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Differential Expression Profile Analysis of DNA Repair Genes in Mycobacterium Leprae by qPCR

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Abstract : Leprosy is a chronic human disease caused by Mycobacterium leprae, that cannot be cultured in vitro. Though treatable with multidrug therapy (MDT), recently, bacteria reported resistance to multiple antibiotics. Targeting DNA replication and repair pathways can serve as the foundation of developing new anti-leprosy drugs. Due to the absence of an axenic culture medium for the propagation of M. leprae, studying cellular processes, especially those belonging to DNA repair pathways, is challenging. Genomic understanding of M. Leprae harbors several protein-coding genes with no previously assigned function known as 'hypothetical proteins'. Here, we report identification and expression of known and hypothetical DNA repair genes from a human skin biopsy and mouse footpads that are involved in base excision repair, direct reversal repair, and SOS response. Initially, a bioinformatics approach was employed based on sequence similarity, identification of known protein domains to screen the hypothetical proteins in the genome of M. leprae, that are potentially related to DNA repair mechanisms. Before testing on clinical samples, pure stocks of bacterial reference DNA of M. leprae (NHDP63 strain) was used to construct standard graphs to validate and identify lower detection limit in the qPCR experiments. Primers were designed to amplify the respective transcripts, and PCR products of the predicted size were obtained. Later, excisional skin biopsies of newly diagnosed untreated, treated, and drug resistance leprosy cases from SIHR & LC hospital, Vellore, India were taken for the extraction of RNA. To determine the presence of the predicted transcripts, cDNA was generated from M. leprae mRNA isolated from clinically confirmed leprosy skin biopsy specimen across all the study groups. Melting curve analysis was performed to determine the integrity of the amplification and to rule out primer-dimer formation. The Ct values obtained from qPCR were fitted to standard curve to determine transcript copy number. Same procedure was applied for M. leprae extracted after processing a footpad of nude mice of drug sensitive and drug resistant strains. 16S rRNA was used as positive control. Of all the 16 genes involved in BER, DR, and SOS, differential expression pattern of the genes was observed in terms of Ct values when compared to human samples; this was because of the different host and its immune response. However, no drastic variation in gene expression levels was observed in human samples except the nth gene. The higher expression of nth gene could be because of the mutations that may be associated with sequence diversity and drug resistance which suggests an important role in the repair mechanism and remains to be explored. In both human and mouse samples, SOS system – lexA and RecA, and BER genes AlkB and Ogt were expressing efficiently to deal with possible DNA damage. Together, the results of the present study suggest that DNA repair genes are constitutively expressed and may provide a reference for molecular diagnosis, therapeutic target selection, determination of treatment and prognostic judgment in M. leprae pathogenesis.

Keywords : DNA repair, human biopsy, hypothetical proteins, mouse footpads, Mycobacterium leprae, qPCR
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