Review

Post-translational modifications in MeHg-induced neurotoxicity☆

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ABSTRACT

Mercury (Hg) exposure remains a major public health concern due to its widespread distribution in the environment. Organic mercurials, such as MeHg, have been extensively investigated especially because of their congenital effects. In this context, studies on the molecular mechanism of MeHg-induced neurotoxicity are pivotal to the understanding of its toxic effects and the development of preventive measures. Post-translational modifications (PTMs) of proteins, such as phosphorylation, ubiquitination, and acetylation are essential for the proper function of proteins and play important roles in the regulation of cellular homeostasis. The rapid and transient nature of many PTMs allows efficient signal transduction in response to stress. This review summarizes the current knowledge of PTMs in MeHg-induced neurotoxicity, including the most commonly PTMs, as well as PTMs induced by oxidative stress and PTMs of antioxidant proteins. Though PTMs represent an important molecular mechanism for maintaining cellular homeostasis and are involved in the neurotoxic effects of MeHg, we are far from understanding the complete picture on their role, and further research is warranted to increase our knowledge of PTMs in MeHg-induced neurotoxicity.

1. Introduction

Mercury (Hg) exposure remains a major public health concern as the metal is present in the environment due to both natural and anthropogenic sources. Within the environment, Hg occurs as both inorganic (elemental or Hg^0, Hg^+ or Hg^{2+}) and organic compounds, such as methylmercury (MeHg) [1]. Organic mercurials, such as MeHg, have received extensive attention due to their ability to cause congenital effects, leading to the development of characteristic neurological symptoms, including mental retardation, cerebellar ataxia and

cerebral palsy, commonly characterized as Fetal Minamata disease (FMD) [2,3]. In adults, toxic effects of environmental level of MeHg are characterized by a long latency period before the appearance of neurotoxic symptoms, which include weight loss, blurred vision and paresthesia, followed by visual field constriction and ataxia [4].

MeHg is produced by biomethylation of inorganic mercury present in aquatic sediments, a reaction catalyzed primarily by aquatic microorganisms [5]. It accumulates up the aquatic food chain, and reaches maximal concentrations in long-lived, predatory fish such as tuna, swordfish, shark and whale [5]. As a consequence, MeHg toxicity represents an ongoing environmental problem to human health, especially in susceptible populations whose diet consists largely of fish and other seafood products [1,6]. As mentioned previously, seafood is the main source of MeHg in the human diet, and about 95% of that ingested is absorbed in the gastrointestinal tract [7]. After absorption, MeHg is ubiquitously distributed, and readily penetrates the central nervous system (CNS) [8]. It can distribute to all brain regions by crossing the blood–brain barrier via the neutral amino acid transport system l as a complex with L-cysteine [9]. The brain has high affinity for MeHg, and concentrations can be 3–6 times greater than those found in blood [10]. In fact, it is well documented that the brain is particularly vulnerable to MeHg, especially the developing CNS [11–13]. In this regard, the most susceptible brain regions to MeHg-mediated injury are the brain cortex and the cerebellum, with particular susceptibility exhibited by cerebellar granule cells (CGC) [2]. The neurotoxic effects of MeHg are largely related to its pro-oxidative properties. MeHg is a soft electrophile that has extremely high affinity for nucleophilic thiol (−SH) and selenol (−SeH) groups, which plays a fundamental role in MeHg-induced toxicity. Indeed, the high affinity of MeHg for −SH and −SeH groups on amino acids such as cysteine and selenocysteine may block critical (catalytic) functional groups and/or alter the structure of a large number of proteins [14], leading to disruption of various intracellular functions, including elevations of reactive oxygen species (ROS) [15–17].

The toxic consequences of MeHg exposure are triggered by its ability to interrupt intracellular homeostasis, such as redox status, glutamine recycling and calcium homeostasis, to name a few. Indeed, the electrophilic properties of MeHg entails that a large pool of cellular molecules could be its candidate targets. Given the pivotal role of post-translational modifications (PTMs) of proteins in the regulation of cellular environment, the understanding of its roles in MeHg-induced neurotoxicity could be fundamental to the knowledge of this metal, facilitating targeted therapies or interventions for MeHg-related injuries of the CNS, including neurodevelopmental disorders early in life and neurodegenerative diseases associated with aging. This review summarizes the most current knowledge regarding the role of PTMs in MeHg-induced neurotoxicity. Considerable attention has been directed toward the most commonly occurring PTMs (protein phosphorylation, protein acetylation, and protein ubiquitination), as well as PTMs induced by oxidative stress and PTMs of antioxidant proteins.

2. Mechanism of MeHg-induced neurotoxicity

2.1. Astrocytes, Ca^{+2} and neurotransmitters

As mentioned earlier, MeHg is a highly and selectively neurotoxic compound, leading to neurological and developmental deficits in the CNS, both in humans and experimental animals [5,18,19]. While MeHg can adversely affect many cell types, a central role of astrocytes in mediating MeHg-induced neurotoxicity had been characterized (Fig. 1) [20]. Indeed, a number of experimental findings suggest that astrocytes represent a preferential site for MeHg accumulation [21–23]. Astrocytes have an essential role in control of extracellular pH and ionic balance, neurotrophic factor secretion, and most importantly, astrocytes are primarily responsible for glutamate uptake [24]. In addition to the inhibition of glutamate uptake by astrocytes, increase in

spontaneous release of glutamate from presynaptic terminals, and inhibition of vesicular glutamate uptake, have also been considered critical phenomena linked to MeHg-mediated excitotoxicity [20,25,26]. These evidences are in line with the increased levels of extracellular glutamate in microdialysis probes implanted in the frontal cortex of adult Wistar rats exposed to MeHg [27]. Earlier studies have demonstrated that MeHg-induced toxic effects can be attenuated by glutamate receptor antagonists [28]. Importantly, during critical CNS developmental windows, MeHg exposure induced disruption of the glutamate homeostasis [29]. Although the molecular mechanisms mediating increased glutamate release and decreased glutamate uptake in MeHg toxicity are not yet fully understood, evidence shows that hydrogen peroxide, whose levels are increased during MeHg exposure, may have a role in the inhibition of astrocyte glutamate transport [30].

The increase of the extracellular glutamate levels with consequent overactivation of N-methyl D-aspartate (NMDA) receptors increases Ca^{2+} influx into neurons, therefore leading to activation of important pathways involved in cell death. In many instances, such effects are mediated by increased generation of ROS in the mitochondria or other intracellular compartments [31,32]. Indeed, studies have shown that MeHg at low micromolar concentrations causes prolonged increases in intracellular Ca^{2+} concentrations [33,34], which may be secondary to increases in extracellular glutamate [23]. Sustained elevated Ca^{2+} levels have been found in various cell types after MeHg exposure, and protective effects of Ca^{2+}-chelators or Ca^{2+}-channel blockers have been reported in vitro and in vivo [33–36]. Furthermore, studies with acutely prepared cerebellar slices showed that multiple sources of Ca^{2+} contributes to the elevation of intracellular Ca^{2+} [37], which is developmentally-dependent [38], and contributes to MeHg-induced stimulation of spontaneous GABA release in cerebellar neurons [37]. Blockage of GABA receptors in hippocampal CA1 neurons is responsible for MeHg's early stimulatory effect on excitatory synaptic transmission [39]. Other experiments have demonstrated that the sensitivity of GABA receptors to MeHg may explain vulnerability of CGC to this organometal [40] (see review [41] on the mechanisms of MeHg-induced alteration of chemical neurotransmission).

Dopaminergic neuron and dopamine metabolism are also important targets of MeHg in various models. In vivo study using microdialysis coupled to high-performance liquid chromatography demonstrated that 6 ppm MeHg induced significant increases in the striatal release of dopamine (DA) and the acidic dopamine (DA) catabolites homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) [42]. The DA release by MeHg in striatum can be prevented by administration of −SH containing compounds [43]. Intrastriatal infusion of 400 μM MeHg markedly increased the extracellular DA levels [44]. MeHg (0.5 ppm) exposure for 14 days during perinatal period caused reduced binding activity of dopamine D2 receptor in the caudate putamen in rats, which correlates with retarded learning ability in adult stage [45]. In vitro study using PC12 cell suggests that the DA release is partly due to the increased synthesis of DA [46] and indirect inhibition of aldehyde dehydrogenase (ALDH) by MeHg [47]. Comparative studies on MeHg and MPP(+) demonstrated that MeHg acts like MPP(+) impairing DA metabolism [48,49]. The researches on MeHg exposure and serotonin (5-HT) system revealed that this is a highly rewarded area, as serotonin levels have been linked to psychiatric disorders. 5-HT uptake in astrocytes was inhibited by treatment of 10 μM MeHg for 30 min [50]. Perinatal exposure of MeHg was associated with decreased monoamine oxidase (MAO) activity in female offspring [51]. A decreased level of extracellular 5-HT was found in MeHg treated zebrafish, which was associated with anxiogenic-like behaviors [52].

2.2. Mitochondrial damage and ROS

The mitochondrion is very well known as the central core of energy metabolism within the cell, producing the major source of cellular ATP through the process of oxidative phosphorylation (OXPHOS). This
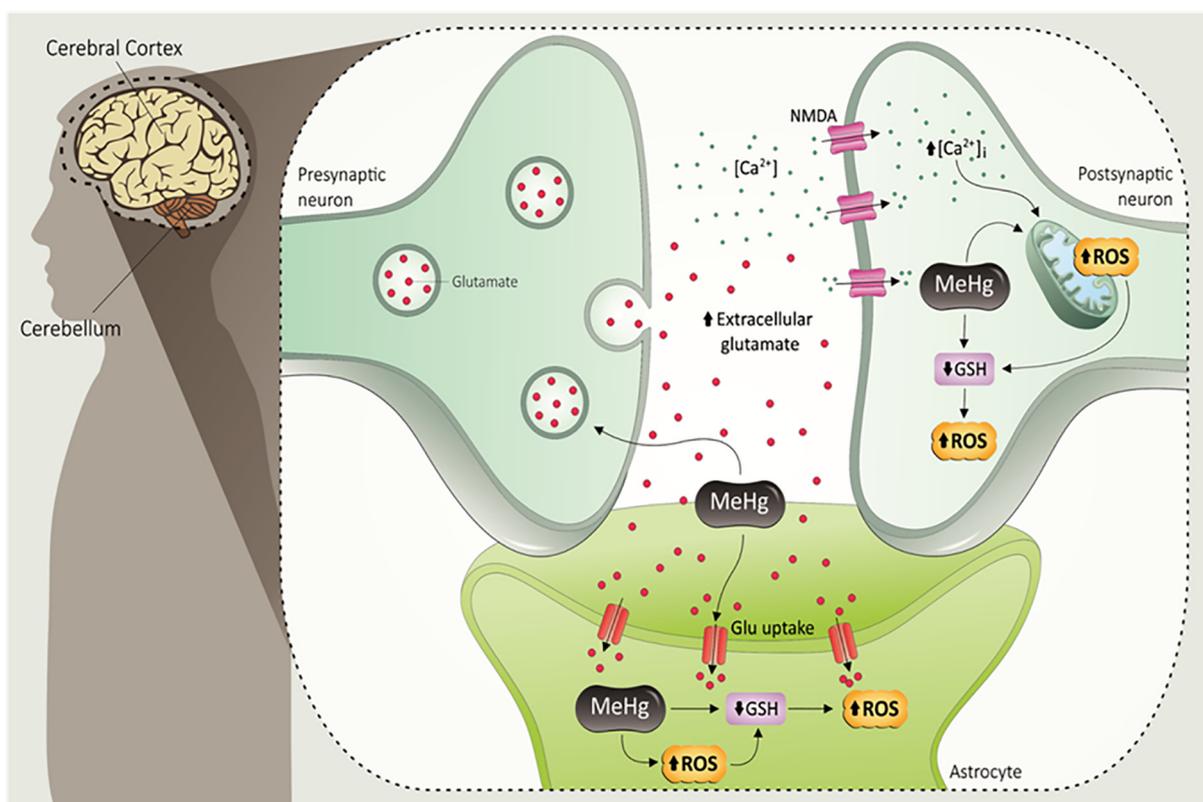
Fig. 1. General mechanisms of MeHg-induced neurotoxicity. MeHg inhibits astrocytic glutamate uptake and enhances glutamate release from presynaptic terminals. The increased extracellular glutamate (GLU) levels lead to overactivation of N-methyl D-aspartate (NMDA)-type glutamate receptor, increasing the influx of Ca^{2+} into postsynaptic neurons. The increased levels of intracellular Ca^{2+} may cause mitochondrial dysfunction and increased reactive oxygen species (ROS) formation. MeHg can affect directly the mitochondrial electron transfer chain (mainly at the level of complexes II–III), also leading to increased ROS generation. MeHg disrupts the redox state of the cells that indirectly depletes GSH, which will further increase ROS production in both astrocytes and neurons.

multifunctional organelle not only maintain and regulate nutrient flow through metabolic pathways, such as fatty acid and glucose oxidation and urea cycle, but also is crucial to thermogenesis, calcium homeostasis, heme and phospholipid biosynthesis, redox signaling and apoptosis [53,54].

Mitochondrial respiration is the main source of constitutive cellular ROS [55], and its significance as a target in the MeHg-induced neurotoxicity is primarily linked to this effect [56–58]. In fact, several studies have shown that the mitochondria are actively involved in the MeHg-induced neurotoxic damage [20]. In this sense, a study from our group using primary astrocytes culture demonstrated that MeHg induces a concentration-dependent impairment in the mitochondrial membrane potential ($\Delta\Psi m$), and this event might be related to increased Ca^{2+} levels triggering ROS formation and increased oxidative stress [20]. Consistent with these observations, MeHg has been shown to promote a concentration/dose- and time-dependent increase in lipid peroxidation and ROS generation, both *in vitro* [25,26] and *in vivo* [27].

It is noteworthy that mitochondria have a large number of redox centers, potentially prompt to react with xenobiotics and metals, which could in turn produce reactive metabolites and ROS [59]. In this context, one key site for both ROS production and ROS damage is the mitochondrial electron transport chain (ETC). The OXPHOS site takes place in the inner mitochondrial membrane and consists of five multi-subunit protein complexes named complex I to V (CI–CV) [60]. Several *in vivo* and *in vitro* studies have shown mitochondrial effects related to MeHg toxicity, which includes alterations in complexes II and III of the mitochondrial ETC [61], depression of respiration and ATP production [62] and swelling of the mitochondrial matrix [57,63,64]. In mice, it was demonstrated that MeHg can disrupt complexes I and II, and reduce the number of mitochondria [65,66]. Corroborating these findings,

using *C. elegans* as an experimental model, our group has also demonstrated that MeHg induces depletion in cellular NAD^+ levels, in addition to oxidative stress and mitochondrial dysfunction. These latter effects were prevented by NAD^+ supplementation prior to MeHg exposure, which is an essential cofactor required for electron transfer reactions. This effect demonstrates the importance of NAD^+ cofactor in the MeHg-induced toxicity [67].

Besides the pivotal role of mitochondria in energy metabolism, these organelles are also directly involved in the regulation of cell death pathways. Neuronal death *via* apoptosis and/or necrosis is commonly a consequence of oxidative damage. Not only the antioxidant response, but also the repair processes to the damaged biomolecules are triggered in response to oxidative injury [68]. It is well known that mitochondria can release several apoptotic mediators, including cytochrome C (cyt C), apoptosis protease-activating factor 1 (Apaf-1) and apoptosis inducing factor (AIF) [69], in addition, mitochondria can be the primary target for several other molecules, especially proteins related to the Bcl-2 family [70]. In this context, it has been demonstrated that MeHg is able to induce apoptosis *via* a classical mitochondrial pathway, where the loss of mitochondrial membrane potential [71] is followed by the release of the pro-apoptotic mediator cyt C [72] and Bax activation [15,73].

The brain has been drawn much attention in MeHg studies due to its high susceptibility to oxidative stress, secondary to its high energy demand and redox-activity [74]. To illustrate that, a study has compared the effects of MeHg on regular murine embryonal carcinoma cells (undifferentiated), as well as in the differentiated neuronal state (after exposition to retinoic acid), evaluating the susceptibility of these cells to oxidative stress and apoptotic cell death. MeHg treatment induced mitochondrial depolarization, cyt C release, and significantly higher

levels of ROS production in differentiated carcinoma cells when compared to undifferentiated cells [75]. In general, it seems that the magnitude of MeHg neurotoxicity and specific consequences of mitochondrial dysfunction likely depend on the dose, cell type and, particularly, the stage of development [75].

3. PTMs - a mechanism to diversify protein functions

The function, structure and stability of proteins in cells are frequently modulated by chemical modifications introduced after translation from RNA. PTMs are important steps in the biosynthesis of proteins, and are known to be essential mechanisms used by eukaryotic cells to diversify their protein functions and dynamically coordinate their signaling networks [76]. Most proteins do not perform their molecular function as unmodified folded polypeptides. In fact, while cognate genes encode the basic biological functions of proteins, the real-time dynamics and regulation of protein structure and function are generally carried out by over 200 protein PTMs that have been described, including phosphorylation, glycosylation, ubiquitination, nitrosylation, methylation, acetylation, lipidation and proteolysis [77,78]. PTMs are found in all types of proteins, from nuclear transcription factors to metabolic enzymes, structural proteins and plasma membrane receptors [77]. These regulatory processes involve an alteration of the original chemical composition of a protein, usually through the covalent addition of a small molecule to one of the amino-acid residues in proteins, usually in the context of a particular sequence motif [79]. By the covalent addition of functional groups or proteins, proteolytic cleavage of regulatory subunits, or degradation of entire proteins, PTMs have been shown to play prominent roles in protein alteration for destruction [80], general regulation [81] and stress response [82], without involvement of genomic, transcriptomic or translational regulation [83].

Protein phosphorylation is one of the most important, prevalent and best-studied PTMs, which is involved in almost every cellular processes including cell cycle, growth and apoptosis, as well as participating in signal transduction pathways [84]. Virtually all types of extracellular signals exert their physiological effects by modulating phosphorylation of specific proteins in target cells [85]. This reversible mechanism is mediated by protein kinases, and consists of the addition of a phosphate group (PO_4) donated from ATP to the polar group R of various amino acids. A complementary group of enzymes, the phosphatases, can remove the attached PO_4 group from a phosphorylated protein substrate, reverting the action of the protein kinases. In eukaryotic cells, this occurs most commonly on serine, followed by threonine and tyrosine residues [86]. It is important to note that an imbalance in this equilibrium may produce hyper- or hypo-phosphorylation of target proteins, resulting in cellular dyshomeostasis and, ultimately, in disease conditions, including Alzheimer's disease (AD) and post-encephalitic Parkinsonism [85].

Protein acetylation occurs on the lysine residue of histone proteins at the N-terminal tail of lysine and is regulated by the enzymes, histone acetylases (HATs), or histone deacetylases (HDACs), and thus is dynamic and reversible [87]. Due to the consumption of Ac-CoA during acetylation and NAD^+ during deacetylation, acetylation interferes with metabolic processes and energy homeostasis. Recent studies not only have linked the process of protein acetylation with a number of diseases but also have shown that amino acid acetylation significantly contributes to the overall pathophysiology of the diseases. Indeed, malfunctioning acetylation machinery can lead to disorders including cancer, neurodegenerative and cardiovascular diseases [88–90].

The covalent attachment of the small, highly conserved, and abundant protein ubiquitin to a variety of cellular proteins (ubiquitination) is one of the most common PTMs in eukaryotic cells. Ubiquitination was initially recognized as a signal for proteasome-mediated protein degradation. Besides regulating protein longevity, ubiquitination is also be involved in the control of many cellular

processes, including protein activity, localization, and interactions [91]. Ubiquitin has a flexible C-terminus, terminating with a glycine residue that *via* an isopeptide bond can be attached covalently with ε-amino groups of lysine residues on substrates [92]. Although the most commonly described sites for this ligation are lysine residues, ubiquitination is also known to occur on the side chains of other amino acids, such as cysteine, serine, and threonine [93]. Alterations of protein ubiquitination have been linked to many diseases including cancer, neurodegenerative, cardiovascular and inflammatory diseases [94].

Although transcriptional induction of stress genes constitutes a major cellular defense program against a variety of stressors, post-translational control directly regulating the activities of preexisting stress proteins provides a faster-acting alternative response [95]. In fact, to add functional diversity and adaption to their alternative environments, proteins may respond to stresses by a transformation of structure and, then, function [96]. The rapid and transient nature of many PTMs allows efficient signal transmission in response to internal and environmental stimuli. On the other hand, the excess of reactive chemical species may be able to post-translationally modify proteins, resulting in the disturbance of cellular redox systems and the incidence of oxidative stress. For instance, cysteine oxidation in cells is an emerging class of redox protein PTMs that creates a range of modifications, which can regulate signal transduction and biological functions [97,98].

4. PTMs in MeHg-induced neurotoxicity

4.1. Phosphorylation

The phosphorylation process is regulated by the activity of different protein kinases and phosphatases. Previous studies have shown that MeHg can modulate protein phosphorylation in the brain cytosol fraction of rats exposed to 10 ppm MeHg for 7 days, and in cultures of cerebellar granule neurons (CGNs) exposed to 5 μM MeHg for 24 h [99–101]. These alterations are developmentally dependent and cell-type specific. Moreover, it was recently shown that MeHg (1 μM, 24 h) can decrease protein tyrosine phosphatase 1B activity in human brain microvascular endothelial cells and pericytes [102], and *in vivo* data have indicated an increase in cerebellar PP2B expression following gestational exposure to 2 ppm MeHg [103]. Overall, MeHg exposure can induce alteration of protein phosphorylation in a concentration, developmental stage- and cell-type dependent manner. In this review, we are focusing on the modulation of mitogen-activated protein kinases (MAPKs), protein kinase A (PKA) and protein kinase C (PKC) following MeHg exposure. Those effects are summarized in Table 1.

In mammalian cells, 14 MAPKs have been characterized. Among them, extracellular signal-regulated kinases 1/2 (ERK1/2), c-Jun N-terminal kinases (JNKs), and p38^{MAPK} isoforms are the foremost studied MAPKs [119,120]. ERKs are important components of the Ras-Raf-MEK signaling pathway that mediate the transduction of intracellular stimuli and gene expression. As a member of the MAPKs family, the phosphorylation of a serine and a threonine residue (in this case: Thr202 and Tyr204) are essential to ERK1/2 activity. Regulation of ERK1/2 phosphorylation by MeHg is contextually dependent. Current evidence supports a notion that MeHg modulates ERK1/2 phosphorylation indirectly rather than directly. Treatment of PC12 cells with nerve growth factor (NGF) for 5 min induced a marked increase of ERK1/2 phosphorylation, which was believed a mechanism for NGF-induced neurite outgrowth [105]. Cotreatment with 0.01–0.30 μM MeHg inhibited NGF-stimulated ERK1/2 phosphorylation with an EC50 of 0.018 μM [105]. The down-regulated ERK1/2 was supposed to be an indirect effect of MeHg on the upstream pathway that regulates ERK1/2 phosphorylation [105]. ERK1/2 phosphorylation in the hippocampus of gestationally exposed young rats (2 ppm MeHg) was down regulated with concomitant hippocampus-mediated behavior abnormity [106]. Furthermore, a time-dependent increase of ERK1/2 phosphorylation

Table 1
Effects of MeHg on different kinases in the CNS.

Protein kinase	Experimental model	Effect	Reference
ERK1/2	PC12 cells	Attenuated NGF-induced ERK1/2 phosphorylation	[104,105]
	In vivo exposure during gestational period in rats	Decreased ERK1/2 phosphorylation in the cerebellum and hippocampus of young rats	[103,106]
	Primary cortical astrocyte culture	Increase ERK1/2 phosphorylation	[107]
	Primary cerebellar neurons	ERK1/2 inhibition attenuated MeHg-induced cell death	[108]
	Neuro-2A cells	Increase ERK1/2 phosphorylation	[109]
		ERK1/2 inhibition attenuated MeHg-induced cell death	[109]
	Repeated administration in adult mice	Increase ERK1/2 phosphorylation in the cerebral cortex	[110]
JNK	Primary cortical astrocyte culture	Increase on JNK phosphorylation	[111]
	Repeated administration in adult mice	Increase on JNK phosphorylation in the cerebral cortex	[112]
	In vivo exposure during gestational period in rats	Decreased JNK phosphorylation in the cerebellum of young rats	[103]
p38^{MAPK}	*In vivo* exposure during gestational period in rats	Increase hippocampal p38^{MAPK} phosphorylation	[106]
	Repeated administration in adult mice	Increase p38^{MAPK} phosphorylation in the cerebral cortex	[110]
	Neuro-2A cells	Increase p38^{MAPK} phosphorylation	[109]
		p38 inhibition attenuated MeHg-induced cell death	[109]
	SH-SY5Y cells	Increase p38^{MAPK} phosphorylation	[113,114]
	Human brain microvascular endothelial cells	Increase p38^{MAPK} phosphorylation	[102]
	Primary cortical astrocyte culture	Increase p38^{MAPK} phosphorylation	[115]
	Primary microglia culture	Increase p38^{MAPK} phosphorylation	[116]
PKC	Repeated administration in adult mice	Decrease PKC activity	[117]
	In vivo exposure during gestational period in rats	Decrease PKC activity	[118]
		Impairment of PKCa and e in the brain	
	PC12 cells	Decrease PKC activity	[105]
	Recombinant PKC	Decrease PKCd and z activity and increase PKCa	[105]
PKA	*In vivo* exposure during gestational period in rats	Increase PKAc expression	[103]
	Repeated administration in adult mice	Increase PKAc activity	[110]

Abbreviations: ERK1/2: extracellular signal-regulated kinase 1/2; JNK: c-Jun N-terminal kinases; PKC: protein kinase C; PKA: Protein kinase A; PKAc: Protein kinase A catalytic subunit.

was observed in primary astrocyte cultures treated with low level of MeHg [107]. In primary culture of rat cerebellar granular cells, inhibition of ERK1/2 phosphorylation ameliorated cell death caused by 30 nM MeHg alone or a combination of 30 nM MeHg and 10 ng/ml BDNF, and the later was shown to accelerate MeHg-induced cell death [108]. In Neuro-2a cells, ERK1/2 phosphorylation was elevated after 0.5 h post-treatment of 3 μM MeHg, and ERK1/2 inhibitor attenuated MeHg-induced cytotoxicity [109].

JNKs are a member of the MAPKs kinase family responsible for phosphorylating serine or threonine residues that are flanked by a carboxy-terminal proline. The JNK family proteins were initially discovered for being strongly activated by cellular stress and for its key role in apoptosis. For this reason, these kinases have also been referred to as stress-activated protein kinases (SAPKs). Indeed, JNK activity is higher in brain tissue when compared to other tissues, which may suggest a pivotal role of this kinase family in CNS function. In the human brain, three JNK genes are expressed and are associated with at least ten splice variants of 46 kDa or 54 kDa JNK proteins. All of these isoforms require a double phosphorylation in a serine and a tyrosine residue for their kinase activity [121,122]. It was recently demonstrated treatment with 3 μM MeHg for 24 h induced increase in JNK phosphorylation as well as TNF-α level in primary astrocytes, which is associated with cytoskeleton remodeling and necrotic death of astrocytes [111]. In other cell models like HepG2 cell, 8 μM MeHg induced a peak elevation of JNK phosphorylation at 8 h of treatment, which is inhibited by cotreatment of 10 μM selenocystine (SeCys), an organoselenium compound that can ameliorate the oxidative damage caused by MeHg [123], while in mouse embryonic fibroblast (MEF) cells, 1 μM MeHg induced a peak elevation of JNK phosphorylation at 4 h of treatment, which is associated with the expression of p62, an autophagy marker which level was increased by MeHg exposure [124]. Upregulation of JNK phosphorylation in the cerebral cortex was found in adult mice exposed to 30 ppm MeHg for 8 weeks, which is associated with an increase in tau-hyperphosphorylation and characteristic neuropathological changes in the deep layer of primary motor cortex and prelimbic cortex [112]. JNK also plays an important role during brain development, such as morphogenesis and neural migration [121,125].

Recently, a decrease in the cerebellar JNK phosphorylation in young rats gestationally exposed to 2 ppm MeHg was shown to be associated with the misregulated redox equilibrium of developing cerebellar cells [103]. Due the complex regulation among all the JNK isoforms and their function in different phases of neural development, further studies are warranted to elucidate the role of JNKs in response to MeHg-induced neurotoxicity.

p38^{MAPK} is a member of the MAPKs family responsive to stress stimuli such as inflammatory cytokines and oxidative stress. There are 4 isoforms of this kinase named α, β, γ and δ. The p38α and β isoforms share 70% of homology, which are ubiquitously expressed in most tissues including the CNS [126]. *In vivo* models including C57BL/6NJcl mice exposed to 30 ppm MeHg for 8 weeks and postnatal 30-day Wistar rats gestationally exposed to 2 ppm MeHg, have shown that MeHg exposure increased p38^{MAPK} phosphorylation [106,110]. However, the consequences of the MeHg-induced p38^{MAPK} phosphorylation seem to be dependent on the cell type and MeHg concentration. The activation of p38^{MAPK} in human brain endothelial cells could mediate a pro-inflammatory response after MeHg treatment [102]. On the other hand, exposure of astrocytes to low concentrations of MeHg is able to increase p38^{MAPK} phosphorylation, leading to release of ATP and interleukin-6, and affording neuronal protection against this metal [115,116]. In Neuro-2a cells [109] and cortical neurons [113], the MeHg-induced increase in p38^{MAPK} phosphorylation and consequent kinase activation, is commonly associated with cell death.

PKC represents an important family of serine/threonine kinase that plays a role in cell growth, differentiation, pos-synaptic transport, neuronal long-term potentiation (LTP), synaptic plasticity and cell survival. The PKC family comprises 12 enzymes that can be divided into three subclasses: the conventional PKC that are sensitive to Ca^{2+} and diacylglycerol, the novel PKCs (also named non-conventional PKC) that are only activated by DAG, and atypical PKCs that are not regulated by Ca^{2+} or DAG [127,128]. *In vivo* data have demonstrated that repeated MeHg administrations (10 ppm) can induce a decrease in overall PKC activity in the mouse brain [117]. Similarly, Haykal-Coates et al. [118] have shown that MeHg exposure during gestational period decreases the cortical PKC activity on the postnatal day 1 and 4, and also impairs

the postnatal ontogeny of PKCα and PKCε in the rat brain. Furthermore, PKC activity *in vitro* was also decreased after MeHg treatment in cerebellar granular neurons, PC12 cells, and rat brain homogenates [105,129]. Further studies using recombinant PKC enzymes have demonstrated an *in vitro* inhibition of the PKC isoforms δ and ζ kinase activity. On the other hand, the PKCα activity was increased after the treatment with a low concentration of MeHg. Since PKCα contains cysteine-rich calcium binding domains and cysteine residues in the catalytic domain of the enzyme, it is speculated that MeHg in low concentrations can mimic the effect of a divalent cation after binding to cysteine (mimicking calcium activation) and stimulate PKCα activity [105].

5′-Cyclic-adenosine monophosphate (cAMP) is an intracellular signaling molecule that regulates many processes in the CNS, such as apoptosis, establishment of neuronal circuitry and plasticity, and other cognitive functions. The cAMP synthesis in the brain is related to the activity of transmembrane adenylyl cyclases (ACs) and soluble ACs which are expressed in both glia and neurons. PKA is positively regulated by cAMP and is one of the main downstream effectors of cAMP signaling. The PKA inactive structure consists of two catalytic subunits (PKAc) and two regulatory subunits (PKAr I and II), which bind together as a heterotetramer. The increase in AC activity upregulates cAMP levels in the intracellular compartment, which binds to PKAr I or PKAr II causing dissociation of PKAc subunit and promotes the kinase activity [130,131]. An increase in the PKAc subunit content in the cerebellum of mice exposed to MeHg during the gestational period has been documented [103]. The study from Fujimura et al. [110] suggested an upregulation in PKA activity after MeHg treatment, and an increase in ser-499 phosphorylation of RAP1-GAP (a specific marker of PKA activity) and ser-133 CREB phosphorylation (a downstream PKA target). This PKA activation could be associated with neural hyperactivity that precedes MeHg-induced neuronal degeneration.

4.2. Ubiquitination and acetylation

As mentioned before, ubiquitin is a 76-amino acid protein that can be conjugated to other proteins, either singly or as polyubiquitin chains. Ubiquitination can target proteins for degradation, but can also modify the protein structure and function. This process is associated with complex cellular machinery that involves the activation of E1 ubiquitin-activating enzymes, E2 ubiquitin conjugates enzymes and E3 ubiquitin ligases. On the other hand, ubiquitin is removed from substrate proteins by a class of enzymes called deubiquitinases (DUBs). The ubiquitin system is central to the regulation of a wide range of cellular processes due to its ability to control protein activity and abundance [132,133]. Interestingly, in an early attempt to search for genes that confer resistance to MeHg, a gene, *CDC34*, that encodes an ubiquitin-conjugating enzyme (E2) was identified to play a protective role in yeast cells following incubation of 0–1.0 μM MeHg for 48 h. CDC34 contains one cysteine residue that is essential for its function and might be a direct target of MeHg, and overexpression of other ubiquitin-conjugating enzymes might confer resistance to MeHg. The later was confirmed by the experiments showed that *Ubc4* and *Ubc 7* also play a protective role in MeHg's toxicity. Moreover, treatment of yeast cells (W303B) with 25 nM MeHg for 90 min could induce increased transcription levels of *CDC34*, *Ubc4* and *Ubc7* [134]. A further exploration found another ubiquitin system enzyme, Rad23, confer resistance to MeHg by its appropriate domain function that binds to the multiubiquitin chain of ubiquitinated proteins [135]. The Skp1/Cullin/F-box protein (SCF) complex contains a F-box protein that binds directly to the target protein for degradation. Genetic screenings found that F-box containing proteins Hrt3, Ylr224w, and Ymr258c confer protection against MeHg's toxicity in yeast cells that incubated with 0–140 nM MeHg for 48 h [136,137]. In the human derived cell line model, Toyama et al. [138] have demonstrated that the treatment of the cell line SH-SY5Y with MeHg (1 μM, 9 h) induced inhibition of one of the enzymes in the DUB

family, specifically binding to Cys152 residue of the ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), and inhibiting the enzyme activity. Furthermore, proteasomic and genomic analyses of MN9D cells exposed to MeHg (1–5 μM, 48 h) revealed an increase in ubiquitin conjugating enzyme E2L3 (UBE2L3) [49]. It has been noted that symptoms of MeHg toxicity share some similarity with those of Parkinson's disease (PD) [139,140]. PD is characterized by the loss of dopaminergic neurons and the presence of inclusion bodies (Lewy body) in brain tissue that is associated with the aging process. Pathological investigation revealed that malfunction of ubiquitin system components or overload of the system implicates in the formation of toxic protein aggregation [141]. A genomic and proteomic study suggests that MeHg and MPP(+) share similar signaling pathways that are involved in the pathogenesis of PD [49]. Thus, further investigations on the role of environmental factors, such as MeHg exposure, in the aberrations of protein ubiquitination system and its relation to the development of neurodegenerative disease are warranted. In addition, animal studies showed that exposure to environmental level of MeHg (0.50–0.59 ppm, 14 days) [142–144] predisposed animals to depression, a debilitating disease that is highly prevalent among US adults [145]. It has been known that MeHg (10 μM, 30 min) treatment of astrocytes significantly inhibited the fluoxetine-sensitive 5-HT uptake [50]. Perinatal MeHg exposure sensitized behavioral response to amphetamine in adult stage [146]. Treatment of MeHg induced anxiogenic behavior in zebrafish, which is associated with decreased level of extracellular 5-HT [52]. Although many drugs and behavior therapies have been developed, there is still a proportion of patients don't respond to either of them, which caused a great number of economic loss and social burdens [147]. A clear understanding and elucidation of the causal relationship between environmental factors, like MeHg exposure, and pathogenesis of depression will improve the prevention and treatment measures of the disease.

The development of an organism is controlled by sequentially happened programming events, among which epigenomic remodeling plays a key role. MeHg are a known neurodevelopmental toxicant, and early life stage demonstrates greater sensitivity to MeHg. As to epigenetic effects, MeHg exposure caused global DNA hypomethylation as well as methylation level changes at specific regions of genes in various models (see review [148]). In a prospective pre-birth cohort study, it was found that methylation at paraoxonase 1 gene (*PON1*) that is relative to prenatal mercury exposure, is associated with cognitive performance in childhood [149]. Experimental studies support that MeHg-induced epigenetic modification might explain the latent neurobehavioral effects of animals developmentally exposed to MeHg [150], thus underpins the theory of "developmental origin of health and disease" [151]. Perinatal exposure to MeHg (0.5 ppm, 14 days) could induce a long-lasting depressive behavior in adult male mice, which might be associated with a decrease in the expression of brain-derived neurotrophic factor (BDNF) and a decrease in histone H3 acetylation [143]. Furthermore, it has been reported that exposure to MeHg (1–100 nM, 24 h) was able to promote epigenetic transgenerational inheritance associated with epimutation in zebrafish [150]. Though it is still unclear whether these are direct effects or indirect effects by modulation of thiol-containing proteins by MeHg, these studies pave the way to the understanding of epigenetic modifications induced by MeHg and its relation to neurodevelopmental disorders.

4.3. PTMs induced by oxidative stress

Redox protein and lipid modifications are essential for normal physiology, as long as ROS are produced under a fine control by specific pathways. Mounting evidence has corroborated that pathological dysfunction arises not because of a non-specific oxidation in biomolecules, but rather as a consequence of damage in the specific signaling domain control which could lead to bigger impacts in the system [152]. In other words, the loss of control of non-physiological redox modifications and the toxic gain of function of oxidized proteins and lipids are the core of
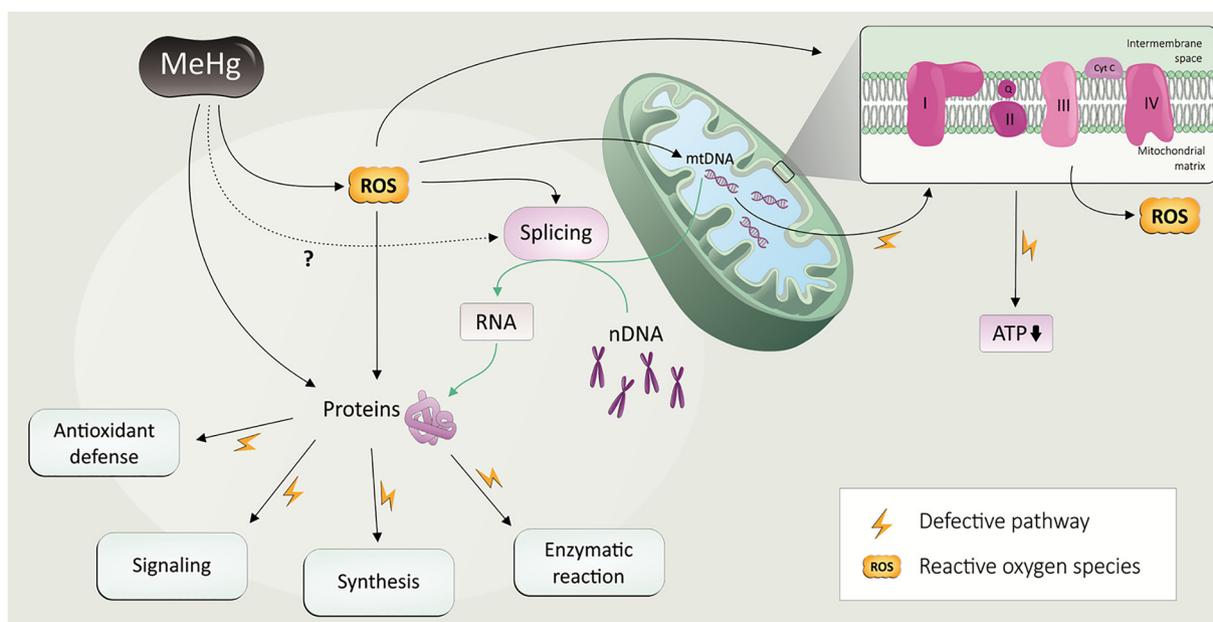
Fig. 2. ROS and MeHg-induced damages of protein synthesis and functions. Reactive oxygen species (ROS) are known to mediate MeHg-induced neurotoxicity. MeHg induce oxidative stress *via* overproduction of ROS and decrease in the synthesis of antioxidants directly. Further, ROS induced by MeHg are able to promote an imbalance in the number of copies of the mtDNA as well as in its quality control of important protein such as the complex I of the mitochondrial respiratory chain, which in turn favor the overproduction of ROS and decrease the ATP synthesis. Several proteins are affected by redox imbalance induced by MeHg: oxidative damage *via* reaction with thiol and selenol groups can cause impairment in the protein function. ROS induced by MeHg alters the proteome of the cell by compromising the splicing and PTM of the proteins involved in antioxidant defense, signaling, synthesis and enzymatic reactions in general. Plain black plain arrows means effect of MeHg exposure based on literature data; dotted line arrow means a likely but not documented event; green arrow means physiological events. Yellow bolt cartoon represents the pathways that are defective due to MeHg exposure.

oxidative stress consequences [157]. Indeed, in order to maintain the redox state of the cell, redox-active molecules promote reversible oxidative PTMs to promote signal transduction and modulation of the cellular function [154,155]. As would be expected, oxidative post-translational modification of proteins has been associated with several CNS pathologies [156–158].

It is well documented that mercury has a strong affinity for thiol and selenol groups, and by binding to these groups mercury can impair protein function [159,160]. The classical decrease in the antioxidant enzyme activities is explained by the inhibition of enzymes such as glutathione reductase (GR), glutathione peroxidases (GPxs), and superoxide dismutases (SODs) [161]. However, recent studies have demonstrated that in addition to a direct effect on proteins and other biomolecules (more details will be discussed in Section 4.4), MeHg can modulate several signaling pathways by PTMs. An interesting study on the activity of CYP1A1, a member of the cytochrome P450 enzymes family, showed that MeHg affects the activity of this enzyme through PTMs. More specifically, the researchers treated human hepatoma HepG2 cells with a halogenated aromatic hydrocarbon (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, named TCDD) that is able to induce the activity of CYP1A1. They found that MeHg (1.25–5 μM, 24 h) was able to inhibit TCDD-mediated induction of CYP1A1 activity by a post-translational mechanism [162]. A study using Neuro-2a cells, a mouse neuroblastoma cell line, showed that in addition to reducing cell viability and increasing oxidative stress damage, MeHg (3 μM, 0.5 h) was also able to increase the phosphorylation of ERK1/2 and p38^{MAPK}. The authors suggest that the mitochondria-dependent apoptotic pathway might be regulated by signals of ROS-mediated ERK1/2 and p38^{MAPK} activation [109]. Furthermore, Petroni and colleagues have demonstrated that neuroblastoma cells (SH-SY5Y) incubated with low concentrations of MeHg (50 nM, 6 h) showed an increase in tau phosphorylation, possibly through an oxidative stress-dependent mechanism [163]. Treatment with antioxidants blocked the MeHg-induced ROS generation and significantly reduced the effects of MeHg on tau phosphorylation.

Moreover, a calpain inhibitor, MDL-28170, attenuated MeHg-induced tau phosphorylation and cytotoxic effects. These findings are consistent with findings by Sakaue et al. who noted that the toxic effects of MeHg in CGC were dependent on calpain activation (30 nM MeHg, 48 h) [164].

In addition, omics studies can provide greater systematic landscape on the toxic effects of MeHg and its affected molecular pathways. A more recent metabolome research using fathead minnow as a model demonstrated that metabolites involved in lipid metabolism and neurotransmission could be significantly altered in fathead minnow larvae exposed to maternally-transferred dietary MeHg (0.72 ppm) [165]. The number of proteomic studies related to MeHg poisoning has been progressively mounting. Li and collaborators [166] used proteomic and high-throughput mRNA sequencing (RNA-seq) to evaluate the cellular responses of human neuroblastoma SK-N-SH cells to MeHg. Interestingly, the authors observed that MeHg alters RNA splicing *via* spliceosome, where a total of 658 aberrant RNA alternative splicing events were observed post-MeHg treatment. Another study using RNA-seq analysis in *C. elegans* (larval 4 stage) exposed to MeHg (10 μM) revealed several changes in the gene transcription. It was found that 541 genes were upregulated and 261 genes down-regulated, encoding proteins related to oxidative stress responses and ER stress pathways [167]. Additionally, a sophisticated proteome study using marmoset to identify and analyze the differential expression induced by MeHg (1.5 ppm, 14 days) showed that from a total of 1045 and 1062 proteins identified in the frontal lobe and occipital lobe, 62 and 89 proteins were altered after MeHg exposure, respectively. Among them, the authors highlight that apolipoprotein E (ApoE) and GPx1 could be possible key proteins targeted by MeHg in occipital lobe [168]. In addition, another study using an aquatic organism (beluga), demonstrated that animals exposed to MeHg (diet containing 0.8 ppm MeHg for 70 days) presented significant changes in the proteome. Some of the identified proteins were involved in metabolism, protein folding, cell division, and signal transduction [61]. Similarly, MeHg effects have been also observed in
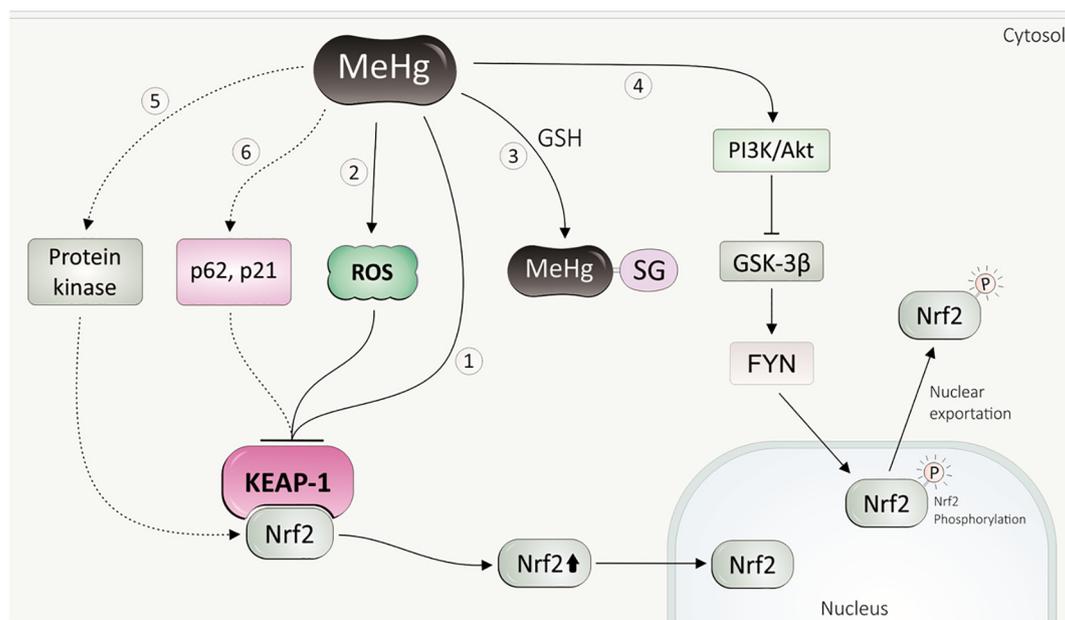
Fig. 3. Mechanism of MeHg-induced Nrf2 activation. (1) MeHg directly binds with thiol groups in Keap1, inhibiting Keap1 activity, thus compromise Nrf2 ubiquitination. (2) MeHg-induced ROS oxidizes Keap1, and leads to disassociation of Nrf2 from Keap1. (3) The MeHg-GSH adduct, a major form of MeHg excretion, can inhibit Keap1. (4) MeHg inhibits Nrf2 nuclear exportation by inhibiting GSK-3 *via* PI3K. (5) Activation of Nrf2 by MeHg can be possible *via* protein kinase pathways. (6) p21, p62 play protective role in MeHg toxicity, which might function through Nrf2 activation. Dashed line presents possible mechanisms.

systems other than CNS. For instance, rats treated with MeHg (oral daily dose of 0.04 mg/kg for 60 days) showed a significant modification in the proteomic profile in the salivary glands, especially in proteins related to structural components of cytoskeleton, metabolic pathways, and redox homeostasis [169].

Several groups have extensively investigated the role of PTMs as modulators of metabolic pathways, and its effects on mitochondrial proteins (Fig. 2) [170]. In fact, PTM enzymes such as deacetylase, desuccinylase and demalonylase and ADP-ribosylase, as well as kinase or phosphatase activity are localized within mitochondria. Furthermore, many mitochondrial PTMs have been described in signal transduction pathways, modulating mitochondrial energy generation, apoptosis, autophagy, metabolism, and tissue response to ischemic injury [171]. As mentioned above, MeHg-induced neurotoxicity is widely associated with excessive generation of ROS in mitochondrial respiration chain, as well as inhibition of antioxidant enzymes [6]. Consequently, the disrupted cellular redox homeostasis leads to oxidative stress, which alters cellular function [172]. In this context, increased oxidative stress seems to alter the quantity of mitochondria per cell as well as the copy number and integrity of mtDNA [74,173]. In fact, it has been demonstrated that mitochondrial redox status correlates with mitochondrial morphology and is also associated with processing of important mitochondrial PTMs in proteins that regulate mitochondrial dynamics, such as optic atrophy 1 (OPA1) proteolysis, ubiquitin proteasome mediated degradation of A-kinase anchor protein 121 (AKAP121), and dynamin-relate protein 1 (DRP1) phosphorylation [174]. MeHg (500 nM, 12 h) was shown to reduce significantly both mtDNA content and mtDNA transcript levels in cultured rat CGNs. These effects could be a result from a ROS-induced mutation in the origins of replication and transcription in MeHg-treated CGNs [175]. Likewise, a study performed to evaluate the effects of MeHg (2.5 μM, 24 h) on myotube formation has demonstrated that along with the reduction in Myogenic factor (MyoG) expression (a transcription factor involved in myocyte differentiation) there was a significant decrease in many other factors involved in mitochondrial biogenesis and mtDNA transcription and translation (mitochondrial Transcription factor A, mitochondrial single-stranded DNA-binding protein and citrate synthase), which may implicate a role for mitochondria in mediating MeHg-induced impairment in myocyte

differentiation and myotube formation [176]. Furthermore, an *in vitro* study using human neural progenitor cells (hNPCs) demonstrated that low-dose MeHg (10 nM, 24 h) was able to upregulate mitochondrial genes transcription followed by higher levels of mtDNA mutations and ROS production [177]. Despite the potential relevance of mitochondrial PTMs in MeHg toxicity, very little was investigated about this phenomenon thus far. Nonetheless, the close proximity of the mtDNA to the main source of ROS generation make mtDNA highly susceptible to oxidative damage, added to this the absence of a histone protection, and relatively less efficient DNA repair mechanisms [178]. It is noteworthy that there is an important communication between the mitochondria and the nucleus. When, mitochondrial function is controlled by the nucleus, a mechanism referred to as 'anterograde regulation' promotes biogenesis and regulation of the mitochondrial activity according to the cellular demand. Also, the nucleus can be controlled by the mitochondria through a 'retrograde response', thus modifying cellular function by reprogramming its metabolism. This mitonuclear communication is an important network which maintains cellular homeostasis in response to stress [179].

MeHg can increase or decrease protein nitrosylation. As a form of PTMs, nitrosylation describes the covalent attachment of nitrogen monoxide (NO) group to the thiol side chain of cysteine. In an experiment to analyze the role of NMDA in MeHg-induced neurotoxicity in the developing cortical neurons, the author found that MeHg (10 ppm, 7 days) induced a marked accumulation of nitrotyrosine, a product of peroxynitrite and L-tyrosine. MK-801, a non-competitive antagonist of NMDA, suppressed accumulation of nitrotyrosine [180]. Another study using SH-SY5Y cells as experimental model showed that treatment with MeHg (1 μM, 24 h) caused 50% reduction of nitrosylation of the catalytic domains of protein disulfide isomerase (PDI) [181].

4.4. PTMs of antioxidant proteins

4.4.1. Nuclear factor erythroid 2–related factor 2 (Nrf2)

Nrf2 functions mainly as a transcription factor to initiate gene expression of phase II detoxifying enzymes to combat oxidative stress resultant from xenobiotics exposure. It has been known that, upon 0.25 μM MeHg exposure, the antioxidant transcription factor Nrf2 is

activated in SH-SY5Y cells [182]. Numerous studies, using different models including cell lines [182], primary cultures [183,184], Nrf2-deficient mice [185], *Drosophila* [186] and *C. elegans* [187,188] verified that Nrf2 plays a pivotal role in mitigating MeHg toxicity. Due to its importance in cellular responses to oxidative stress, Nrf2 activity is regulated by complex pathways upon MeHg exposure (Fig. 3). A recent study from our group, using primary cortical astrocytes as an experimental model, showed that exposure to 5 μM MeHg for 6 h induced decreased transcription as well as nuclear protein levels of the Src family kinase, Fyn, which is a negative regulator of Nrf2 nuclear translocation by phosphorylation of Nrf2 [189]. Based on the concomitant finding that the phosphorylation levels of Akt were increased by MeHg treatment, we suggested that the Akt/GSK-3β/Fyn pathway is one of mechanisms of MeHg-induced Nrf2 activation. Another study using primary culture of neural progenitor cell showed that, at low level of MeHg (10 nM), GSK-3β was up-regulated 3 h after exposure, and that GSK-3β inhibitors protected neural progenitor cell death against MeHg cytotoxicity [190]. Interestingly, it has been known that GSK-3β can directly phosphorylate Nrf2 to signal its nuclear exclusion [191]. Although there is no direct evidence yet, the possibility that GSK-3β is a negative regulator of MeHg-induced Nrf2 activation should be considered. In the same experiment, we also found that MeHg decreased the level of Kelch-like ECH-associated protein 1 (Keap1), a major negative regulator of Nrf2 [189]. Nrf2/Keap1 complex facilitates ubiquitination of Nrf2, and the disassociation of Nrf2 from Keap1 is believed to be a cause, at least in part for the increased levels of Nrf2. There are two sequences in Nrf2 that are the binding sites for Keap1, namely, the high-affinity ETGE and low-affinity DLG motifs. Both of the sequences are bound by different Keap1 sub-units, and are required for Cul3–Rbx1 to ubiquitylate Nrf2. The ubiquitylation of Nrf2 follows a cycle that the formation of Nrf2/Keap1 complex enables Cul3–Rbx1 to ubiquitinate Nrf2, and consequently the released Keap1 can bind to a new Nrf2 molecule [192].

It was originally suggested that the release of Nrf2 from Keap1 is a result of oxidative modifications of thiol groups of the two proteins [193], and it is possible that MeHg-induced activation of Nrf2 was mediated by increased levels of ROS. Due to the fact that Keap1 contains at least 25 cysteine residues and Nrf2 has 7 cysteines, later experiments revealed that a direct binding of MeHg with thiol groups in Keap1 is also involved in MeHg-dependent activation of Nrf2. By using matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF/MS) analysis, three cysteine residues (Cys151, Cys368, and Cys489) in Keap1 were identified as binding sites for MeHg. As Cys151 is one of essential residues for regulating Nrf2 function [194], the author concluded that binding of MeHg with Cys151 in Keap1 potentially causes a structural alteration, releasing Nrf2 (see review [195]). The same group also found that the MeHg-SG adduct, formed by the reaction of MeHg with GSH, can modify Keap1, activating Nrf2 [196]. The above conclusion supported a notion that it is not oxidization of Keap1 but conformational changes in Keap1 that result in release of Nrf2. Since oxidative stress plays a major role in MeHg toxicity, we cannot preclude the possibility that increased oxidative species upon MeHg exposure leads to protein oxidation, thus causes alterations of binding activities of Keap1 or Nrf2.

As far as we know, Nrf2 activity is largely controlled by Keap1, and it is believed that proteins that can compete with Keap1 to bind Nrf2 are potential positive regulators of Nrf2. Chemically, many biomolecules, including proteins and low-molecular weight molecules, are potential targets of MeHg due to its electrophilic property. Some of these proteins or molecules are modified by MeHg, either directly or indirectly, and may participate in the molecular networks that modulate MeHg toxicity. p21, also known as Waf1 or Cip1, is a cyclin-dependent kinase inhibitor, and experiments with primary embryonic cells (2 μM MeHg exposure for 24 h) and adult mice (10 ppm MeHg exposure for 4 weeks) showed that both mRNA and protein levels of p21 are markedly increased following MeHg exposure [197]. Later experiment also

showed that p21 mRNA was up-regulated in neural stem cells exposed to low level of MeHg (2.5 or 5.0 nM for 48 h) [198]. It has been shown that p21 can bind with the DLG and ETGE motifs in Nrf2 via its KRR motif, competing with Keap1 to compromise Nrf2 ubiquitination [199]. Moreover, p62 is believed to be a competitor of Keap1 for binding to Nrf2 [200], and experiments in MEFs cells (1 μM MeHg exposure for 4 h), astrocytes (1 μM MeHg exposure for 6 h), and SH-SY5Y (1 μM MeHg exposure for 24 h) showed that p62 was up-regulated and plays a protective role in MeHg toxicity [129,201,202,]. Therefore, whether these molecules function via Nrf2 activation pathway warrants further investigations.

The activation of Nrf2 by MeHg is far more complex than originally thought. An experiment with SH-SY5Y cells showed that carbon monoxide (CO) and its producing enzyme heme oxygenase-2 (HO-2) protects cells against MeHg cytotoxicity (1 μM, 24 h) by Nrf2 activation, and a possible mechanism accounting for this observation is CO activation of Nrf2 via MAPK or ERK pathway [203]. As noted earlier, different signaling pathways and kinases are involved in toxic consequences upon MeHg exposure, and these kinases, such as PKC, PI3K, MAPKs, are also involved in modulation of Nrf2 activity [204]. The functional importance of Nrf2 in antioxidant response, especially in MeHg toxicity, warrants further investigations on its role and regulation.

4.4.2. Glutathione system

The glutathione system includes reduced glutathione (GSH) and oxidized forms of glutathione (GSSG), the enzymes that are required for GSH synthesis and enzymes such as glutathione *s*-transferases (GSTs) and GPxs that use GSH in the antioxidant defense activities. GSH is a tripeptide that plays an essential role in antioxidant defenses. Due to its high concentration in cells, GSH may act as the first target for ROS generated by MeHg [205,206]. MeHg exposure causes GSH depletion and a decreased ratio of GSH/GSSG, a consequence of the pro-oxidative events caused by MeHg. Notably, the abundance of GSH in the brain tissue is in the low millimolar range [207], whereas a nanomolar range of MeHg can induce neurotoxicity. Therefore, these events are not consequence of the simple interaction between MeHg and GSH, but rather resultant from the increased levels of oxidants that are secondary to inhibition of antioxidant selenoenzymes, such as GPx and TrxR after MeHg exposure [6,208]. MeHg-GSH adduct is the major form of detoxification. There is a large body of epidemiology studies corroborating that polymorphisms in GSH-related genes was associated with body burdens of MeHg, and a recent epidemiology study showed that polymorphism in glutamate cysteine ligase (GCL) has a significant association with hair Hg content [209]. Experimental evidence has shown that GCL transcription levels in the placenta of C57Bl/6 mice were up-regulated after exposure to 1 ppm MeHg for 32 days [210].

Selenium (Se) is an essential nutrient required by selenoproteins. GPxs represent a family of peroxide-detoxifying enzymes; most of them are selenoproteins whose catalytic activity depends on the reducing power of a selenol group located at the active site. MeHg preferentially targets selenol-containing molecules than thiol-containing molecules (see review [14]), and reduced activity of GPxs is an important pro-oxidative event in MeHg neurotoxicity. Experimental data showed that GPx activity was significantly inhibited in mice after 21-day exposure of 40 ppm MeHg. Incubation of isolated mitochondrial with exogenous GPx totally blocked MeHg-induced lipid peroxidation. Moreover, the inhibitory effect of 1 μM MeHg exposure for 24 h on GPx activity was observed in SH-SY5Y cells [211]. Although the inhibitory effects of MeHg on GPxs' activity was believed to be the chemical modification of the selenol group in the protein by MeHg, experiments on selenium supplementation did not find a full recovery of enzyme activity [66,212]. The reason behind this might be that MeHg can sequester selenium into nonbioavailable forms, and the enzyme activity can't be fully reversed due to the sustained structural modification. Of particular importance, the protective effect of synthetic organic selenium

compounds against MeHg toxicity has received much attention. Study with slices of cerebral cortex of rats revealed that 1 μM MeHg exposure for 30 min induced hyperphosphorylation of high molecular weight neurofilament subunit (NF-H). Both ebselen (5 μM) and diphenyl diselenide (15 μM), which mimic GPx activity, presented a protective effect against MeHg-induced hyperphosphorylation of NF-H [213]. Experiments with primary cortical astrocytes showed that pretreatment with ebselen (10 μM) partially reversed the 1 μM MeHg-induced astrocytic inhibition of glutamine uptake and reversed the 1 μM MeHg-induced changes in mitochondrial membrane potential. Meanwhile, ebselen pretreatment inhibited MeHg-induced phosphorylation of ERK and blocked MeHg-induced activation of caspase-3 [107].

4.4.3. Thioredoxin system

The thioredoxin system comprises thioredoxin (Trx), thioredoxin reductase (TrxR) and NADPH [214]. The Trx system is the major system responsible for maintaining the redox state of cells and this function involves thiol reduction mediated by selenol groups in TrxRs. TrxR is an early target of MeHg. Using human neuroblastoma cells as an *in vitro* model, TrxR activity was significantly decreased by MeHg, with 1 μM this metal causing 50% inhibition in TrxR activity [215]. MeHg can bind to selenocysteine residues present in the catalytic site of TrxR, in turn, causing enzyme inhibition that can compromise the redox state of cells. MeHg caused a significant inhibition of TrxR in a tissue-dependent manner. *In vitro* assay with purified enzyme from brain, liver and kidney of mice showed that 0.05 μM MeHg caused a significant inhibition of TrxR in brain, but the net effect was lower than that in liver or kidney [216]. *In vivo* experiment also showed that exposure to 5 ppm MeHg for 24 h caused a significant inhibitory effect on TrxR activity in liver and kidney, but not in brain [216]. Study in zebra-seabreams showed that both TrxR and Trx were affected by exposure to 0.5–2 ppb MeHg for 28 days. The thioredoxin system in the brain was also affected. In contrast to TrxR, Trx was only affected in the later stages of exposure [217]. In SH-SY5Y cells, the oxidation of Trx1 can be induced at dosage of 5 μM MeHg. Depletion of GSH is directly linked to increased oxidation of Trx1. Meanwhile, the phosphorylation of apoptosis signaling kinase 1 (ASK-1) was increased [160]. Different species of mercurial exert varied inhibitory effect on Trx. A previous *in vitro* study showed that Hg^{2+} can make a disulfide bridge between adjacent thiols and promotes dimerization of Trx molecules, whereas MeHg can only bind to one thiol at a time, making Hg^{2+} a stronger inhibitor of Trx [218].

Although MeHg presents significant pro-oxidative properties (mainly due to its electrophilic profile) and has been reported to cause several PTMs, a clear mechanistic relationship between both phenomena is lacking in the literature. Moreover, it is not clear if the effects of MeHg on antioxidant systems represent a relevant cause of PTMs. Although there is an evident need for further research on this topic, recent data begin to emerge in the literature. In this regard, a recent *in vitro* study with cultured SH-SY5Y cells has shown that, due to its affinity for nucleophilic groups, MeHg can cause *S*-mercuration (which represents a PTM), and that this phenomenon was responsible for the modulation of signaling pathways involved in cell survival and apoptosis [219]. Thus, MeHg-induced PTMs can be a direct consequence of its electrophilic/oxidant effects toward nucleophilic groups of biomolecules. On the other hand, it is important to mention that the well-reported inhibitory effects of MeHg on GPx [211] may lead to increased levels of hydroperoxides [29,220]. Because hydrogen peroxide (H_2O_2) presents a central role in regulating several signaling pathways through the reaction with cysteine regulatory switches [221], one may posit that the increased hydroperoxide levels resulting from MeHg-mediated GPx inhibition may contribute to oxidative PTMs that modulate myriad of signaling pathways. In this scenario, it is noteworthy that cells present diverse thiol protein sensors, with different reactivity of their sulfhydryl groups toward H_2O_2, being activated by different concentrations and times of exposure to H_2O_2 [222]. Thus, it is provocative to hypothesize

that several of the PTMs induced by MeHg can represent a secondary (indirect) consequence of its effects toward antioxidant systems. However, this is a newly opened research field that deserves significant efforts in order to elucidate the relationship between the effects of MeHg on antioxidant systems and its ability to cause PTM. Taking into account that (i) the pro-oxidative effects of MeHg can also lead (even indirectly) to increased levels of NO and GSSG [63,223,224], and that (ii) *S*-nitrosylation [225] and glutathionylation [226] also represent pivotal signaling PTMs modulating both physiological and pathological events, a potential role of *S*-nitrosylation and glutathionylation in MeHg-induced toxicity cannot be ruled out, representing a promising new research field.

5. Conclusions and perspectives

MeHg exposure continues to remain an environmental health burden due to its toxic effects, especially on the brain. Population whose diet consists largely of fish is at the greatest risk for exposure. The developing fetus and children are susceptible to its neurotoxic effects. Studies on the molecular mechanism of MeHg-neurotoxicity are pivotal to our understanding of its toxic effects and the development of preventive measures. MeHg is a soft electrophile that has extremely high affinity for nucleophilic thiol (−SH) and selenol (−SeH) groups, thus a large pool of cellular molecules could be its candidate targets. PTMs, such phosphorylation, ubiquitination, and acetylation, are essential mechanisms in the biosynthesis of proteins and play pivotal role in the regulation of cellular environment. MeHg modulates the activities of MAPKs (ERK1/2, JNK, p38^{MAPK}), PKA and PKC, which are important in modulating pathways related to its neurotoxicity, such as cellular redox status, cell differentiation, and cell death. Mitochondrial damage and increased ROS generation caused by MeHg are important steps in the process of PTMs of proteins that mark MeHg neurotoxicity. Nfr2 is important in combating oxidative stress, and many pathways have been proved to be involved in MeHg-induced activation of Nrf2. Accumulated data shows that antioxidant proteins (such as GPxs and TrxR) are likely to be modulated by MeHg *via* a PTMs mechanism. Although researches on MeHg-neurotoxicity have illustrated many important aspects of its mechanism, such as oxidative stress, calcium dyshomeostasis, and disrupted glutamine recycling, *etc.*, we still lack knowledge on the molecular events triggered by MeHg. For example, cells contain more than 19,000 −SH containing proteins and 25 selenoproteins, however, we do not know with which of these proteins MeHg directly interacts. The PTMs modification can be a result of direct inhibition of the proteins by MeHg or can be indirect either inhibition or activation *via* modulation of up-stream thiol- or selenol-containing proteins. Since PTMs is a conserved and essential process for maintaining cellular homeostasis and function, and many newly defined cellular machineries are carried out by proteins that are modified by multiple PTMs mechanisms, current investigations should offer new insight on the relation between PTMs and MeHg neurotoxicity.

Conflicts of interest

The authors declare no conflict of interest.

Transparency document

The Transparency document associated with this article can be found, in online version.

Acknowledgments

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