Arecoline, an alkaloid of betel quid, inhibits p53-mediated DDB2 expression and DNA repair in human head and neck cancer cells

Jau-Ling Huang¹, Yu-Chiu Wang²,³, Ka-Wo Lee⁴, Hsing-Han Lu¹,², Yuan-Jen Lin², Long-Fong Chen², Chang-Shen Lin²

¹Chang Jung Christian University, Department of Bioscience Technology, Tainan, Taiwan ²Kaohsiung Medical University, Graduate Institute of Medicine, Kaohsiung, Taiwan ³Kaohsiung Medical University Hospital, Department of Surgery, Kaohsiung, Taiwan ⁴Kaohsiung Medical University Hospital, Department of Otolaryngology, Kaohsiung, Taiwan

Introduction: Arecoline is the major alkaloid in areca nut, which is carcinogenic to human (IARC class 1). Areca nut is an essential constituent of betel quid (BQ) and is the fourth most common addictive substance in the world. Epidemiological studies show that BQ-chewing significantly contributes to the occurrence of head and neck cancer (HNC). Arecoline exhibits obvious genotoxic activity, such as induction of DNA damage, chromosome breaks, and micronucleus formation; however, the underlying mechanism is not fully elucidated.

Objective: Previously, we have reported that arecoline (0.3 mM) inhibits nucleotide excision repair (NER) in a p53-dependent manner. Here we further investigated the effect of arecoline on p53-regulated NER genes, DDB2 and XPC.

Methods: Gene expression was examined using western blot and quantitative RT-PCR. NER capacity was measured by host cell reactivation (HCR) assay. The effect of arecoline on p53’s DNA-binding and transactivation domains was elucidated by luciferase-reporter and chromatin immunoprecipitation (ChIP) assays. A cell model under long-term arecoline exposure was established by daily repetitive treatment of arecoline (6-8 h/day for 60 days). DDB2 expression in BQ-exposed HNC specimens was evaluated using quantitative RT-PCR after approval of Institute Review Board. RNA sequencing data was retrieved from the Cancer Genome Atlas (TCGA) data portal and analyzed using SPSS software.

Results: Arecoline (0.3 mM, 24 h) specifically inhibited the expression of DDB2, but not that of XPC in HNC cells. HCR assay showed that ectopic DDB2 expression restored NER activity in arecoline-treated cells, suggesting that DDB2 downregulation was critical for arecoline-mediated NER inhibition. Luciferase-reporter and ChIP assays showed that arecoline inhibited p53-induced DDB2 promoter activity through the DNA-binding but not transactivation domain. Both NER and DDB2 promoter activities declined in the arecoline long-term exposed cells. DDB2 mRNA level was downregulated in HNC specimens with a history of BQ exposure but not in those without BQ exposure (TCGA cohort).

Conclusion: Arecoline inhibits NER capacity through downregulating 53-regulated DDB2 expression, which may contribute to HNC development.

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