Proteins purified from Mycobacterium tuberculosis MDR and Susceptible clinical isolates: Identification by proteomics approach

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ABSTRACT

Aims and objectives: Tuberculosis (TB) remains a major global health problem. The incidence of multidrug-resistant tuberculosis (MDR-TB) has increased in recent years. To combat the disease, novel intervention strategies effective against drug-resistant and susceptible sub-populations of Mycobacterium tuberculosis are urgently required as adducts in present treatment regimen. This study has employed a proteomic approach, which is a direct method, to identify proteins from resistant M. tuberculosis isolates compared with sensitive isolates.

Methods: The clinical isolates M. tuberculosis strains were provided by the TB bank of Pasteur Institute of Iran. The bacilli were cultured on 7H9 broth with ADC enrichment at 37 °C for 3–4 weeks (late log phase). Cells were washed with PBS, suspended in sonication buffer (10 mM DTT, 1 μg/ml DNase, 100 mM Glycerol, 10 mM Triton X100, 20 mM EDTA, 50 mM Tris-HCl, 1 mM PMSF, 0.02% sodium azide, pH 7.4) and then were subjected to sonication for 1 h at 50 Hz, in ice, and subsequently centrifuged at 5000 rpm for 45 min at 4 °C. Proteins were precipitated by adding refrigerated ethanol or saturated ammonium sulfate to the supernatant. Pellet resuspended in PBS and then dialyzed for 24 h against PBS pH 7.4.

IEF was carried out using the Ettan IEFphor 3 isoelectric focusing system. Immobilized pH gradient IPG strips of pH 4–7 and length 11 cm were rehydrated overnight at 20 °C with 500 μg protein which was mixed with rehydration buffer. Proteins were separated in second dimension on 12% SDS-PAGE in a vertical electrophoretic dual gel. 2D gels were analyzed using Image Master, Melanie 7.0 software. Mass spectrometry was performed using Autoflex II TOF/TOF. Protein sequences were retrieved from Tuberculist server hosted by Pasteur Institute, Paris.

Results: Mass spectrometry and bioinformatic characterization of both drug-resistant and sensitive isolates of M. tuberculosis showed that the majority of commonly expressed/upregulated proteins belonged to the cellular metabolism and respiration category.
(Rv0866, Rv3057c, Rv3248c, Rv1133c, Rv0462, and Rv1876). One hypothetical protein (Rv2744c) and two membrane and cell wall fraction proteins (Rv0379, Rv1886c) were identified. Protein spot 1 was 60 kDa chaperonin-2/GroEl-2 (Rv0440). This essential gene prevents misfolding and promotes the refolding and proper assembly of unfolded/misfolded polypeptides generated under stress conditions. Further, another spot Rv2013c (HSP16.3/HSPX) has been shown to be induced under oxygen-deficient conditions. Its role in maintenance of long-term viability during latent, asymptomatic infections and in replication during initial infection has also been proposed.

Conclusion: Such information could be helpful for the development of newer therapeutic agents or of diagnostic markers for better treatment or diagnosis of TB. This study extends the list of the potential determinants of differences in virulence between the two isolates (MDR and susceptible TB) and adds to the current understanding of MTB pathogenesis.

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