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Chronic MeHg exposure modifies the histone H3K4me3 epigenetic landscape in Caenorhabditis elegans

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Epigenetics
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A B S T R A C T

Methylmercury (MeHg) is a persistent environmental pollutant that occurs in the food chain, at occupational sites, and via medical procedures. Exposure in humans and animal models results in renal, neuro, and reproductive toxicities. In this study, we demonstrate that chronic exposure to MeHg (10 μM) causes epigenetic landscape modifications of histone H3K4 trimethylation (H3K4me3) marks in Caenorhabditis elegans using chromatin immunoprecipitation sequencing (ChIP-seq). The modifications correspond to the locations of 1467 genes with enhanced and 508 genes with reduced signals. Among enhanced genes are those encoding glutathione-S-transferases, lipocalin-related protein and a cuticular collagen. ChIP-seq enhancement of these genes was confirmed with increased mRNA expression levels revealed by qRT-PCR. Furthermore, we observed enhancement of H3K4me3 marks in these genes in animals exposed to MeHg in utero and assayed at L4 stage. In utero exposure enhanced marks without alterations in mRNA expression except for the lpr-5 gene. Finally, knockdown of lipocalin-related protein gene lpr-5, which is involved in intercellular signaling, and cuticular collagen gene dpy-7, structural component of the cuticle, by RNA interference (RNAi) resulted in increased lethality of animals after MeHg exposure. Our results provide new data on the epigenetic landscape changes elicited by MeHg exposure, as well as describe a unique model for studying in utero effects of heavy metals. Together, these findings may help to understand the toxicological effects of MeHg at the molecular level.

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1. Introduction

Methylmercury (MeHg) is an environmental pollutant and serious public health concern with exposure occurring through ingestion of contaminated fish and seafood, or via occupational and environmental routes (Clarkson, 1983). MeHg is a potent teratogen, retinotoxin, and neurotoxin that poses a risk during gestational, postnatal and adult developmental stages. Because of its strong affinity to SH- groups, MeHg binds the amino acid L-cysteine and is transported to tissues as a L-amino acid substrate (Yin et al., 2008). It crosses the blood-brain barrier and accumulates amino acid L-cysteine and is transported to tissues as a L-amino acid sub-

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2. Materials and methods

2.1. C. elegans maintenance

C. elegans wild-type (WT) Bristol N2 and RNAi-sensitive mutant NL2099 (rfl-3(pk1426)) strains used in this study were obtained from the Caenorhabditis Genetics Center (St. Paul, MA USA) and maintained at 20 °C on nematode growth media (NGM) plates containing OP-50 bacteria according to standard protocols (Brenner, 1974).

2.2. Synchronization, treatment and larval development assay

Synchronization of the worms was carried out using potassium hypochlorite solution to bleach the gravid adults. Isolated embryos were washed 4 × with M9 buffer and to obtain synchronized population incubated in M9 buffer with gentle rocking for 1 h at a room temperature. For chronic exposure in ChiP-seq, RNA-Seq and qRT-PCR experiments L1 larvae animals were placed on NGM plates ± MeHg (0, 10 μM) (CH₃HgCl, Sigma-Aldrich, St Louis, MO) and allowed to grow at 20 °C until reaching L4 stage just before adulthood. For acute MeHg treatment animals were grown until L4 stage and transferred onto an NGM plates containing MeHg (25 μM) for 4 hours. MeHg-containing plates were collected, washed 4 × with sterile water, centrifuged several times at 2000 rpm for 1 min to obtain tightly packed pellet and frozen immediately. Frozen material was ground using a cryo mortar, suspended in 1% formaldehyde in PBS solution to cross-link proteins to DNA and chromatin was sheared into a 150–800 base pair size range using a Branson ultrasonic dismembrator (Thermo Fisher Scientific, Waltham, USA). ChiP reactions were carried out according to Rechtsteiner et al. (2010) using ~20 μg of chromatin and 3 μl of H3K4me3 (Active Motif, Carlsbad, CA) antibody per reaction, leaving 10% of a starting material as input. Rabbit IgG antibody (Sigma, St Louis, MO) or no antibody was used for a negative control. ChiP reaction was incubated with antibody at 4 °C overnight.

2.3. Chromatin immunoprecipitation (ChiP) from L4 larvae animals

For IP reaction control and MeHg-treated L4 larvae worms were collected, washed 4 × with sterile water, centrifuged several times at 2000 rpm for 1 min to obtain tightly packed pellet and frozen immediately. Frozen material was ground using a cryo mortar, suspended in 1% formaldehyde in PBS solution to cross-link proteins to DNA and chromatin was sheared into a 150–800 base pair size range using a Branson ultrasonic dismembrator (Thermo Fisher Scientific, Waltham, USA). ChiP reactions were carried out according to Rechtsteiner et al. (2010) using ~20 μg of chromatin and 3 μl of H3K4me3 (Active Motif, Carlsbad, CA) antibody per reaction, leaving 10% of a starting material as input. Rabbit IgG antibody (Sigma, St Louis, MO) or no antibody was used for a negative control. ChiP reaction was incubated with antibody at 4 °C overnight. Protein G-coupled Dynabeads (Thermo Fisher Scientific) were added and incubated for 2 h with rotation. Magnetic beads were extracted using DynaMag-2 magnet (Thermo Fisher Scientific) and washed at room temperature in FA buffer (50 mM HEPES, pH 7.5/ 1 mM EDTA, pH 8/1% TRITON X-100/0.1% sodium deoxycholate/150 mM NaCl) twice for 5 min, FA buffer with 500 mM NaCl once for 5 min, FA buffer with 1 M NaCl once settling beads right away, TEL buffer (0.25 M LiCl/1% NP-40/1% sodium deoxycholate/1 mM EDTA/10 mM Tris–HCl, pH 8.0) once for 5 min and TE buffer twice for 5 min. Eluted, RNase A/proteinas K treated and overnight reverse-crosslinked samples were cleaned using QiAquick PCR purification kit (Qiagen, Hilden, Germany). Antibody affinity was tested by qPCR method using act-1 and act-4 genes as a positive control and intergenic region sequences from chromosomes IV and X (primer sequences are shown in Table 1). The ChiP-seq experiments were performed in duplicate and DNAs immunoprecipitated were sequenced at the Millard and Muriel Jacobs Genetics and Genomics Laboratory of California Institute of Technology (Pasadena, CA, U.S.A.) on an Illumina HiSeq 2500 instrument. Sequences were obtained in single read mode.

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For IP reaction control and MeHg-treated L4 larvae worms were collected, washed 4 × with sterile water, centrifuged several times at 2000 rpm for 1 min to obtain tightly packed pellet and frozen immediately. Frozen material was ground using a cryo mortar, suspended in 1% formaldehyde in PBS solution to cross-link proteins to DNA and chromatin was sheared into a 150–800 base pair size range using a Branson ultrasonic dismembrator (Thermo Fisher Scientific, Waltham, USA). ChiP reactions were carried out according to Rechtsteiner et al. (2010) using ~20 μg of chromatin and 3 μl of H3K4me3 (Active Motif, Carlsbad, CA) antibody per reaction, leaving 10% of a starting material as input. Rabbit IgG antibody (Sigma, St Louis, MO) or no antibody was used for a negative control. ChiP reaction was incubated with antibody at 4 °C overnight. Protein G-coupled Dynabeads (Thermo Fisher Scientific) were added and incubated for 2 h with rotation. Magnetic beads were extracted using DynaMag-2 magnet (Thermo Fisher Scientific) and washed at room temperature in FA buffer (50 mM HEPES, pH 7.5/ 1 mM EDTA, pH 8/1% TRITON X-100/0.1% sodium deoxycholate/150 mM NaCl) twice for 5 min, FA buffer with 500 mM NaCl once for 5 min, FA buffer with 1 M NaCl once settling beads right away, TEL buffer (0.25 M LiCl/1% NP-40/1% sodium deoxycholate/1 mM EDTA/10 mM Tris–HCl, pH 8.0) once for 5 min and TE buffer twice for 5 min. Eluted, RNase A/proteinas K treated and overnight reverse-crosslinked samples were cleaned using QiAquick PCR purification kit (Qiagen, Hilden, Germany). Antibody affinity was tested by qPCR method using act-1 and act-4 genes as a positive control and intergenic region sequences from chromosomes IV and X (primer sequences are shown in Table 1). The ChiP-seq experiments were performed in duplicate and DNAs immunoprecipitated were sequenced at the Millard and Muriel Jacobs Genetics and Genomics Laboratory of California Institute of Technology (Pasadena, CA, U.S.A.) on an Illumina HiSeq 2500 instrument. Sequences were obtained in single read mode.
2.4. ChIP-seq analysis

The number of reads obtained from MeHg non-treated duplicate samples were 34.7M/48.9M and 41.7M/29.3M from the IgG control and H3K4me3 samples, respectively. The number of reads, obtained from MeHg-treated samples were 31.0M/27.6M and 24.8M/27.5M from the IgG control and H3K4me3 samples, respectively. Reads were aligned to the C. elegans genome (WS220) using Bowtie 1.1.2 (Trapnell et al., 2009) allowing at most 2 mismatches and 10 alignments per read and reporting reads in the best stratum (–best and –strata). The percentage of mapped reads with at least one reported alignment ranged from 6–38% for all H2O/MeHg treatment samples. The alignments were processed using MACS2 peak calling algorithm with false discovery rate- corrected p-values (q-values) of <0.01. A total of 2704 peaks were identified in IgG/H3K4me3 for MeHg-non-treated samples and 4098 peaks were identified in IgG/H3K4me3 for MeHg-treated samples. The nearest gene for the peaks was obtained using ChiPpeakAnno (Zhu et al., 2010). Gene enrichment analysis was performed using DAVID Functional Annotation Tool 6.7 (Huang et al., 2009).

2.5. RNA isolation and cDNA synthesis

Both control and MeHg-treated L4 stage worms were collected, washed 4× with sterile water and placed immediately into Trizol solution (Gibco-BRL, Gaithersberg, MD). Total RNA was isolated according to manufacturer’s protocol using RiboPure RNA purification kit and quantitated on a Nanodrop device (Thermo Scientific, Wilmington, DE, USA). cDNA was synthesized using RevertAid RT reverse transcription kit (Thermo Scientific) according to manufacturer’s protocol using 0.5 μg of total RNA as a template.

2.6. Quantitative real-time PCR (qRT-PCR)

Several genes were selected for verification using qRT-PCR method. Both worm ChIP and cDNA samples from isolated total RNAs were used for confirmation reactions. Gene specific oligonucleotide primers for qRT-PCR were designed using Primer-BLAST (Ye et al., 2012) and obtained from Oligomer OY (Helsinki, Finland). Maxima SYBR green qPCR Master mix (Thermo Fisher Scientific) was used for amplification reactions according to the manufacturer’s protocol. Reactions were performed in iCycler 1.0 system (Biorad, Hercules, CA, USA). Three independent biological replicates in technical duplicates were used for this analysis. Quantitative differences were calculated using delta-deltaCT method (Livak and Schmittgen, 2001) normalized to act-1 (gene expression) or input (ChIP reactions). Sequences for all primer sets used are listed in Table 1.

2.7. RNA-mediated interference and whole body vulnerability assay

RNA-mediated interference (RNAi) was performed as previously described (Rudgalvyte et al., 2016) using RNase III-deficient Escherichia coli bacteria strain HT115 (DE3) carrying L4440 vector with the gene fragments (lpr-5 and dpy-7) (GeneService, Source BioScience, PLC, Nottingham, UK). Animals were exposed to MeHg (0, 10 or 20 μM) for 24 or 48 h. Worms were considered dead if a touch on the anterior end of the body with a wire pick did not cause any movement. Experiments were repeated at three different times in triplicate technical replicates each time, using 50 animals per replicate. Results are presented as an average ± SD.

2.8. Statistics

The results are presented as the average of 3 independent replicates ± S.D. For gene expression analysis fold changes were calculated using ΔΔCT method. The statistical analysis was performed using Student’s t-test.

3. Results

3.1. ChIP-seq analysis of C. elegans chronically exposed to MeHg

In this study, 1975 genes with a modified H3K4me3 pattern were uncovered of which 1467 had an increased level of methylation and 508 had a reduced methylation level. The complete gene lists can be found in Supplementary Table 1. Among increased H3K4me3 genes we observed were cytochrome c encoding cyc-2,1 family members of the glutathione S-transferases (gst-1, gst-5, gst-6, gst-10, gst-38), mitochondria-specific chaperone hsp-60, unfolded protein response (UPR)-related CAMP-dependent transcription factor atr-6 and lipocalin-related protein lpr-5. In the decreased methylation list, we uncovered another UPR sensor protein kinase ire-1, Ca2+-binding ER membrane protein cxx-1 and NAD-dependent protein deacetylase sir-2.1.

The data from ChIP-seq were compared to our previous RNA-Seq data where gene expression changes following MeHg treatment were investigated (Rudgalvyte et al., 2013). Overall, we found overlap of 43 genes up-regulated by RNA-Seq and increased methylation by ChIP-seq and 7 genes in down-regulated/decreased methylation lists (Table 2). We then compared mapped reads for selected genes that were on the overlapping lists: gst-5, gst-38, lpr-5, and dpy-7 (Fig. 1). A large number of the 43 genes up regulated by RNA-seq and enhanced in H3K4me3 were from specific gene families that form or affect structures in the cuticle. These include dumpy (dpy-3, dpy-7, dpy-9, dpy-8, dpy-5), molting (mlt-11, mlt-10, mlt-8), collagen (col-176, col-110, col-129), blister ( bli-1, bli-2), long ( lon-3), and roller ( rol-6) gene families (Table 2).

3.2. Functional annotation and GO enrichment analysis for H3K4me3 associated genes

In order to identify biological themes over-represented in our gene lists, DAVID functional annotation tool was used to annotate and extract gene groups that displayed significant (p<0.05) enrichment of Gene Ontology (GO) biological processes, cellular compartments, or molecular functions (Fig. 2 and Supplementary Table 2). Enriched biological processes that were identified in lists for enriched H3K4me3 marks were: nematode larval development (209 genes), positive regulation of growth rate (181 genes), collagen and cuticlein-based cuticle development (19 genes), protein transport (30 genes), and translation (29 genes). A number of increased histone activity genes belong to the cellular component cytoplasm (134 genes), intracellular organelle (191 genes), nucleus (96 genes), mitochondrial part (14 genes) groups including ATP synthesis proteins ATP-synthase G homolog, ATP-synthase subunit, cytochrome c, and mitochondria-specific hsp-60. The most over-represented biological processes for reduced H3K4me3 were genitalia development (45 genes), positive regulation of growth rate (78 genes), meiosis (15 genes), and organ morphogenesis (21 genes). Enriched cellular component groups for decreased H3K4me3 were intracellular organelle (73), cytoplasm (43 genes), nucleus (43 genes), mitochondrial inner membrane (5 genes), and endoplasmic reticulum (8 genes). Interestingly, the GO category for positive regulation of growth rate was found on both enriched and reduced gene lists.

3.3. Confirmation of H3K4me3 methylation pattern changes by ChIP and qRT-PCR at selected gene regions in MeHg exposed animals

We selected a number of genes to verify the increased methylation level of H3K4me3 using ChIP and qRT-PCR methods. These included 2 glutathione S-transferases gst-5 and gst-38, lipocalin-related protein lpr-5, cuticular collagen coding dpy-7, UPR-related CAMP-dependent transcription factor atr-6, and UPR sensor protein kinase ire-1. In addition to histone methylation pattern analysis, we performed gene expression analysis to determine if changes in methylation affected gene expression. Of all the genes tested, only ire-1 showed no significant
changes in both methylation and gene expression levels (data not shown). We observed significant increases in the rest of the selected H3K4me3 associated genes. Significant (≥2-fold change and p < 0.05) H3K4 trimethylation and gene expression changes were observed in gst-5 (3.2-fold H3K4 trimethylation/8.2-fold gene expression), gst-38 (2.7-fold/18.7-fold), lpr-5 (3.2-fold/4.7-fold), dpy-7 (3.5-fold/1.3-fold), and aff-6 (2.7-fold/1.1-fold) genes (Fig. 3a).

### 3.4. Persistence of H3K4me3 signal enrichment after in utero MeHg exposure

In order to determine if MeHg exposure induces effects in methylation pattern in utero that persists over a long-term period, we isolated embryos from gravid adults exposed to MeHg (10 μM) for 96 h. Embryos were allowed to grow until the L4 larval stage free of MeHg and analyzed. Although animals exposed in utero to MeHg did not superficially appear different from controls, development was slightly delayed (Fig. 3b).

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It has been shown that MeHg exposure induces ROS production in C. elegans and activates a cellular stress response (Vanduyn et al., 2010). We determined if early embryonic in utero MeHg exposure can modulate later stress responses to MeHg. We measured expression levels of several glutathione S-transferases (GSTs) and heat shock proteins (HSPs) that are known stress response genes after acute MeHg exposure (4 h, 25 μM) in naive or in utero pre-exposed animals. We observed a significant increase (p < 0.05) in gst-4 (7.5-fold in naive/6.6-fold in utero pre-exposed), gst-5 (6.6-fold/7.8-fold) and gst-38 (7.2-fold/7.7-fold) gene expression. These results suggest that a previous in utero exposure to MeHg does not influence response to later acute MeHg exposure. No changes were observed in the expression of gst-1 in any samples. No differences between samples were observed in hsp-4 and hsp-6 genes (Fig. 3d).

### 3.5. Acute MeHg exposure increases GST and HSP gene expression in both naive and in utero pre-exposed animals

The lipocalin-related protein lpr-5 and cuticular collagen dpy-7 were selected from our gene lists to determine if these genes have an effect on animal vulnerability in presence of MeHg. RNAi experiments were performed to knock-down expressions of these genes. Since lpr-5 knockdown animals suffer from body rupture during later reproductive stages, we decided to score death or survival after 24 h of exposure to eliminate additional effects of gene knock-down. Dpy-7 animals were scored for death after 48 h of MeHg exposure. Our study revealed that RNAi of lpr-5 and dpy-7 resulted in increased vulnerability to death following MeHg exposure. After 24 h of MeHg exposure (20 μM), 25% of lpr-5 knockdown animals were found dead, while WT animals tolerated the toxicant (Fig. 4a). The decrease of dpy-7 expression resulted in a death of 63% (10 μM) and 97% (20 μM) of animals while only 3% (10 μM) and 5% (20 μM) of animals experienced death in WT control group (Fig. 4b). Results are presented as an average of three independent experiments ± S.D., p < 0.05. These results suggest that expression of lpr-5 and dpy-7 are essential in the inhibition of MeHg-induced animal death.

### 4. Discussion

We performed global chromatin immunoprecipitation sequencing (ChIP-Seq) to uncover H3K4me3 enriched and reduced regions after chronic MeHg exposure (10 μM) in the nematode C. elegans. Our results show enrichment in several glutathione S-transferases (e.g. gst-1, gst-5, gst-6, gst-10 and gst-38), mitochondria-specific chaperon hsp-60, and unfolded protein response (UPR)-related cAMP-dependent transcription factor af-6. Our results support previously reported UPR involvement in MeHg-induced toxicity (Rudgalvye et al., 2013) and gene expression changes in GST family following MeHg exposure (Vanduyn et al., 2010; Rudgalvye et al., 2013). Consistent with these results, a previous study has shown that prenatal exposure to MeHg can developmentally retard the glutathione system in mouse brain (Montgomery et al., 2008). Studies investigating the epigenetic regulation of the Gstz1 locus in developing mouse liver also show low levels of DNA
methylation and H3K27me3 gene reduction marks and enrichment of H3K4me2 gene activation marks (Cui et al., 2010). Our results showing both increases in mRNA expression levels and H3K4me3 marks in several GST genes suggests that our *C. elegans* model may correspond some elements of epigenetic regulation observed in higher organisms.

Although data in this study was obtained using the concentration of MeHg higher than normally can be encountered in the environment, previous studies using more environmentally relevant concentration for cell line treatment that correspond to Hg levels in the brain of fish consumers (Ceccatelli et al., 2013) and studying dental professionals (Goodrich et al., 2013) revealed epigenetic changes as the effect of MeHg toxicity, confirming that MeHg is an epigenetic toxicant. Using higher concentrations for animal treatment might help to uncover a wider range of pathways involved in MeHg-induced stress response and may also detect adverse effects sooner.

In addition, our study provides evidence for widespread epigenetic changes elicited by MeHg that can be correlated with changes in gene expression. We demonstrate that for some of the genes, these epigenetic changes persist. A previous study has shown that epigenetic changes are associated with long lasting adverse effects in stem cells induced by MeHg and these can be inherited (Bose et al., 2012). Other studies have shown epigenetic effects of mercury exposure in human tissues (Cardenas et al., 2015; Maccani et al., 2015). However, these studies looked at non target tissues such as umbilical cord blood (Cardenas et al., 2015) or placenta (Maccani et al., 2015) and direct epigenetic effects on the offspring remain unknown. In contrast to the human studies where offspring were not studied directly, mice studies (Pilones et al., 2009; Montgomery et al., 2008; Stringari et al., 2008) looked at tissues or behaviors in individual adult animals exposed to mercury *in utero* during the sensitive gestation period, however, epigenetic analyses were not performed. Our studies with *C. elegans* were aimed to bridge these two approaches where we were able to expose animals *in utero*, demonstrate a developmental phenotype, and provide evidence of epigenetic changes in specific genes directly in the affected animal after the *in utero* exposure. The results of long lasting increase in H3K4me3 signals but non-correlation with RNA expression levels after *in utero* exposure suggests complex gene regulatory mechanisms. Other histone modifications or epigenetic changes (methylated DNA, miRNA, etc.) may ultimately compensate for long lasting marks. Many more epigenetic marks that are associated with increased or decreased transcription will need to be explored in future studies to discern the changes that ultimately result in RNA expression. We also observed that while H3K4me3 marks remain after *in utero* exposure, they do not confer an additive effect.

We observed upregulation and epigenetic activation of gene *lpr-5* that belongs to the calycin sub-family of lipocalin-related proteins expressed in *C. elegans* in all stages from embryos to adults. RNAi

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**Fig. 1.** ChIP-seq and RNA-seq in control treated and MeHg treated animals. (a) Chip-seq peaks were identified using H3K4Me3 antibody or input DNA as a control. MeHg treatment increased H3K4me3 signals in genes *gst-5*, *gst-38*. (b) Peaks from *lpr-5* and *dpy-7* genes. ChIP-Seq data (lines 1–3) and the corresponding genes were compared with RNA-Seq data (lines 4–5). Gene structure is shown at the bottom. Images were generated by Integrated Genome Viewer 2.3.
knockdown of this gene resulted in decreased animal survival after MeHg exposure. Moreover, reduced lpr-5 activity caused an egg laying defective phenotype in control animals causing body rupture. An lpr-5 promoter-GFP reporter transgene indicates that this gene is expressed in seam cells of the hypodermis (Thoemke et al., 2005). Our data suggests lpr-5 involvement in the development of intact epithelium and knock down of this gene may result in lost protection from environmental toxicants. In support of this we previously found that lpr-5 RNAi also made animals more sensitive to MnCl2 toxicity (Rudgalvyte et al., 2016). In humans, neutrophil gelatinase-associated lipocalin (NGAL) has been proposed as a biomarker for renal injury that may occur during transplants, bypass surgeries, or xenobiotic toxicity including mercury (Mishra et al., 2005). Another lipocalin-related protein lpr-1 was found to be involved in excretory system development in C. elegans (Stone et al., 2009) and suggests another structure that may be affected by MeHg. Whether the excretory system of C. elegans mimics the renal system of humans remains to be seen, yet it appears that the lipocalin related (LPR) proteins are affected in both systems. Further work on defining the function of LPR family genes is necessary to determine how closely excretory systems are between humans and C. elegans.

In addition, inactivation of cuticular collagen dpy-7 resulted in increased animal lethality after MeHg exposure. Another study has shown increased sensitivity to bisphenol A of dpy-7 mutant animals (Watanabe et al., 2005) further suggesting that intact epithelium may play an important role in animal protection against environmental toxicants. DPY-7 is highly conserved across species and homologous to human Isoform 2 of Collagen alpha-1(XVII) chain. These results would predict that dyp-7 mutants would be sensitive to a large range of toxins. Coupled with results from lpr-5, the loss of epithelial integrity may allow easier absorption of toxicants and could explain the increased sensitivity of lpr-5 and dpy-7 to MeHg in C. elegans. In support of this,
we observed that a large proportion of cuticle proteins were both enhanced in H3K4me3 marks and upregulated in gene expression. These included a family of dumpy (dyp), molting (mlt), blister (bit), long (lon), roller (rol) and collagen (col) genes (Table 2). This was also seen in the enriched GO biological process groups (Fig. 2, Supplementary Table 2). As MeHg may bind to cysteine residues on the cuticle surface, cuticular genes may provide the first response to environmental toxicants in our exposure system. Loss of epithelial integrity is a fairly severe response to high level MeHg toxicity that might not be a major concern for vertebrates affected by lower environmental concentrations. Yet, in more severe poisoning cases, dermal toxicity or epithelium exposure may be an important and overlooked aspect of MeHg’s harmful effects.

5. Conclusion

To our knowledge, this is the first global histone 3 at lysine 4 trimethylation pattern analysis following acute MeHg exposure. Our study presents new insights into molecular effects of MeHg at the epigenetic level that are manifested at transcriptional levels. Moreover, our results support the notion that MeHg causes long-lasting effects via in utero exposure and suggests a novel model for studying transgenerational effects of heavy metals. Our investigation also provides a new source of data for further studies for molecular toxicologists to investigate specific genes that are affected following methyl mercury exposure. Finally, we highlight effects on genes important for cuticle formation and structure that are sensitive to MeHg exposure.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cbpc.2016.10.001.

Conflict of interests

The authors declare that they have no conflicts of interest.

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Fig. 4. Role of lpr-5 and dpy-7 gene in mediating MeHg induced lethality. (a) The RNAi-sensitive strain N2L099 (ryf-3) was grown for 48 h on plates with the lpr-5 RNAi bacteria clone or empty vector (WT control) containing bacteria at L4 stage and transferred onto MeHg (10 μM or 20 μM as indicated) plates for 24 h (lpr-5). (b) RNAi assay for 48 h with dpy-7 bacteria. The number of dead worms was then scored. The experiment was repeated three times in triplicates counting at least 50 animals per replicate. Results are presented as average ± SD. **RNAi (lpr-5 or dpy-7) is significantly different from WT control, t-test, p < 0.05. Figures were created using GraphPad Prism software.

References


