrane space [14]. Out of several members of the family, some are involved in apoptosis inhibition that includes Bcl-2, Bcl-xL, and Mcl-1, and others such as Bax, Bak, Bid, Bim and Puma are involved in apoptosis activation [15-17]. The proteins that are released due to mitochondrial outer membrane permeabilization includes cytochrome c, AIF, Smac/DIABLO, Omi/Htra2, and Endo G, that have well established role in caspase-dependent and independent cell death [18]. In particular, during intrinsic pathway of apoptosis, the release of cytochrome c results in the formation of apoptosome which activates procaspase-9, thereby activating executioner caspase like caspase-3, caspase-6 and caspase-7 [6]. Interestingly, in a study performed by Yuan and group in 1998, it was identified that the mitochondria plays an important role in amplification of extrinsic apoptosis signaling pathway [19]. It was reported that Bid, BH3 domain-containing pro-apoptotic Bcl-2 family member, is a specific substrate of active caspase-8 during extrinsic pathway of apoptosis. Bid is localized in the cytosol, while after cleavage with caspase-8, Bid is truncated (t-Bid) and translocates to mitochondria to transduce apoptotic signal, thereby leading to release of proapoptotic proteins from mitochondria to cytosol. Release of proapoptotic factors from mitochondria results in activation of caspase-9 and the subsequent intrinsic pathway of apoptosis. Thus, during extrinsic pathway of apoptosis, caspase-8 acts upon mitochondria via t-Bid to amplify the apoptosis cascade by activation of the intrinsic pathway of apoptosis. Thus, mitochondria acts to coordinate the existing crosstalk between the two pathways of apoptosis. To add further, studies performed by different groups including ours, have suggested that during stress signaling, caspase-2 activation occurs upstream of MMP followed by the release of cytochrome c, leading to caspase-9 activation by the Apaf-1 apoptosome in the intrinsic pathways of apoptosis in a stimuli specific manner [20, 21]. It has been identified that caspase-2 mediates mitochondrial damage via Bcl-2 family proteins and other mechanism. Furthermore, it has been identified that caspase-2 mediates mitochondrial damage via Bcl-2 family proteins and other mechanism and caspase-2 can get activated downstream of mitochondrial damage, a self-amplifying loop of mutual activation exist. Taken together, as proposed by Green and Kroemer in 1989 mitochondria and caspases engage in a self-amplifying pathway of mutual activation during apoptosis. However, in regard to the role of mitochondria in caspase activation and apoptosis induction, it is important to mention that in Caenorhabditis elegans, as well as Drosophila melanogaster, mitochondrial involvement is not observed in the process of caspase activation and induction of apoptosis [22]. Thus, involvement of mitochondrial pathway, more specifically intrinsic pathways of apoptosis that results in downstream caspase induction seems more specific to mammalian system. Furthermore, mitochondria not only play a role in mediating the intrinsic pathway of apoptosis but also extrinsic pathway of apoptosis. Further identification of the

mediators of the regulatory loop between caspases and mitochondria may be beneficial in understanding the process of apoptosis that plays an important role in a variety of physiological and pathological processes.

3. Mitochondrial Provides a Platform for Activation of Caspases

Caspases, synthesized as inactive pro-enzymes are activated via cleavage to generate proteolytically active fragments during apoptosis and to perform other non-apoptotic functions [6]**.** The localization of caspases in its active or inactive form has been extensively studied due to its importance in various physiological and pathological conditions. It has been identified that caspases (both active and inactive) are localized in cytosol and other subcellular compartments including nucleus, Golgi apparatus, endoplasmic reticulum and mitochondria [23]. It has been suggested that the localization of caspases in a particular compartment in apoptotic and non-apoptotic conditions promote its function as a major sensor of local stress and to perform specific functions against distinct pathways regulated by different modulators of apoptosis.

In a variety of cell types, inactive i.e. the proforms of caspases like procaspase-2, procaspase-3, procaspase-8, and procaspase-9 have been reported to be present in the mitochondria [24-28]. Furthermore, besides inactive, active forms of caspases are also localized in mitochondria. Various groups have reported the presence of procaspase-2 and -3 in mitochondria that translocates to nucleus upon activation [23, 29, 30]. Activated forms of caspase-2, caspase-3, caspase-7 and caspase-9 have been detected in the mitochondria [24, 30]. Still, the presence of caspases in mitochondria has remained debatable. Van Loo et. al., (2002) in their study were unable to detect the presence of active or inactive forms of caspases in the mitochondrial fractions. However, these differences may be due to cell specific variations and experimental conditions [31].

An interesting question arises, how procaspases like caspase-2, caspase-3, caspase-8 and caspase-9 are activated in mitochondria and how the active forms of caspases are translocated in mitochondria to perform their functions. Study performed by Chandra and Tang identified that caspase-9 is activated in the cytosol and then translocates to mitochondria. Further, it has been suggested that translocated active caspases may subsequently activate the mitochondrial procaspases in the organelle [32]. It is still not very clear how the active caspases reaches inside mitochondria, however, it has been proposed that the active caspases might possess a cryptic targeting sequence**.** Additionally, Mancini et. al., in their study identified that caspase-3 precursor localizes in the mitochondria of healthy cells but is absent from this site in the apoptotic cells [25]. Later, in an interesting study, Gonzalvez et. al., showed a novel and unexpected role for mitochondria in particular, mitochondrion-specific lipid cardiolipin (CL), in the activation of the apical caspase-8 in cells that require the mitochondrial amplificatory loop [33]. Their study identified that caspase-8 gets activated upon translocation to the mitochondrion. Mitochondrial cardiolipin provides a platform for the binding of caspase-8 to become activated by proximity. The activated fragments of caspase-8 gets inserted and stabilized on the mitochondrial membrane where it leads to the cleavage of Bid on the surface of mitochondria which is also the target organelle for t-Bid. This finding further indicate a model for localized activation of molecules and compartmentalization of the signaling. For instance compartmentalization of Bid to mitochondria could prevent it from being mistargeted to other intracellular membranes, such as endoplasmic reticulum [34]**.**

Thus, based on various experiments it has been suggested that 1) active caspases translocate to mitochondria and activate other caspases or perform various other proteolytic functions, 2) direct pro-caspases activation takes place within the mitochondrial intermembrane space, and 3) localization of inactive caspases on mitochondrial membrane that provides a platform for its activation. Now the question arises, what is the potential biological function of mitochondrial localized procaspases or active caspases. Multiple piece of evidences suggest that the activation of caspases in the mitochondria may play a role in apoptosis. Firstly, the activated caspases in the mitochondria can activate residual or other resident procaspases in the organelle and thus establishes a positive feedback amplification mechanism. Secondly, they may proteolytically regulate some mitochondrial proteins and thus regulate some of the mitochondrial functions. Based on a variety of reports, it is possible that active caspase-3 as well as caspase-9 in the mitochondria may specifically degrade some mitochondrial substrates such as the mitochondrial respiratory chain protein complexes. Thirdly, the mitochondrial active caspases may further disintegrate mitochondrial integrity and function such as facilitating MMP, cytochrome c release, and generation of ROS. Thus, presence of active as well as pro caspases in the mitochondrion can play a variety of roles in apoptotic as well non-apoptotic processes and needs further investigation.

4. Association of Caspases and Mitochondria in Regulation of Autophagy

Autophagy, a homeostatic and catabolic degradation process, is required for the development, growth and maintenance of an organism. During autophagy cellular proteins and organelles are engulfed by autophagosomes, digested in lysosomes, and recycled to sustain cellular metabolism. Autophagy is also induced during a variety of pathological processes where it plays a role in survival or cell death depending upon the factors like cell type and stimuli. Autophagy is a well regulated process and a number of proteins have been identified that play an important role in the regulation of autophagy [35]. A role for mitochondria has long been implicated in the process of autophagy [36]. Growing amount of evidences are also

indicating towards a role of caspase in regulation of autophagy [37, 38]. Recent reports have further suggested that caspases may regulate autophagy via regulating mitochondrial pathways. In this regard, our group has recently identified a role for caspase-2 in the regulation of autophagy under non stressed conditions [39]. Upon induction of mitochondrial stress, loss of caspase-2 further promotes protective autophagy in different cell types [21, 39]. We also observed that in the absence of caspase-2, mitochondria further elongates under conditions of mild mitochondrial oxidative stress whereas during prolonged conditions the mitochondria are removed by autophagy (unpublished data). We and others have also reported that during stressed conditions, in the absence of mitochondrial-mediated apoptosis, autophagy is the preferred pathway and promotes survival [4, 21, 39, 40]. However, our study did not provide a direct link between caspases and mitochondria. In this regard, recent reports have identified a direct link between mitochondria and caspases in regulation of autophagy. Gorski and group identified a novel role for caspases in regulation of autophagy via mitochondrion under both basal and nutrient stress conditions *in vivo* [41]. They identified that *Drosophila* effector caspase, *Drosophila* caspase 1 (Dcp-1), regulates mitochondrial morphology by localizing within mitochondria. In the absence of Dcp-1 mitochondria elongates and the levels of the mitochondrial adenine nucleotide translocase stress-sensitive B (SesB) and adenosine triphosphate (ATP) increases whereas a significant reduction in autophagic flux is observed in the absence of the effector caspase, Dcp-1. It is important to mention that in *Drosophila*, mitochondria are not involved in downstream activation of caspase to regulate the process of apoptosis, which is in contrast to the mammalian system where mitochondria play crucial role in a variety of apoptotic processes. In another study, Sun et. al., identified that that caspase-1 activation that mainly plays a role during inflammation, plays a role in autophagy regulation by reducing the mitochondrial respiration and ROS and by increasing subsequent clearance of mitochondria in hepatocytes after hypoxia/ reoxygenation [42]. In another study, a role for caspase-9 was identified in regulation of mitochondrial functions and autophagy. The study identified loss of caspase-9 function leads to impairment of apoptosome formation thereby enabling mitochondrial functions and promotes autophagy. However, it was not clear how caspsae-9 modulates mitochondrial function [43]. Further, an interesting study published in Cell Death & Disease by Wirawan et. al., suggested that Beclin-1 a pro-autophagic protein is cleaved by caspases, as a result, it loses its pro-autophagic activity [44]. The fragment of Beclin-a formed acquires a new function. The C-terminal fragment but not N-terminal of Beclin-1 acquires the property to get inserted in the mitochondria, induce the release of proapoptotic factors from mitochondria thereby amplifying the mitochondrial mediated apoptosis activity. However, in a recent study, Yu et. al., identified that in the absence of melanoma 2 (AIM2) and nucleotide-binding oligomerization domain-like receptor pyrin domain-containing protein 3 (NLRP3), inflammasomes trigger caspase-1–dependent mitochondrial damage and caspase-1 inhibits mitophagy to amplify mitochondrial damage, mediated in part by cleavage of the key mitophagy regulator Parkin [45]. Thus, based on recent studies, an important role for caspases in modulating autophagy via modulating mitochondrial functions is emerging. However, interaction between caspases and mitochondria during autophagy needs further investigation for proper understanding and consideration from therapeutic implications.

5. Mitochondrial Fusion and Fission and Caspases

To meet the metabolic demands and to regulate other cellular functions like cell death, redox signaling, oxidative stress generation; mitochondria responds dynamically and undergo changes in shape, structure and ultrastructural architecture. This dynamicity is regulated by fusion and fission of mitochondria and an aberration in the process has been associated with a variety of diseases including aging and neurodegenerative disease [46]. Extensive mitochondrial fragmentation has been observed during apoptosis [47]. An essential role of mitochondrial fission in caspase activation and thereby cell death is well established and inhibition of the mitochondrial fission machinery blocks or delays cell death [48]. Interestingly, emerging data also indicates a role for caspases in regulating mitochondria dynamics. In a study conducted by Peng et. al., role for caspases in the regulatory mechanisms of mitochondrial dynamics was identified [49]. Their group studied the effects of squamocin, a known apoptosis inducer on CHO cells. Their study further demonstrated that the inhibitors of caspase-8 and caspase-9 can partially rescue mitochondrial fragmentation by interacting with mitochondrial fusion-fission regulatory proteins to influence morphology and functions of mitochondria. It was proposed that caspase-8 and caspase-9 activation leads to activation of caspase-3, which in turn degrades Bcl-2. Reduction of Bcl-2 favors Bax translocation to mitochondria and promotes mitochondrial fission [50, 51]. Further, Loucks et. al. (2008) also showed that caspase-8 can directly inhibit OPA1, a positive regulatory protein for mitochondrial fusion. Thus, besides a role for mitochondrial fission in caspase activation, a role of caspases in controlling mitochondrial dynamics cannot be underscored and may be additionally responsible for the feedback amplification loop between mitochondria and caspases signaling during apoptotic and non-apoptotic processes.

6. Role of Caspases in Disruption of Mitochondrial Membrane Potential and Generation of ROS

A variety of functions performed by mitochondria that includes ATP generation, Ca^{2+} homeostasis, lipid metabolism, protein import e.t.c. depends on the maintenance of $\Delta\Psi m$ [52]**.** Depending on the extent, the loss of $\Delta\Psi m$ has been associated

with disruption of mitochondrial functions, ROS generation and cell death. For long, it has been well established that during apoptosis, MMP results in the release of cytochrome c and subsequent caspase-9 and executioner caspase activation, leading to cell death by apoptosis. In this process, a role for proapoptotic members of the Bcl-2 family induced permeabilization of the mitochondrial outer membrane [14, 53] is well established. Besides undergoing changes in outer mitochondrial membrane permeabilization, mitochondria also loses its ΔΨm, functions and generates higher levels of ROS. On one hand where it is well established that in most of the circumstances caspase-9 activation is downstream to MMP, caspase-8 and caspase-2 have been shown to act upstream to the events associated with MMP, still, the relationship between the of loss of ΔΨm and caspases remains obscure. In this regard, studies performed by Waterhouse et. al., in 2001 demonstrated that in the absence of caspase activation ΔΨm does not change and remains intact [54]. Further, studies performed by different groups identified an involvement of caspase in mediating these mitochondrial changes. In an interesting study performed by Douglas Green and his group, it was identified that the activation of caspases results in a rapid loss of ΔΨm and ROS generation [14]. Importantly, their study also suggested that after mitochondrial outer membrane permeabilization and the activation of caspases, the caspases target complexes I and II of the electron transport chain. This results in a sustained loss of ΔΨm and production of ROS, both of which may then contribute to the rapid dismantling of the cell. Further, it has been observed by different groups that in the absence or inhibition of caspases, ΔΨm is maintained after cytochrome c release, suggesting an important role of caspases in regulation of ΔΨm during stress conditions and mitochondria may be among the earliest targets of caspase activation [54-57]. It is important to note that in the absence of mitochondrial outer membrane permeabilization, active caspases do not alter ΔΨm and mitochondrial outer membrane permeabilization itself does not interfere with the function of the electron transport chain unless caspases are subsequently activated. Further, the findings by Cepero et. al., in 2005 demonstrated that during intrinsic cell death stimulation, caspase-9 and effector caspases have sequential and distinct effects on mitochondria [58]. Caspase-9 can prevent accessibility of cytochrome c to complex III in the mitochondria, resulting in an increase in ROS production, but in the presence of effector caspase activity, ROS production is terminated. Later on Brentnall et. al. identified the role of caspase-9-mediated cleavage of Bid on ROS production [59]. Based on these observations it appears that caspases-mediated remodeling of mitochondria results in an amplification loop that may set a point of no return from the cell death pathways (Fig. 1). However, studies on the role of caspases in modulating ΔΨm and ROS during non-apoptotic processes are lacking and needs to be investigated further.

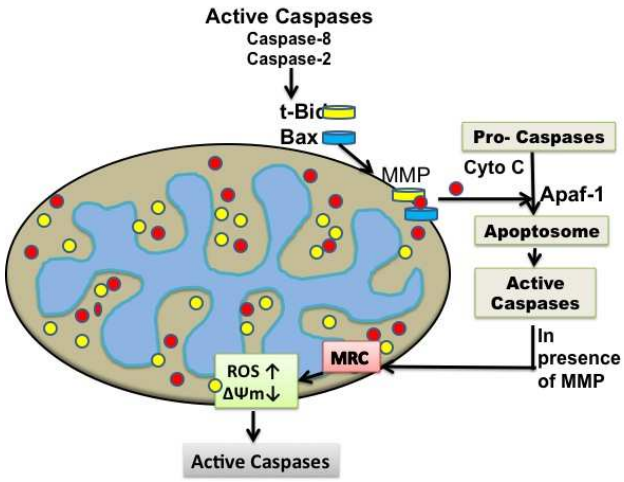

Figure 1. *Interaction between mitochondria and caspases to regulate mitochondrial membrane potential and ROS: Active caspases like caspase-8 and caspase-2 activated via intrinsic or extrinsic or caspases regulate mitochondrial membrane permeabilization by modulating BH3 family members (activation of Bax and Bid) thereby leading to release of pro-apoptotic molecules including cytochrome c, from mitochondria. Release of cytochrome c and other such molecules leads to activation of caspases like caspase 9 and other effectors caspases. These activated caspases further lead to loss of ΔΨm and generation of ROS via a variety of mechanism that includes cleavage of mitochondrial respiratory chain complex (MRC) proteins. An increase in ROS and decrease in ΔΨm further leads to increase in caspases activation resulting in a positive feedback mechanism of regulation.*

7. Association Between Caspases and Mitochondria Regulates Immune Response

In order to survive, organisms develop immune responses against invading microorganisms and to eliminate the infection. Caspases not only play an important role during apoptosis but also regulate host responses to invaders and injury. The inflammatory caspases mainly caspase-1, caspase-4, and caspase-5 are associated with immune response against pathogens and their activation occurs upon assembly of an intracellular complex, designated as inflammasome/ NOD-like receptor family, pyrin domain (NALPs). This results in the cleavage and activation of the proinflammatory cytokines like IL-1β and IL-18. Thus, caspases are activated in response to sensors of pathogens or danger, they are also regulated to terminate the inflammatory response and control cell survival [60]. However, how these inflammasomes are activated is still not well understood. Based upon growing amount of literature, a role for mitochondria is proposed in the proper function of the innate and adaptive immune system as well as immune signaling pathways [61]. It has been demonstrated that dysfunctional mitochondria generate ROS, which is required for inflammasome activation, thus leading to activation of caspases [62]. Studies have also suggested a role for mitochondria in providing a platform for innate antiviral signaling and in orchestrating the innate immune response for disruption of homeostasis. Additionally, mitochondria influence antiviral signaling via the production of ROS [63]. NLRP3

translocates from the endoplasmic reticulum to the mitochondrion when activated and mitochondrion-derived ROS are required for activation of NLRP3 inflammasome [64]. Interestingly, an association between mitochondria and caspases in mediating inflammatory response against viral infection has recently been identified. It has been proposed that caspases are responsible for immunologically silent cell death where as necrotic cell death results in the release of molecules with proinflammatory properties, collectively termed damage-associated molecular patterns (DAMPs) or alarmins [65]. These DAMPs contribute to the recruitment and activation of inflammatory cells of the immune system such as granulocytes and monocytes/ macrophages. In a recently published study in "Cell" by Rongvaux and his group, a novel mechanism by which dying cells expose an intracellular DAMP that activates a cell intrinsic innate immune response has been identified in regulation of antiviral immunity [66]. Their study identified a novel mechanism by which mitochondria and downstream proapoptotic caspases regulate the activation of antiviral immunity. Bax/Bak dependent apoptosis is generally considered as a noninflammatory type of cell death, where as their study identified that Bax and Bak contribute actively in induction of the type I interferons (IFNs). Type I IFNs (IFNa and IFNb) are cytokines of major importance for the innate antiviral response by promoting the capacity to interfere with every step of viral replication, and, as a consequence, the IFN response results in the establishment of a cellular state of viral resistance [67]. Rongvaux et. al. identified that the induction of IFNs is mediated in a mitochondrial DNA-dependent manner that is regulated by caspase-activation. In their study it was demonstrated that besides Bax and Bak translocation to mitochondria, an activation of caspases is essential to induced cell death without inducing an inflammatory reaction. Their study highlighted an association between caspases and mitochondrial interaction to determine the fate of dying with or without generating immune response. Their results indicated that in the absence of caspases, type-I IFNs are induced in response to viral infections where as in the presence of caspases, required to maintain this type of cell death immunologically silent. In the absence of active caspases, mitochondrial outer membrane permeabilization by Bax and Bak results in the expression of IFNs. Further, in a study performed by Maadidi et. al. (2013), it was identified that SFV, a positive-sense ssRNA virus, stimulates the formation of a novel mitochondrial platform consisting of the innate immune signaling component MAVS and the initiator caspase-8 [68]. The purpose of this platform is to trigger caspase-3 activation and apoptosis in an entirely Bax/Bak- and death receptor/FADD–independent manner. The signaling pathway leading to this event is initiated by dsRNA, transiently formed during the RNA replication cycle of the virus.

8. Caspases and Mitochondria During Cellular Differentiation

Cell specialization is the defining hallmark of multicellular life forms that results from the process commonly referred to as differentiation. A role for the process of apoptosis has been identified during differentiation [69]. More generally, it has been suggested that the process of differentiation is regulated by a subtle balance of repression and activation between proliferation and apoptosis. Besides playing a role in apoptosis induction during the process of differentiation, various reports have identified an involvement of caspases in differentiation of a variety of cell types that includes differentiation of skeletal myoblasts, lens cell, erythrocyte, platelets and keratinocytes; the process is regarded as caspase-mediated incomplete apoptotic process. [70-73]. A role for mitochondria is also well defined in the process of differentiation [74]. Various groups have also identified an association between mitochondria and caspases in regulating the process of differentiation. In a study performed by Murray et. al., a novel mechanism was identified during muscle cell differentiation which demonstrated an involvement of mitochondrial pathway in caspase activation during the differentiation of skeletal myoblasts into myotubes; where mitochondrial pathway lead to activation of caspase-9 and caspase-3 but was not associated with any detectable release of cytochrome *c* or Smac (Diablo) or activation of apoptotic cell death [75]. Further, Polakowska and his group demonstrated that keratinocyte differentiation involves components of the mitochondria- and caspase-dependent apoptotic machinery [76]. Their results demonstrated that the differentiation process in epidermal keratinocytes utilizes process involving mitochondrial changes followed by cleaved caspase-3 in terminally differentiating keratinocytes and may contribute to the degradation process in the late stages of epidermal cell differentiation. In another study performed by Sanders and Parker, a non-apoptotic association between caspase-9 and mitochondria to induce differentiation of secondary lens fiber cells from the lens epithelium was identified [77]. Later, in an interesting study, Sordet et. al., identified activation of caspase-3 and caspase-9 downstream to mitochondria in human peripheral blood monocytes induced to differentiate into macrophages in response to macrophage colony stimulating factor and was not associated with cell death [78]. Later, in an interesting study Arama et. al., in 2004 identified that caspase activity is required for spermatid individualization in Drosophila melanogaster [79]. Activation of the effector caspase drICE in spermatids requires one of the two cytochrome c genes of Drosophila, cyt-c-d. from mitochondria. How caspase activity is restrained and guided to influence differentiation without inducing cell death awaits future studies.

9. Conclusions and Future Prospective

Mitochondria are major players in a variety of physiological as well as pathological processes. Similarly, caspases, best known for their role in apoptosis are also emerging as an important modulators in both pathological and physiological processes. Thus, mitochondria and caspases are potential therapeutic targets in a variety of diseases and disorders that

include neuro-degeneration, aging, cancer, of the two may affect the function of the other and may effect a variety of functions regulated by either caspases or mitochondria (Fig. 2). Since, the role for both caspases and mitochondria are emerging in regulation of a variety of processes whether individually or in association, it becomes important to delineate the pathways more specific to cell types, stimuli and the key molecules involved in cross regulation.

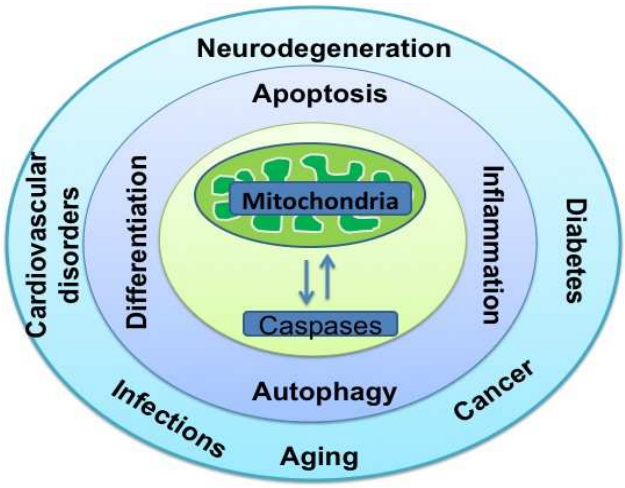

Figure 2. *Interaction between mitochondria and caspases in the regulation of variety of processes: Mitochondria and caspases interact with each other to regulate a variety of processes including apoptosis, autophagy, inflammation, differentiation etc. Any dysregulation in this regulation may lead to a variety of diseases including neurodegeneration, cardiovascular disorder infection, aging, cancer and diabetes.*

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