

ORAL PRESENTATION

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Generation of differentially regulated genes in *Mycobacterium tuberculosis* isolates from cerebrospinal fluid and respiratory secretions using suppression subtractive hybridization

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Background

Suppression Subtractive Hybridization (SSH) is an established method for separating DNA molecules that distinguish two closely related DNA samples, which has become a powerful method for generating subtracted cDNA libraries. In this study, we postulated that SSH could be used to identify significant genetic differences between *Mycobacterium tuberculosis* causing meningitis and *M. tuberculosis* causing pulmonary tuberculosis.

Hypothesis

The unique DNA patterns present only in cerebrospinal fluid (CSF) TB isolates might be markers for neurotropism and neurovirulence in some strains of *M. tuberculosis*.

Methods

Bacterial DNAs were isolated and SSH was performed using PCR-Select Bacterial Genome Subtraction Kit (Clontech). Twenty subtracted DNAs were generated from 3 CSF isolates and 3 respiratory isolates. Molecular cloning was done on the subtracted DNAs and the positive isolates were extracted, purified and sequenced for analysis.

Results

So far, 9 of the subtracted DNAs have been successfully cloned into pGEM-T vector, resulting in 21 positive clones. Two preliminary BLASTP results revealed several mycobacterial PE/PPE proteins which play a role in the evasion of host immune responses, possibly via antigenic

variation, together with conserved hypothetical proteins with unknown function.

Conclusion

Further studies are needed to determine the role of these subtracted DNAs.

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