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Commentary

Proteomics of multidrug resistant *Mycobacterium tuberculosis* clinical isolates: A peep show on mechanism of drug resistance & perhaps more

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*Mtb*) has emerged as one of the deadliest of bacterial communicable diseases faced by mankind. According to the 2014 WHO report, nine million new cases of TB and 0.36 million deaths including HIV positive cases were recorded[1]. Despite the availability of antibiotics, childhood vaccine and advances in understanding the biology of *Mtb* and the host immune responses to this stealth pathogen, this *Mycobacterium* continues to present an insurmountable challenge. Emergence of drug resistant strains of *Mtb* threatens the effectiveness of the disease control programmes in India and other countries[2]. *M. tuberculosis* lacks resistance plasmids and does not acquire drug resistance through horizontal gene transfer. Intrinsic drug resistance is primarily due to the limited penetration of the thick, lipid rich cell wall of *Mtb* by the antibiotics. While the mechanism of multidrug resistance remains elusive, recent evidences suggest that multiple mechanisms are exploited by the bacterium[3]. Moreover, drug modifying or inactivating enzymes and efflux pumping mechanisms have also been reported[3-5]. Clinically relevant resistance to *Mtb* results from the acquisition of spontaneous mutations including novel mutations in *rpoB, katG*, *inhA, gyrA* and *gyrB* loci and the selection of the mutants by the drug treatment to which the resistance has originated[6,7]. A better understanding of the mechanistic basis of drug resistance will be potentially important in designing new treatment strategies for multidrug resistant (MDR)-TB.

The article by Singh *et al*[8] in this issue identifies important changes in the proteome of multidrug resistant tuberculosis caused due to non-compliance with the standard chemotherapy. The authors performed proteomic analysis of the sequential clinical isolates of *Mtb* from a 22 yr old "treatment non-compliant" male patient and identified 27 proteins that were upregulated in the drug resistant clinical isolates. Importantly, Singh *et al*[8] observed that five different protein spots representing chaperonin protein dnaK HSP70 (2 spots), hypothetical protein Rv2004, antigen 84 (wag31) and bfrA were consistently upregulated in all the three drug resistant isolates examined. Among the 27 proteins up-regulated in drug resistant isolates, about 30 per cent of the proteins have roles in intermediary metabolism and respiration function, as per TubercuList database (*http://tuberculist.epfl.ch/*). Expectedly, the protein levels of drug pressure responsive chaperonin proteins dnaK HSP70 (Rv350) and groEL2 (Rv440) were elevated in the drug resistant isolates[9]. *M. tuberculosis* aconitase (Rv1475c), another protein identified by Singh and his colleagues, was earlier shown to be a bifunctional enzyme[10] with roles in tricarboxylic acid (TCA) cycle, and also displayed the ability to respond to iron levels by binding to both mycobacterial and mammalian transcripts harbouring iron responsive elements. Interestingly, their observation that Rv1475c protein is specifically over-expressed in *Mtb* clinical isolate resistant to rifampicin, isoniazid, ethambutol and kanamcyin points to a link between iron homeostasis and development of MDR in tuberculosis[11]. The glycogen accumulation regulator protein GarA, another protein found to be upregulated in drug resistant isolates by Singh *et al*[8], was earlier reported to have a key role in TCA cycle and glutamate metabolism and was required for intracellular growth of *Mtb* in macrophages[12]. Consistent with the elevated GarA levels, the glycogen levels in all the three drug resistant isolates were enhanced after seven days of culture. *Mtb* growth regulator, Wag31 (Rv2145c), also found to be overexpressed at both transcript and protein levels in drug resistant clinical isolates, was consistent with a previous study[13]. The observed difference in mobility of Wag31 between sensitive and resistant isolates points to the role of post-translational protein modifications.

Another important aspect of this study was that drug resistance information was derived from the proteomic analysis of sequential clinical isolates from a single tuberculosis patient with increasing resistance to standard chemotherapy. Such analysis is likely to provide more realistic information than data obtained from the drug resistant strains developed in the laboratory. This will also help identify the mutations acquired by *Mtb* due to increasing drug resistance rather than merely highlighting the differences caused due to genotype-environment interaction as in the case of drug resistant *Mtb* isolates from multiple individuals. While the identification of eight hypothetical proteins overexpressed in drug resistant clinical isolates is significant, ascribing a function(s) to them, particularly in the context of development of multidrug resistance, would have important implications.

The data presented in this study should be interpreted with circumspect. As rightly acknowledged by the authors, the overexpression of several proteins observed in this study may not be exclusively due to the increasing drug resistance but could be a result of host specific stress or extended growth period in the patient or a combination of both. The overexpression of chaperonin protein dnaK HSP70 lends support to such an argument. The technical problems, including sensitivity and resolution issues, encountered by these investigators also raise questions about the truly representative proteome profile of the MDR isolates.

Considering the growing incidences of extensively drug resistant (XDR)-TB in India, it is imperative to carry out more such studies with sequential multidrug resistant clinical isolates, for such information will likely not only provide important mechanistic cues for the development of novel anti-tuberculosis drugs in future but could also serve as potential biomarker for drug resistance and dormancy.

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References

1. World Health Organization. Global tuberculosis, report 2014, WHO/HTon/TB/2014.08. Available from: *www.who.int/tb/publications/global_report/en/*, accessed on December 27, 2014.
2. Gandhi NR, Nunn P, Dheda K, Schaaf HS, Zignol M, van Soolingen D, et al. Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. *Lancet* 2010; *375* : 1830-43.
3. Muller B, Borrell S, Rose G, Gagneux S. The heterogeneous evolution of multidrug-resistant *Mycobacterium tuberculosis*. *Trends Genet* 2013; *29* : 160-9.
4. Gupta AK, Chauhan DS, Srivastava K, Das R, Batra S, Mittal M, *et al.* Estimation of efflux mediated multi-drug resistance and its correlation with expression levels of two major efflux pumps in mycobacteria. *J Commun Dis* 2006; *38* : 246-54.
5. Siddiqi N, Das R, Pathak N, Banerjee S, Ahmed N, Katoch VM, *et al. Mycobacterium tuberculosis* isolate with a distinct genomic identity overexpresses a tap-like efflux pump. *Infection* 2004; *32*: 109-11.
6. Siddiqi N, Shamim M, Hussain S, Choudhary RK, Ahmed N, Prachee, *et al.* Molecular characterization of multidrug-resistant isolates of *Mycobacterium tuberculosis* from patients in North India. *Antimicrob Agents Chemother* 2002; *46* : 443-50.
7. Siddiqi N, Shamim M, Jain NK, Rattan A, Amin A, Katoch VM, *et al.* Molecular genetic analysis of multi-drug resistance in Indian isolates of *Mycobacterium tuberculosis*. *Mem Inst Oswaldo Cruz* 1998; *93* : 589-94.
8. Singh A, Gopinath K, Sharma P, Bisht D, Sharma P, Singh N, *et al.* Comparative proteomic analysis of sequential isolates of *Mycobacterium tuberculosis* from a patient with pulmonary tuberculosis turning form drug sensitive to multidrug resistant. *Indian J Med Res* 2015; *141* : 27-45.
9. Cardoso K, Gandra RF, Wisniewski ES, Osaku CA, Kadowaki MK, Felipach-Neto V, *et al.* DnaK and GroEL are induced in response to antibiotic and heat shock in *Acinetobacter baumannii. J Med Microbiol* 2010; *59* : 1061-8.
10. Banerjee S, Nandyala AK, Raviprasad P, Ahmed N, Hasnain SE. Iron-dependent RNA-binding activity of *Mycobacterium tuberculosis* aconitase. *J Bacteriol* 2007; *189* : 4046-52.
11. Pandey R, Rodriguez GM. A ferritin mutant of Mycobacterium tuberculosis is highly susceptible to killing by antibiotics and is unable to establish a chronic infection in mice. *Infect Immun* 2012; *80* : 3650-9.
12. Ventura M, Rieck B, Boldrin F, Degiacomi G, Bellinzoni M, Barilone N, *et al.* GarA is an essential regulator of metabolism in *Mycobacterium tuberculosis. Mol Microbiol* 2013; *90* : 356-66.
13. Jiang X, Zhang W, Gao F, Huang Y, Lv C, Wang H. Comparison of the proteome of isoniazid-resistant and -susceptible strains of *Mycobacterium tuberculosis. Microb Drug Resist* 2006; *12* : 231-8.