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Characteristics, functions, and applications of metallothionein in aquatic vertebrates

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The documents on Metallothioneins (MTs) in aquatic creatures, especially focusing on their function as biomarkers in environmental monitoring programmes, are vast and increasing. There are, however, few papers to summary the physiological role of MTs in aquatic organisms especially on development. The multifaceted roles of MTs include involvement in homeostasis, protection against heavy metals and oxidant damages, and metabolic regulation, sequestration and/or redox control. In this paper, we have collected published information on MTs in aquatic organisms—pisces, amphibians, mammals, etc., and analyzed their function in these aquatic animals. MTs have four main functions in aquatic vertebrate. They are respectively bioaccumulation of toxic metals and detoxification, homeostatic regulation of metals, protection against oxidative stress and neuroprotective mechanism. MTs separate in different tissues and they have various distributions in different tissues of aquatic vertebrate, including liver, gills, kidney, testes, and brain. MTs can be induced by a variety of environmental and physiological factors, among which, heavy metals are the main kind of MTs inducers in aquatic vertebrate. Here we pay more attention on the essential metals copper (Cu) and zinc (Zn) and the non-essential metals cadmium (Cd), silver (Ag), lead (Pb), and mercury (Hg).

Keywords: heavy metals, metallothionein, homeostasis, detoxification, aquatic vertebrates

INTRODUCTION

The MTs were initially found in equine renal cortex (Margoshes and Vallee, 1957). With respect to aquatic species, in the marine fish *Sebastes seboides* MTs were first revealed by Olafson and Thompson (1974), who described the occurrence of low molecular weight, cadmium-binding proteins. MTs or Metallothionein-like protein (MTLP) have since been demonstrated in many vertebrates including fish (Roeva et al., 1999).

MTs play an important role in processes of cellular protection from actions of harmful agents (metals, free radicals, etc.) and in mechanisms controlling growth, differentiation and proliferation of cells, explicating their nuclear vs. cytoplasmic localization (Dziegiel, 2004). MTs are not only cytoplasmic protein, but also accumulate in lysosomes, yet could be transported to the nucleus and to the intermembrane space of mitochondria (Tsujikawa et al., 1991; Ye et al., 2001). Notably, they are also not merely intracellular, and could be exported from cells and absorbed by other cells via a receptor-mediated mechanism, in which the protein remains in an endocytotic compartment and the metal could be transported to the cytosol (Moltedo et al., 2000; Wolff et al., 2006; Hao et al., 2007).

STRUCTURE CHARACTERISTICS OF MTs

The distinguishing trait of MTs is that one-third of their amino acids are cysteines. Templeton and Cherian (1991) found that the behavior of MTs is dependent on the chemistry of the thiol group, such that any metal sharing stoichiometric characteristics with copper or zinc, may also link to MTs. Until quite recently, the most notable characteristics of the primary structure of all MTs was the Cys-Cys, Cys-X-Cys, and Cys-X-X-Cys structural motifs, herein X stands for an amino acid residue except Cys. Furthermore, these motifs display a strong affinity to bind metal ions such as Cu, Zn, Cd, and Hg (Vallee, 1991).

Lower organisms synthesize monodominial MTs that bind monovalent mentals, whereas higher organisms have bidominial MTs that bind divalent metals. Vertebrate MTs show a bidominial structure with divalent metals as Zn(II) or Cd(II). In vertebrate MTs, the β-domain contains an M3Cys9 cluster and the α-domain contains an M4Cys11 cluster, herein M stands for Zn(II) or Cd(II) (Arseniev et al., 1988; Robbins et al., 1991). Each Zn(II) ions in the clusters of MT is in a tetrathiolate coordination environment. However, seven Zn(II) ions locate in different environment, one type of zinc ions has two terminal sulfurs and two bridging sulfurs, another type of zinc ions has three bridging sulfurs (Vasák et al., 1981). The different environment leads to affinity variation over four orders of magnitude. Thus, the process of metal-binding occurs via a sequential, non-cooperative mechanism (Krezel and Maret, 2007).

FUNCTIONS OF MTs

In the past few years, MTs were suggested to act in the processes such as apoptosis, regulation of neuronal growth, and protection against free radicals and other oxidants (Vasák and Hasler, 2000).

Abbreviations: FHM, Fathead minnows; MeHg, Methylmercury; MTLP, Metallothionein-like protein; MTF-1, Metal regulatory transcription factor 1; MRE, metallothionein promoter region; ROS, reactive oxygen species.

Gradually, the facts that MTs are involved in the homeostatic regulation of metals which provides a reservoir of metals for other metalloproteins or metalloenzymes, in heavy metal detoxification, in protection of tissues against various forms of oxidative injuries and transferring of essential metals (Coyle et al., 2002; Baird et al., 2006) (**Figure 1**). All the phenomena could be elucidated by chemical property of the thiolate bond (Suzuki et al., 2002). Some deep-sea fish species like *Alepocephalus rostratus* sequester heavy metals like Hg and Ag bound to MT under the synergy effects of Se (Siscar et al., 2014). *Caretta caretta* and *Chelonia mydas* also secretes mucus containing MT proteins that decrease Cu and Cd concentrations in hepatic and renal (Andreani et al., 2008). Generally, the MT expression level is dose-dependent on heavy metals. However, the response of MT to metals is not positively correlated when the amount of metals overdose (Walker et al., 2014).

In mammals the disruption of the MT-I and MT-II genes and interruption of MTs synthesis resulted in a loss of tolerance to Cd (Masters et al., 1994). Furthermore, important activities of MTs are not limited to metal ion homeostasis, but also include the enhancement of cellular survival and tissue regeneration, metabolic activity, and the blunting of pathways that promote inflammatory or apoptotic responses (Swindell, 2010). Hepatic MTs inhibit apoptosis by regulating caspase-3 activity in *Carassius auratus gibelio* (Falfushynska et al., 2014). MT also protect testis and liver in the marine teleost *Gobius niger* from Cd effects (Migliarini et al., 2005).

Organisms with the induction of MTs could tolerate oxidation stress better, because MTs have effects of removing hydroxyl ($OH^{\cdot}$) and super oxide (O^{2-}) radicals (Amiard et al., 2006). Because of a high content of cysteine, MTs can also function as an antioxidant in aquatic vertebrates (Xie and Klerks, 2004). In addition, another strong evidence is that metallothionein gene expression is regulated by oxidative stress (Glen, 1999). Thus, the synthesis of MTs could be regarded as a step of oxidative stress. Besides, MTs are involved in anti-oxidation effects. The response of MT to Tributyltin (TBT) is not positively correlated and the latter has led to marine pollution which induced testicular toxicity (Mitra et al., 2013).

In *Channa punctata* MTs are proposed to have a free-radical-scavenging activity, and their expression may be regulated in response to stress and chemical exposure (Atif et al., 2008). Besides, MTs are also involved in cytochrome induced oxidative damages in the brain, such investigation has been carried out in rainbow trout (Erdoğan et al., 2011).

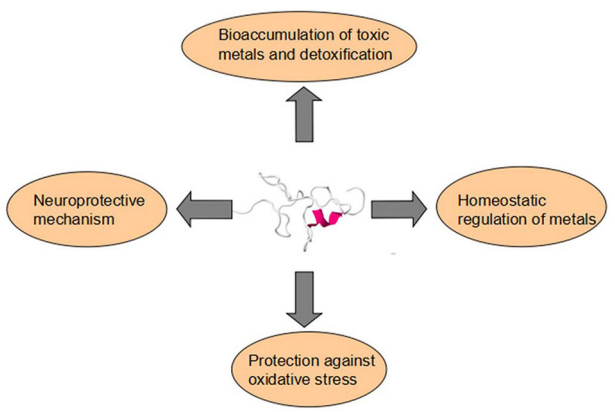

FIGURE 1 | Metallothioneins have four main functions in aquatic vertebrate. They are respectively bioaccumulation of toxic metals and detoxification, homeostatic regulation of metals, protection against oxidative stress, and neuroprotective mechanism.

THE STRUCTURAL ANALYSIS OF *METALLOTHIONEIN*

Hemibarbus mylodon metallothionein cDNA possesses the common features of vertebrate MTs including the high rate of Cys residues as Cys-X-Cys or Cys-Cys forms in the conserved positions. Genomic structure of *H. mylodon metallothionein* revealed the conserved characteristics of the tripartite exon/intron structure with the conserved splicing junction rule (GT/AG) (Cho et al., 2008). *H. mylodon metallothionein* cDNA shows three putative polyadenylation signals in the 3′-UTR, implying the possible processing of the mRNA species with different lengths (Cho et al., 2008). Existence of multiple polyadenylation signals in *metallothionein* mRNA were also found in other teleost species including common carp (Chan et al., 2004), zebrafish (Chen et al., 2004), and crucian carp (Ren et al., 2006). The 5′-flanking sequences of *H. mylodon metallothionein* shared a high degree of homology with previously known vertebrate *metallothionein* especially on the conserved motifs and/or the core sequences for the binding of known transcriptional factors such as TFIID, AP-1, Sp1, and HNF-5 (Haq et al., 2003; Lin et al., 2004; Ren et al., 2006). Metal response elements (MREs) play an important role in binding target for the transcription activating protein factor MRE-binding transcription factor-1 (MTF-1), controlling both basal and heavy metal-induced *metallothionein* transcription (Haq et al., 2003). Moreover, the transcriptional activation of *H. mylodon metallothionein* during metal exposures followed a dose-and/or time-dependent manner (Cho et al., 2008).

The localization of mRNAs to different compartments of the cell allows the synthesis of proteins as to where they are needed, and signals within the 3′ UTR have been implicated in such addressing particularly for *metallothionein* (Mickleburgh et al., 2004). Some studies perspicuously indicated that abnormal 3′ UTR modified by deletion or mutation may induce a rapid degradation of the *metallothionein* mRNA (Muhlrad and Parker, 1999; Levadoux-Martin et al., 2001). Some previous studies demonstrated that a tandem repeat of a CACC could be essential for the transcript localization (Chabanon et al., 2004; Nury et al., 2005).

TRANSCRIPTIONAL REGULATION OF *METALLOTHIONEIN*

Metal-induced regulation of *metallothionein* has been described in several recent reviews (Miles et al., 2000). MTF-1 is important in the regulation of a group of genes that play a key role in cellular response to various stressors (Lichtlen et al., 2001). MREs are known in multiple copies in the *metallothionein* promoter region, and they appear to be variable in their response to metal-induced transcription. Interestingly, Zn, Cd and Bi ions could activate the promoter of the *metallothionein* via MREs (Palmiter, 1994).

However, there are other potential pathways of metal induction, since protein kinase C inhibitors have been found to inhibit

Zn and Cd induction of MTs in Chinese hamster cells (Yu et al., 1997). Moreover, MREs can interact with various nuclear proteins that either activate or inhibit transcription (Tang et al., 1999; Miles et al., 2000; Ogra et al., 2001). There is evidence that several MREs could respond directly to hypoxia and oxidants, possibly via MTF-1, although these conditions may also separate Zn from protein ligands which could then activate MTF-1 (Murphy et al., 1999). A similar combination of inflammatory factors has been discovered to facilitate the MTs and acute phase response in mice following restraint stress (Hernández et al., 2000). Nucleotide sequences except MREs in the *metallothionein* promoter have been found to respond to glucocorticoids (Plisov et al., 1994; Kelly et al., 1997), interleukin-6 (IL-6) (Lee et al., 1999), phorbol esters (Angel et al., 1987), and hydrogen peroxide (Dalton et al., 1994). Reactive oxygen intermediates produced during the inflammatory response may induce MTs via multiple pathways, including directly stimulating an antioxidant response element and specific MREs in the promoter region as well as by events correlating with various seconds-messenger protein kinase pathways (Arizono et al., 1993) (**Table 1**).

FOUND IN AQUATIC VERTEBRATE

THE INDUCTION OF MTs

MTs are also induced by other agents, such as hormones, pharmaceuticals, alcohols, cytokines, alkylating agents, irradiation, infection, reactive oxygen species, and other diverse chemical treatments (Waalkes and Goering, 1990). Nonetheless, some isoforms are relatively not sensitive to these inducers (Samson and Gedamu, 1998).

Piscator (1964) found increased metallothionein levels in the liver of rabbits exposed to Cd. Zn is the most effective inducer of *metallothionein* transcription, while non-toxic Cu levels do not induce MTs, although it is often bound to MTs *in vivo* (Munger et al., 1985). This is in contrast to the binding affinities. Therefore, the capability to induce MTs could not be reflected by the binding affinity of the metal to MTs (Zafarullah et al., 1989). Cd is another usual metal inducer of MTs, *Coho salmon* receiving low dose of Cd exposure result in induction of MT in liver, gill and olfactory tissues respectively and the effects can be superposed (Espinoza et al., 2012).

In addition to chemical treatments, MTs are also in response to physical treatment. Crucian carp (*Carassius cuvieri*) exposed to air-pumbing stress reveal time-dependent induction of MT-like metal-binding proteins (Muto et al., 1999).

Table 1 | List of the published studies on regulation pathways of metallothionein transcription and expression.

Regulation pathways of metallothionein	References
Metal-induced regulation of metallothionevia MREs	Miles et al., 2000
Protein kinase C inhibitors inhibit Zn and Cd induction of MTs	Yu et al., 1997
MREs interact with various nuclear proteins that either activate or inhibit transcription	Miles et al., 2000
MREs respond directly to hypoxia and oxidants, possibly via MTF-1	Murphy et al., 1999
A similar combination of inflammatory factors facilitate the MTs and acute phase response in mice following restraint stress	Hernández et al., 2000
Nucleotide sequences except MREs in the metallothionein promoter respond to glucocorticoids	Plisov et al., 1994; Kelly et al., 1997
Interleukin-6 (IL-6)	Lee et al., 1999
Phorbol esters	Angel et al., 1987
Hydrogen peroxide	Dalton et al., 1994
Reactive oxygen intermediates correlate with various seconds-messenger protein kinase pathways	Arizono et al., 1993

THE SATURATION OF DETOXIFICATION MECHANISM

Such a saturation of expression is consistent with previous observation on the responses of MTs and other metalloenzymes during acute exposure to relatively high doses of heavy metals (De Smet et al., 2001; Yan and Chan, 2004; Cheung et al., 2005; Cho et al., 2006). It is worth noting that not only metal dose but also duration time should be taken into account (Van Campenhout et al., 2010). However, in some fish species, like the yellow perch (*Perca flacescens*), such threshold exposure concentration is not appreciable (Campbell et al., 2005).

MT DISTRIBUTION IN DIFFERENT TISSUES OF AQUATIC VERTEBRATE

Heavy metals accumulate in tissues of aquatic animals and hence heavy metals detected in tissues of aquatic animals can reflect the exposures (Kalay et al., 1999; Canli and Atli, 2003; Yılmaz, 2003, 2005) (**Figure 3**). Much of the variation in trace metal tissue concentrations in aquatic organisms has been ascribed to the diversity in size as well as age of individuals (Farkas et al., 2003), sex (Al-Yousuf et al., 2000), and feeding habits (Canli et al., 2001; Yılmaz, 2005). Ubiquitous detection of *metallothionein* mRNA in various of tissues was not surprising, because many other previous findings have established the extensive tissue distribution in fish species (Chan et al., 2004; Lin et al., 2004). Tissue-specific rising of *metallothionein* mRNA under metal exposures might have been due to the different rates of influx/efflux of metal ions relying on tissue types, which was also associated with the compensative effects of other metal-binding proteins (e.g., superoxide dismutase) of which levels were largely different among tissues (Kock et al., 1995; Cho et al., 2005; Nam et al., 2006).

HEPATOPANCREAS

Experimental animals treated with high doses of Cd led to morphological and functional changes in this organ (Goering and Klaassen, 1984). High MTs amount in liver but low in muscular tissues have been observed in some fish species (Chan et al., 2004; Lin et al., 2004).

In *H. mylodon*, liver was more sensitive to metal ions than kidney and gill (Cho et al., 2008), and the increase of hepatic *metallothionein* mRNA was dose-dependent in other studies (Langston et al., 2002; Lin et al., 2004; Cho et al., 2005). Induction of hepatic MTs by Cd was time-dependent but transient. Sudden increase of metal-binding proteins or metalloenzymes in short

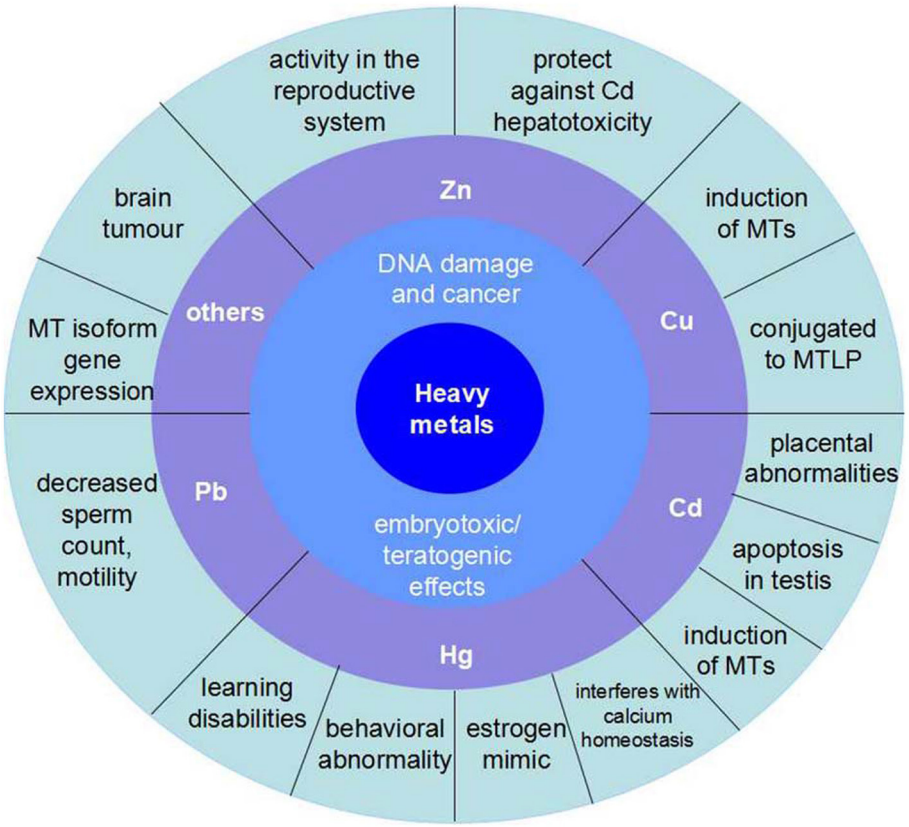

FIGURE 2 | The influence of main kinds of heavy metals on aquatic vertebrate. Zn has activity in the reproductive system and protects against Cd hepatotoxicity. Cu is a kind of induction of MTs and conjugated to MTLP. Cd is the course of placental abnormalities and apoptosis in testis, besides, it is also another induction of MTs. Hg mainly has damages to nervous system, which would reduce the ability of learning and result in behavioral abnormality. In addition, the organic Hg is a kind of estrogen mimic and interferes with calcium homeostasis. Pb can decreases sperm count and motility.

period and then decrease down in longer exposures has been found before, and it might be due to the "indication of saturation" or "acclimation process" of the organisms to tissue loads resulted from accumulated metals (Langston et al., 2002; Basha and Rani, 2003; Cho et al., 2006).

GILL

Although gills are thought to be not only the first target organ for acute metal toxicity in fish but also the predominant absorption site of waterborne toxicants, metal accumulation, and MTs induction in fish gills have been revealed to be very species-specific, with some dispute on fish species (Boeck et al., 2003). Piscine gills comprise a diversity of cell-types such as pavement cells, chloride cells, mucous cells and respiratory cells, and the major cell type dedicated to MTs expression in response to metal exposure are chloride cells although there have been species-specific variations (Dang et al., 2001). In *H. mylodon*, gill showed a quite low sensitiveness to most metal exposures, despite its relatively high basal level of *metallothionein* mRNA (Cho et al., 2008).

KIDNEY

Along with liver, piscine kidney was an universal target to investigate metal toxicity and MTs expression, since this organ has been often reported to be one of main sites for the high accumulation of metals especially during acute phase, although the direct relationship between tissue burden (tissue metal concentration) and MTs expression has not been shown (Hollis et al., 2001; Szebedinszky et al., 2001; Cho et al., 2005). Long-term, even low-level, exposure to this metal also results in kidney destroy characterized by tubular dysfunction (Elinder et al., 1987). Increased nephrotoxicity of Cd in animals provided with Zn-deficient diet may be a consequence of the augmented renal Cd deposition and reduced synthesis of MTs, noted in these conditions of exposure (Fox et al., 1984; Waalkes, 1986; Panemangalore, 1993). The action of Zn can contribute to a reducing amount of Cd bound to MTs because Cd^{2+} ions are bound in the kidney cytosol by Zn-induced MTs (Squibb and Fowler, 1984; Sato and Nagai, 1989; Suzuki et al., 1990; Liu et al., 1992, 1994).

TESTIS

Some investigation indicated that testicular MTs does not act in the defense of the testes by Zn and that some other mechanisms must be involved (Waalkes et al., 1988; Wahba et al., 1994). In the black goby *Gobius niger*, it not only shows the toxic effect of Cd on hepatic tissue, but also suggests its potency as apoptotic factor in the testis (Migliarini et al., 2005).

NERVOUS TISSUE

MT gene is also expressed in the Xenopus central nervous system. MT transcripts were found in several fractions, especially in cellular bodies of periventricular regions (Durliat et al., 1999). This pattern is very similar to most other studied vertebrates (Hao et al., 1994; Choudhuri et al., 1995). This suggests that MT may play similar functions in all vertebrate brains.

In the study on brain tissue of ringed seal, there was apparently little relationship between MT levels in brain and other tissues (Sonne et al., 2009). There are two possible courses, first, blood brain barrier may be rather impermeable for metals and subsequently resulting in low MT concentrations. Second, there is a brain-specific form of MT which is supposed not to be affected by metal stress (Uchida et al., 1991).

THE INFLUENCE OF SOME HEAVY METALS ON AQUATIC VERTEBRATE

Organisms inhabiting contaminated waters present fairly high metal concentrations. Heavy metal venting into the marine environment can impair both marine species diversity and ecosystems, owing to their toxicity and accumulative behavior (Matta et al., 1999) (**Figure 2**).

Heavy metals are harmful materials that would lead to DNA damage and cancer (Valko et al., 2006). A distinguished increase of DNA breaks was found in peripheral blood cells of *Oreochromis niloticus* exposed to water samples including chromium (Matsumoto et al., 2006). Heavy metals such as lead, nickel, and zinc, among other metals, available in water and in the branchial, muscle, and hepatic tissues of two fish species was not followed by change in micronucleated erythrocyte frequency (Koca et al., 2008). These effects may be on account of the strong toxicity that high concentration of heavy metals may exert on fish cells (Cavas et al., 2005).

Heavy metals may cause embryotoxic/teratogenic effects via affecting cellular physiology (Calevro et al., 1998). Metal accumulation relies on tissue metabolism and differences in their chemical environment, including presence of various ligands, each with different metal-binding characteristics (Campenhout et al., 2004). Different extent of tolerance of each fish species to different heavy metals may also induce different expression level of MTs (Boeck et al., 2003; Kim et al., 2012). Notably, differential regulation of *metallothionein* by various metals has been revealed in many other fish species (Cho et al., 2005). Besides, the reason for metal-specific potential for MTs induction could generally been interpreted by the different availability of each metal ion in each organism or cell type (Kock et al., 1995; Olsvik et al., 2001).

In the previous study, the toxicity of metals was much more severe in younger fingerings (3-month-old) than juveniles (9-month-old) of *H. mylodon*, and sub-lethal doses of waterborne metals for juveniles led to remarkable mortality of younger fingerings, which was obviously proportional to the increasing doses (Cho et al., 2008).

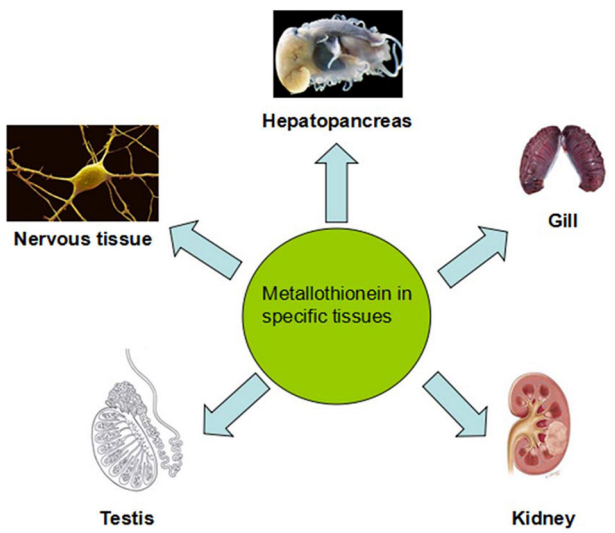

FIGURE 3 | Metallothionein separates in different tissues and they have variety distributions in different tissues of aquatic vertebrate. Experimental animals treated with high doses of Cd led to morphological and functional changes in hepatopancreas and high MTs amount in liver. Heavy metal accumulation and MTs induction in fish gills have been revealed to be very species-specific. For example, the major cell type dedicated to MTs expression in response to metal exposure are chloride cells. Kidney was an universal target to investigate metal toxicity and MTs expression. Even low-level exposure to Cd also results in kidney destroy. Testicular MTs may not act in the defense of the testes by Zn and that some other mechanisms must be involved in. MT gene expresses in the central nervous system to protect against the MeHg insults.

ZINC

It was presumed that molecular mechanism of Zn activity in the reproductive system may be associated with metal binding MTs (Kheradmand et al., 2010). Under the non-exposed condition, MTs are known to be linked principally by Zn, and higher accumulation of Zn might be required to induce additional MTs synthesis (Zhang and Wang, 2005). Zn can also prevent the pancreas from toxicity of Cd (Suzuki et al., 1990).

It has been demonstrated that pre-exposed Zn prior to Cd protects against Cd-induced liver toxicity, including lipid peroxidation and cell damage, even utilizing lethal doses of Cd (Goering and Klaassen, 1984; Khan et al., 1991; Kudo et al., 1991). Zn induces MTs synthesis with following changes in the hepatic subcellular Cd distribution (Goering and Klaassen, 1984). A shielding action of Zn against Cd hepatotoxicity has also been exhibited in *in vitro* studies (Chan and Cherian, 1992).

COPPER

The induction of MTs was partially ascribed to the Cu regulation process (McCarter and Roch, 1984; Boeck et al., 2003), and MTs may be in favor of Cu storage. For instance, in black sea bream *Acanthopagrus Schlegeli*, it appeared that MTs concentration increased 4.1-fold in the gastrointestine, 2.0-fold in the gills, and 1.8-fold in the carcass, compared with the extent of Cu accumulation (4.2-fold in the gastrointestine, 2.3-fold in the gills, and 1.5-fold in the carcass, separately) in fish (Dang et al., 2009). It was well-known that excess Cu ions are disruptors for ion homeostasis especially in gills of fish species, which might lead to a significant adverse effect on the viability of the fish (Dang et al., 2001). It was also observed that an increase

in hepatic Cu conjugated to MTLP in perch *(Perca fluviatilis)* exposed to Cu (Hogstrand and Haux, 1991; Kraemer et al., 2005). Increased levels of MT mRNA levels were also found in the zebrafish (*Danio rerio*) larvae and a liver cell-line under exposure to Cu_2O nanoparticle and $CuCl_2$ (Chen et al., 2011).

CADMIUM

Cd is one of the most toxic heavy metals and its toxicity has been widely investigated and reported. This metal is a serious environmental and occupational contaminant and may cause a severe hazard to the health of man and animals (Staessen et al., 1999). And the mortality and MTs levels were not directly related across groups exposed to different metals: the highest induction of *metallothionein* mRNA was achieved by Cd rather than Cu (Cho et al., 2008).

Cd appears to cause a variety of adverse reproductive effects in humans and experimental animals. Even low-level exposure to Cd, the metal still has its accumulation in placenta, placental abnormalities, decrease in birth weight, embryonic growth retardation, and malformations (Kuhnert et al., 1987; Fréry et al., 1993; Wier et al., 1993; Milnerowicz and Zasławski, 1995; Kantola et al., 2000). In addition, Cd exposure also results in pathological conditions in liver, testis, brain, and nervous system, kidney, spleen, and bone marrow (Shen and Sangiah, 1995; Yamano et al., 1998). Cadmium exposure can also lead to apoptosis in testes of rat, mouse liver, and human T-cells (Xu et al., 1996; Habeebu et al., 1998; Tsangaris and Tzortzatou-Stathopoulou, 1998). Cadmium appears to inhibit microtubule sliding in bovine sperm axoneme (Kanous et al., 1993).

Therefore, the strong Cd-inducibility of MTs from *Panulirus argus* in the nervous tissue might suggest an available mechanism that would make this tissue from the lobster to treat with this highly neurotoxic contaminant. And the role of MTs in nervous tissue of vertebrates has been suggested (Kramer et al., 1996; Tallkvist et al., 2002). Cd was the most potential for MTs induction among three metals (i.e., Cd, Cu, and Zn) in *H. mylodon*, and this outcome was consistent with previous discoveries that Cd was one of very powerful inducers for *metallothionein* transcription in fish (De Smet et al., 2001; Hermesz et al., 2001; Chan et al., 2006; Cho et al., 2008). It had been also suggested that transcriptional regulation of *metallothionein* in the fingerings of this species should be very sensitive to Cd, and any potential harmful stress to this species origin from the Cd pollution at much lower concentration than 0.1 mM in wild habitats may be detected by tracing the MTs expression in fingerlings if the exposure is extended (Cho et al., 2008).

MERCURY

After emission, inorganic Hg is methylated by microbes and enters aquatic food chains. Methylmercury (MeHg) is the most toxic form of Hg, and almost all (95–99%) Hg in fish is MeHg (Grieb et al., 1990). Notably, the highest concentrations of MeHg are found in piscivorous fish and wildlife (Spry and Wiener, 1991).

MeHg is neurotoxic, particularly in developing nervous systems, and has been related to many different neurological problems, from learning disabilities and behavioral abnormality to death (Zelikoff et al., 1995). Fathead minnows (FHM), *Pimephales promelas*, if fed MeHg-contaminated diets, showed a delay in spawning, a decline in spawning activity, and a decline in the quantities of eggs laid along with increasing MeHg (Hammerschmidt et al., 2002). Besides, dietary MeHg also damages gonadal development in walleye *Sander vitreus* (Friedmann et al., 1996) and walking catfish *Clarias batrachus* (Kirubagaran and Joy, 1988, 1992) and leads to testicular atrophy in guppies (Wester, 1991). And there is some evidence suggesting that MeHg inhibits sex hormones that cause secondary sex characteristics and stimulate gonadal development and gametogenesis. Specifically, MeHg could interfere with vitellogenesis (Kirubagaran and Joy, 1988) and spermatogenesis (Kirubagaran and Joy, 1992). In another study, it was found that MeHg exposure decreased phospholipid content in ovarian tissue of fish and implied that this may contribute to inhibition of vitellogenin synthesis in the liver (Kirubagaran and Joy, 1995). In previous studies, exposure to environmentally related concentrations of MeHg suppressed gonadal development and estrogen production in female FHM and testosterone in male FHM (Drevnick and Sandheinrich, 2003). Therefore, MeHg may function as an endocrine disruptor by binding to estrogen receptors and acting virtually as an estrogen mimic (Klaper et al., 2006). In addition, MeHg appears to affect bone cells, inducing hypercalcemia in goldfish and interferes with calcium homeostasis (Suzuki et al., 2004).

LEAD

Lead (Pb) is extensive used chemical for the preparation of large industry and household-based products. The toxicity of lead compounds, like all other heavy metals, was related to different disorders in humans (Saxena et al., 1986). More importantly, it has been recently noticed that lead compounds could result in oxidative stress in various tissues along with the generation of reactive oxygen species (ROS) (Quinlan et al., 1988; Acharya and Acharya, 1997; Hsu et al., 1998). In general, ROS are involved in impairing the polyunsaturated fatty acids of the membrane phospholipids of the cells causing defects of cellular functions (Halliwell and Gutteridge, 1985). Pb exposure was also related with tissue oxidative damage in chicks (Donaldson and Leeming, 1984) and rats (Sandhir et al., 1994).

Pb-treatment has been known to associate with decreased sperm count, motility, and increased morphological abnormalities in animals (McGivern et al., 1991; Sokol and Berman, 1991) and humans (Lerda, 1992; Alexander et al., 1996). A report demonstrated that Pb exposure stimulated ROS generation in rat spermatozoa, which was negatively correlated with fertility (Hsu et al., 1997). Pb exposure might descend defense ability of sperm to the oxidative stress and enhance the ROS generation, reduce sperm motility and oocyte penetration capability in rat (Hsu et al., 1998). More evidences have revealed that testicular physiologies, basically characterized by spermatogenesis process, could be at least partially controlled by reactive oxygen-dependent mechanisms (Koizumi and Li, 1992). It is well-known that ROS could cause chromosomal aberrations by mutating certain gene segments, resulting in abnormal sperm population and/or dramatically reduce in sperm count (Eyden et al., 1978; Hartwing

et al., 1990; Koizumi et al., 1992; Hsu et al., 1998). For instance, chromosomal aberrations, increased sperm abnormalities and decreased sperm count profile in lead-exposed mice (Usha et al., 2003).

OTHER HEAVY METALS AND ARSENIC (AR)

Tin-protoporphyrin regulates heme oxygenase and metallothionein gene expression through heme-hemopexin interaction, since tin-protoporphyrin is a kind of heme analog (Piotrowski and Szymańska, 1976). After 7 days of exposure to nickel chloride, MT accumulation in sexually immature sea bass *Dicentrarchus labrax* increased with a marked synergetic effect (Banni et al., 2011). The study of rainbow trout presented that cobalt exposure result in increased expression levels of metallothionein genes. Since the organism like fishes do not have ability to completely discharge or decompose heavy metals, they tend to bio-accumulate the non-eliminated metals rather than decompose or discharge heavy metals, which may lead to fish death at last. Bismuth is known to induce the synthesis of renal metallothionein (Disilvestro et al., 1996).

Fisrt, inducation of Ar was found in rat hepatic metallothionein *in vivo* (Albores et al., 1992). Complex interactions also were found between Ar and human metallothionein (Toyama et al., 2002; Ngu and Stillman, 2006). Recently, influence of As on MT isoform gene expression in Human Glioblastoma cells was clearly demonstrated, As may be related with brain tumor and type II cell death (Falnoga et al., 2012).

THE CONTRIBUTION OF MTs TO AQUATIC ANIMAL ESPECIALLY ON DEVELOPMENT

MTs appear to play a predominant role in the metabolism and detoxification of Cd in marine organisms (George et al., 1996). During oxidative stress, synthesis of MTs may increase several times (Thornalley and Vasak, 1985) to prevent the cells from cytotoxicity (Aschner et al., 1998) and DNA damage (Cai et al., 1995).

In another study, basal level of *H. mylodon metallothionein* mRNA reached highest in ovary, indicating that the rich *metallothionein* mRNAs in ovary would be transported to early embryos (Cho et al., 2008). The transmitted *metallothionein* mRNAs may act a critical role in the fine regulation of metal homeostasis which is one of primary requirements for normal embryogenesis and early larval development (Chen et al., 2004).

APPLICATION OF MTs AS A BIOMARKER FOR EVALUATING THE AQUATIC ENVIRONMENT

Owing to its highly inducible expression during exposures to various heavy metals, MTs have been paid much attention as a potential biomarker to monitor the heavy metal pollution of aquatic ecosystem, a major receptor of pollutants especially with relatively high amount of heavy metals (Langston et al., 2002; Tom et al., 2004; Cho et al., 2005). Nevertheless, the application of MTs to biomarker assay needs large evaluation of a number of abiotic and biotic factors such as salinity, pH, temperature, seasonality, fish age, sex, and reproductive cycle that may affect the expression of MTs (Chen et al., 2004; Marijić and Raspor, 2006).

CONCLUSIONS

We reviewed the recent advances of characteristics, functions and applications of metallothionein in aquatic vertebrates, the general function of MT, transcription regulation, induction factors, and the influence on the development of aquatic vertebrates were summarized. MTs could be induced by various physiological and toxicological stimuli, such as oxidative stress (suggesting that *in vivo* they may inactivate hydroxyl radicals), cytokines, chemicals, and heat as well as heavy metals. Sensitive regulation of the MTs expression by heavy metal exposures would make it possible to use the transcriptional quantification of the present MTs as a biomarker for evaluating metal pollutions. We then focus on the role of MTs in different organs and tissues. Marine heavy metal pollution is increasingly serious, which leads to oxidative stress, cell apoptosis, and neuron damage in marine vertebrates and human who eat seafood. MTs can protect aquatic vertebrates from harmful metals especially during development. More importantly, the study indicates a novel way to protect human from polluted sea products.

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