— **Review** —

The Role of Mitochondrial Chaperone Tumor Necrosis Factor-associated Protein 1 (TRAP1) in the Regulation of Apoptosis

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An increase of mitochondrial membrane permeability is one of the key events in apoptosis, since it leads to the release of mitochondrial apoptogenic factors, such as cytochrome *c*, into the cytoplasm that activate downstream target of apoptotic cell death. Bcl-2 family is one of the best-characterized proteins that directly regulate mitochondrial functions. A major role of the Bcl-2 family of proteins is to alter mitochondrial membrane permeability, thus controlling the release of caspase-activating cytochrome *c*. Recent reports describe about involvement of interesting apoptogenic regulators other than Bcl-2 family in regulation of mitochondrial function. Tumor necrosis factor receptor-associated protein 1 (TRAP1) is a member of the heat-shock family of mitochondrial proteins, and substantially homologous to members of the 90-kDa families of heat-shock proteins (HSP90). TRAP1 seems to have specific functions differ from those of other members of the HSP90 family. Downregulation of TRAP1 expression enhances the release of cytochrome *c* from mitochondria. Moreover, reactive oxygen species (ROS) are involved in the regulation of the TRAP1 expression, indicating that TRAP1 is a sensor that involved in ROS mediated regulation of apoptosis. Here, we describe the mechanisms underlying the regulation of mitochondrial functions during apoptosis by TRAP1.

Key words —— apoptosis, Bcl-2, heat shock protein, mitochondria, oxidative stress, TRAP1

INTRODUCTION

Apoptosis is a form of programmed cell death, and originally defined in terms of characteristic changes in cell morphology. It has been implicated in a variety of biological process, such as embryogenesis, regulation of the immune system, cytotoxic cell killing of virally infected cells, and the elimination of damaged cells. So dysregulation of programmed cell death leads to a variety of diseases in humans, including certain neurodegenerative diseases and cancer.

The molecular basis of apoptosis have been extensively studied over the last 10 years, and revealed several important mechanisms. The mitochondria play a crucial role in the apoptotic cell death by releasing various apoptogenic proteins, including cytochrome *c*, into the cytoplasm.[1] In the process of apoptosis, release of cytochrome *c* into the cytoplasm activates death-driving proteolytic enzymes known as caspases, which in turn cleave a set of cellular proteins and cause cell death.[2,3] These mitochondrial functions is regulated by several sets of factors, among which the best characterized is the Bcl-2 family.[4,5] The Bcl-2 family of proteins regulates the changes in mitochondrial functions during apoptosis. A major role of the Bcl-2 family of proteins is to alter mitochondrial membrane permeability, thus controlling the release of caspase-activating cytochrome *c*. Although the mitochondrial contribution to apoptotic cell death is well established, the detailed mechanisms underlying the increase of outer mitochondrial membrane permeability during apoptosis, and how this process is controlled are still to be determined.

Interestingly, damage to cells can induce one of two opposing responses. Apoptotic cell death re-

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moves damaged cells to prevent inflammation and stress responses induce several heat shock proteins (HSPs) to prevent cells from damage. Interactions between these two pathways determine the fate of a cell, having a profound effect on the biological consequences of stress. HSPs, also called stress proteins, are highly conserved proteins that is induced by various kinds of stimuli.[6] Many data accumulated in the recent years clearly demonstrate that HSPs have essential properties that are involved in the regulation of apoptotic process. This review is primarily focused on the function of tumor necrosis factor receptor-associated protein 1 (TRAP1), a member of the heat-shock family of mitochondrial proteins, and also discusses about the mechanism of the regulation of mitochondrial function by TRAP1.

A MITOCHONDRIAL ROLE IN CELL DEATH

In recent years, the role of mitochondria in cell death has received considerable attention. The changes in mitochondrial membrane permeability are one of crucial events in cell death, including apoptosis and necrosis. Mitochondria play an important role in apoptotic process of mammalian cells by releasing apoptogenic proteins into the cytoplasm.[1,7] Most of the mitochondrial apoptogenic factors are mainly localized to the intermembrane space between the outer and inner mitochondrial membranes. Therefore, an increase in the permeability of these membranes is required for the release of apoptogenic factors. Cytochrome *c* is the first characterized apoptogenic molecules shown to be released from the mitochondrial intermembrane space and to be actively involved in apoptotic cell death. Once released in the cytosol, cytochrome *c* is binds to an adaptor protein, Apaf-1, which self-oligomerizes and recruits pro-caspase-9 to form complex. The protein complex consist of cytochrome *c*, Apaf-1 and caspase-9 is called the apoptosome. Activated caspase-9 cleaves and activates effector caspases such as caspase-3, then degrade a variety of target substrates and cause cell death.[2] Other mitochondrial proteins, such as apoptosis inducing factor (AIF), Smac/DIABLO, endonuclease G and Omi/HtrA2, were also found to undergo release during apoptosis and have been implicated in various aspects of the cell death process.[8]

Apoptogenic factors are released from mitochondria as a results of the opening of the permeability transition pore (PTP). Opening of the PTP is postulated to the results in the loss of mitochondrial membrane potential, swelling of the mitochondrial matrix and rupture of the outer membrane, allowing the release of proteins from the intermembrane space.[9,10] This process has been termed the mitochondrial membrane permeability transition (MPT). The MPT can be triggered under various conditions, such as elevated matrix Ca^{2+}, especially when this is accompanied by oxidative stress and depleted adenine nucleotides. Although the molecular mechanisms of the MPT are largely unknown, the most widely accepted working hypothesis is that it occurs after the opening of a channel complex that is known as PTP, which putatively consists of the voltage-dependent anion channel (VDAC: a channel in the mitochondrial outer membrane), the adenine nucleotide translocator (ANT: inner membrane channel), cyclophilin D (Cyp D), and possibly other molecule(s) (Fig. 1).[11] VDAC is a component of the PTP complex and interacts with other components including ANT and plays a important role in mammalian apoptosis by regulating the permeability of the mitochondrial membrane. Since permeability transition is controlled by cellular and mitochondrial conditions such as redox state, Ca^{2+} lev-

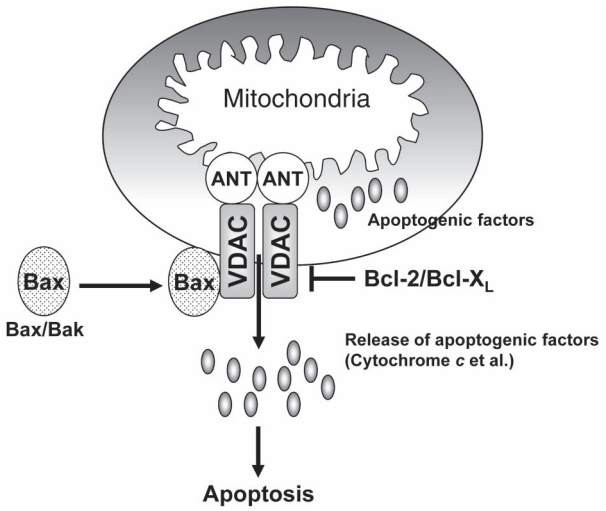

Fig. 1. Mitochondrial Function in Apoptosis (PTP model)

An increase in the permeability of the outer mitochondrial membrane is important for apoptotic process. The PTP complex consists of several transmembrane proteins with core components VDAC in the outer mitochondrial membrane and ANT in the inner mitochondrial membrane. Activated Bax/Bak interact with VDAC of the PTP complex and induce its opening resulting in the release of intermembrane space proteins such as cytochrome *c*. Anti-apoptotic Bcl-2 proteins, such as Bcl-2 and Bcl-x_L, prevent the increase in mitochondrial membrane permeability by ihibiting the opening of the PTP.

els and respiration, it appears that VDAC is regulated indirectly by these conditions. VDAC has three isoforms, including VDAC1, 2 and 3. The polyclonal antibodies of VDAC that prevent activity of these isoforms, have been shown to inhibit the Ca^{2+}-induced MPT, suggesting an important role of VDAC in the regulation of MPT.[12] The ANTs also have isoforms of ANT1 and ANT2 in mouse and are considered to be a crucial role for the MPT. However, recent report describes the possible or partial involvement of ANTs in the regulation of MPT. Mitochondria lacking ANT could still be induced to undergo permeability transition, resulting in release of cytochrome *c*, although the triggering Ca^{2+} concentration was slightly increased, suggesting that ANTs are non-essential structural components of the MPT, although they do contribute to its regulation.[13] Cyp D is a cyclophilin family, which has peptidyl prolyl-cis, trans-isomerase (PPIase) activity and possesses a important role in protein folding. The putative role of Cyp D in the MPT was firstly suggested by the finding that the MPT is blocked by cyclosporine A, a specific inhibitor of the PPIase activity of cyclophilins.[14] Recently, significant progress has been made by studies performed with Cyp D-deficient mice at several laboratories, which have demonstrated that Cyp D is essential for the MPT to occur and that the Cyp D-dependent MPT regulates some forms of necrotic, but not apoptotic, cell death.[15] By analyzing Cyp D-deficient mice in more detail, some interesting physiological roles of the MTP should be obtained.

MPT during apoptosis is regulated directly by the Bcl-2 family of proteins.[1] Bcl-2 was firstly shown to play a role in cell survival by a study on cell death induced by deprivation of interleukin (IL)-3 in lymphoid cell line and subsequently reported that Bcl-2 also inhibits cell death induced by various stimuli such as a chemotherapeutic agent, ethanol and heat shock, indicating Bcl-2 as a negative regulator of cell death.[16, 17] On the basis of various structural and functional characteristics, the Bcl-2 family of proteins is divided into three subfamilies. Anti-apoptotic (Bcl-2, Bcl-x_L, Bcl-w, Mcl-1, Boo, Bcl-B), the multi-domain pro-apoptotic (Bax, Bak Bok Bcl-rambo), and Bcl-2 homology (BH) 3-only protein subfamily (Bik, Bad, Bid, Bim, Noxa, Bnip3, Bmf, Puma) are characterized by the presence of BH domains.[18–20] Anti-apoptotic members of the Bcl-2 family inhibit the release of apoptogenic factors such as cytochrome *c* and Smac/Diablo from mitochondria, whereas pro-apoptotic members including BH3-only protein subfamily promote the release of these factors. The major function of Bcl-2 family members is to regulate directly mitochondrial membrane permeability and thereby regulate release of apoptogenic molecules from the intermembrane space into the cytoplasm (reviewed by Tsujimoto and Shimizu).[21, 22] Anti-apoptotic subfamily, such as Bcl-2 and Bcl-x_L, prevent the Bax/Bak-induced increase of mitochondrial membrane permeability by direct interaction with these pro-apoptotic members of this family, and also inhibit the MPT itself.[23, 24] As described above, VDAC plays an essential role in mammalian apoptosis and MPT.[12] However, involvement of ANT in MPT is unclear because ANT deficient mitochondria still underwent the MPT.[13] So VDAC might be a key functional target of Bcl-2 family of proteins for the regulation of MPT. Indeed, Bcl-2 is able to inhibit the activity of VDAC (Fig. 1).[23] Anti-apoptotic members of the Bcl-2 family, such as Bcl-2 and Bcl-x_L might prevent the induction of MPT by inhibiting VDAC or other functional proteins for MPT.

ROLE OF HSP FAMILY IN THE REGULATION OF APOPTOSIS

Apoptosis is induced by a variety of stimuli, such as growth factor withdrawal, hypoxia, heat shock and DNA damage. Heat shock is one of extracellular stimuli that are involved in the induction of apoptosis and it results in the synthesis of a set of HSPs. Interestingly, these stimuli, described above, induce the expression of HSPs, suggesting that induction and accumulation of HSPs have essential role in regulation of apoptosis. These proteins form a large family, which includes HSP90, HSP70, HSP60, and other small HSPs, and they play important roles in various aspects of cell homeostasis by functioning as molecular chaperones.[25, 26] The most conserved and best-studied class of HSPs is the HSP70 family. HSP70 protects cells from a number of apoptotic stimuli, such as heat shock, radiation, oxidative stress, withdrawal of growth factors, chemotherapeutic agents, ceramide, and tumor necrosis factor (TNF).[27–31] HSP90 appears to be involved in the inhibition of apoptosis by suppressing the cytochrome *c*-mediated oligomerization of Apaf-1.[32] A similar mechanism for suppression of apoptosis that involves HSP27, another member of the HSP family, has also been reported. HSP27

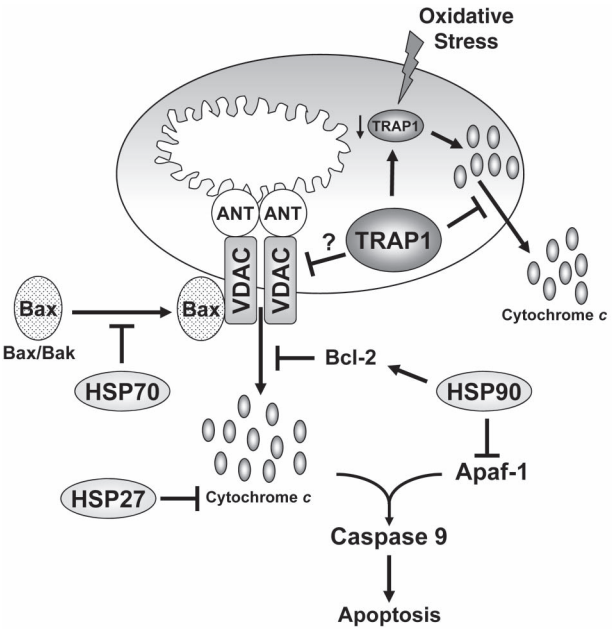

Fig. 2. Schematic Representation of HSPs and TRAP1 Function in Apoptosis

HSPs are able to regulate apoptosis induction by interacting with several key proteins. HSP70 inhibits Bax translocation to mitochondria, and prevent mitochondrial outer membrane permeability. HSP27 blocks the activation of caspases by directly sequestering cytochrome *c* when released from mitochondria. HSP90 prevents cytochrome *c*-mediated oligomerization of Apaf-1 and inhibits the activation of procaspase-9. TRAP1 seems to be involved in the regulation of cytochrome *c* release from mitochondria. At the mitochondrial level, TRAP1 may act as a sensor of oxidative stress.

blocks etoposide-induced apoptosis.[33] These observations suggest that HSPs might play important roles in the regulation of apoptosis. Especially, some of these HSPs have a crucial roles in mitochondrial function.

HSP70, associated with HSP40, inhibits Bax translocation to mitochondria, and prevent mitochondrial outer membrane permeability (Fig. 2).[34] Thus HSP70 inhibits the release of cytochrome *c* and that of other apoptogenic molecules such as AIF. HSP27 blocks etoposide-induced apoptosis by preventing the cytochrome *c* and dATP-triggered activity of caspase-9, downstream of the release of cytochrome *c* (Fig. 2).[33] HSP27 has also been shown to prevent the mitochondrial release of Smac/DIABLO that is one of mitochondrial XIAP antagonist, and thereby to confer resistance of myeloma cells to dexamethasone.[35] By contrast to the functions of HSP70 and HSP27, which act in many different steps or apoptotic pathways, HSP90 appears to operate via a more specific inhibitory mechanism. Inhibition of binding of Akt to HSP90 leads to the dephosphorylation and inactivation of Akt, resulting in the increased sensitivity of cells to the induction of apoptosis.[36] As described above, a recent report describes the negative regulation of the cytochrome *c*-mediated oligomerization of Apaf-1 and the activation of procaspase-9 by HSP90 (Fig. 2).[32]

TRAP1 was initially identified as a type I TNF receptor-binding protein by yeast two-hybrid screening, which is an efficient method for studying interactions among proteins.[37, 38] An analysis of the cDNA sequence revealed that human TRAP1 is identical to HSP75, which is a member of the HSP family of molecular chaperones that interact with the retinoblastoma protein during mitosis and after heat shock.[39] TRAP1 is substantially homologous to members of the HSP90 and is expressed both in transformed cells and in a wide variety of normal tissues.[37] However, TRAP1 does not form a stable complex with the classic co-chaperones of HSP90, such as p23 and Hop,[40] even though HSP90 is able to form multiprotein complexes with these co-chaperones. Moreover, immunofluorescence experiments showed that human TRAP1 is localized in mitochondria and, indeed, mitochondrial localization sequences have been found at the amino terminus of this protein.[40] Thus, it appears that TRAP1 has specific functions for mitochondria that differ from those of other members of the HSP90 family.

INVOLVEMENT OF TRAP1 IN MITOCHONDRIAL FUNCTION

We found that expression of TRAP1 was significantly suppressed upon treatment of human cancer cells DMS114 with apoptosis inducer, such as etoposide and β-Hydroxyisovalerylshikonin (β-HIVS), a compound isolated from the traditional oriental medicinal herb *Lithospermum radix* (Fig. 3).[41] The suppression of TRAP1 expression in mitochondria during apoptosis prompted us to study the possible role of TRAP1 in mitochondria and to examine the regulatory relationship between the TRAP1 expression and the mitochondrial function. Although TRAP1 was identified originally as a TNF receptor-binding protein,[37] which suggested that one of the cellular targets of TRAP1 might be a transmembrane cell-surface protein, recent work has demonstrated that TRAP1 is localized to mitochondria.[40] However, it has been reported that TRAP1 is also present in the cytoplasm,

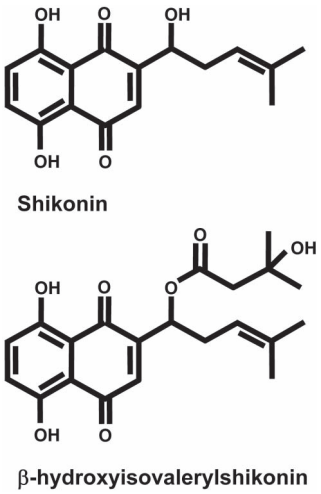

Fig. 3. Chemical Structures of Shikonin and Its Derivative β-HIVS

where it interacts with the retinoblastoma protein during mitosis or heat shock.[39] Indeed, we investigated the subcellular localization of TRAP1 by subcellular fractionation, and detected TRAP1 in the mitochondrial fraction but not in isolated nuclei or in the cytosolic fraction in human lung cancer DMS114 cells. When the cells were treated with either β-HIVS or VP16, suppression of the expression of TRAP1 by small interfering RNA enhanced the release of cytochrome *c* from mitochondria. While cytochrome *c* is released from mitochondria into the cytosol in response to apoptotic stimuli, the expression of TRAP1 in mitochondria was suppressed without the apparent release of TRAP1 into the cytosolic fraction during apoptosis, suggesting that changes in the level of expression of TRAP1 in mitochondria might be responsible for the release of cytochrome *c* (Fig. 2).[41] This hypothesis is also supported by our cell-free system using isolated mitochondria. However, how does TRAP1 regulate the mitochondrial function or release of cytochrome *c* from mitochondria? Previous study demonstrated that TRAP1 associated with one of PTP proteins, such as VDAC.[42] As described above, the VDAC play a crucial role in the regulation of MPT,[12] whereas the ANT might not be important.[13] TRAP1 might directly modulate release of apoptogenic molecules from mitochondria through the association of VDAC (Fig. 2).

It has been reported that β-HIVS inhibits the activity of several tyrosine kinases, such as v-Src and a receptor for epidermal growth factor *in vitro*,[43] and that it exerts its apoptosis-inducing activity via the inhibition of tyrosine kinases.[44] Thus, it is possible that tyrosine kinases might be direct targets of β-HIVS in the induction of apoptosis. Indeed, when DMS114 cells were exposed to β-HIVS, the extent of tyrosine phosphorylation of proteins was reduced while the number of apoptotic cells increased. These results suggest that expression of TRAP1 might be regulated by the tyrosine kinases upstream of TRAP1. However, genistein and STI571, two specific inhibitors of protein tyrosine kinases, did not suppress the expression of TRAP1, even though they significantly inhibited the activity of tyrosine kinases.[41] Moreover, VP16, an inhibitor of topoisomerase II that does not inhibit the activity of tyrosine kinases, suppressed the expression of TRAP1,[41] suggesting that inhibition of the activity of tyrosine kinases is not involved in the regulation of TRAP1 expression. However, it is noteworthy that N-Acetyl-cysteine, a scavenger of reactive oxygen species (ROS), efficiently prevented apoptosis induced by β-HIVS and VP16 but not apoptosis induced by camptothecin and cisplatin. Furthermore, the β-HIVS-induced suppression of the expression of TRAP1 was blocked by N-acetyl cysteine, indicating the involvement of ROS in the regulation of TRAP1 expression. TRAP1 might be a sensor or important molecule that is able to link the relationship between regulation of apoptosis and oxidative stress (Fig. 2).

POSSIBLE FUNCTION OF TRAP1 IN DISEASES

Mitochondria are involved in many cellular processes and dysfunctions of mitochondria have been associated with a number of physiological conditions, including several diseases. Ischemia and subsequent reperfusion cause ROS overproduction by mitochondria, and then leading to an increase in oxidative stress.[45] Overexpression of mitochondrial TRAP1 in rat brain protects mitochondria, reduces oxidative stress, and protects from focal ischemia,[46] suggesting that targeting mitochondria for protection may be a useful strategy to reduce ischemic brain injury. Another report describe about the relationship between osteoarthritis (OA) and TRAP1 expression. OA, the most common age-related cartilage and joint pathology, is a slowly progressive degenerative disease characterized by degradation of the matrix and cell death, which result in a gradual loss of articular cartilage integrity.[47] To identify new mitochondrial proteins

related with OA pathogenesis, the differential mitochondrial protein profile of osteoarthritic human articular chondrocytes was analyzed, and confirmed the increase of TRAP1 in OA chondrocytes, hypothesizing that the increased presence of TRAP1 in OA tissue might be a compensatory output displayed by chondrocyte mitochondria to hold out a high oxidative stress environment. These recent reports describe above indicate the involvement of TRAP1 in oxidative stress-mediated diseases caused by mitochondrial dysfunction.

Parkinson disease (PD) is the most frequent neuro degenerative disorder, characterized by the selective loss of dopaminergic neurons in the substantia nigra, and also accompanied by the accumulation of Lewy bodies, which are abnormal structures inside nerve cells that contain some proteins such as α-synuclein and Parkin.[48] The cause of PD is unclear, however, there seems to be both genetic and environmental factors. Exposure to environmental toxins that prevent the mitochondrial electron transport complex I (which is involved in the production of the cellular energy source, ATP) can cause PD-like phenotypes in animal models.[49] Indeed, activity of the mitochondrial complex I is impaired in patients with PD.[50] Moreover, chemicals that induce production of ROS or that inhibit the mitochondrial electron transport complex I is able to induce PD in humans.[51] These findings suggest that mitochondria, which control apoptotic process, play an important role in PD pathogenesis. Recent genetic studies have identified several genes associated with the familial form of PD. Among them, direct relationship between mitochondrial function and PD comes from the discovery of PTEN induced putative kinase 1 (PINK1) mutations in some patients.[52] Mutations in the PINK1 gene were originally discovered in three pedigrees with recessively inherited PD.[52–54] PINK1 encodes a 581 amino acid protein with a N-terminal sequence acting as a mitochondrial localization signal and a kinase domain with the enzymatic activity to phosphorylate serine/threonine amino acid residue.[52] Recent study by Pridgeon *et al.* offer the molecular basis of the regulation of TRAP1 function in PD.[55] They attempt to clarify the mechanism between a familial from of PD and the mutation in the PINK1 gene,[55] and they have identified TRAP1 as a cellular substrate for PINK1 kinase (Fig. 4). PINK1 binds and colocalizes with TRAP1 in the mitochondria and phosphorylates TRAP1 both *in vitro* and *in vivo*. They also showed that PINK1 protects

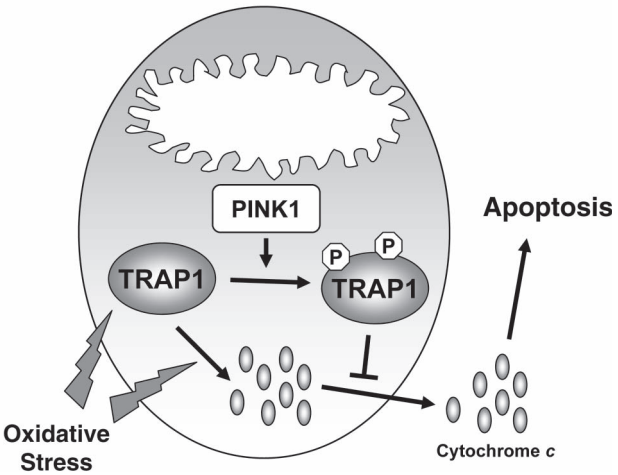

Fig. 4. Involvement of TRAP1 in Parkinson Disease Pathogenesis

PINK1 gene was originally discovered as mutation gene in three pedigrees with recessively inherited Parkinson disease. PINK1 protein has a mitochondrial localization signal and a kinase domain with the enzymatic activity to phosphorylate serine/threonine amino acid residue. TRAP1 is one of cellular substrate for PINK1 kinase. It is likely that PINK1 phosphorylate TRAP1, and the phophorylated TRAP1 prevents the release of cytochrome *c* from the mitochondrial intermembrane space into the cytoplasm.

against oxidative-stress-induced cell death by suppressing cytochrome *c* release from mitochondria, and this protective action of PINK1 depends on its kinase activity to phosphorylate TRAP1, suggesting a novel pathway by which PINK1 phosphorylates downstream effector TRAP1 to prevent oxidative-stress-induced apoptosis and implicate the dysregulation of this mitochondrial pathway in PD pathogenesis (Fig. 4). Further studies must be performed to investigate how phosphorylated TRAP1 regulate the release of cytochrome *c* from mitochondria, and involve mitochondrial dysfunction in PD.

CONCLUSION

HSPs form a large family, and play a crucial role in regulation of apoptosis by various different mechanisms. Several apoptotic stimuli result in the synthesis of a set of HSPs. Especially, some of these HSPs have an important role in mitochondrial function, and it would be of interest to clarify their function in mitochondria. TRAP1 is a mitochondrial protein, and substantially homologous to members of the HSP90. This heat-shock family protein has specific functions differ from those of other members of the HSP90 family. One of interest TRAP1 function is involvement of protection of ox-

idative stress-induced cell death by the suppression of cytochrome *c* release from mitochondria. Thus, TRAP1 appears to act as a sensor of oxidative stress and regulate ROS-mediated apoptosis through the changes in the mitochondrial function.

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