World Academy of Science, Engineering and Technology
International Journal of Biotechnology and Bioengineering
Vol:10, No:09, 2016

Possible Involvement of DNA-methyltransferase and Histone Deacetylase in the Regulation of Virulence Potential of Acanthamoeba castellanii

Authors : Yi H. Wong, Li L. Chan, Chee O. Leong, Stephen Ambu, Joon W. Mak, Priyadashi S. Sahu

Abstract : Background: Acanthamoeba is a free-living opportunistic protist which is ubiquitously distributed in the environment. Virulent Acanthamoeba can cause fatal encephalitis in immunocompromised patients and potential blinding keratitis in immunocompetent contact lens wearers. Approximately 24 species have been identified but only the A. castellanii, A. polyphaga and A. culbertsoni are commonly associated with human infections. Until to date, the precise molecular basis for Acanthamoeba pathogenesis remains unclear. Previous studies reported that Acanthamoeba virulence can be diminished through prolonged axenic culture but revived through serial mouse passages. As no clear explanation on this reversible pathogenesis is established, hereby, we postulate that the epigenetic regulators, DNA-methyltransferases (DNMT) and histone-deacetylases (HDAC), could possibly be involved in granting the virulence plasticity of Acanthamoeba spp. Methods: Four rounds of mouse passages were conducted to revive the virulence potential of the virulence-attenuated Acanthamoeba castellanii strain (ATCC 50492). Briefly, each mouse (n=6/group) was inoculated intraperitoneally with Acanthamoebae cells (2x 105 trophozoites/mouse) and incubated for 2 months. Acanthamoebae cells were isolated from infected mouse organs by culture method and subjected to subsequent mouse passage. In vitro cytopathic, encystment and gelatinolytic assays were conducted to evaluate the virulence characteristics of Acanthamoebae isolates for each passage. PCR primers which targeted on the 2 members (DNMT1 and DNMT2) and 5 members (HDAC1 to 5) of the DNMT and HDAC gene families respectively were custom designed. Quantitative real-time PCR (qPCR) was performed to detect and quantify the relative expression of the two gene families in each Acanthamoeba isolates. Beta-tubulin of A. castellanii (Genbank accession no: XP_004353728) was included as housekeeping gene for data normalisation. PCR mixtures were also analyzed by electrophoresis for amplicons detection. All statistical analyses were performed using the paired one-tailed Student’s t test. Results: Our pathogenicity tests showed that the virulence-reactivated Acanthamoeba had a higher degree of cytopathic effect on vero cells, a better resistance to encystment challenge and a higher gelatinolytic activity which was catalysed by serine protease. qPCR assay showed that DNMT1 expression was significantly higher in the virulence-reactivated compared to the virulence-attenuated Acanthamoeba strain ($p \leq 0.01$). The specificity of primers which targeted on DNMT1 was confirmed by sequence analysis of PCR amplicons, which showed a 97% similarity to the published DNA-methyltransferase gene of A. castellanii (GenBank accession no: XM_004332804.1). Out of the five primer pairs which targeted on the HDAC family genes, only HDAC4 expression was significantly difference between the two variant strains. In contrast to DNMT1, HDAC4 expression was much higher in the virulence-attenuated Acanthamoeba strain. Conclusion: Our mouse passages had successfully restored the virulence of the attenuated strain. Our findings suggested that DNA-methyltransferase (DNMT1) and histone deacetylase (HDAC4) expressions are associated with virulence potential of Acanthamoeba spp.

Keywords : acanthamoeba, DNA-methyltransferase, histone deacetylase, virulence-associated proteins
Conference Title : ICMB 2016 : International Conference on Microbiology and Biotechnology
Conference Location : Singapore, Singapore
Conference Dates : September 08-09, 2016