Chemosphere 191 (2018) 302–314

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Modulation of *sestrin* confers protection to Cr(VI) induced neuronal cell death in *Drosophila melanogaster*

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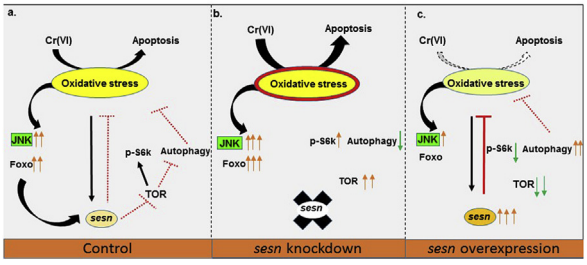
HIGHLIGHTS

- Cr(VI) exposure leads to apoptosis in neuronal cells by increasing oxidative stress.
- The predominance of Foxo in the nucleus of brain cells of Cr(VI) exposed larvae.
- Nuclear localization of Foxo was poor in *sesn*-overexpression larvae.
- Cr(VI) induced *sesn* expression follows JNK/Foxo signaling pathway.
- *sesn* protects brain cells from Cr(VI) induced adversities by increasing autophagy.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 August 2017
Received in revised form
23 September 2017
Accepted 6 October 2017
Available online 7 October 2017

Handling Editor: Jim Lazorchak

Keywords:
Hexavalent chromium
Sestrin
Oxidative stress
Autophagy
Drosophila
Apoptosis

ABSTRACT

Increased oxidative stress is one of the major causes of hexavalent chromium [Cr(VI)], a heavy metal with diverse applications and environmental presence, induced neuronal adversities in exposed organism including *Drosophila*. Sestrin (*sesn*), an oxidative stress responsive gene, emerges as a novel player in the management of oxidative stress response. It is reported to be regulated by Target of rapamycin (TOR) and the former regulates autophagy and plays an important role in the prevention of neurodegeneration. Due to limited information regarding the role of *sesn* in chemical induced cellular adversities, it was hypothesized that modulation of *sesn* may improve the Cr(VI) induced neuronal adversities in *Drosophila*. Upon exposure of Cr(VI) (5.0–20.0 μg/ml) to *D. melanogaster* larvae (w^{1118}; background control), neuronal cell death was observed at 20.0 μg/ml of Cr(VI) concentration which was found to be reversed by targeted *sesn* overexpression (*Elav-GAL4>UAS-sesn*) in those cells of exposed organism by the induction of autophagy concomitant with decreased reactive oxygen species (ROS) level, *p*-Foxo-, *p*-JNK- and *p*-Akt-levels with decreased apoptosis. Conversely, after *sesn* knockdown (*Elav-GAL4>UAS-sesn*^{RNAi}) in neuronal cells, they become more vulnerable to oxidative stress and apoptosis. Furthermore, knockdown of *sesn* in neuronal cells of exposed organism resulted in decreased autophagy with increased TOR and p-S6k levels while overexpression of *sesn* led to their decreased levels suggestive of decreased anabolic and increased catabolic activity in neuronal cells shifting energy towards the augmentation of cellular repair. Taken together, the study suggests therapeutic implications of *sesn* against chemical induced neuronal adversities in an organism.

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https://doi.org/10.1016/j.chemosphere.2017.10.037
0045-6535/

1. Introduction

Chromium (Cr), a well-known heavy metal, exists in Cr(VI) to trivalent-[Cr(III)] oxidation states in the environment and is reported to cause detrimental effects on an organism. Its industrial use such as leather tanning, chrome plating, steel industries and mines for various applications is well reported (Shelnutt et al., 2007). Its release in the environment is more than 10^5 tons annually which is of concern (Gadd and White, 1993; Zhitkovich et al., 1998). Reduction of Cr(VI) to Cr(III) leads to various reactive Cr intermediates and free radical generation (Borthiry et al., 2007, 2008) which results in oxidative stress. Cr(VI), with induction of OS in the brain (Carson et al., 1986), also causes cerebral cortex degeneration in rabbits (Mathur et al., 1977; Carson et al., 1986). Further, Cr(VI) was reported to inhibit acetylcholinesterase activity in rats leading to oxidative damage to their brain (Moshtaghie et al., 2004; Soudani et al., 2012). It also causes neuronal cell death, via generation of reactive oxygen species (ROS) (Dashti et al., 2015; Singh and Chowdhuri, 2017).

Every organism present in the environment is exposed to different types of stressors in their day-to-day life. Since the survival of an organism and its reproduction is one of the essentials to maintain ecological balance, organisms have developed strategies to defend themselves against these stressors. Oxidative stress happens due to an imbalance in maintaining free radicals and as a part of its management cells have both enzymatic and non-enzymatic components (Birben et al., 2012; Kabel, 2014). A number of genes and their products have also been identified to have various roles in maintaining redox balance in the cells (Tomanek, 2015).

sesn, an oxidative stress responsive gene (Budanov et al., 2004), is evolutionary conserved and was reported to confer protection against stress (Lee et al., 2010). In mammals, it has three paralogs while one *sesn* gene has been reported in *Drosophila* (Budanov et al., 2004). It has been reported that Sesn restores the functions of peroxidase peroxiredoxin (Prx), considering the homology of its N-terminal domain to bacterial alkyl hydroperoxide reductase (AhpD) (Velasco-Miguel et al., 1999; Budanov et al., 2004). Sesn plays two independent functions such as ROS regulation and promotion of autophagy via mTOR regulation (Budanov et al., 2010). *sesn* expression was found to be highly misregulated in the brain of human immune deficiency virus-associated neuronal disorders causing increased oxidative stress (Soontornniyomkij et al., 2012). Sesn protein levels were found to be higher in the patients suffering from Alzheimer's and Parkinson's disease (Soontornniyomkij et al., 2012; Zhou et al., 2013; Rai et al., 2016) suggesting its role in neurotoxicity. Thus, Sesn malfunction may be associated with neuropathology.

From a toxicological perspective, the study of stress tolerance of an organism against xenobiotic assault is of importance and in this context, genes involved in the regulation of oxidative stress play an important role in combating toxic chemicals. *sesn* being an oxidative stress responsive gene can fit in this role. Considering the above, in one of the microarray experiments previously carried out by our laboratory, upregulation of *sesn* was observed in Cr(VI) exposed *Drosophila* larvae (Mishra et al., 2011, 2013) which supports further studies of its response to chemical exposure. Exposure to Cr(VI) induced neuronal cell death in *D. melanogaster* (Singh and Chowdhuri, 2017). Considering the above, the present study aims to examine the role of *sesn* in Cr(VI) induced neuronal cell adversities in a genetically tractable model, *Drosophila*. The use of fruit flies raises limited ethical concerns regarding animal testing (Adams et al., 2000) and has been used for toxicological studies and has been modeled for human disease like phenotypes (Mukhopadhyay et al., 2006; Gupta et al., 2010; Shukla et al., 2014, 2016). The study provides evidence that *sesn* modulation has a positive effect on Cr(VI) induced neuronal cell adversities.

2. Materials and methods

2.1. Fly strains

Fly stocks w^{1118} (white-eyed base stock that serves as a genetic background control of thousands of transposon insertion as well as isogenic deficiency lines available), *UAS-sesn* [(P{XP} $Sesn^{d04539}$); expresses *sesn* under the control of UAS wherein *sesn* overexpression can be achieved after crossing this strain with a driver-GAL4 strain; The Exelixis Collection at the Harvard Medical School] (Lee et al., 2010) and *UAS-*$sesn^{RNAi}$ [(P{KK107937}VIE-260B); expresses dsRNA for RNA interference of *sesn* under the control of UAS wherein *sesn* knockdown is achieved after crossing this strain with a driver-GAL4 strain; Vienna *Drosophila* RNAi Center], were used for the study. Necessary genotypes such as over-expression (*Elav-GAL4>UAS-sesn*) or knockdown (*Elav-GAL4>UAS-*$sesn^{RNAi}$) specifically in *Drosophila* brain was achieved by genetically crossing *UAS-GAL4* strains with *Elav-GAL4* (Elav is a pan neuronal driver which is used for the targeted expression of a gene of interest in the nervous system) driver line. *Tb>UAS-sesn* and *Tb>UAS-*$sesn^{RNAi}$ were used as genetic controls of overexpression and knockdown strains respectively. All fly strains and their different developmental stages were maintained at 24 ± 1 °C on standard *Drosophila* food medium consisting of agar, corn flour, sugar, yeast, nepagin and propionic acid.

2.2. Treatment schedule

Analytical grade potassium dichromate ($K_2Cr_2O_7$) (HIMEDIA Laboratories Ltd., India) was used for the study. Based on previously published reports (Aravindhan et al., 2004; Sharma et al., 2012; Mishra et al., 2013; Singh and Chowdhuri, 2017), three different concentrations of Cr(VI) (5.0, 10.0 and 20.0 μg/ml) that have environmental relevance were chosen. The anthrapyrazolone inhibitor SP600125 (Sigma Aldrich, USA) (100.0 μM) was used to suppress activity of Jun N-terminal kinase. Larvae of 80 ± 1 h and 56 ± 1 h were fed on Cr(VI) contaminated food for 24 and 48 h. The control group received normal food.

2.3. Semi-quantitative PCR and quantitative real-time reverse transcriptase PCR (qPCR)

The expression profile of the genes was carried out by Semi-quantitative PCR and qPCR assay as described below:

2.3.1. RNA extraction

Total RNA was isolated from brain ganglia (40 nos.) of *Drosophila* larvae using RNAZol (Molecular Research Center, USA) essentially following the manufacturer's instructions. The purity of RNA was confirmed by measuring the optical density of the extracted RNA samples on a Nano Drop® ND-1000 UV–Vis Spectrophotometer (Wilmington, USA) at OD_{260}/OD_{280} (>1.8) and an OD_{260}/OD_{230} (>2).

2.3.2. cDNA synthesis

cDNA synthesis was performed by using Revert Aid™ H Minus first strand cDNA synthesis kit (Fermentas, USA) essentially following the manufacturer's protocol.

2.3.3. Semi-quantitative PCR

The cDNA from each sample was amplified by PCR on a thermocycler (Eppendorf; Germany) using gene specific primer pairs mentioned in Table S1. The PCR reaction mixture contained 1 × Taq