Deciphering DNA methyltransferase inhibitor mediated transgenerational effects on Daphnia: high-throughput analyses and Adverse Outcome Pathway assembly

Presentation · May 2020

DOI: 10.13140/RG.2.2.20230.19522

6 authors, including:

You Song
Norwegian Institute for Water Research
58 PUBLICATIONS **319** CITATIONS
SEE PROFILE

Jorke Harmen Kamstra
Utrecht University
77 PUBLICATIONS **2,045** CITATIONS
SEE PROFILE

Yang Cao
Örebro University, School of Medical Sciences
232 PUBLICATIONS **1,681** CITATIONS
SEE PROFILE

Marc Anglès d'Auriac
Norwegian Institute for Water Research
65 PUBLICATIONS **169** CITATIONS
SEE PROFILE

Deciphering DNA methyltransferase inhibitor mediated transgenerational effects on *Daphnia*: high-throughput analyses and Adverse Outcome Pathway assembly

You Song[1], Jorke H. Kamstra[2], Yang Cao[3],Marc Anglès d'Auriac[1], Jana Asselman[4], Nikolai Friberg[1]

[1] Norwegian Institute for Water Research (NIVA), Gaustadalléen 21, N-0349 OSLO, Norway; [2] Institute for Risk Assessment Sciences (IRAS), Utrecht University, PO Box 80177, NL-3508 TD Utrecht, The Netherlands; [3] Clinical Epidemiology and Biostatistics, School of Medical Sciences, Örebro University, 70182 Örebro, Sweden; [4] Blue Growth Research Lab, Ghent University, Bluebridge building, Ostend Sciene Park 1, 8400 Ostend, Belgium
E-mail contact: you.song@niva.no

1. Introduction

Epigenetic marks can in many cases reflect the life-time exposure history of an organism to environmental stressors [1]. Among the epigenetic marks, DNA methylation is considered tightly related to the control of gene expression and has been frequently used as an (eco)toxicological biomarker to indicate effects of epigenetic modulators. While the rapid development of the OMICS techniques allows measurements of genome-wide DNA methylation and gene expression profiles, the high costs for such analyses still limit our ability to fully understand the epigenetic changes across doses/concentrations, exposure durations and multiple generations in a population. In addition to the OMICS tools, targeted high-throughput (HT) epigenetic bioassays are also needed, allowing the inclusion of more life stages and exposure conditions to yield comparative concentration-response data on a temporal scale. Such data can greatly facilitate the development of systems toxicological models, such as Adverse Outcome Pathways (AOPs) for increased prediction of toxic effects across stressors and taxa [2]. The present study has therefore integrated and refined several HT bioassays to: 1) understand the relationships between chemical-mediated DNA methyltransferase (DNMT) inhibition, DNA promoter methylation, gene body methylation, gene expression and multigenerational reproduction; 2) develop a novel epigenetic AOP for DNMT inhibitor-mediated reproduction effects in aquatic organisms.

2. Materials and methods

The water flea *Daphnia magna* was used as the model organism. A widely-used DNA methyltransferase (DNMT)-inhibiting pharmaceutical, 5-azacydine was used as the prototypical stressor in the present study. Briefly, synchronized (14-15d) adult female *D. magna* were exposed to 0 (control), 5, 10, 20, 40 and 80 µM of 5-azacytidine for 7 days and recovered in clean media for another 7 days. F1-F3 offspring were sub-cultured in clean media to investigate potential transgenerational effects (Fig. 1).

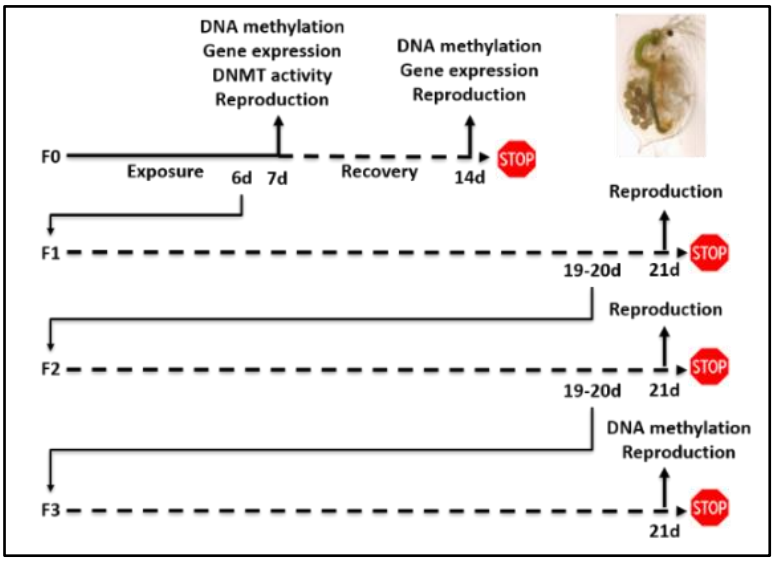

Figure 1: Experimental setup for investigating the transgenerational effects of 5-azacytidine on Daphnia magna by high-throughput analyses.

A high-throughput enzyme-linked immunosorbent assay (ELISA) was employed to determine the total activity of DNMTs after exposure. High-throughput methylation-sensitive high-resolution melt analysis (MS-HRM) was used to measure locus-spefic DNA methylation of 16 genes in F0 after exposure and recovery, and in F3. High-throughput quantitative real-time RT-PCR (qPCR) was used to determine the transcriptional changes of 34 genes in F0 after exposure and recovery. The test genes are well-known biomarker genes or key regulators involved in major biological pathways such as regulation of DNA methylation, one-carbon metabolism, DNA repair, cell cycle regulation, apoptosis, oxidative stress response, cell migration, calcium signaling and small sugar metabolism. Cumulative fecundity (total number of viable offspring) was recorderd for each generation.

3. Results and discussion

After 7-day exposure, the total DNMT activity in F0 *D. magna* decreased in a concentration-dependent manner, with 80 µM 5-azacytidine caused significant reduction in DNMT activity. The majority of the test genes showed reduced promoter methylation and increased transcription in F0 after 7-day exposure, whereas increased promoter methylation and down-regulation in F0 after recovery (Fig. 2A). In F3, most of the genes displayed increased promoter methylation with the exception of tet methylcytosine dioxygenase 2 (*Tet2*), caspase 2 (*Casp2*) and galactose-1-phosphate uridylyltransferase (*Galt*). The cumulative fecundity decreased in a concentration-dependent manner in F0 after exposure and recovery, and in the succesive generations (Fig. 2B). On the basis of the results, a conceptual AOP on DNMT inhibition leading to oocyte apoptosis associated population decline was assembled and submitted to the AOPWiki (https://aopwiki.org/aops/290).

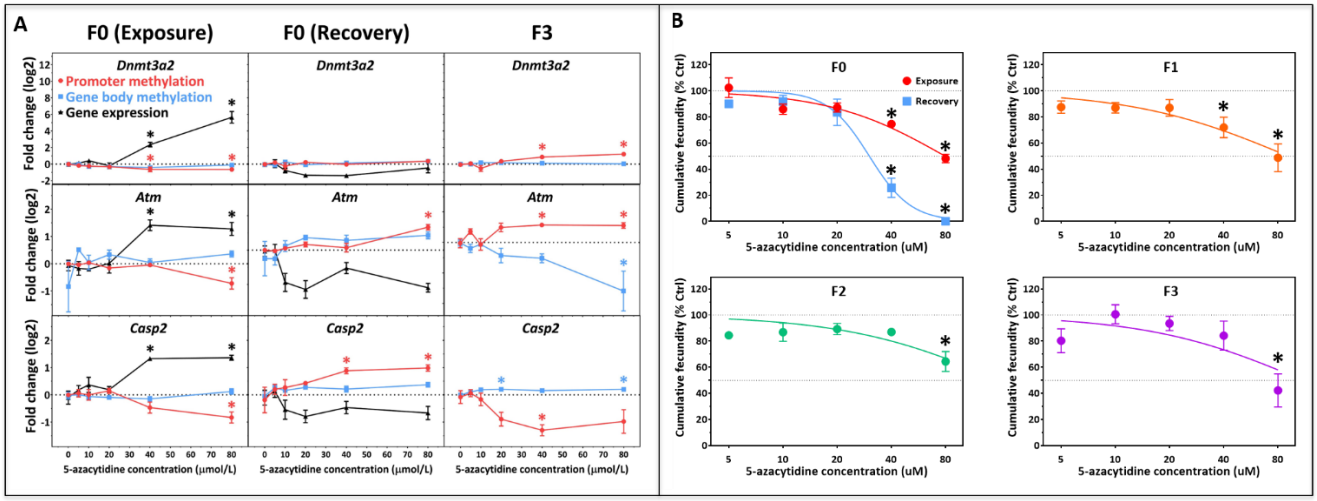

Figure 2: DNA promoter methylation (A-red), gene body methylation (A-blue) and gene expression (A-black) of DNA methyltransferase 3A2 (Dnmt3a2), ataxia telangiectasia mutated serine/threonine kinase (Atm) and caspase 2 (Casp2) as examples, and the cumulative fecundity (B) of F0-F3 Daphnia magna after exposure to 5-azacytidine.

4. Conclusions

The present study has developed a novel analytical pipeline for targeted high-throughput analyses of epigenetic effects, and has unravelled the relationships between DNMT inhibition, DNA promoter methylation, gene body methylation, gene expression and transgenerational reproduction in *D. magna* exposed to 5-azacytidine. The world's first epigenetic AOP was proposed for understanding and predicting chemical-mediated transgenerational population effects in aquatic organisms.

5. References

[1] Mirbahai, L., Chipman, J.K., 2014. Epigenetic memory of environmental organisms: A reflection of lifetime stressor exposures. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 764-765, 10-17.

[2] Ankley, G. T., Bennett, R. S., Erickson, R. J., Hoff, D. J., Hornung, M. W., Johnson, R. D., Mount, D. R., Nichols, J. W., Russom, C. L., Schmieder, P. K., Serrrano, J. A., Tietge, J. E. and Villeneuve, D. L. (2010), Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. Environmental Toxicology and Chemistry, 29: 730-741.

Acknowledgement - This project was funded by the NIVA institutional funding scheme "Strategic Institutional Initiatives programme (SIS) for Restoration Ecology". The modeling work was supported by the Research Council of Norway (RCN) funded "RiskAOP" project (Project# 301397) and infrastructure supported by the NIVA Computational Toxicology Program (NCTP, www.niva.no/nctp).