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Transcriptomic Analysis of Acanthamoeba castellanii Virulence Alteration by Epigenetic DNA Methylation

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Abstract : Background: Acanthamoeba is a genus of amoebae which lives as a free-living in nature or as a human pathogen that causes severe brain and eye infections. Virulence potential of Acanthamoeba is not constant and can change with growth conditions. DNA methylation, an epigenetic process which adds methyl groups to DNA, is used by eukaryotic cells, including several human parasites to control their gene expression. We used qPCR, siRNA gene silencing, and RNA sequencing (RNA-Seq) to study DNA-methyltransferase gene family (DNMT) in order to indicate the possibility of its involvement in programming Acanthamoeba virulence potential. Methods: A virulence-attenuated Acanthamoeba isolate (designation: ATCC; original isolate: ATCC 50492) was subjected to mouse passages to restore its pathogenicity; a virulence-reactivated isolate (designation: AC/5) was generated. Several established factors associated with Acanthamoeba virulence phenotype were examined to confirm the succession of reactivation process. Differential gene expression of DNMT between ATCC and AC/5 isolates was performed by qPCR. Silencing on DNMT gene expression in AC/5 isolate was achieved by siRNA duplex. Total RNAs extracted from ATCC, AC/5, and siRNA-treated (designation: si-146) were subjected to RNA-Seq for comparative transcriptomic analysis in order to identify the genome-wide effect of DNMT in regulating Acanthamoeba gene expression. qPCR was performed to validate the RNA-Seq results. Results: Physiological and cytophatic assays demonstrated an increased in virulence potential of AC/5 isolate after mouse passages. DNMT gene expression was significantly higher in AC/5 compared to ATCC isolate ($p \leq 0.01$) by qPCR. si-146 duplex reduced DNMT gene expression in AC/5 isolate by 30%. Comparative transcriptome analysis identified the differentially expressed genes, with 3768 genes in AC/5 vs ATCC isolate; 2102 genes in si-146 vs AC/5 isolate and 3422 genes in si-146 vs ATCC isolate, respectively (fold-change of ≥ 2 or ≤ 0.5, p-value adjusted (padj) < 0.05). Of these, 840 and 1262 genes were upregulated and downregulated, respectively, in si-146 vs AC/5 isolate. Eukaryotic orthologous group (KOG) assignments revealed a higher percentage of downregulated gene expression in si-146 compared to AC/5 isolate, were related to posttranslational modification, signal transduction and energy production. Gene Ontology (GO) terms for those downregulated genes shown were associated with transport activity, oxidation-reduction process, and metabolic process. Among these downregulated genes were putative genes encoded for heat shock proteins, transporters, ubiquitin-related proteins, proteins for vesicular trafficking (small GTPases), and oxidoreductases. Functional analysis of similar predicted proteins had been described in other parasitic protozoa for their survival and pathogenicity. Decreased expression of these genes in si146-treated isolate may account in part for Acanthamoeba reduced pathogenicity. qPCR on 6 selected genes upregulated in AC/5 compared to ATCC isolate corroborated the RNA sequencing findings, indicating a good concordance between these two analyses. Conclusion: To the best of our knowledge, this study represents the first genome-wide analysis of DNA methylation and its effects on gene expression in Acanthamoeba spp. The present data indicate that DNA methylation has substantial effect on global gene expression, allowing further dissection of the genome-wide effects of DNA-methyltransferase gene in regulating Acanthamoeba pathogenicity.

Keywords : Acanthamoeba, DNA methylation, RNA sequencing, virulence
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