Genomics in amoebiasis

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Abstract

The extensive use of molecular tools and sequencing data has heralded the age of genomics in the diagnosis of many diseases including parasitic infections like amoebiasis. Amoebiasis, caused by Entamoeba histolytica is a significant cause of morbidity and mortality, especially in developing countries. Amoebiasis has a worldwide distribution affecting more than 50 million people every year. The draft genome sequence of E. histolytica (strain HM1:IMSS) was published and analyzed in 2005 by Loftus etal. and was subsequently re-assembled and re-annotated by Lorenzi etal. in 2010 and Clark etal. in 2007. It was one of the first protist genomes to be sequenced. A whole-genome shotgun method was used to sequence parasite genome as a better choice for sequencing. The genome assembly consists of 20,800,560 base pairs of DNA in 1496 scaffolds. The genome is very AT-rich (approximately 75% AT) and quite gene-rich: around half of all assembled sequence is predicted to be coding sequence, with 8333 annotated genes. Entamoeba dispar, the closest described relative of E.histolytica is morphologically indistinguishable from it .The size of its genome has also found to be similar to that of E.histolytica. Molecular tools have revolutionized the diagnosis of many parasitic diseases including amoebiasis. Now, polymerase chain reaction (PCR) is the method of choice for clinical and epidemiological studies of E.histolytica. The importance of carrying out a multiplex PCR capable of differentiating between Entamoeba histolytica, Entamoeba dispar and Entamoeba moshkovskii at the species level lies in the fact that although these three species are indistinguishable by stool microscopy, only E.histolytica can cause invasive amoebiasis and needs to be treated. Several PCR assays designed to differentiate E. histolytica from E. dispar have been described. Most of them targeted either the small subunit ribosomal RNA gene or specific episomal repeats of the species.

Our center, JIPMER is one of the very centers in the country which carry out multiplex PCR to differentiate among the different species of Entamoeba. Our center was the first to detect E.histolytica DNA in urine by nested multiplex PCR targeting the 16S like rRNA gene.Thus it reported that kidney barrier is permeable to E.histolytica DNA which is excreted in urine and that this can be used as a prognostic marker to access the course of the disease following therapy. The Loop-mediated Isothermal Amplification (LAMP), first developed by Notomi et al in 2000 amplifies DNA with high specificity, efficiency and rapidity under isothermal conditions. The LAMP technique employs 4 specially designed primers that recognise 6 distinct sequences on the target DNA. It has been found that levels of sensitivity and specificity of the LAMP assay is similar to those of nested PCR and can be useful for clinical detection and active surveillance of E. histolytica parasites in countries where amebiasis is endemic. To date DNA microarray (also commonly known as DNA chip or biochip) are the most popular methodology to determine genome-wide expression profile (transcriptome). DNA microarrays represent a very versatile tool that allows measuring mRNA expression of large numbers of genes simultaneously. Microarrays technology has been successfully applied in E. histolytica to obtain transcriptional profiling in several conditions, including virulence, stress and DNA damage, during cyst-trophozoites conversion and to evaluate the role of epigenetic control on gene expression.The newer tools of genomics and proteomics have helped in making rapid strides in gaining knowledge about the evolution of various species of Entamoeba and the virulence of E.histolytica. It is expected that these tools will prove helpful in evaluating newer vaccine candidates for this important public health problem.