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Histone methylation-associated transgenerational inheritance of reproductive defects in *Caenorhabditis elegans* exposed to crude oil under various exposure scenarios

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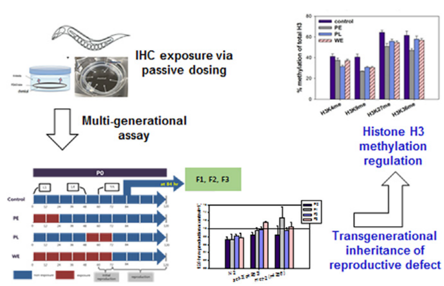
HIGHLIGHTS

- Transgenerational toxicity of Iranian heavy crude oil (IHC) was investigated in the nematode *Caenorhabditis elegans* in the four consecutive generations under different exposure scenarios.
- *C. elegans* reproduction potential was inhibited by the IHC in the unexposed generations and in the exposed parental generation.
- Whole-life exposure condition exhibited transgenerational inheritance of defective reproduction.
- Decreased methylation of histone H3K9 was found in the exposed generation; however, a heritable diminution in reproduction did not occur in the H3K9 histone methyltransferase defective mutant.
- Reproductive toxicity caused by IHC exposure was found to be transmitted to subsequent unexposed generations, and methylation of histone H3K9 seems to be involved in it.

GRAPHICAL ABSTRACT



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ABSTRACT

As part of a study to explore the long-term effects of the Hebei Spirit oil spill accident, transgenerational toxicity and associated epigenetic changes were investigated in the nematode *Caenorhabditis elegans*. Under experimental conditions, worms were exposed to Iranian heavy crude oil (IHC) under three different scenarios: partial early-life exposure (PE), partial late-life exposure (PL), and whole-life exposure (WE). Growth, reproduction, and histone methylation were monitored in the exposed parental worms (P0) and in three consecutive unexposed offspring generations ($F_{1\text{-}3}$). Reproductive potential in the exposed P0 generation in the WE treatment group was reduced; additionally, it was inhibited in the unexposed offspring generations of the P0 worms. This suggests that there was transgenerational inheritance of defective reproduction. Comparison of developmental periods of exposure showed that IHC-

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treated worms in the PL group had a greater reduction in reproductive capacity than those in the PE group. Decreased methylation of histone H3 (H3K9) was found in the IHC-exposed parental generation. A heritable reduction in reproductive capacity occurred in wildtype *N2* but was not found in a H3K9 histone methyltransferase (HMT) mutant, *met-2*(*n4256*), suggesting a potential role for HMT in transgenerational toxicity. Our results suggest that the reproductive toxicity after IHC exposure could be heritable and that histone methylation is associated with the transmission of the inherited phenotype.

1. Introduction

Epigenetics, the transgenerational transfer of phenotypic characteristics without modification of gene sequences, is a burgeoning area of study in many disciplines of biology (Lim and Brunet, 2013; Kelly, 2014). Known epigenetic mechanisms include DNA methylation, histone modification, and non-coding RNA regulation (Kelly, 2014). Environmental changes may affect the unexposed progeny of exposed generations, with genes showing aberrant silencing or expression, without an associated gene sequence change, for many generations (Lim and Brunet, 2013; Kelly, 2014). A growing body of scientific evidence indicates that exposure to environmental chemicals may contribute to the deregulation of epigenetic mechanisms (Vandegehuchte et al., 2009a, 2009b; Menzel et al., 2011; Manikkam et al., 2012; Lim and Brunet, 2013; Norouzitallab et al., 2014) with reports of multi- and transgenerational effects of environmental chemicals, such as biocides, pesticides, endocrine-disrupting chemicals, and fuel mixtures, in many species (Peterson et al., 2003; Anway et al., 2005; Titus-Ernstoff et al., 2010; Bruner-Tran and Osteen, 2011; Guerrero-Bosagna and Skinner, 2012). However, the relationship between the overarching concept of epigenetics and interesting transgenerational phenomena that alter the physiological phenotype of subsequent generations remains enigmatic and somewhat ill defined.

The nematode *Caenorhabditis elegans* is a valuable model for studying transgenerational effects because of its short life cycle and ease of maintenance under laboratory conditions. Hence, many studies on multi- and transgenerational toxicities of diverse environmental stresses have been performed in this species (Kim et al., 2013; Tauffenberger and Parker, 2014; Zhao et al., 2014, 2015; Jobson et al., 2015; Liu et al., 2015). In addition, the contribution of epigenetic changes to transgenerational effects has been investigated, and potential molecular mechanisms have been suggested (Lim and Brunet, 2013). In *C. elegans*, histone methylation plays a crucial role in epigenetic memory and chromatin structural alterations (Kelly, 2014). Histone methyltransferases (HMTs) regulate the methylation of lysine in histone H3, and differences in the methylation status of lysine can lead to changes in gene transcription activities (Bassett and Barnett, 2014). HMTs of histone H3 are also reported to play a considerable role in epigenetic memory (Kelly, 2014).

In December 2007, the oil tanker Hebei Spirit spilled about 10,800 tons of oil into the Yellow Sea off Taean, South Korea. This historical oil spill accident had both short-term and long-term effects on human and ecological health (Peterson et al., 2003; Zock et al., 2007; Guterman, 2009; Hong et al., 2012; Incardona et al., 2012; Jiang et al., 2012; Noh et al., 2015). However, there have been little studies on whether the oil spill had the potential to cause multi- and/or transgenerational effects on exposed organisms.

This study was initiated to gain insights into the long-term consequences of the Hebei Spirit oil spill by investigating the transgenerational inheritance of toxicity after the exposure of *C. elegans* to Iranian heavy crude oil (IHC) and to analyze the associated epigenetic changes. IHC was chosen as the model crude oil because it was one of the main components of the Hebei Spirit oil spill (Yim et al., 2012). *C. elegans* specimens were exposed to IHC by a passive dosing method, in which the dissolved aqueous concentration of hydrophobic hydrocarbons from IHC was maintained at a constant level throughout the experiment. The effects of early-life chemical exposure on health in later life have recently been receiving much attention (Head et al., 2012). In this context, the responses of the worms to exposure during different periods of their life cycle were compared. Transgenerational toxicity was also investigated in the IHC-exposed parental (P0) generation and in their unexposed progeny (F_{1-3}). Finally, to identify the epigenetic mechanism in IHC-induced transgenerational toxicity, methylation of histone H3 was investigated in the *C. elegans* exposed generation (P0). The role of histone H3 methylation in transgenerational toxicity was further investigated by the analysis of *C. elegans* with loss-of-function mutations of genes encoding HMTs and histone demethylase (HDM).

2. Materials and methods

2.1. C. elegans maintenance

C. elegans was cultured in Petri dishes on nematode growth medium (NGM) at 20 °C and fed OP50 strain *Escherichia coli* according to a standard protocol (Brenner, 1974). The life cycle of *C. elegans* comprises the embryonic stage, four larval stages (L1-L4), and adulthood. A synchronously developing population of worms was used in the various experiments to eliminate any variation due to age differences. To produce age-synchronized cultures, eggs from mature adults were isolated using 10% hypochlorite solution, followed by rinsing with M9 buffer (4.2 mM Na_2HPO_4, 2.2 mM KH_2PO_4, 86 mM NaCl, and 1 mM $MgSO_4 \cdot 7H_2O$). Synchronized L1 stage worms were then allowed to hatch from eggs in S-basal (0.1 M NaCl, 0.05 M KH_2PO_4, pH 6.0) without a food source, resulting in a synchronized population. For multigenerational analyses, *C. elegans* L1 larvae were cultured in complete K-media (0.032 M KCl, 0.051 M NaCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, and 13 mM cholesterol). Wildtype (N2) and mutant strains were provided by the Caenorhabditis Genetics Center (MN, USA). A list of the mutant strains employed in this study is presented in Table S1.

2.2. Iranian heavy crude oil (IHC)

The IHC used as the model crude oil in this study was provided by Taean Environmental Health Center (Taean, Korea). A detailed analysis of the chemical composition of IHC has been reported previously (Yim et al., 2011; Kang et al., 2014). The polydimethylsiloxane (PDMS) tubing used for the passive dosing exposures was purchased from Dong-Bang Silicone Inc. (Seoul, Korea).