Tuberculosis 115 (2019) 154–160

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: www.elsevier.com/locate/tube

Molecular Aspects

Proteomic changes in *Mycobacterium tuberculosis* H37Rv under hyperglycemic conditions favour its growth through altered expression of Tgs3(Rv3234c) and supportive proteins (Rv0547c, AcrA1 and Mpa)

Jyoti Kundu[a], Arpana Verma[a], Indu Verma[a], Sanjay K. Bhadada[b], Sadhna Sharma[a,*]

[a] Department of Biochemistry, Post Graduate Institute of Medical Education and Research, Chandigarh, India
[b] Department of Endocrinology, Post Graduate Institute of Medical Education and Research, Chandigarh, India

ARTICLE INFO

Keywords:
Hyperglycemia
Mycobacterium tuberculosis H37Rv
Proteome
Tuberculosis

ABSTRACT

Diabetes affects the presentation of tuberculosis including delayed clearance of the bacteria from host cells, however, the molecular changes which help survival of phagocytosed mycobacterium in the diabetic host are still not clear. The effect of *in vitro* high glucose concentrations on the proteome of the phagocytosed mycobacterium isolated from the human monocytic THP1 cell line derived macrophages has been investigated in the present study. Concurrent tuberculosis and hyperglycemia conditions were mimicked by growing *M. tuberculosis* infected THP1 cells under high glucose conditions. Phagocytosed bacilli were isolated 5 days post infection. Proteomics analysis of the isolated bacilli was done by two-dimensional gel electrophoresis followed by mass spectrometry. A total of 224 ± 18 protein spots were obtained out of which 10 were found to be differentially expressed under high glucose concentrations in comparison to normal glucose concentration. Further, identity of all the ten proteins namely Tgs3, Rv0547c, AcrA1, EsxU, Rv2219, Mpa, Rv2308, ORN, LucA, and Rv1414 was elucidated by peptide mass finger printing using Matrix-assisted laser desorption and ionization-mass spectrometry (MALDI/MS) assisted with MASCOT software. Though Tgs3, Rv0547c, AcrA1 and Mpa proteins have been demonstrated to play a major role in lipid metabolism under nitric oxide stress conditions, the functional role of rest of the differentially expressed proteins remains to be elucidated. Under hyperglycemic conditions in the host cells, differential expression of these proteins might help in the better survival of mycobacteria and can further act as suitable targets to design novel drugs for more effective therapy for comorbid tuberculosis and diabetes.

1. Introduction

Mycobacterium tuberculosis is considered to be one of the nearly perfect pathogens. It resides in the phagosomal compartments of host macrophages defying the host's immune response [1] and can switch between asymptomatic i.e. metabolically inert latent form to metabolically active disease-causing form. It is a well-known fact that diabetes is one of the important risk factors for the activation of latent tuberculosis [2]. No alternative treatment is available for the co-pathogenesis to avoid the extended chemotherapy regimen. Therefore, it becomes important to find out the new drug targets that can help in better clearance of the pathogen during this co-pathogenesis. Intracellular adaptation of this pathogen in diabetes can be interpreted with the detailed analysis of mycobacterial proteome under high glucose conditions which mimics the hyperglycemia prevailing in a diabetic host. A recent study has revealed several phagocytosed mycobacterial factors which might play a crucial role in co-pathogenesis of TB and HIV by modulating the host environment thus leading to increased viral load in the host which explains synergism between two diseases [3]. Upon phagocytosis, several molecular switches are turned on and off as a protective strategy by *M. tuberculosis*. Intracellular growth of mycobacterium can occur because of failure of phagosome lysosomal fusion [1]. In order to adapt to the intracellular conditions, *M. tuberculosis* survives and grows inside the phagosomes. Recently it has been shown that Serine/threonine protein kinases *PknL* play major role in the adaptation of *M. tuberculosis* to survive inside the cells by slowing its growth [4]. Mycobacterium thrives well in the intraphagosomal acidic environment of macrophage probably by developing some acid resistance mechanism through intrabacterial pH maintenance system [5]. Monahan et al., 2001 utilized the proteomics approach and identified altered expression of several mycobacterial proteins like 16 kDaα-crystallin, GroEL-1 and GroEL-2, a hypothetical

* Corresponding author.
E-mail address: sadhnabiochem@gmail.com (S. Sharma).

https://doi.org/10.1016/j.tube.2019.03.006
Received 10 January 2018; Received in revised form 16 March 2019; Accepted 18 March 2019

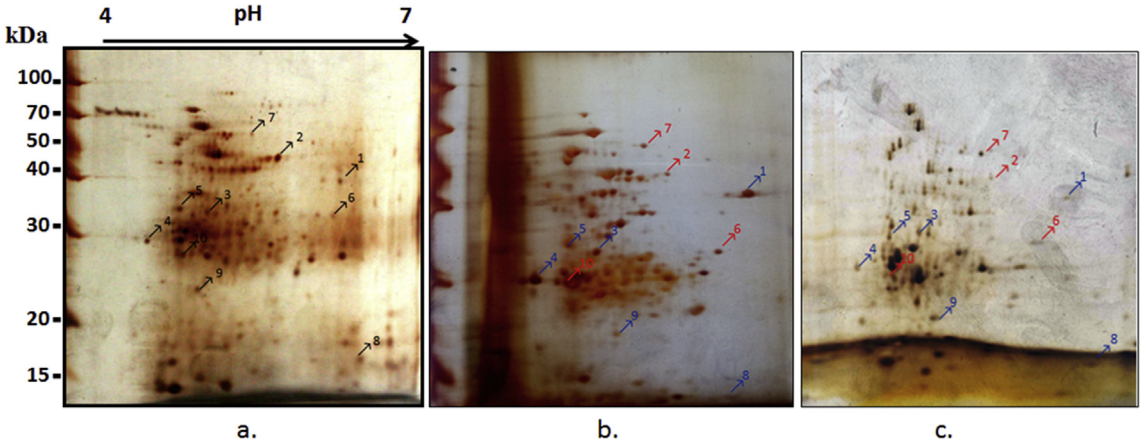

Fig. 1. 2D-PAGE images of the phagocytosed *M. tuberculosis* H37Rv. *M. tuberculosis* H37Rv were isolated from infected macrophages grown under normoglycemic and hyperglycemic conditions. 2D-PAGE of phagocytosed *M. tuberculosis* proteins isolated from macrophages grown in the presence of 5.5 mM glucose (normal glucose) (a), in the presence of 15 mM glucose (high glucose) (b), and in the presence of 25 mM glucose (high glucose) (c). Marked spots indicated by arrows depict the position and identity of the differentially expressed proteins as detected by software analysis. Spots marked with red coloured arrows indicate downregulated proteins and blue arrows indicate upregulated proteins. Marked spots were selected for identification by MALDI-TOF mass spectrometry. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

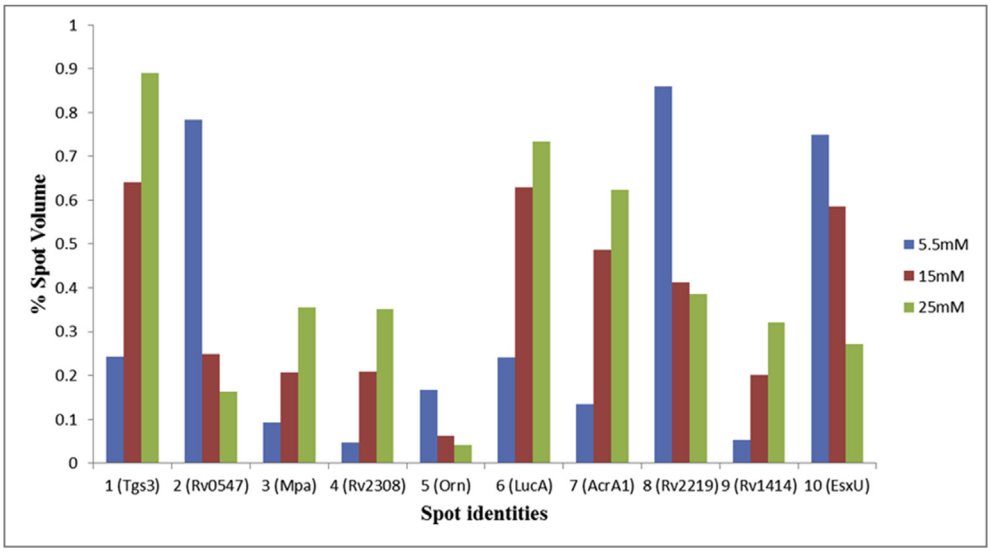

Fig. 2. Histogram of spot intensity of proteins extracted from phagocytosed *M. tuberculosis*. Spot intensities are expressed as %spot volume. These spots were found to be differentially expressed in phagocytosed *M. tuberculosis* under high glucose conditions (15 mM and 25 mM) as compared to normal glucose conditions (5.5 mM).

protein (Rv2623), InhA and elongation factor Tu upon phagocytosis by the macrophages [6]. In the present study, proteomic analysis was carried out in the phagocytosed mycobacterium under high glucose conditions as compared to normal glucose conditions in THP1 monocytes derived macrophages. This cell line was chosen because it mimics the human alveolar macrophages [7]. Proteomics of mycobacterium in diabetes like conditions using 2DE-MALDI/MS approach and classical BLAST database search was studied. Expression of few proteins was found to be differentially regulated which could be responsible for the better adaptation of mycobacterium under hyperglycemia in a diabetic host in the comorbid tuberculosis and diabetes cases.

2. Materials and methods

2.1. M.tuberculosis H37Rv and THP1 cell line culture and maintenance

M.tuberculosis H37Rv (NCTC-7416) originally procured from National collection of type culture, London, UK was cultured in Sauton's media under shaking conditions at 150 rpm and maintained in sterile Lowenstein Jensen medium. *M. tuberculosis* cell suspension was passed 5–6 times through 26G needle in order to disperse the bacterial cells and enumerated by comparing with Mac-farlands standards. Bacilli were further preserved at −80 °C as 20% glycerol stocks and used directly for inducing the infection. THP1 cells (procured from National Centre for Cell Sciences, Pune, India; cell line repository) were cultured in suspension phase in antibiotic free FBS supplemented RPMI1640 medium at a density of 2×10^6 cells/mL under 5%CO_2 in humidified incubator at 37 °C. THP1 monocytes were induced to macrophages with 20 nM (12 ng/ml) phorbol 12-myristate13 acetate (PMA) for 24 h in antibiotic-free RPMI medium. After 24 h induction, medium was replaced with fresh antibiotic-free RPMI media with different glucose concentrations (5.5 mM = 100 mg/dL, 15 mM = 270 mg/dL, and 25 mM = 450 mg/dL) to mimic the normoglycemic (5.5 mM) and intermediate hyperglycemic (15 mM) and uncontrolled hyperglycemic (25 mM) conditions [8,9]. All the three groups of cells were simultaneously infected with *M. tuberculosis* H37Rv (2×10^7 cells/mL, MOI = 10 bacilli per cell) and then further incubated for 12 h. In order to kill the non-phagocytosed extracellular mycobacteria, after 12 h of infection amikacin (50 μg/mL) was added to the medium for 2 h and then replaced with fresh glucose containing RPMI. Further, cells were incubated for 5days in humidified CO_2 incubator [10].

Table 1
Identification of the differentially expressed spots by MALDI/MS.

Spot No.	Protein	Mr	pI	Sequence coverage %	Mascot score	Function and Cellular location	% Sequence similarity with *M.tuberculosis H37Rv*
1	Tgs3(Rv3234c)	28.9	6.2	38	31	Triacylglycerol synthesis and accumulation, better survival under hypoxic and NO stress conditions [15], Transmembrane protein	100%
2	Rv0547c	37.83	5.1	39	44	Possible Oxidoreductase, Transmembrane protein	100%
3	Mpa(Rv2115c)	28.36	4.2	13	44	Ubiquitin-like protein involved in the proteasome pathway of *Mycobacterium tuberculosis*, plays role in resistance to RNI produced by macrophages and other cell types [16,17], Transmembrane protein	100%
4	Rv2308	26.67	4.2	23	26	Uncharacterized protein, Function not known	100%
5	Orn	23.42	4.3	7	35	Intermediary metabolism and respiration, Cytoplasm	100%
6	LucA(Rv3723)	27.35	6.8	9	47	Required for the import of both fatty acids and cholesterol during growth in macrophages [18], Transmembrane protein	100%
7	AcrA1 (Rv3391)	63.3	5.8	17	21	Short chain dehydrogenase, Virulence, detoxification and adaptation to stress, Cytoplasm	100%
8	Rv2219	11.06	4.9	66	50	Function not known, Transmembrane protein	100%
9	Rv1414	14.3	4.7	12	25	Uncharacterized transmembrane protein, Function not known	100%
10	EsxU(Rv3445c)	8.3	4.9	32	18	Putative role as virulence factor, Cytoplasm	100%

2.2. Isolation of phagocytosed mycobacteria

After 5 days of incubation, phagocytosed mycobacteria were isolated from THP1 cells by lysing with 0.5% SDS-PBS at 37 °C for 10 min and centrifugation at 5000 × *g* for 20min. Pure mycobacterium pellet was obtained by washing the pellet 4–5 times with 0.1%SDS, 0.1%Tween 80-PBS.

2.3. CFU enumeration

Human monocytic leukemic cell line THP1 derived macrophages grown at a density of 1×10^5 cells/well in six well plates were infected with *M. tuberculosis H37Rv* at a multiplicity of infection 10 bacilli per macrophage (MOI 10:1) and media was simultaneously replaced with three different concentrations of glucose i. e 5.5 mM = 100 mg/dL, 15 mM = 270 mg/dL, and 25 mM = 450 mg/dL in triplicate. After 12 h of macrophage infection with *M. tuberculosis* H37Rv, cells were washed to remove the unattached bacilli and fresh media was added with the respective glucose concentrations. Macrophages from each group were lysed with 0.1% SDS in PBS at 12 h, 24 h and 48 h post infection and plated on 7H11 OADC supplemented agar plates for CFU enumeration. CFUs enumerated for 12 h post infection indicated the number of bacilli initially phagocytosed by cells under different glucose concentrations. The intracellular replication of bacilli was assessed by CFU enumeration at 24 h and 48 h post infection.

In vitro study was performed to demonstrate the lipid accumulation by nile red staining. Briefly, log phase *M. tuberculosis* H37Rv was cultured in Sauton's media supplemented with different glucose concentrations (5.5 mM, 15 mM and 25 mM) till 5days. After 5 days, bacilli were harvested, fixed and Auramine-O/Nile Red dual fluorescent staining was performed [11]. Cells were visualized under fluorescent microscope at 100× at excitation/emission of 460/520 nm for auramine and 550nm/650 nm for nile red.

2.4. Preparation, quantification and purification of mycobacterial proteins

Cells were lysed and proteins were isolated according to the recommended protocol with slight modifications [12]. Briefly, mycobacterial pellet was suspended (0.2 g/ml) in lysis buffer (50 mM Tris/HCl, pH 7.4 with 10 mM $MgCl_2$, 1 mM PMSF and 1 mM EGTA) and sonicated for 30min intermittently with 30s ON and 30s OFF cycle on ice. Lysates were clarified by centrifugation at 12,000 × *g* for 30 min. Protein concentration was estimated in the supernatant using protein estimation kit from Pierce. Phagocytosed mycobacterial proteins (100 μg) were purified and precipitated by Bio-Rad protein purification kit. The protein pellet thus obtained was dissolved in two-dimensional rehydration buffer (8 M Urea, 2% CHAPS, 0.002% Bromophenol Blue, 3 mg/ml dithiothreitol (DTT) and 1 μL IPG carrier buffer (pH4-7).

2.5. Two-dimensional gel electrophoresis (2-DE)

Proteins were focused on the IPG strips using in-gel rehydration method [13] with slight modifications to a total voltage of 30 kVh. Each protein sample was run in triplicate. After equilibration with dithiothreitol and Iodoacetic acid (IAA), second dimension was run on 12% SDS-PAGE at constant voltage of 150 V for 3–4 h. Gels were stained with silver nitrate to visualize protein spots. Images of gels were acquired by Chemidoc (BIO-RAD) using Quantity One software (BIO-RAD, Hercules, CA, USA). Gel Images were analyzed using Image master Platinum6 software from GE Healthcare. For this, data were normalized by expressing protein abundance as percent spot volume relative to volume of total protein in the gel (%spot volume). Gel images were also manually checked for artifactual, merged, and missed spots. An equal amount of protein was loaded in all the gels. Differentially expressed proteins in terms of %spot volume that showed a change of at least 1.5 fold in comparison to normal glucose concentration group were

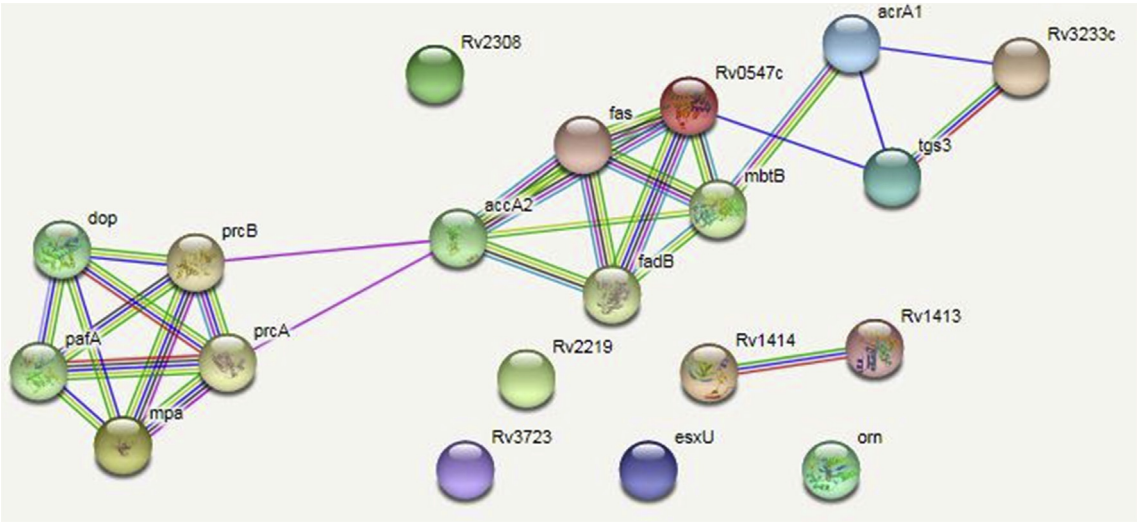

Fig. 3. String analysis showing the interaction network between the identified differentially expressed spots. Nodes in the image show modulated proteins and their predicted functional partner proteins. Edges represent protein-protein interactions in terms of shared functions and necessarily do not mean physical binding of the proteins. Each edge colour represents different type of interaction between two proteins (Light blue colour for known interactions from curated databases, Pink for known experimentally determined interactions, Green for gene neighbourhood, red for gene fusions, blue for gene co-occurrence, yellow for text mining, black for co-expression, and grey for protein homology). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

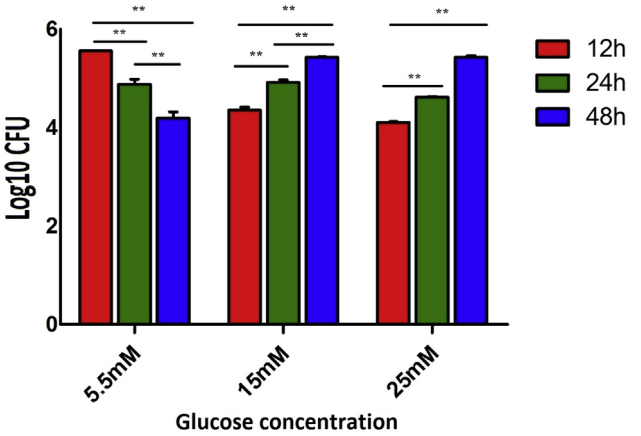

Fig. 4. Log_{10} CFUs of *M. tuberculosis* H37Rvin the THP1 macrophages under different glucose conditions at 12, 24, and 48 h post infection. Results are mean ± SD of 3 independent experiments at each time point.$^{**}p < 0.01$ indicates significant variation in CFUs at 24 h and 48 h in comparison to initially phagocytosed mycobacteria (12 h) at the respective glucose concentrations.

identified and functionally annotated by mass spectrometry.

2.6. In-gel trypsin digestion and peptide mass finger printing

Differentially expressed protein spots were manually picked and in-gel trypsin digestion was carried out [14]. Further, digested samples were desalted and concentrated using C-18 ZipTips (Millipore, Billerica, MA, USA). The desalted purified peptide solution thus obtained was mixed with an equal volume of matrix (a saturated a-cyano-4-hydroxy cinnamic acid solution in 50% acetonitrile, 0.3% TFA). Two microliter of the mixture was applied to the MALDI-target plate. Mass spectra were acquired using Autoflex II TOF/TOF 50 (BruckerDaltonic GmbH, Leipzig, Germany) in positive reflectron mode, in detection range of 500–3000 m/z. External calibration to a spectrum, acquired for a mixture of peptides with masses ranging from 1046 to 2465Da was done prior to acquisition. Peak detection of proteins was done by analyzing the proteolytic masses through Flex Analysis v.2.4 programme. Further peak analysis was done by Mascot software (Matrix Science, U.K). Peptide mass tolerance was set to 100 ppm with carbamidomethylcystein set as fixed modification, oxidation of methionine as variable modification and one missed cleavage site was allowed. The peptides with high signal to noise ratio were subjected to MS/MS analysis and confirmed by matching with *Mycobacterium tuberculosis* complex database.

2.7. Bioinformatics analysis of the identified proteins

The proteins identified by MS/MS analysis were further analyzed for their known functions, cellular location and interaction with other proteins by searching BLASTp, Uniprot, Swissprot, NCBIprot, Tuberculist and STRING databases.

2.8. Statistical analysis

All experiments were performed in triplicate. Two way ANOVA was used to determine the level of significance by using the GraphPad Prism package, version 5.0 (GraphPad Software, San Diego, USA).

3. Results

3.1. Ex-vivo THP1 macrophage model of tuberculosis and hyperglycemia

In order to find out the signature proteins from the phagocytosed *M. tuberculosis* H37Rv in concurrent tuberculosis and diabetes conditions, *ex-vivo* THP1 macrophage model of tuberculosis and hyperglycemia was developed. Cells were found to be adherent and morphology was normal till 5days of infection. THP1 cell viability was never found to be below 90% under experimental conditions. THP1 monocyte derived macrophages were exposed to *M. tuberculosis* infection and different glucose concentrations i. e 5.5 mM glucose (normal glucose concentration), 15 mM and 25 mM glucose (high glucose concentration) and the isolated proteins from phagocytosed mycobacteria were processed for comparative 2DE-PAGE analysis.

3.2. Computational analysis of the 2D-PAGE images of phagocytosed mycobacterial proteins

Comparative phagocytosed mycobacterial proteome analysis was

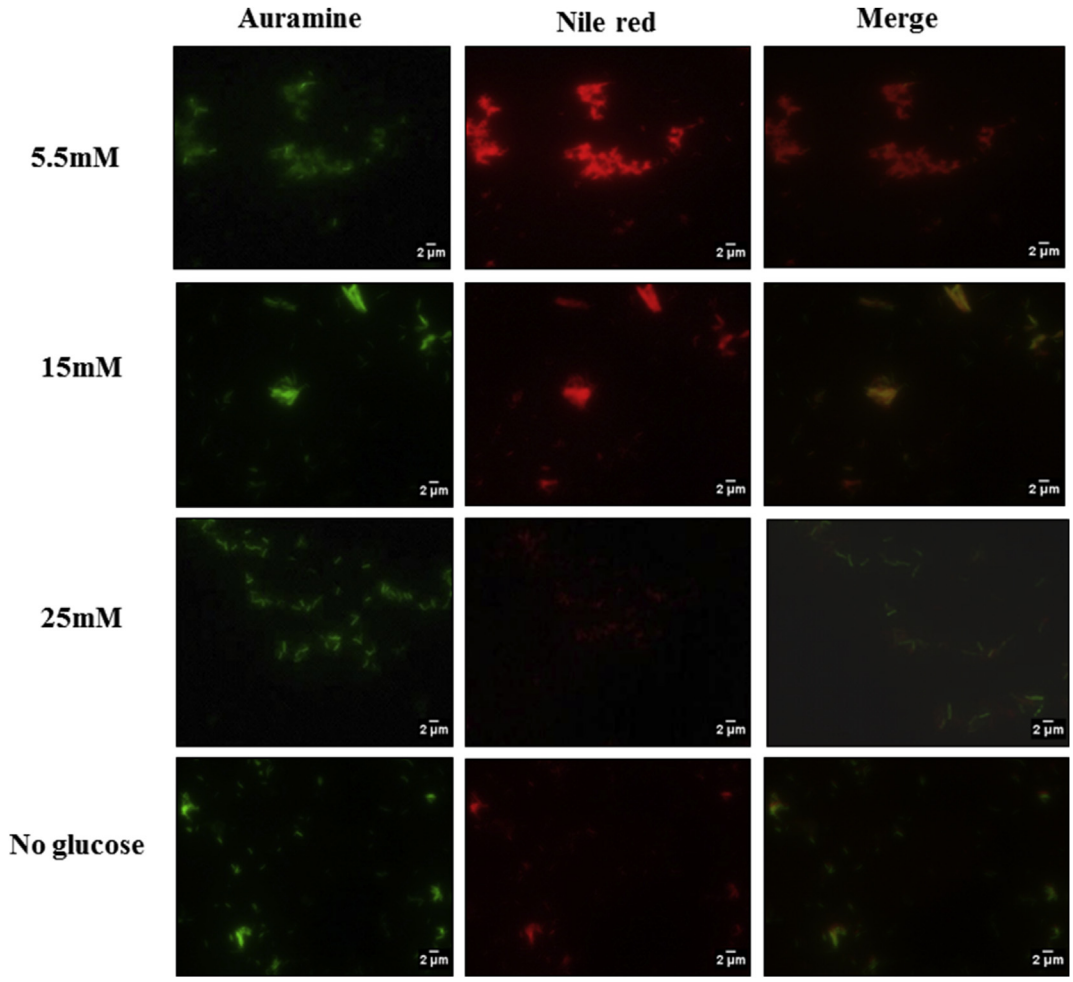

Fig. 5. Representative images of Auramine O/Nile red staining of *M. tuberculosis* H37Rv in the presence different glucose concentrations. *M. tuberculosis* H37Rv was cultured *in vitro* supplemented with different glucose concentrations (no glucose, 5.5 mM, 15 mM and 25 mM) till 5days after which bacilli were stained with Auramine-O/Nile Red and visualized under fluorescent microscope at 100×. Bar = 2 micrometer for each image. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

done within three groups based upon the glucose concentration (5.5 mM, 15 mM, and 25 mM) in which THP1 derived macrophages mimicked the only TB and TBDM conditions. A total of 224 ± 18 spots were identified on the 2D gel after elimination of artifacts and merged spots. Under both the high glucose conditions (15 mM and 25 mM) in comparison to normal glucose conditions (5.5 mM), expression of 10 protein spots was found to be modulated (Fig. 1) in terms of spot intensity (represented as %spot volume of at least > 1.5 fold difference) in 2D gels. Six proteins were upregulated (Tgs3, Mpa, Rv2308, LucA, AcrA1 and Rv1414) and 4 proteins (Rv0547c, Orn, Rv2219 and EsxU) were downregulated in the presence of high glucose concentrations in comparison to normal glucose conditions. Effect of hyperglycemia in terms of protein expression modulation was more pronounced in terms of both over and underexpression between normal and high glucose conditions, but the difference of spot intensities between 15 mM and 25 mM hyperglycemia was less than 1.5 fold, thus non-significant. Fig. 2 represents the histogram of the percentage spot volume of each over-/underexpressed protein at both the high glucose concentrations as compared to normal glucose conditions.

3.3. Identification, functional annotations and BLASTp search of the differentially expressed protein spots

BLASTp was performed to get the percentage sequence similarity with *M. tuberculosis* H37Rv. The identified proteins showed 100% similarity with *M. tuberculosis* H37Rv. Functional characterization of all the proteins and their subcellular localization was performed using Tuberculist, Swissprot, Uniprot and NCBIprot databases. Table 1 shows the details of the identified spots in terms of protein names with molecular weights (Mr), isoelectric points (pI), percentage sequence coverage of the peptide ions, Mascot score, protein functions with their cellular location and percentage sequence similarity of the identified peptides with the *M. tuberculosis* H37Rv.

3.4. In silico protein-protein interaction of the identified protein spots

The STRING database was used to analyze the networks of the protein-protein interaction among the characterized proteins (Fig. 3). Out of the ten identified proteins Tgs3, AcrA1, Rv0547c, and Mpa were found to be functionally interacting as depicted by bold blue lines. These four proteins are known to help *M. tuberculosis* H37Rv survive under nutrient starvation and excess of reactive nitrogen intermediates in the surroundings. Remaining proteins Rv2219, EsxU(Rv3445c), Rv2308, Orn, Rv3723(LucA) and Rv1414 were not connected to these proteins through the known genomic or functional interactions. Out of these, LucA(Rv3723) protein is known to coordinate import of both fatty acids and cholesterol during growth in macrophages [18] and EsxU(Rv3445c) is known to modulate host immune response to the mycobacterium [19].

3.5. Intracellular mycobacterial growth in infected macrophages

Initial phagocytosis by the macrophages under high glucose concentration was observed to be decreased in comparison to normal glucose conditions. However, intracellular replication of bacilli was observed to be increased in the presence of high concentration of glucose (both 15 mM and 25 mM) as compared to normal glucose concentration (5.5 mM) at 24 h and 48 h post infection (Fig. 4). Further, *in vitro* nile red staining of mycobacteria grown in the presence of different concentrations of glucose indicated relatively less staining of the cells with the dye at higher glucose concentrations (15 mM and 25 mM) as compared to normal (Fig. 5). These findings reflect utilization of lipids as energy source for cell replication in the presence of higher concentration of glucose.

4. Discussion

In vivo proteomic studies of the bacteria during infection of the host can provide insight into the proteins relevant to intracellular survival and manipulation of host cell. Due to differences in the clinical presentation of TB while in co-pathogenesis with diabetes, it is expected that *M. tuberculosis* might have developed some adaptive protein modifications under the dual pathogenesis conditions within the host macrophages. Using the gel-based proteomics approach, out of ten proteins, four (Tgs3, AcrA1, Rv0547c and Mpa) were found to be interacting at genomic and functional level as predicted by STRING analysis. Tgs3(Rv3234c), a trans membrane protein is is one of the 15 Tgs proteins that has a crucial role in triacylglycerol synthesis and its accumulation. It is also required for better survival of *M. tuberculosis* H37Rv under hypoxic and NO stress conditions [15,20,21]. It was found to be upregulated under high glucose conditions in comparison to the normal conditions in the present study. High glucose has also been reported to favour the accumulation and synthesis of cholesterol in the THP1 cells leading to formation of foam cells [22]. Another protein identified as Mpa-ATPase, which is a proteasome associated ATPase along with another protein Rv2097c(PafA) confer defense mechanism against lethal effects of nitrosative and oxidative stress by activated macrophages and other cell types [16,17,23]. This protein was found to be upregulated under high glucose conditions in comparison to normal glucose conditions thus providing support for the better survival of *M. tuberculosis* under high glucose conditions as represented by increased CFU count as well. MT0572(Rv0547c), a possible oxidoreductase is known to be functionally associated with Tgs3 protein and another hypothetical protein Rv3740c which is known to have triacylglycerol synthase function and is involved in the synthesis of triacylglycerols from diole in and long-chain fatty acyl-CoA in *E. coli*. Also it functions as a wax synthase, as it incorporates palmityl alcohol into wax esters in the presence of palmitoyl-CoA (Tuberculist). However, its function in mycobacterial survival is not known. Another protein identified and found to be interacting with Tgs3 and Rv0547c is AcrA1(Rv3391), which is a multi-functional short chain dehydrogenase with fatty acyl-CoA reductase activity in C-terminal part and also known to facilitate accumulation of fatty acids along with other Tgs proteins in mycobacteria. Its expression was observed to be upregulated in the mycobacteria isolated from the macrophages grown under high glucose concentrations. These four proteins known to interact at genomic and functional level (Fig. 3), have roles in fatty acid metabolism and could be responsible for protecting mycobacterium from oxidative and nitrosative stress conditions. Increased Tgs 3 protein expression along with an increase in CFU count under high glucose conditions, are supported by the previous study by Low et al., 2009 showing that replicating bacteria can simultaneously utilize lipids as energy source instead of storing the lipids [24]. Further, Nile red staining of mycobacteria grown *in vitro* under higher glucose conditions (25 mM) showed less accumulation of lipids indicating simultaneous utilization of lipids to provide energy for active replication of bacteria (Fig. 5). Remaining six proteins, namely EsxU, Rv2219, Orn, Rv2308, LucA and Rv1414 were not found to be interacting through STRING pathway to the above mentioned four functionally interactive proteins i.e. Tgs3, AcrA1, Rv0547c and Mpa. But it does not decrease the importance of these proteins. These six proteins may have some significant function in the pathogenesis of *M. tuberculosis* however the functional involvement of these proteins has still not been elucidated in the co-pathogenesis of tuberculosis and diabetes. Based on this study, it can be predicted that these proteins might be involved in essential pathways responsible for survival of the pathogen under co-pathogenesis conditions like synthesis of its energy stores and protection from nitrosative and oxidative stress inside the host macrophages which might be co-related with delayed clearance of *M. tuberculosis* in concurrent tuberculosis and diabetic conditions. Further *in vivo* validation studies at genomic and transcriptomic levels might support our proteomics findings in case of dual pathogenesis of tuberculosis and diabetes.

5. Conclusion

Differential expression of the mycobacterial proteins observed under conditions mimicking tuberculosis and diabetes may help in designing more effective treatment strategies against copathogenesis conditions.

Acknowledgments

Research fellowship from University Grants commission, New Delhi, India is highly acknowledged.

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tube.2019.03.006.

References

[1] Vergne I, Chua J, Singh SB, Deretic V. Cell biology of mycobacterium tuberculosis phagosome. Annu Rev Cell Dev Biol 2004;20(1):367–94. 2004/11.
[2] Jeon CY, Murray MB. Correction: diabetes mellitus increases the risk of active tuberculosis: a systematic review of 13 observational studies. PLoS Med 2008;5(8):e181. 2008/08/26.
[3] Ganji R, Dhali S, Rizvi A, Sankati S, Vemula MH, Mahajan G, et al. Proteomics approach to understand reduced clearance of mycobacteria and high viral titers during HIV-mycobacteria co-infection. Cell Microbiol 2015;18(3):355–68. 2015/10/07.
[4] Refaya AK, Sharma D, Kumar V, Bisht D, Narayanan S. A Serine/threonine kinase PknL, is involved in the adaptive response of Mycobacterium tuberculosis. Microbiol Res 2016;190:1–11. 2016/09.
[5] Vandal OH, Pierini LM, Schnappinger D, Nathan CF, Ehrt S. A membrane protein preserves intrabacterial pH in intraphagosomal Mycobacterium tuberculosis. Nat Med 2008;14(8):849–54. 2008/07/20.
[6] Monahan IM, Butcher PD, Betts J, Banerjee DK. Differential expression of mycobacterial proteins following phagocytosis by macrophages. Microbiology 2001;147(2):459–71. 2001/02/01.
[7] Riendeau CJ, Kornfeld H. THP-1 cell apoptosis in response to mycobacterial infection. Infect Immun 2003;71(1):254–9. 2003/01/01.
[8] Grosick R, Alvarado A, Romero-Sandoval E. (148) Optimizing an in vitro model using THP-1 macrophages to study pain, inflammation, and wound healing in the context of diabetes. J Pain 2017;18(4):S13. 2017/04.
[9] Pal PB, Sonowal H, Shukla K, Srivastava SK, Ramana KV. Aldose reductase mediates NLRP3 inflammasome–initiated innate immune response in hyperglycemia-induced Thp1 monocytes and male mice. Endocrinology 2017;158(10):3661–75. 2017/07/28.
[10] Singhal N, Sharma P, Kumar M, Joshi B, Bisht D. Analysis of intracellular expressed proteins of Mycobacterium tuberculosis clinical isolates. Proteome Sci 2012;10(1):14.
[11] Garton NJ, Christensen H, Minnikin DE, Adegbola RA, Barer MR. Intracellular lipophilic inclusions of mycobacteria in vitro and in sputum. Microbiology 2002;148:2951–8.
[12] Brodie AF, Kalra VK, Lee S-h, Cohen NS. Properties of energy-transducing systems in different types of membrane preparations from Mycobacterium phlei-preparation, resolution, and reconstitution. Biomembranes Part F: Bioenergetics: Oxidative

Phosphorylation: Elsevier; 1979. p. 175–200.
[13] Görg A. High-Resolution two-dimensional electrophoresis of proteins using immobilized pH gradients. Cell Biology: Elsevier; 1994. p. 231–42.
[14] Speicher D. Gel-based proteome analysis. Current protocols in protein science. John Wiley & Sons, Inc.; 2003. p. 22.0.1-.0.2.
[15] Daniel J, Deb C, Dubey VS, Sirakova TD, Abomoelak B, Morbidoni HR, et al. Induction of a novel class of diacylglycerol acyltransferases and triacylglycerol accumulation in Mycobacterium tuberculosis as it goes into a dormancy-like state in culture. J Bacteriol 2004;186(15):5017–30. 2004/07/19.
[16] Gandotra S, Lebron MB, Ehrt S. The Mycobacterium tuberculosis proteasome active site threonine is essential for persistence yet dispensable for replication and resistance to nitric oxide. PLoS Pathog 2010;6(8):e1001040. 2010/08/12.
[17] Burns KE, Liu W-T, Boshoff HIM, Dorrestein PC, Barry CE. Proteasomal protein degradation in mycobacteria is dependent upon a prokaryotic ubiquitin-like protein. J Biol Chem 2008;284(5):3069–75. 2008/11/21.
[18] Nazarova EV, Montague CR, La T, Wilburn KM, Sukumar N, Lee W, et al. Rv3723/LucA coordinates fatty acid and cholesterol uptake in Mycobacterium tuberculosis. eLife 2017:6. 2017/06/27.
[19] Uplekar S, Heym B, Friocourt V, Rougemont J, Cole ST. Comparative genomics of esx genes from clinical isolates of Mycobacterium tuberculosis provides evidence for gene conversion and epitope variation. Infect Immun 2011;79(10):4042–9. 2011/08/01.
[20] Peyron P, Vaubourgeix J, Poquet Y, Levillain F, Botanch C, Bardou F, et al. Foamy macrophages from tuberculous patients' granulomas constitute a nutrient-rich reservoir for M. tuberculosis persistence. PLoS Pathog 2008;4(11):e1000204. 2008/11/11.
[21] Russell DG, Cardona P-J, Kim M-J, Allain S, Altare F. Foamy macrophages and the progression of the human tuberculosis granuloma. Nat Immunol 2009;10(9):943–8. 2009/08/19.
[22] Naito T, Oikawa S, Kotake H, Hayasaka K, Toyota T. Effect of glucose concentration on foam cell formation in THP-1 cells. J Atheroscler Thromb 2001;8(2):55–62.
[23] Darwin KH. The proteasome of Mycobacterium tuberculosis is required for resistance to nitric oxide. Science 2003;302(5652):1963–6. 2003/12/12.
[24] Low KL, Rao PSS, Shui G, Bendt AK, Pethe K, Dick T, et al. Triacylglycerol utilization is required for regrowth of in vitro hypoxic nonreplicating Mycobacterium bovis Bacillus calmette-guerin. J Bacteriol 2009;191(16):5037–43. 2009/06/12.