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Epigenetic alterations induced by aflatoxin B_1: An *in vitro* and *in vivo* approach with emphasis on enhancer of zeste homologue-2/p21 axis

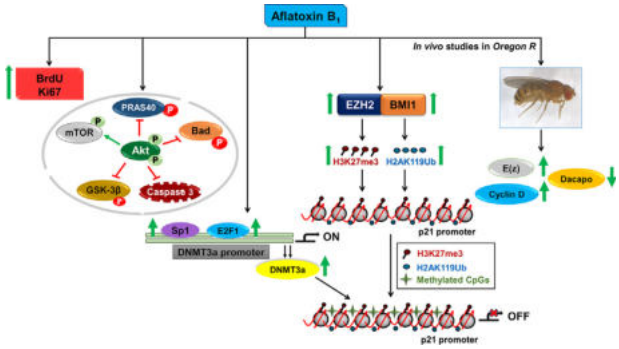
Priyanka Soni [a], Md. Sajid Ghufran [a,1], Shilpa Olakkaran [b], Gurushankara Hunasanahally Puttaswamygowda [b], Govinda Rao Duddukuri [a], Santosh R. Kanade [c,*]

[a] Department of Biochemistry and Molecular Biology, School of Biological Sciences, Central University of Kerala, Tejaswini Hills, Periye, Kasargod 671316, Kerala, India
[b] Department of Zoology, School of Biological Sciences, Central University of Kerala, Tejaswini Hills, Periye, Kasargod 671316, Kerala, India
[c] Department of Plant Science, School of Life Science, University of Hyderabad, Prof. C. R. Rao Road Gachibowli, Hyderabad 500046, India

HIGHLIGHTS

- AFB_1 increases the proliferation of cells by activating Akt and its downstream signaling molecules.
- AFB_1 mediated expression of polycomb group proteins transcriptionally repress the cell cycle regulator p21.
- EZH2 mediated chromatin remodeling enhanced the p21 promoter methylation and reduced the p21 expression.
- AFB_1 upregulated the expression of E(z), Cyclin D and downregulated Dacapo expression in *Drosophila melanogaster*.

GRAPHICAL ABSTRACT



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ABSTRACT

The potent environmental toxicant aflatoxin B_1 (AFB_1), is a group I carcinogen reported to induce the expression of many cancer associated proteins. Epigenetic alterations such as DNA methylation and histone modifications play vital role in AFB_1-mediated carcinogenesis. These epigenetic modifications may result in the recruitment of specific proteins and transcription factors to the promoter region and regulate gene expression. Here we show that AFB_1, at lower concentrations (100 and 1000 nM) induced proliferation in L-132 and HaCaT cells with activation of the Akt pathway, which ultimately steered abnormal proliferation and transmission of survival signals. We demonstrated a significant reduction in the expression of p21 with a remarkable increase in the expression of cyclin D1 that correlated with increased methylation of CpG dinucleotides in p21 proximal promoter, while cyclin D1 promoter remained unmethylated. The chromatin immunoprecipitation results revealed the enrichment of DNMT3a and H3K27me3 repressive marks on the p21 proximal promoter where EZH2 mediated H3K27me3 mark enhanced the binding of DNMT3a at the promoter and further contributed to the transcriptional inactivation. The overall study provided the novel information on the impact of AFB_1 on p21 inactivation *via* EZH2 and promoter methylation which is known to be a vital process in proliferation. Furthermore, AFB_1 induced the expression of EZH2 analogue protein E(z), cyclin D1 analogue cyclin D and decreased the expression of p21 analogue Dacapo in *Drosophila melanogaster*. Interestingly, the aggressiveness in their expression upon re-exposure in successive generations suggested first hand perspectives on multigenerational epigenetic memory.

Abbreviations: DNMTs, DNA methyltransferases; PcG, polycomb group proteins; PRC, polycomb repressive complex; EZH2, enhancer of zeste homologue 2; BMI1, B cell specific Moloney murine leukemia virus integration site 1; CDK, cyclin dependent kinase; HOX genes, Homeobox genes; E(z), enhancer of Zeste.

* Corresponding author.
E-mail address: san@uohyd.ac.in (S.R. Kanade).
[1] Present address: Department of Chemistry, Faculty of Natural and Computational Sciences, Gambella University, Gambella, P.O. Box 126, Federal Republic of Ethiopia.

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