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Focused review

Arsenic–glutathione conjugate transport by the human multidrug resistance proteins (MRPs/ABCCs)

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ABSTRACT

Millions of people world-wide are chronically exposed to inorganic forms of the environmental toxicant arsenic in drinking water. This has led to a public health crisis because arsenic is a human carcinogen, and causes a myriad of other adverse health effects. In order to prevent and treat arsenic-induced toxicity it is critical to understand the cellular handling of this metalloid. A large body of literature describes the importance of the cellular tripeptide glutathione (γ-Glu-Cys-Gly,GSH/GS) in the excretion of arsenic. The triglutathione conjugate of arsenite [$As^{III}(GS)_3$] and the diglutathione conjugate of monomethylarsonous acid [$MMA^{III}(GS)_2$] have been isolated from rat bile and mouse urine, and account for the majority of excreted arsenic, suggesting these are important transportable forms. The ATP-binding cassette (ABC) transporter proteins, multidrug resistance protein 1 (MRP1/*ABCC1*) and the related protein MRP2 (*ABCC2*), are thought to play an important role in arsenic detoxification through the cellular efflux of arsenic–GSH conjugates. Current knowledge on the cellular handling of arsenic with a special emphasis on the transport pathways of the arsenic–GSH conjugates $As^{III}(GS)_3$, $MMA^{III}(GS)_2$, and dimethylarsenic glutathione $DMA^{III}(GS)$, as well as, the seleno-bis(*S*-glutathionyl) arsinium ion $[(GS)_2AsSe]^-$ are reviewed.

1. Introduction

1.1. Arsenic

Inorganic arsenic (arsenate [As^V] and arsenite [As^{III}]) is a multi-target human carcinogen: chronic exposure is associated with increased incidences of skin, lung, and bladder tumors [1–3]. In addition, exposure to inorganic arsenic is associated with peripheral vascular disease, neurological disorders, and diabetes mellitus [4]. Millions of people worldwide are chronically exposed to nonanthropogenic sources of As^V and As^{III} in their drinking water, the most common exposure route for adverse health effects [1,2,5,6]. Arsenic-based compounds are also used in chemotherapy. Arsenic trioxide (As_2O_3) has been approved for treating both newly diagnosed and relapsed acute promyelocytic leukemia, with high remission rates [7]. Furthermore, As_2O_3 and another arsenical, dimethylarsenic glutathione [$DMA^{III}(GS)$] are in clinical trials for the treatment of multiple hematological and solid tumors [8–10]. Despite the recognized dangers of environmental arsenic exposure and its use in chemotherapy, certain key pathways involved in arsenic metabolism, excretion, and carcinogenesis remain poorly understood.

Studies of inorganic arsenic-exposed human populations show large inter-individual differences in susceptibility to carcinogenesis, however, the genetic basis for this variation is not known [11,12]. In order to prevent and treat arsenic-induced toxicity it is critical to understand the underlying molecular mechanisms that render a person susceptible. Arsenic metabolism and excretion pathways are complex. Although multiple pathways are likely responsible for the inter-individual differences, the relevance of transport proteins has become increasingly evident [4,13,14]. The focus of this review article is the cellular handling of inorganic and methylated arsenic species with a special emphasis on arsenic-GSH conjugates and how they are transported out of the cell.

1.2. ATP-binding cassette transporter proteins

The human genome encodes for 48 ATP-binding cassette (ABC) proteins which are organized into seven subfamilies (A–G) [15]. Several of these genes are involved in well-defined genetic disorders [16,17]. ABC transporters are responsible for the active transport of a wide variety of compounds, including phospholipids, peptides, and amino acids as well as drugs and toxicants [18]. A typical mammalian ABC transporter protein is composed of four structural domains: two polytopic membrane-spanning domains (MSDs) and two nucleotide-binding domains (NBDs) (Fig. 1) [19]. Several members of subfamily C, including the multidrug resistance protein 1 (MRP1/*ABCC1*) and MRP2 (*ABCC2*), contain an additional amino-

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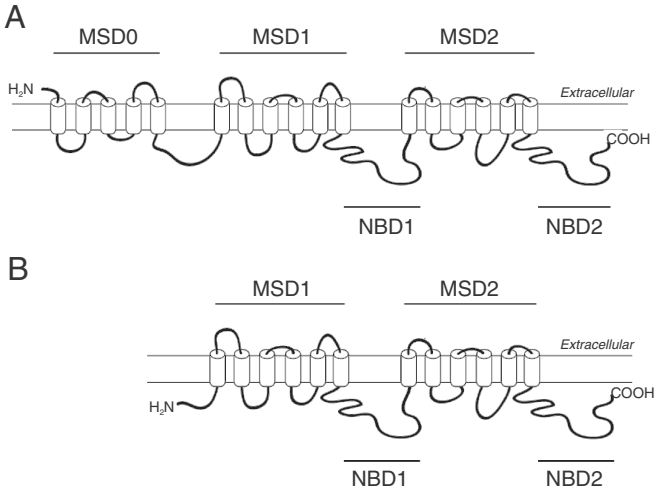

Fig. 1. Predicted secondary structure for the MRPs that (*A*) contain the characteristic third NH_2-terminal membrane spanning domain (MSD0) (MRP1, MRP2, MRP3, MRP6, and MRP7) and (*B*) the smaller MRPs with the more typical ABC four domain structure that lacks the extension (MRP4, MRP5, MRP8, and MRP9).

terminal MSD (MSD0) (Fig. 1) [20]. The NBDs are required to provide energy for the movement of substrates across membranes and contain three characteristic motifs: Walker A, Walker B and the ABC signature motif (or C motif). The Walker A and B motifs are conserved in all ABC proteins as well as other ATP-binding proteins, while the C motif is unique to ABC proteins [21]. In all ABC proteins, two NBDs associate as a head to tail dimer, with ATP sandwiched between the A and B motifs of one monomer and the C-motif of the other monomer [22].

1.2.1. ATP-binding cassette transporters and tissue defense

P-glycoprotein (*ABCB1*) was the first mammalian ABC transporter to be discovered [23]. It was isolated based on its ability to confer multidrug resistance through an ATP-dependent decrease in cellular drug accumulation. More recently identified ABC proteins that confer drug resistance include MRP1 and the breast cancer resistance protein 1 (BCRP/*ABCG2*). The isolation of MRP1 facilitated the discovery of eight more genes within the same ABC subfamily (subfamily C) MRP2 (*ABCC2*), MRP3 (*ABCC3*), MRP4 (*ABCC4*), MRP5 (*ABCC5*), MRP6 (*ABCC6*), MRP7 (*ABCC10*), MRP8 (*ABCC11*), and MRP9 (*ABCC12*), of which at least five: MRP2, MRP3, MRP4, MRP5, and MRP7, are potentially involved in mediating drug resistance [20,24–27]. ABC transport protein nomenclature dictates that human ABC transporters are referred to using capital letters (e.g., MRP1), while rodent forms are referred to using first letter capitalized followed by lower case (e.g., Mrp1).

In addition to their role in anticancer drug resistance, P-glycoprotein, BCRP, and several MRPs are known to be important for protecting tissues from xenobiotic accumulation and toxicity [28–30]. P-glycoprotein substrates are generally large, hydrophobic or amphipathic, neutrally or positively charged, aromatic ring containing structures, and do not include GSH-conjugates [31]. Although mice lacking the two murine Abcb1 genes (*Abcb1a*/*Abcb1b*) were found to be more sensitive to As^{III} and accumulated more total arsenic in certain tissues than wild-type animals [32,33], it is highly unlikely that P-glycoprotein transports arsenic-GSH conjugates [28], and P-glycoprotein will not be discussed further here. To date there is no evidence to suggest that BCRP plays a role in protecting tissues from any form of arsenic, therefore, BCRP also will not be discussed further. Thus, the ABC proteins that have an established role in protecting cells from inorganic and methylated arsenic species through the efflux of arsenic-GSH conjugates are MRP1 and MRP2, and are the major focus of this review.

1.2.2. MRP1 and MRP2 substrate specificity and tissue distribution

A major distinguishing feature of the substrate specificities of the various MRPs, compared to P-glycoprotein, is the ability of the MRPs to transport a broad range of GSH-, glucuronide-, and sulfate-conjugated organic anions [20,28,34]. MRP1 and MRP2 act synergistically with several phase II conjugating enzymes [including glutathione transferases (GSTs)] to confer resistance to the toxicities of electrophilic drugs and carcinogens [35–38]. However, MRP1 and MRP2 require GSH (γ-Glu-Cys-Gly) not only for the transport of GSH-conjugated molecules but also for the co-transport of certain unconjugated compounds, including the chemotherapeutics vincristine, vinblastine, etoposide, and doxorubicin [34,39–45]. The reducing thiol group of GSH (in its Cys residue) is not required for the enhancement or facilitation of GSH-dependent transport by MRP1 or MRP2 because nonreducing GSH analogs such as *S*-methyl GSH and/or ophthalmic acid (γ-Glu-Abu [aminobutyrate]-Gly) can substitute functionally [41,46–49].

MRP1 is expressed in most tissues throughout the body, with relatively high levels found in the lungs, testes, and kidneys; however, its levels are almost undetectable in healthy human liver [24,50,51]. MRP1 localizes to the basolateral surface of epithelia and the apical surface of brain capillaries, generally resulting in the efflux of MRP1 substrates into the blood. MRP2 is localized to the apical surface of epithelial and endothelial cells, primarily in sanctuary sites such as the blood–brain barrier, placenta, liver, gut, and kidney; thus, MRP2 is important for the absorption, distribution, and elimination of xenobiotics and their metabolites [28,52,53].

2. Biotransformation and transport pathways of arsenic

2.1. Cellular methylation and uptake of arsenic

2.1.1. Methylation of arsenic

Arsenic undergoes extensive methylation in humans and most other mammals, with the known exceptions of marmoset, tamarine and squirrel monkeys, chimpanzees and guinea pigs [54,55]. The original and generally well accepted methylation pathway involves the enzymatic reduction of pentavalent arsenicals followed by the oxidative methylation of trivalent species [56]. Conversely, several more recent studies have suggested that trivalent arsenicals undergo reductive (instead of oxidative) methylation while complexed with GSH or protein [57,58]. Regardless of the precise mechanistic pathway, within the cell As^{V} is reduced to As^{III} and in humans the methylated products: methylarsonic acid (MMA^{V}), methylarsonous acid (MMA^{III}), dimethylarsinic acid (DMA^{V}) and dimethylarsinous acid (DMA^{III}) are formed (Fig. 2). Further methylation results in the production of trimethylarsine oxide ($TMA^{V}O$) and trimethylarsine (TMA^{III}) [13]. Methylation requires the enzyme arsenic (+3 oxidation state) methyltransferase (AS3MT), the methyl donating co-factor *S*-adenosylmethionine (SAM) and a reducing agent, which under physiological conditions is most likely GSH.

The role of methylation in arsenic detoxification is complex and somewhat controversial. Comparison of species that do and do not methylate arsenic suggests that methylation increases the rate of whole body clearance of arsenic [54], and it has been generally thought that methylation is a detoxification process. However, in vitro studies have revealed that the trivalent methylated species MMA^{III} and DMA^{III} are substantially more potent toxicants than As^{III} resulting in methylation being considered an activation pathway [59,60]. In contrast, disruption of the *As3mt* gene in mice resulted in a considerable increase in arsenic whole body accumulation, and the *As3mt*(−/−) mice are more sensitive to the toxicity of As^{III} than *As3mt*(+/+) mice, suggesting that methylation is nonetheless protective, at least during short term exposures [61–63].

Genetic differences in the metabolic and transport pathways for arsenic could contribute to the observed variations in arsenic-induced susceptibility to cancer. In humans, the excretion of arsenic